

EXPERIMENTAL ACTINOBACILLUS SEMINIS MASTITIS IN EWES

by

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A THESIS

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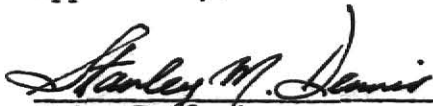
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#### DEDICATION

PRAISE BE TO ALLAH

This thesis is dedicated to my  
parents for their love, good wishes  
and continual encouragement and to  
my family in the Libyan sense.

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| ABSTRACT                                       |      |

## INTRODUCTION

Ovine mastitis occurs in all sheep raising countries and often causes serious losses. It usually appears within a few days to a few weeks after lambing and approximately 5 to 10% of ewes are affected; death is common. In the Mediterranean region, ovine mastitis is economically important. In Egypt, for example, the incidence reported varied from 3.4 to 12.6%.

While bovine mastitis has been extensively studied, ovine mastitis has received little attention. The ewe has, however, been used frequently as a model for investigating various aspects of bovine mastitis. Many infective agents have been incriminated as causes of ovine mastitis and although the agents tend to vary from country to country; Staphylococcus aureus, Pasteurella hemolytica, and Streptococcus agalactiae are common pathogens.

A Gram-negative pleomorphic, facultative, aerobic organism in sheep that still remains to be characterized and classified is believed to be indistinguishable from Histophilus ovis. It has been recovered from cases of suppurative epididymitis in rams, polytenosynovitis and pyemia in lambs, and mastitis in ewes. Further studies are required on the etiology and epizootiology of these conditions.<sup>a</sup>

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<sup>a</sup>Report of the FAO/OIE International Conference on Sheep Diseases, Rome, September, 1966, p. 17.

A similar organism, Actinobacillus seminis, has been recently identified as a cause of ovine epididymitis. A. seminis has also been isolated from fetal membranes, uterus, fetal abomasal contents and from cases of purulent polyarthrititis and posthitis. Experimentally, A. seminis caused acute gangrenous mastitis in lactating ewes, and synovitis and interstitial pneumonia in lambs.

The incidence and significance of A. seminis infection in sheep is unknown. It has been reported from Australia, New Zealand, the United States, and South Africa. Little is known of the sources of infection and means of transmission of A. seminis within or between sheep flocks under natural conditions. Actinobacillosis is considered to be primarily a disease of sexually mature rams manifested by acute to chronic epididymitis. The isolation of A. seminis from semen of a bull with bilateral epididymitis and from an aborted goat has raised the question of the host range of A. seminis and the possible role of these animals in the epizootiology of ovine actinobacillosis.

The role of the ewe in the transmission cycle of A. seminis infection is uncertain. The ewe is considered to play a passive role in venereal transmission but there is a suggestion that there may be ewe-to-ram lamb transmission with infection localizing in a predilection site until sexual maturity. Is the udder a possible source of infection to the neonatal ram?

The objective of this study was to determine the sequential pathological changes in the ovine mammary gland following experimental A. seminis infection.

## REVIEW OF LITERATURE

## Introduction

Mastitis, inflammation of the mammary gland, is characterized by physical, chemical and usually bacteriological changes in milk, and by pathological changes in the glandular tissue. Important milk alterations involve color, consistency, presence of clots, and numerous leukocytes (Blood et al 1979).

The literature on ovine mastitis has been reviewed by Pegrefffi (1963) and Landau and Tamarin (1974). It is usually caused by infective agents and the primary agent is often associated with other microorganisms (Pegrefffi 1963). Generally, there is a degree of correlation between the clinical form and bacterial cause (Jensen 1974). Research on ovine mastitis has been primarily involved etiology and prevention.

Based on cause and epizootiology, ovine mastitis has been classified as primary or secondary (Pegrefffi 1963; Lagneau 1970). Primary mastitis was divided, clinically or etiologically, into seven and secondary mastitis into three (Pegrefffi 1963).

## Etiology

Many infective agents have been incriminated in ovine mastitis (Jensen 1974; Blood et al 1979).

|                                   |                                                                                                                                                           |
|-----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Staphylococcus aureus</u>      | (Nocard 1887; Leyshon 1929; Hauke 1960; Tsonev and Mateev 1961; Saeter and Eieland 1961; Quinlivan 1968; Hungerford 1970; El Etreby and Abdel-Hamid 1970) |
| <u>Pasteurella hemolytica</u>     | (Marsh 1932; Tunnicliff 1949; Tsonev and Mateev 1961; Saeter and Eieland 1961; Quinlivan 1968)                                                            |
| <u>Streptococcus agalactiae</u>   | (Tsonev and Mateev 1961; Saeter and Eieland 1961; Quinlivan 1968; Filev 1973)                                                                             |
| <u>Streptococcus uberis</u>       | (Pisanu and Manca 1964; Filev 1973)                                                                                                                       |
| <u>Streptococcus dysgalactiae</u> | (Filev 1973)                                                                                                                                              |
| <u>Escherichia coli</u>           | (Tsonev and Mateev 1961; Saeter and Eieland 1961; Quinlivan 1968)                                                                                         |
| <u>Histophilus ovis</u>           | (Roberts 1956)                                                                                                                                            |
| <u>Actinobacillus lignieresii</u> | (Laws and Elder 1969)                                                                                                                                     |
| <u>Clostridium welchii</u>        | (Tsonev and Mateev 1961)                                                                                                                                  |
| <u>Corynebacterium pyogenes</u>   | (Marsh 1965; Quinlivan 1968; El Etreby and Abdel-Hamid 1970)                                                                                              |

Corynebacterium pseudotuberculosis

(Hungerford 1970)

Mycoplasma agalactiae(Watson et al 1968)Brucella melitensis

(Pegrefffi 1963; Lagneau 1970)

Mycobacterium tuberculosis

(Pegrefffi 1963; Lagneau 1970)

Nocardia asteroides

(Pegrefffi 1963; Lagneau 1970)

Candida krusei(El-Bahri et al 1970)Cryptococcus neoformans

(Abdallah 1969)

The earliest record of an isolate from ovine mastitis was by Nocard (1887). He recovered a Gram-positive coccus from a case of gangrenous mastitis and named it Micrococcus mastitidis (now known as S. aureus). S. aureus is the major reported cause of ovine mastitis (Hauke 1960; Tsonev and Mateev 1961; Saeter and Eieland 1961; Quinlivan 1968).

The incidence of ovine mastitis is highest around parturition and during the first three weeks postpartum. A significant relationship between stage of lactation and cause was observed in New Zealand (Quinlivan 1968). Around lambing, the predominant causes found in order were S. aureus, streptococci, E. coli, and P. hemolytica but after weaning streptococci, P. hemolytica, C. pyogenes and S. aureus were mainly involved.

In Egypt, S. aureus, C. pyogenes and C. pseudotuberculosis were incriminated as the main causes of ovine

mastitis (El Etreby and Abdel-Hamid 1970). C. pyogenes and C. pseudotuberculosis have been reported elsewhere mainly as causes of udder abscesses (Hungerford 1970; Blood et al 1979). C. pyogenes has been encountered as a secondary invader in cases of chronic pasteurella mastitis (Marsh 1965).

Experimental ovine mastitis has been induced by injection of bacteria such as S. aureus, C. pyogenes and C. pseudotuberculosis (El Etreby and Abdel-Hamid 1970); Actinobacillus seminis (Watt et al 1970); Mycobacterium spp (Richardson 1971); and P. multocida (Dorobantu 1975).

In conclusion, it is evident that a number of micro-organisms are capable of causing ovine mastitis, primarily S. aureus, P. hemolytica, streptococci, and E. coli. In ewes kept for milk, S. aureus is the major cause of gangrenous mastitis. In wool and meat producing breeds, however, P. hemolytica and other bacteria are the major causes (Landau and Tamarin 1974).

#### STAPHYLOCOCCAL MASTITIS

Staphylococcal or gangrenous mastitis is important in all sheep raising countries especially where milk production is a major goal (Tamarin 1972). Gangrenous mastitis may

occur in all sheep breeds especially between lambing and weaning (Jensen 1974).

#### Cause

S. aureus is the cause of most cases of gangrenous mastitis (Jensen 1974). Tamarin (1972) studied various pathobiological properties of 72 strains of S. aureus isolated from cases of ovine mastitis.

#### Pathogenesis

Staphylococcal mastitis occurs primarily when nursing lambs become strong enough to cause trauma to the teats and when ewes are returned to pastures (Heidrich and Renk 1967). S. aureus gains entry to the mammary gland mainly through the teat canal. Skin lesions in the teat enable the staphylococci to grow and to penetrate the teat canal, multiply and rapidly spread throughout the gland via the lactiferous ducts (Jensen 1974).

S. aureus infection of the mammary gland produces three forms of mastitis: peracute, acute and chronic. Peracute staphylococcal mastitis is characterized by gangrene due to the necrotizing effect of the vasoactive alpha toxin (Brown and Scherer 1958; Blood et al 1979). The affected ewe may die from toxemia and shock; the dead

tissue may slough after 2 to 3 days (Jensen 1974). This form of staphylococcal mastitis often occurs during early lactation.

Acute and chronic staphylococcal mastitis usually occurs during the later stages of lactation or during the dry period. The pathogenesis is essentially the same, the only difference being involvement. In the acute form, the small ducts are quickly blocked by fibrin clots and the obstructed area becomes more severely involved. In the chronic form, inflammation is restricted to the ductal epithelium and periductal fibrosis that leads to blockage and atrophy of the drained area (Blood et al 1979).

#### Clinical Signs

Affected ewes usually have a systemic reaction and a temperature of 105 to 107 F following an incubation period of 1 or 2 days. Enlargement of the affected mammary gland, lameness and separation from the flock are usually observed (Leyshon 1929; Jensen 1974).

El Etreby and Abdel-Hamid (1970) infected 5 ewes with S. aureus via the teat canal. One ewe developed a febrile reaction and severe systemic disturbances within 48 hours post inoculation (PI). The infected mammary gland became swollen, hot and tender. The ewe was comatose with a sub-normal temperature on day 3 PI. The other 4 ewes, however,

had only slight systemic involvement. Milk production was greatly reduced in all ewes. Quinlivan (1968) in a survey on ovine mastitis found that 27 of 33 ewes from which S. aureus was recovered had acute mastitis with hot swollen udders and 16 were pyrexia. Most infections were unilateral and 80% of untreated ewes died within 1 to 4 days.

#### Gross Lesions

With staphylococcal mastitis, the affected mammary gland at day 2 to 3 PI was swollen and edematous, the edema extending anteriorly to the periglandular abdomen, and the skin discolored and blackish especially around the teat. This may be followed by tissue fragmentation and sloughing (Jensen 1974).

The mammary gland of 1 of 5 ewes experimentally infected with S. aureus became markedly enlarged and firm and the skin was purple to dark blue (El Etreby and Abdel-Hamid 1970). On incision, the tissues were dark blue to purple and the subcutaneous tissues and interlobular septa were markedly edematous. The supramammary lymph node was enlarged, congested and edematous. The affected gland of the other 4 ewes was hot, swollen and tender, and when slaughtered on day 18 to 20 PI was either slightly enlarged, firm and elastic, or somewhat smaller and indurated with apparent lobulation and thickening of the interlobular septa.

## Microscopic Findings

The histopathological changes in ovine mastitis are essentially acute, taking in consideration the type of infection, intensity and duration of inflammatory process. There are certain features that appear to be common to all cases of mastitis at least during the earlier stages; intense congestion, exudate rich in fibrin that thickens the interstitial tissues, and leukocytic infiltration (Leyshon 1929). Generally, in staphylococcal mastitis, as S. aureus persists within intraacinar tissue, there is severe coagulative necrosis and thrombosis of the veins (Blood et al 1979).

Histopathological examination of ovine mammary glands infected experimentally with S. aureus revealed edema and neutrophilic infiltration of the interstitial tissue, duct system, and supramammary lymph node, and extensive phagocytosis (Hawk et al 1963). In a ewe slaughtered 72 hours PI with S. aureus, the affected gland was edematous, contained abundant serous exudate, and had focal areas of leukocytic infiltration (El Etreby and Abdel-Hamid 1970). The blood vessel walls were infiltrated with serous exudate and numerous leukocytes. Thrombosis of the interlobular arteries and veins and major veins at the dorsal aspects of the inoculated gland was prominent. Some lobules had severe leukocytic infiltration that masked the acinar boundaries, and other lobules were focally necrotic. Four other ewes

developed mild mastitis that resulted in mammary atrophy (El Etreby and Abdel-Hamid 1970).

### PASTEURELLA MASTITIS

Pasteurella mastitis (hard mastitis or "blue bag") is characterized by sudden onset and acute systemic disturbances (Marsh 1965). It is more prevalent in summer, especially in ewes on open range and when lambs are 3 to 4 months old (Marsh 1932).

#### Cause

Hard mastitis is caused by P. hemolytica, a Gram-negative, bipolar rod. P. hemolytica commonly inhabits the upper respiratory tract of normal sheep and the lungs of pneumonic sheep (Jensen 1974). During the early stages of mastitis, P. hemolytica may be isolated from the milk in pure culture but later other bacteria such as S. aureus or C. pyogenes often invade the affected mammary tissue. The disease is readily reproduced by mammary infusion of P. hemolytica (Tunnickliff 1949).

## Pathogenesis

Infection with P. hemolytica is considered to occur following teat trauma caused by vigorous nursing by large lambs (Blood et al 1979). Following entry into the lactiferous sinus, pasteurellae spread throughout the gland and initiate an acute serofibrinous inflammatory reaction and milk secretion ceases (Jensen 1974). Death resulted from toxemia and shock. In surviving ewes, the infection became purulent and localized as an abscess that involved most of the affected gland that may rupture and discharge its infective contents to the exterior. This was the most common termination; the affected gland may become gangrenous (Tunnickliff 1949). In surviving ewes, the affected gland was nonfunctional.

## Clinical Signs

Within 1 to 2 days, affected ewes have a temperature of 105 to 107 F, they separate from the flock, are anorexic, forbid nursing, and because of pain walk with reluctance and lameness (Jensen 1974). The lameness occurs early and is an important sign for detecting affected ewes.

Most infections are unilateral. The affected gland is acutely swollen, hot, painful, firm, and is red during the early stages but may become cyanotic and cold in the

late stages. The milk secretion rapidly diminishes and becomes serous and flocculent. Surviving ewes often develop udder abscesses that may rupture and gland becomes fibrosed (Marsh 1932; Tunnickliff 1949; Jensen 1974). The mortality rate in untreated ewes is 50%, with death occurring within 1 to 4 days.

#### Gross Lesions

Gross lesions of pasteurella mastitis are confined to the affected gland that is enlarged and firm. In fatal cases, the affected gland is bluish. In subacute and chronic pasteurellosis, an udder abscess or abscesses may be present (Marsh 1932; Tunnickliff 1949).

#### Microscopic Findings

Histopathological examination of ovine mammary tissue infected with P. hemolytica revealed hyperemia, hemorrhage, and partial or complete destruction and desquamation of acinar epithelium, and numerous small bacilli in many areas (Marsh 1932; Tunnickliff 1949). Leyshon (1929) found that leukocytic infiltration was not as marked as in other types of ovine mastitis and little or no necrosis of glandular tissue.

## MYCOPLASMA MASTITIS

Ovine mycoplasma mastitis or contagious agalactia is an acute or chronic disease of sheep and goats. It is characterized by septicemia and localization in eyes, joints, udder and pregnant uterus. Contagious agalactia is a major economic disease in endemic areas of Southern Europe, North Africa, Middle East, and Asia. Economic losses result from deaths, debilitation, lowered milk production, abortions, perinatal lamb mortality, and expensive control programs (Jubb and Kennedy 1970; Jensen 1974).

All breeds and sexes are susceptible to contagious agalactia. Pregnant ewes, especially during the last trimester, are the most susceptible (Jensen 1974).

### Cause

Mycoplasma agalactia, the specific agent of contagious agalactia, is pleomorphic and without a cell wall. It is stained negatively by the Gram method and is better illustrated by Giemsa stain or by darkfield microscopy (Jensen 1974).

## Pathogenesis

M. agalactia is transmitted by ingestion via contaminated feed or water or by conjunctival contamination, and results in septicemia and localization of infection in the udder, eyes, joints and gravid uterus (Jensen 1974). In the udder, M. agalactia causes an inflammatory reaction in the interstitial tissue and secondary changes in the acini and gradual fibrosis (Jubb and Kennedy 1970).

## Clinical Signs

M. agalactia infection is characterized by septicemia and pyrexia (106-108 F), and some deaths. With organ localization signs of keratitis and arthritis appear and in ewes, mastitis and abortion. The milk becomes alkaline (from pH 6.8 to 7.8) and yellow and, on standing, separates into a light green supernatant and a viscous sediment. The affected udder gradually atrophies and milk production diminishes (Marsh 1965; Jensen 1974).

## Gross Lesions

The udder lesions vary with the degree of development of M. agalactia infection. One or both mammary glands gradually atrophy (Heidrich and Renk 1967; Watson et al 1968; Jensen 1974).

## Microscopic Findings

The chronic inflammatory reaction in the stroma is evident by increasing fibrosis and acinar replacement (Jubb and Kennedy 1970; Jensen 1974).

## AN UNIDENTIFIED GRAM-NEGATIVE BACILLUS

An unidentified, Gram-negative sheep pathogen was first recorded in New Zealand from suppurative epididymitis in young rams (Dodd and Hartley 1955). Later in New Zealand, a similar organism was incriminated as a cause of suppurative synovitis and pyemia in lambs and mastitis in an ewe (Kater et al 1962). In 1956 in Western Australia a similar organism was isolated from a ewe dying from acute mastitis, it was named Histophilus ovis n. sp. by Roberts (1956).

In Eastern Australia Baynes and Simmons (1960) isolated A. seminis. A. seminis epididymitis was clinically and pathologically indistinguishable from that due to Brucella ovis, the major cause of ovine epididymitis (Baynes and Simmons 1968; Bruner and Gillespie 1977; Jensen 1974).

H. 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 1978a). Infection by H. ovis and A. seminis may be indistinguishable by complement

fixation testing unless serums of positive cases are tested against both antigens and a significant difference in titer to each organism is found (Rahaley 1978b).

The unidentified Gram-negative bacillus recorded in scattered reports may be the same organism called H. ovis, A. seminis and Actinobacillus sp. They may be different strains of A. seminis. Because of similarity in cultural characteristics and pathogenicity of H. ovis and A. seminis (Watt et al 1970; Rahaley and White 1977), a serological comparison between H. ovis, A. seminis and B. ovis carried out by cross-adsorption complement fixation, revealed incomplete relationship existing between H. ovis and A. seminis (Rahaley 1978b).

#### HISTOPHILUS OVIS MASTITIS

H. ovis was first isolated from cases of ovine mastitis in Western Australia (Roberts 1956). A similar but unidentified, pleomorphic, Gram-negative bacillus was isolated from cases of suppurative epididymitis in rams (Dodd and Hartley 1955) and from cases of synovitis and pyemia in lambs and mastitis in an ewe in New Zealand (Kater et al 1962). A similar organism was isolated in Eastern Australia from semen and ampullae of a ram with unilateral periepididymorchitis (Claxton and Everett 1966) and from cases of

synovitis, septicemia and abscesses in sheep (Rahaley and White 1977). Rahaley (1978a) experimentally reproduced lesions in lambs following intravenous and intranasal inoculation of H. ovis.

Two days after injecting H. ovis into a mammary gland of 2 dry ewes, the affected glands were swollen, hot, tense and painful. One gland had a turbid watery fluid with flakes of pus and the other had bloodstained fluid (Roberts 1956). The author commented that clinically H. ovis mastitis was similar to staphylococcal mastitis.

#### ACTINOBACILLUS SEMINIS INFECTION

##### Historical

Actinobacillus seminis was initially isolated from semen of rams with epididymitis (Baynes and Simmons 1960). As the organism was isolated from semen, the name A. seminis n. sp. was proposed. Since then, A. seminis has been isolated from cases of ovine epididymitis in the U.S.A. (Livingston and Hardy 1964), Australia (Simmons et al 1966; Baynes and Simmons 1968), South Africa (Van Tonder and Bolton 1968; Worthington and Bosman 1968; Van Tonder 1973), and New Zealand (Bruere et al 1977).

A. seminis has been isolated from sites other than epididymis and semen. Simmons et al (1966) reported isolating A. seminis from fetal membranes, vagina, and uterus of an ewe and from the abomasal contents of a lamb. Watt et al (1970) isolated an organism closely resembling A. seminis from cases of purulent polyarthrititis and posthitis in 4 sheep flocks in western Australia. Van Tonder (1973) reported isolating A. seminis from sporadic cases of abortion in Dorper ewes in South Africa. A. seminis had also been recovered from bull semen (Van Tonder and Bolton 1970), an Angora goat, and an Afrikaner and Friesland bull, and abortion in a Boer goat (Van Tonder 1973).

Acute gangrenous mastitis was experimentally produced in 2 lactating ewes via injection of A. seminis into the teat canal (Watt et al 1970). Intravenous inoculation of ewes with A. seminis during mid to late pregnancy resulted in abortion and premature births (Smith and Hughes 1974).

#### Bacteriological Characteristics of A. seminis

A. seminis is a Gram-negative, nonmotile, nonsporing pleomorphic bacillus (Bruner and Gillespie 1977). In semen smears, A. seminis occurs extracellularly in chains or palisades and intracellularly in neutrophils (Baynes and Simmons 1960, 1968; Livingston and Hardy 1964).

A. seminis grows under aerobic and microaerophilic conditions but is more luxuriant under the latter. Primary isolation and subsequent growth is enhanced by serum enriched media. A. seminis is not active biochemically; it does not ferment sugars, is indol-negative, does not hydrolyze urea or utilize citrate, does not reduce nitrate or lyse coagulated blood, and is catalase-positive (Baynes and Simmons 1960).

Laboratory mice and guinea pigs were found not to be susceptible to A. seminis injected intraperitoneally and intramuscularly (Baynes and Simmons 1960).

#### Pathogenicity of A. seminis

A. seminis has been isolated from ovine epididymitis (Baynes and Simmons 1960; Livingston and Hardy 1964; Van Tonder 1973), bovine epididymo-orchitis (Van Tonder 1973), ovine and caprine abortion (Van Tonder 1973), and ovine polyarthrititis and posthitis (Watt et al 1970).

Experimentally, A. seminis has caused epididymitis and infection of the accessory sex organs (Baynes and Simmons 1960, 1968; Al-Katib 1980), gangrenous mastitis (Watt et al 1970), abortion and perinatal lamb mortality (Simmons et al 1966; Smith and Hughes 1974), synovitis and interstitial pneumonia (Ogunjumo 1980).

The only report of A. seminis as a possible cause of ovine mastitis was from Western Australia (Watt et al 1970). The right mammary gland of 2 lactating ewes was injected with 0.2 ml of an overnight broth culture of A. seminis. Both ewes developed severe gangrenous mastitis characterized by fever and recumbency by day 3 PI. A. seminis was isolated from the milk of one ewe and from the udder of the other postmortem. The first ewe recovered following treatment with penicillin and streptomycin.

#### ANATOMY OF OVINE UDDER

##### Gross Characteristics

The udder is located in the inguinal region between hindlegs and consists of two mammary glands, each drained by a teat with a streak canal opened to the exterior by a funnel-shaped orifice (Turner 1952). The skin covering the udder is of fine texture and is covered with fine hair excluding the teats.

Blood is supplied by the mammary artery, a branch of the external pudendal artery, and drained by two veins, the external pudendal and the subcutaneous abdominal or milk vein. The lymphatics drain to the supramammary lymph node on each side of the udder and then to a lymph duct

that follows the pudendal blood vessels. Innervation of the udder is supplied by the inguinal nerves (Schmidt 1971).

### Histologic Features

Mammary glands are specialized organs of ectodermal origin (Dellmann and Brown 1976). It is considered to be a modified apocrine sweat gland with a compound branched tubulo-alveolar arrangement (Gibbons 1938; Banks 1974; Dellmann 1976). Histologically, the mammary gland is composed of glandular tissue or parenchyma and interstitial tissue or stroma, the latter being more prominent in the nonfunctional stage. Each mammary gland contains lobes and lobules consisting of acini and interlobular ducts (Dellmann and Brown 1976).

#### 1. Parenchyma

Mammary parenchyma is composed of secretory units and an excretory system. Each secretory unit consists of an acinus or alveolus and its duct and groups of acini form lobules separated by connective tissue septa (Dellmann and Brown 1976).

Acini are drained by terminal intralobular ducts that open into an interlobular duct within the connective tissue septa where several meet to form the lactiferous or lobar duct that drains a lobe of the mammary gland. Several

lactiferous ducts empty into the lactiferous sinus that extends into the teat. The teat or teat cistern opens to the exterior by a single teat canal or streak canal (Banks 1974; Dellmann and Brown 1976).

## 2. Acinus

Each mammary lobule is composed of numerous spherical secretory structures, acini or alveoli. Each acinus is lined a single layer of simple cuboidal to columnar epithelium with surface microvilli surrounding a cavity or lumen. The secretory epithelium is surrounded by a basement membrane and myoepithelial cells (Feldman 1961; Schmidt 1971; Patton and Jensen 1976; Dellmann and Brown 1976). The myoepithelial cells contract under the influence of oxytocin and force the acinar contents into the duct system (Dellmann and Brown 1976).

The acini are well supplied with blood by a dense capillary network within the surrounding interstitial tissue. The lobular acini are essentially separated by basement membrane, blood vessels and lymphatics (Schmidt 1971; Patton and Jensen 1976).

Secretory epithelial cells lining acini vary markedly in size and shape during the various stages of function. As the acinar lumen fills with milk, the acinus stretches and flattens the epithelial cells (Schmidt 1971; Dellmann

and Brown 1976). It has been stated that these cells are often irregular and vary in height within an acinus. The nuclei are usually located towards the basement membrane (Gibbons 1938).

Cellular asymmetry is imposed by the acinar structure and 3 different cell sides have been described: basal oriented towards the interacinar space, circulation and lymph; apical facing the lumen; and lateral towards the adjacent cells. This gives the secretory epithelium 3 different structural-functional status, respectively: transport of metabolites into the cell, milk secretion, and cell-to-cell communication and interaction (Patton and Jensen 1976).

### 3. Duct System

The duct system is designed to transport milk from acini to the lactiferous sinus for delivery from the gland. The system begins with terminal intralobular ducts that drain 1 or 2 acini. The intralobular ducts are lined by nonsecretory simple cuboidal epithelium resting on a basement membrane and often surrounded by myoepithelial cells. The duct lumen has a distinct rounded appearance. Intralobular ducts enter the interlobular septa and become interlobular ducts lined by 2 layers of cuboidal to columnar epithelium. The lumen of interlobular ducts are more elongated and have folds. Interlobular ducts merge with

other ducts and drain into the lactiferous lobar duct within the lobar connective tissue. The lactiferous duct is also lined by two layers of cuboidal to columnar epithelial cells and its wall contains smooth muscle. The lactiferous ducts drain into a single lactiferous sinus lined by stratified columnar epithelium and surrounded by smooth muscle fibers and an elastic fiber system continuous with that around the ducts. The duct system terminates by extending into the teat. Milk is delivered to the exterior via the teat or streak canal. The teat canal is lined by keratinized stratified squamous epithelium (Schmidt 1971; Dellmann and Brown 1976). Keratin is reported to play an important role in hindering the entry of bacteria through the teat canal (Schmidt 1971). The teat wall contains little smooth muscle but more than cattle, and considerable amounts of elastic connective tissue (Turner 1952; Schmidt 1971).

#### 4. Stroma

The secretory acini and ducts of the mammary gland are supported by interstitial tissue containing blood and lymph vessels and nerves. Acini are surrounded by a fine network of reticular and elastic fibers intermingled with dense capillaries. Dense connective tissue septa divide the mammary gland into functional lobules. The septa contain interlobular ducts and larger blood and lymph vessels.

Smooth muscle fibers are associated with the larger ducts (Banks 1974; Dellmann and Brown 1976).

#### 5. Nonfunctional Glands

The parenchyma in nonlactating glands is greatly reduced and the interstitial tissue is more extensive. The secretory acini are reduced in size and number and the lumen are almost obliterated (Gibbons 1938; Dellmann and Brown 1976).

## MATERIALS AND METHODS

## Experimental Ewes

Ten Rambouillet cross ewes, 4 to 5 years old, 7 lactating and 3 nonlactating, were ear tagged and randomly divided into two groups, 8 test and 2 controls. The ewes were dewormed with Levasole<sup>a</sup> two weeks before being moved to 2 isolation rooms with a controlled environment of 70 F, one for test ewes and one for controls. Each ewe was examined clinically with special emphasis on the udder and its secretion. Only ewes with sound symmetrical, palpable udders without evidence of abnormal mammary secretion were used. The rectal temperature, heart rate, and respiratory rate were monitored twice daily for two days prior to inoculation. The ewes were each fed 2 lb. of a balanced ration containing pelleted alfalfa twice daily.

## Preparation of Inoculum

A. seminis (strain #15768) was obtained from the American Type Culture collection. Frozen beads of A. seminis stock culture were transferred to brain heart infusion (BHI) broth<sup>b</sup> containing 10% bovine serum and incubated for 48 hours at 37 C in an atmosphere of 10% CO<sub>2</sub>,

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<sup>a</sup>Pitman-Moore, Inc., Washington Crossing, NJ 08560.

<sup>b</sup>Difco Laboratories, Detroit, MI.

relative humidity 55%. The culture was checked for purity by Gram stain and by plating on 5% bovine blood BHI agar (BBA) plates. Typical small dewdrop-like colonies of A. seminis were inoculated into small flat flasks containing 30 ml of BHI broth with 10% inactivated bovine serum. The flasks were incubated in an atmosphere of 10% CO<sub>2</sub> for 24 hours at 37 C after being carefully laid horizontally. The cultures were checked for purity by Gram stain, by plating on 5% BBA plates, and by specific immunofluorescence (Ajai 1980). The bacterial concentration was determined spectrophotometrically<sup>c</sup> at optical density 1.5 and wavelength 540 mu to be  $2 \times 10^9$  CFU/ml.

#### Inoculation of Ewes

The left teat of each ewe was cleansed with alcoholic detergent solution and disinfected with 70T ethanol. Each test ewe was inoculated with 2 ml of the 24 hour broth culture of A. seminis via the left teat canal. The control ewes were inoculated with 2 ml of sterile BHI broth. The right mammary glands of the test and control ewes served as additional controls.

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<sup>c</sup>Bausch and Lomb Spectronic 20.

## Postinoculation Procedures

Test and control ewes were examined twice daily for clinical signs of fever, anorexia, pain, depression, lameness, reluctance to move, weight loss, and changes in fecal consistency. Rectal temperature, heart rate and respiratory rate were recorded. The udder of each ewe was carefully examined for signs of swelling, heat and pain, and for any change in color, consistency or secretion.

The test ewes were randomly selected and euthanatized by intravenous injection of T61 solution<sup>d</sup> as follows: 2 on day 3 post inoculation (PI) and 1 each on day 4, 5, 6, 7, 8 and 9 PI. One control ewe was euthanatized on day 10 and the other on day 11 PI.

## Necropsy Examination

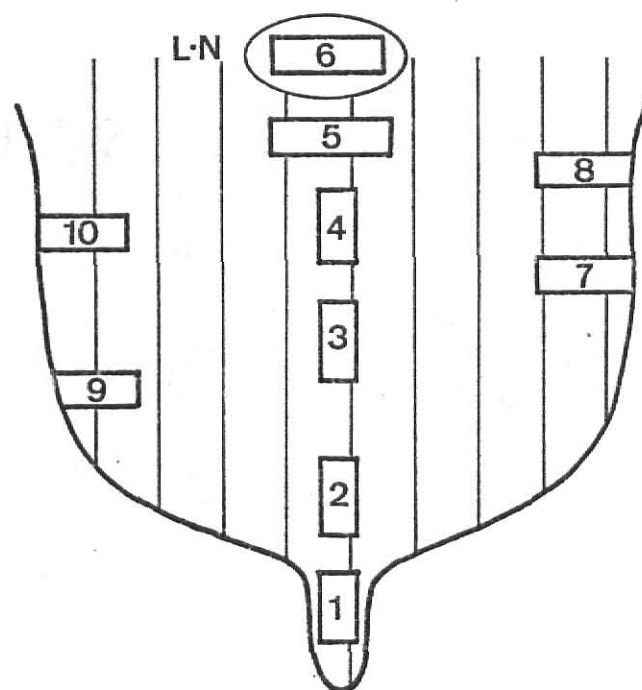
Each ewe was necropsied immediately after euthanasia by a standardized technique and all observations were recorded on a form designed for that purpose (Appendix A). The mammary glands were carefully dissected and gross lesions were photographed.

Each mammary gland was sliced longitudinally into 1 to 1.5 cm wide sections that were fixed in 10% buffered neutral

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<sup>d</sup>National Laboratories Corporation, Somerville, NJ 08876.

formalin (BNF) in separate containers. The fixed slices from each mammary gland were then trimmed and 10 tissue blocks were cut (9 mammary and 1 supramammary lymph node) as indicated in the diagram below.



The tissue blocks were processed in an autotechnicon<sup>e</sup> cut at 6  $\mu$  and stained with hematoxylin and eosin (H&E). Special stains included Brown and Brenn and Giemsa for bacteria, periodic acid-Schiff (PAS) and PAS diastase for eosinophilic bodies in the acini, and trichrome and von Kossa for collagen and mineralization, respectively.

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<sup>e</sup>Technicon Instruments Corporation, Tarrytown, NY 10591.

## Bacteriological Examination

The mammary glands, heart blood, lungs, liver, kidneys, uterus, brain, and major joints of test and control ewes were cultured for A. seminis. The surface of each organ was sterilized by a red hot spatula and the contents removed by a sterile syringe or sterile swab after the organ was opened by a sterile scalpel, and plated on 5% BBA plates. The scalpel was placed in 70% ethanol and flamed between sampling. The plates were incubated at 37 C in an atmosphere of 10% CO<sub>2</sub> for 72 hours. The cultures were checked daily for A. seminis colonies. Isolates suspected of being A. seminis were identified by criteria described by Baynes and Simmons (1960) and confirmed by macroscopic slide agglutination (Kabat et al 1964) or by immunofluorescence (Ajai 1980).

## RESULTS

## Clinical Observations

The 8 test ewes inoculated with A. seminis had some increases in body temperature (up to 3.2 F) for the first 12 to 24 hours PI that varied from ewe to ewe and returned to normal by 36 to 60 hours PI (Appendix B). No significant changes in heart or respiratory rates were observed. Swinging or abduction of the left hind leg was observed, especially when walking. This was more obvious in lactating ewes.

None of the control mammary glands (uninoculated right side of test and control ewes and inoculated left gland of the control ewes) were visibly affected (Fig 1).

Changes in the inoculated udders included enlargement, heat, pain, firmness and slight to moderate erythema of the skin, especially around the base of the teat. The size of the inoculated glands increased 3 to 5 fold compared to the uninoculated side (Table 1). Partial to complete firmness of the udder was noted in ewes after 72 hours PI. The induration became progressively firmer until the experiment terminated. Cessation of normal secretion was evident from 12 hours PI. The affected mammary glands were firm but yielding and an off color or cloudy watery fluid mixed with whitish floccules was expressed. The mammary secretion progressively changed to a thick, viscid, whitish-yellow creamy or yellowish-green caseous material.

None of the test or control ewes exhibited any loss of appetite.

#### Bacteriological Findings

A. seminis was cultured from all inoculated mammary glands at the time of necropsy but not from uninoculated glands, heart, blood, liver, kidneys, lung, joints, uterus or brain.

#### Gross Findings

The inoculated mammary glands of all test ewes infected with A. seminis were enlarged, turgid or indurated with areas of consolidation (Table 1). The affected glands lacked the typical spongy structure and compartmentation was obvious. They contained whitish-creamy or greenish-yellow viscid exudate in contrast to clear milk from the uninoculated glands of the lactating and watery fluid from nonlactating control ewes (Fig 3).

Day 3 PI - The inoculated gland of the lactating ewe (#7809) was turgid and enlarged 5 times that of the uninoculated half. On cut surface, the gland was hyperemic, edematous and filled with a viscid yellowish, caseous exudate mixed with cloudy watery fluid. The inoculated gland of the

TABLE 1--Summary of Gross Pathological Findings in Ewes Inoculated with *A. seminis* via the Teat Canal

| Ewe No.         | Days PI | Lactation Status | Udder       |                          | Hyperemia | Edematous | Milk or Exudate                                   |              | Supramammary Lymph Node         |
|-----------------|---------|------------------|-------------|--------------------------|-----------|-----------|---------------------------------------------------|--------------|---------------------------------|
|                 |         |                  | Enlargement | Consistency              |           |           | Consistency                                       | Amount       |                                 |
| 7809            | 3       | L                | 5X          | Turgid                   | +         | +         | Creamy, caseous and some watery fluid             | Copious      | Slightly enlarged and edematous |
| 7735            | 3       | NL               | 3X          | Consolidated             | +         | +         | Turbid and watery                                 | Little       | Slightly enlarged and edematous |
| 7814            | 4       | L                | 5X          | Turgid                   | +         | +         | Creamy, caseous and some watery fluid             | Copious      | Slightly enlarged and edematous |
| 7812            | 5       | L                | 3X          | Firm                     | +         | +         | Creamy and viscid                                 | Moderate     | Slightly enlarged and edematous |
| 7808            | 6       | L                | 4X          | Turgid                   | +         | -         | Greenish-yellow caseous, some turbid watery fluid | Moderate     | Slightly enlarged and edematous |
| 7810            | 7       | L                | 4X          | Firm, some consolidation | +         | -         | Greenish-yellow, creamy to caseous                | Moderate     | Slightly enlarged and edematous |
| 7736            | 8       | LL               | 3X          | Consolidated             | -         | -         | Greenish yellow, viscid                           | Reduced      | Normal appearance               |
| 7745            | 9       | NL               | 2X          | More consolidated        | -         | -         | Greenish yellow, viscid                           | Reduced      | Normal appearance               |
| <b>Controls</b> |         |                  |             |                          |           |           |                                                   |              |                                 |
| 7734            | 10      | L                | Nil         | Normal                   | -         | -         | Normal milk                                       | Copious      | Normal appearance               |
| 7737            | 11      | NL               | Nil         | Small flaccid            | -         | -         | Honey-like and watery                             | Small amount | Normal appearance               |

L = lactating + = positive

LL = late lactation + = slight

NL = nonlactating - = negative

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Fig 1 - Udder of control ewe at day 5 PI. Note the symmetrical flaccid glands.

Fig 2 - Udder of test ewe at day 5 PI. Note the enlarged left gland with swollen erect teat.

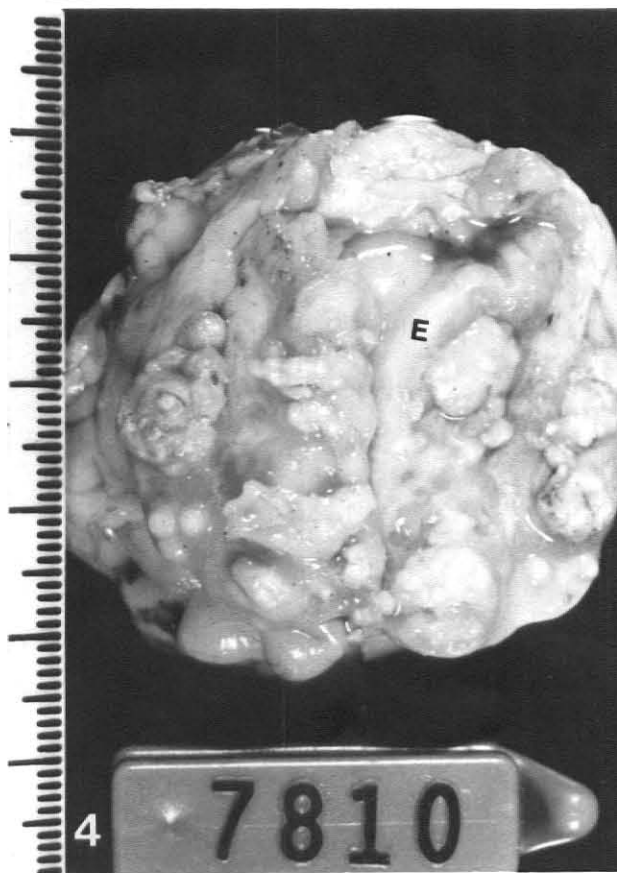
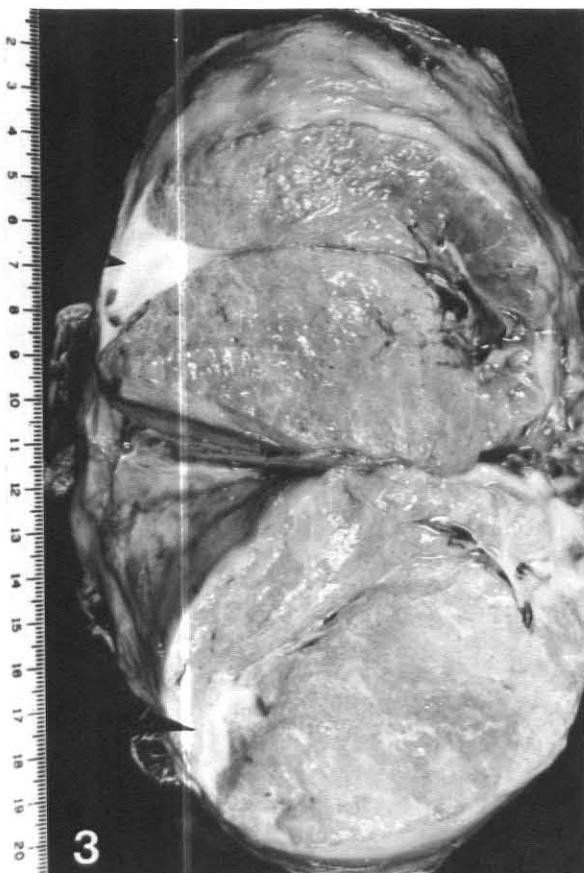
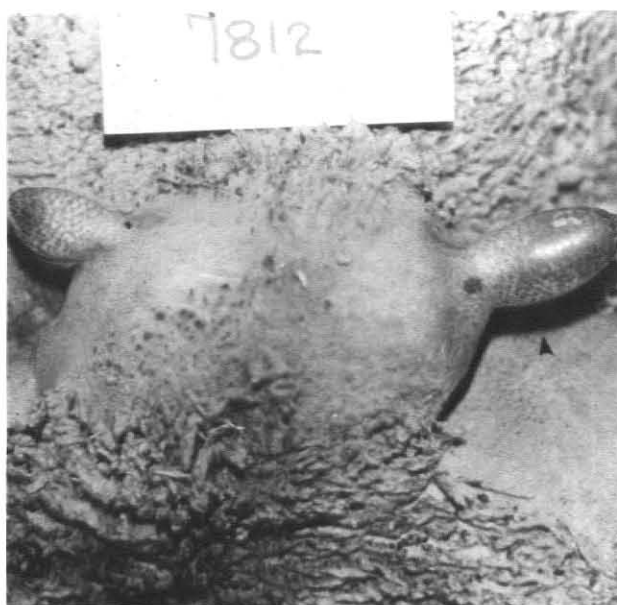
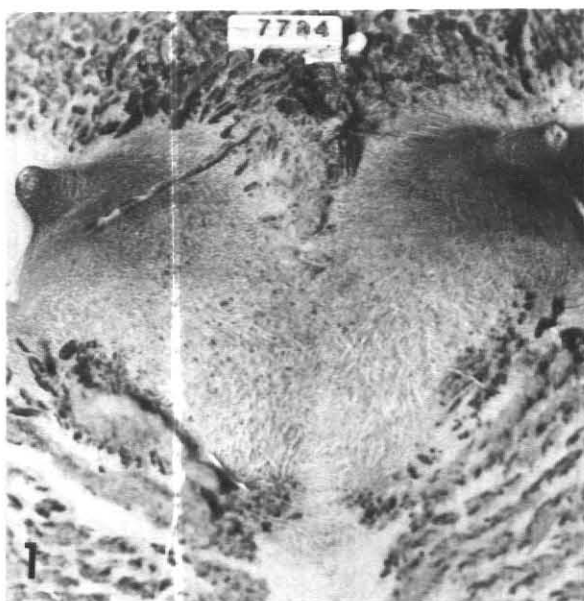
Fig 3 - Udder of control ewe in Fig 1 at the time of necropsy on day 10 PI. Note the normal spongy appearing mammary tissue with normal milk (arrows).

Fig 4 - Infected mammary gland at day 7 PI opened to illustrate swelling and edema (E) and thick caseous exudate.

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nonlactating test ewe (#7735) was enlarged 3 times that of the soft, flaccid uninoculated side. The changes were similar to those of #7809 except that the gland was firmer and contained little exudate. The supramammary lymph node in both ewes was slightly enlarged and edematous.

Day 4 PI - The gross findings in ewe #7814 were similar to those at 72 hours PI.

Day 5 PI - The inoculated gland (ewe #7812) was enlarged 3 times and was firm (Fig 2). The supramammary lymph node was slightly enlarged and edematous.

Day 6 PI - The inoculated gland (ewe #7808) was turgid and enlarged 4 times normal. Upon incision, variation in lobar involvement was evident; some contained greenish-yellow caseous exudate and others had turbid watery contents. The supramammary lymph node changes were similar to the earlier periods.

Day 7 PI - Findings in ewe #7810 were similar to those at day 6 PI except for partial areas of consolidation. The contents were creamy and viscid (Fig 4) uninoculated right side was filled with normal milk.

Day 8 PI - The gland (ewe #7736) was enlarged 3 times its preinoculated size and was consolidated. On cut surface,

the affected areas were pale and distinct, and filled with thick greenish-yellow exudate. The supramammary lymph node appeared normal.

Day 9 PI - Findings in ewe #7745 were similar to ewe #7736 at day 8 except the affected gland was more consolidated.

#### Microscopic Findings

The mammary glands of the control ewes and uninoculated right mammary glands of the test ewes and the associated supramammary lymph nodes had no pathological changes (Fig 5-8). No significant changes were found in the teats of both test and control ewes. The findings in the inoculated left mammary gland of test and control ewes are tabulated in Appendix C.

Day 3 PI - Changes in the lactating gland inoculated with A. seminis (ewe #7809) were neutrophilic infiltration of acini and lactiferous ducts mixed with proteinaceous material, desquamated epithelial cells and some mononuclear cells (Fig 9-12). The ductal and acinar epithelium had degenerative changes and mild leukocytic infiltration. There was some focal ductal hyperplasia of the epithelium. The interstitium, especially intralobular, was thickened by serofibrinous exudate and various degrees of neutrophilic infiltration and a few mononuclear cells (Fig 10).

Fig 5 - Day 3 PI. Photomicrograph of uninoculated lactating mammary gland of a test ewe. Note the normal acini containing milk and the intralobular interstitial tissue. H&E. X330.

Fig 6 - Day 3 PI. Photomicrograph of uninoculated non-lactating mammary gland. Note absence of pathological changes, branched lobular appearance, stroma, and a number of corpora amylacea. H&E. X66.

Fig 7 - Day 6 PI. Photomicrograph of uninoculated lactating gland. Note absence of lesions and normal acini and ducts. H&E. X165.

Fig 8 - Day 10 PI. Photomicrograph of lactating gland of a control ewe (Fig 1). Note normal appearance and lack of lesions. H&E. X165.

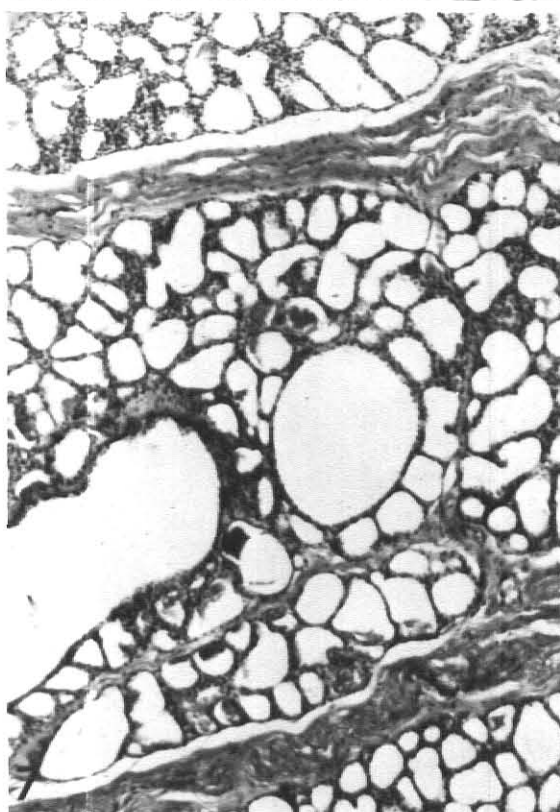
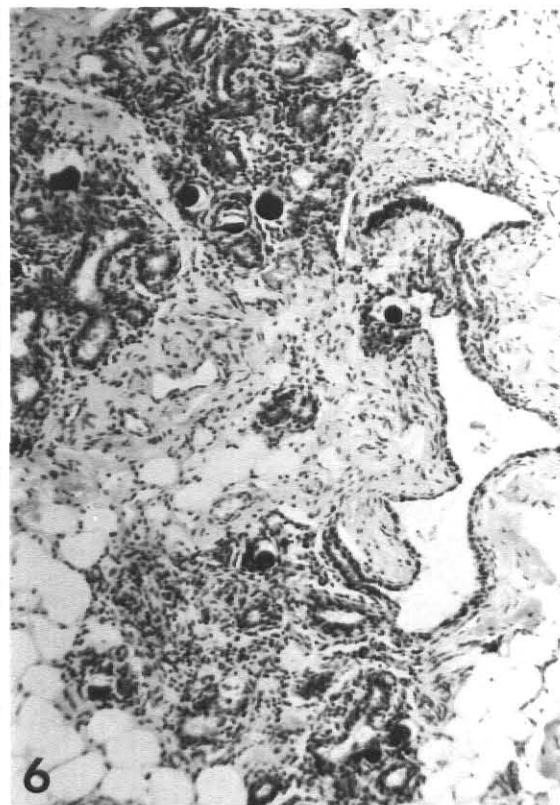
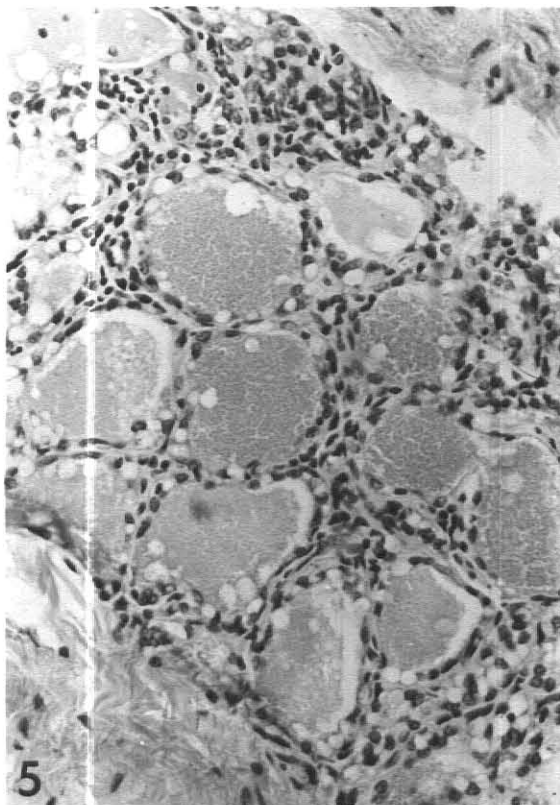
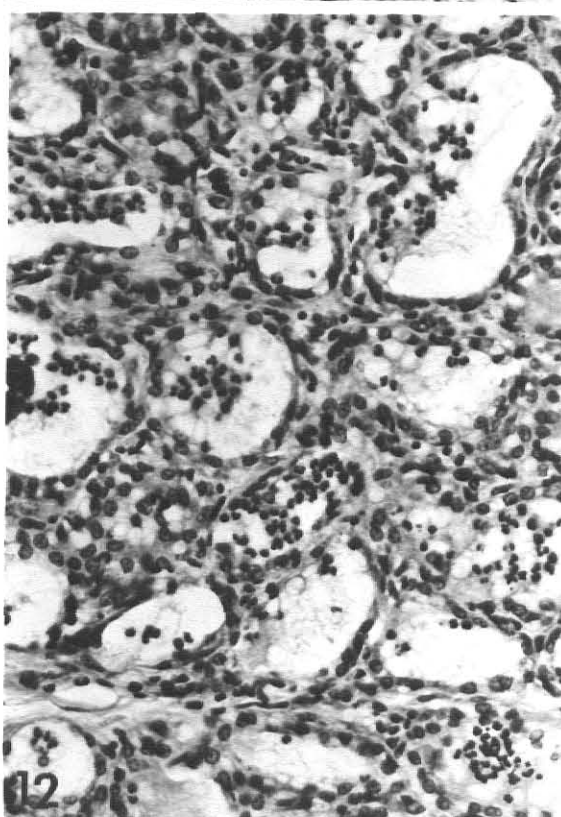
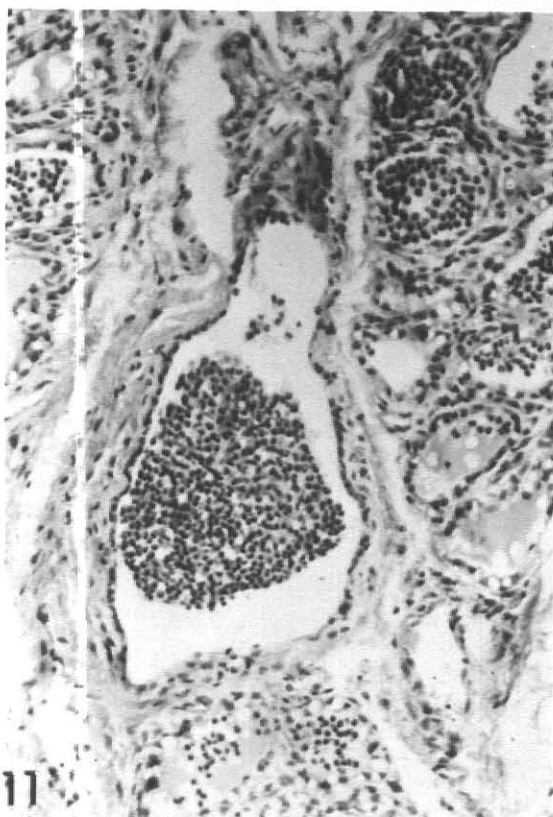
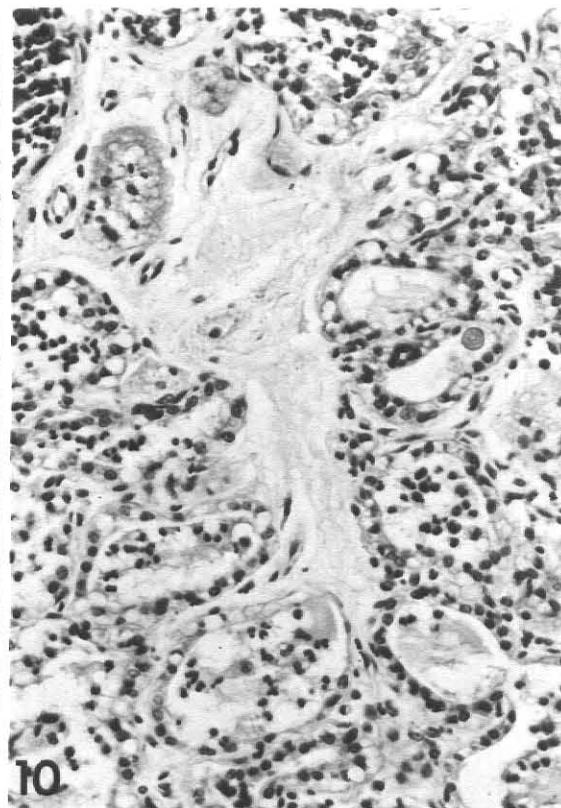
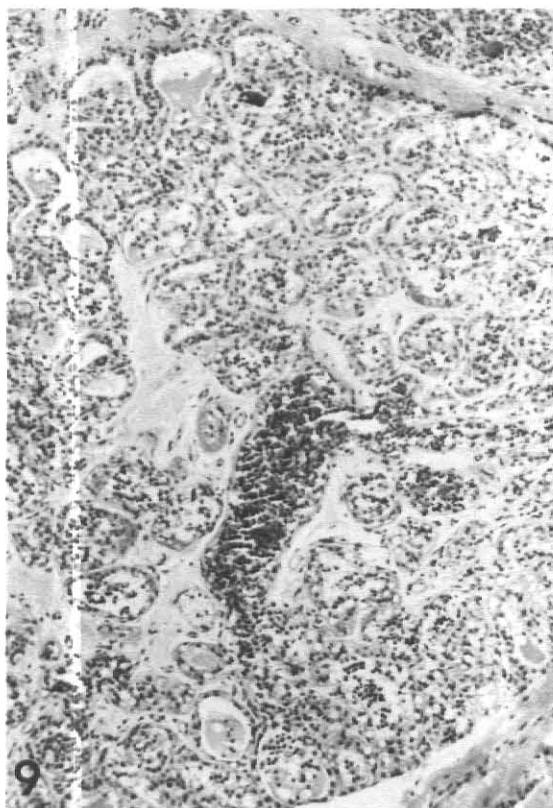


Fig 9 - Day 3 PI. Photomicrograph of lactating gland inoculated with A. seminis. Note separation of interstitial tissue by serofibrinous exudate and neutrophil infiltration of acini and lactiferous duct. H&E. X66.

Fig 10 - Day 3 PI. Photomicrograph of mammary gland inoculated with A. seminis. Note serofibrinous exudation of interstitium, early neutrophilic infiltration of acini and acinar epithelium, and some epithelial desquamation. H&E. X165.

Fig 11 - Day 3 PI. Photomicrograph of A. seminis infected mammary gland. Note neutrophils in acini and duct and variation in acinar involvement, and interstitial edema. H&E. X165.

Fig 12 - Higher magnification of Fig 11. Note variation in acinar involvement, vacuolation of acinar epithelium, and infiltration of the interstitium by inflammatory cells. H&E. X330.



Many lobules and some acini in affected lobules appeared normal, either empty or full of secretion. A few bacteria were observed, mainly within phagocytes.

Tissues from the inoculated and uninoculated nonlactating glands (ewe #7735) revealed involution with branched ducts being prominent. There was increased connective tissue stroma, lymphocytes and plasma cells. The reaction to A. seminis was more diffuse and severe than that of the lactating gland at day 3 PI (Fig 13-16). The changes were more severe in the ductal system than in other parts of the gland. There was diffuse infiltration of neutrophils in the lumen of ducts and remaining acini mixed with deeply eosinophilic proteinaceous material, desquamated epithelial cells, cellular debris, and mononuclear cells (Fig 15 and 16). Changes in ductal and acinar epithelium included degeneration and vacuolation, leukocytic infiltration, dissociation, necrosis, desquamation, hyperplasia and slight metaplasia. The interstitial tissue, especially intralobular, was infiltrated by serofibrinous exudation (Fig 13, 14, 16) and leukocytes. Corpora amylacea were more numerous and varied in size and staining intensity. A few small foci of necrosis were present.

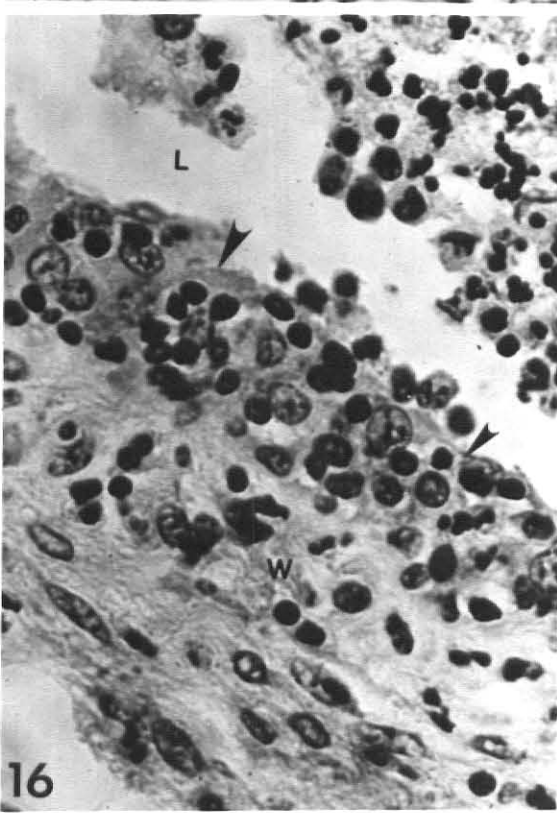
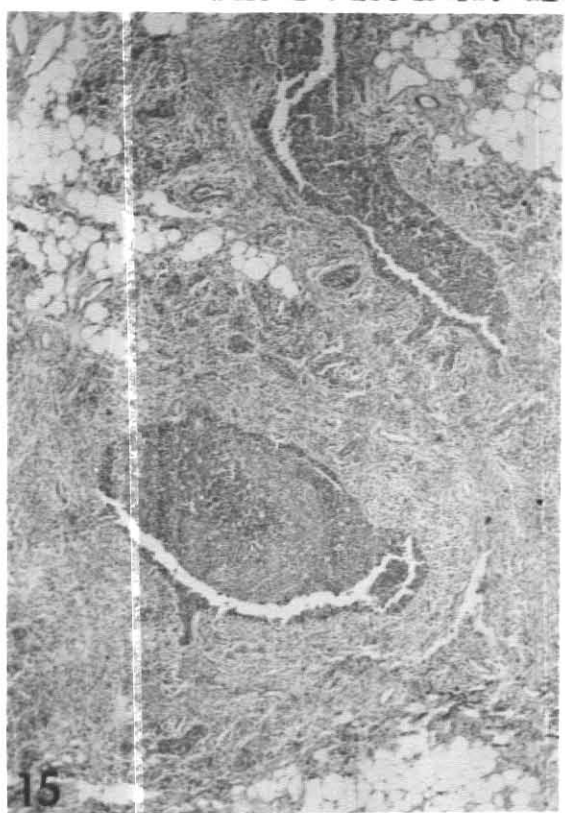
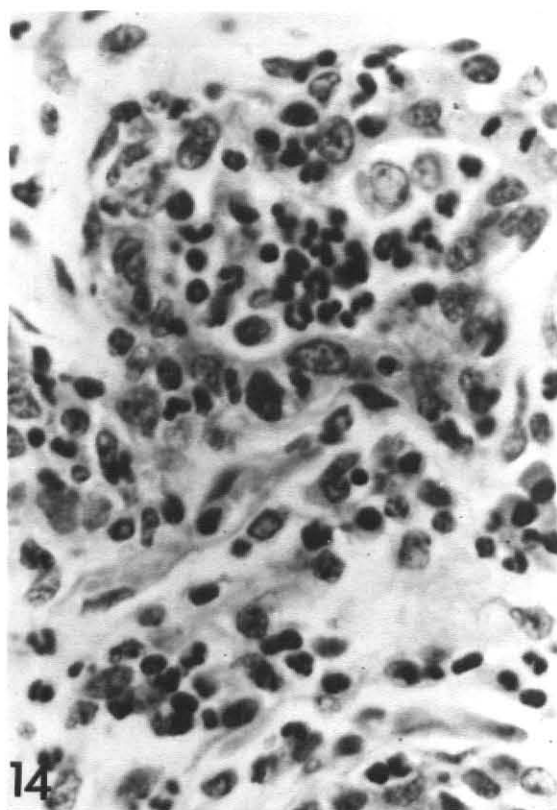
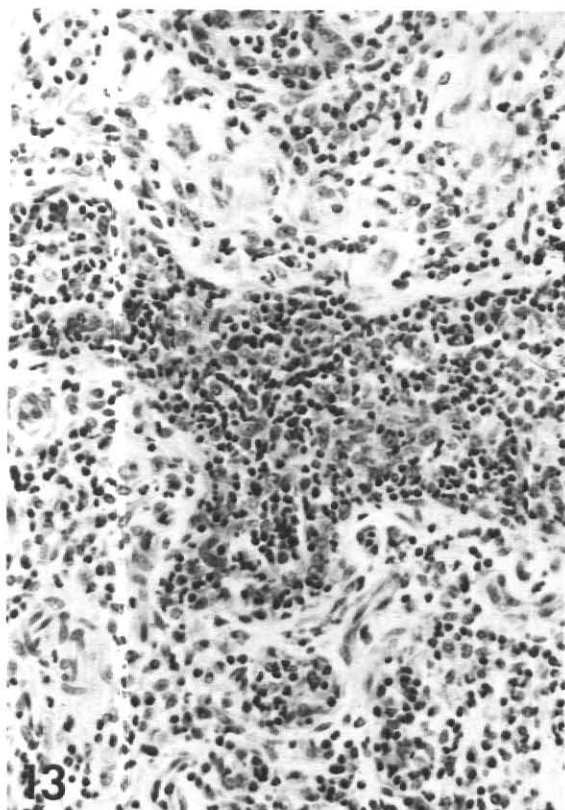
Venous thrombi were occasionally observed (Fig 46) in both ewes. The supramammary lymph nodes were edematous and reactive.

Fig 13 - Day 3 PI. Photomicrograph of a nonlactating gland infected by A. seminis. Note duct filled with exudate, neutrophils and desquamated epithelial cells. The interstitium is separated by edema and leukocytes. H&E. X165.

Fig 14 - Higher magnification of Fig 13 illustrating involvement of the acinar wall and lumen. Note dissociation of the epithelium and infiltrating leukocytes, and serofibrinous exudation and leukocytic infiltration of the interstitium. H&E. X660.

Fig 15 - Day 3 PI. Photomicrograph of nonlactating mammary gland infected with A. seminis. Note the extensive galactophoritis. H&E. X66.

Fig 16 - Higher magnification of Fig 15 illustrating involvement of a large duct. Note exudate in lumen (L) and leukocytes infiltrating the epithelium (arrows) and basement membrane (w). H&E. X660.



Day 4 PI - The changes observed in ewe #7814 were similar to those in ewe #7809 except that the inflammatory reaction was more extensive and severe (Fig 17-20; Appendix D). The lactiferous ducts and acini were distended with a highly cellular exudate containing leukocytes and desquamated epithelium (Fig 17 and 19). Areas of necrosis with loss of architecture and some hemorrhage were present (Fig 20). Venous thrombi were evident (Fig 47).

Day 5 PI - Lesions in ewe #7812 varied greatly from one area to another within the inoculated gland. Although the reaction was primarily purulent (Fig 21), early signs of a sub-acute nature were observed (Fig 23 and 24). Changes in the duct and acini contents, epithelium and interstitial tissue were similar to the findings in the earlier ewes (Fig 21 and 22). In addition, there were focal areas of necrosis with early healing by granulation tissue, and fibroplasia in the interstitium. Severe changes were observed in the larger lactiferous ducts with hyperplastic to metaplastic epithelium, leukocytic infiltration of the epithelium, and periductal fibrosis (Fig 23 and 24).

Day 6 PI - The lesions in ewe #7808 varied from one lobule to another, both in degree and stage of reaction. Normal lobules were present. The less affected lobules had lesions similar to those at day 3 PI (Fig 25, 26, 31). The most

Fig 17 - Day 4 PI. Acini filled with fluid rich in leukocytes and desquamated epithelial cells and protein; the interacinar interstitial tissue is widened by serofibrinous exudate and some degenerating leukocytes. H&E. X330.

Fig 18 - Day 4 PI. Lactiferous duct packed with disintegrating leukocytes, exudate and desquamated epithelium. Note epithelial vacuolation (arrows) and periductal tissue separation due to serofibrinous exudation (E) and inflammatory cell infiltration. H&E. X165.

Fig 19 - Higher magnification of Fig 18. Duct filled with protein, exudate, numerous disintegrating leukocytes, and desquamated epithelial cells. Note vacuolation of the epithelium (arrows) and leukocytic infiltration. H&E. X330.

Fig 20 - Day 4 PI. Section of mammary gland with necrosis and loss of normal architecture and hemorrhage (arrows). H&E. X165.

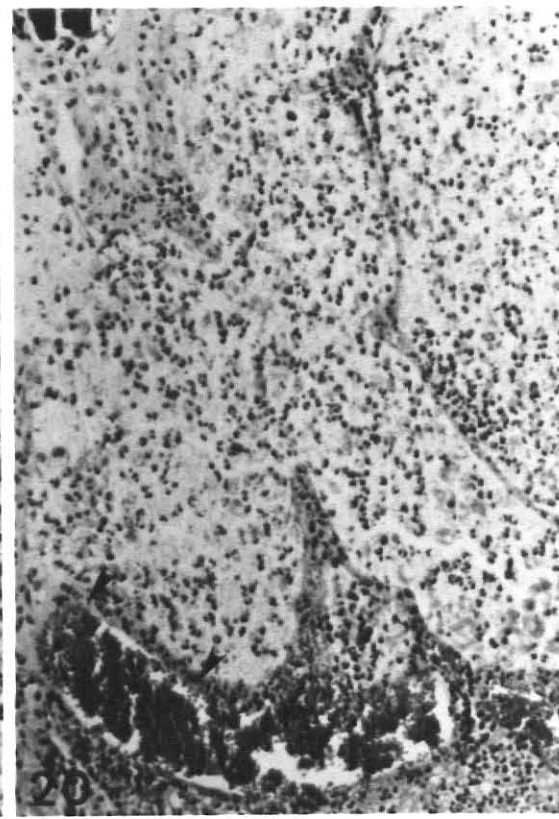
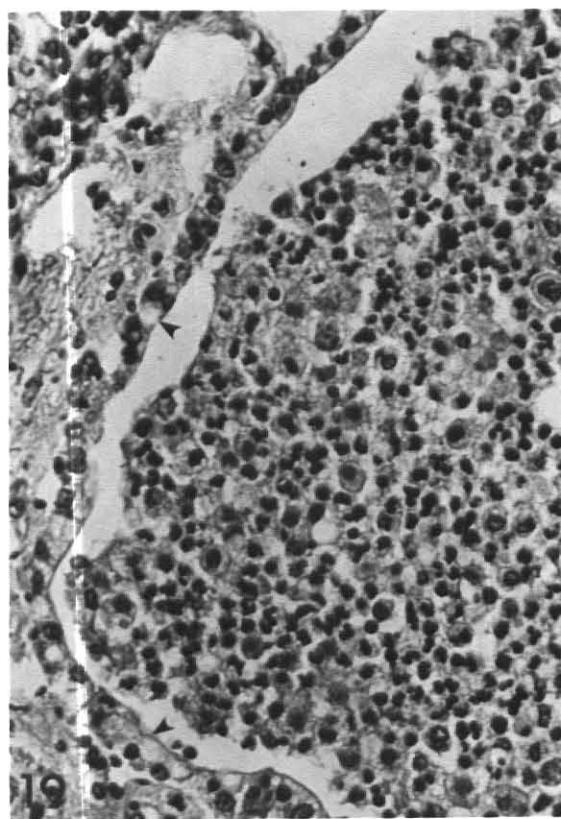
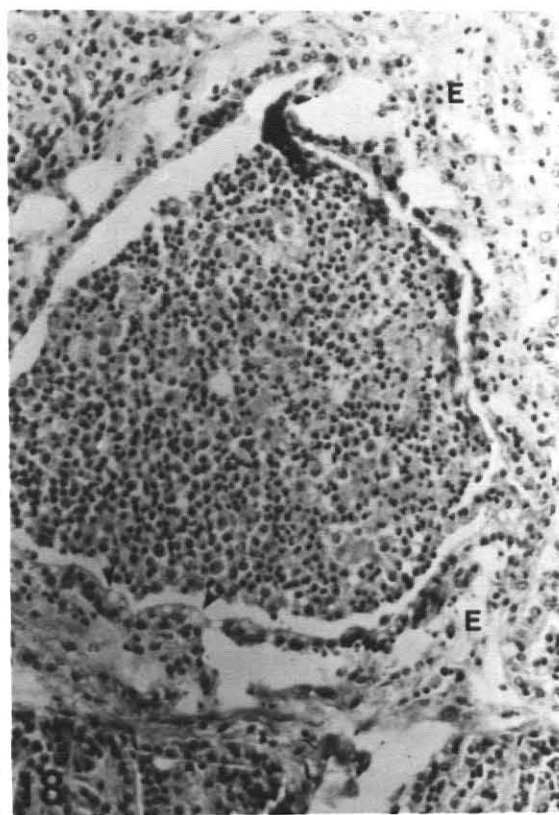
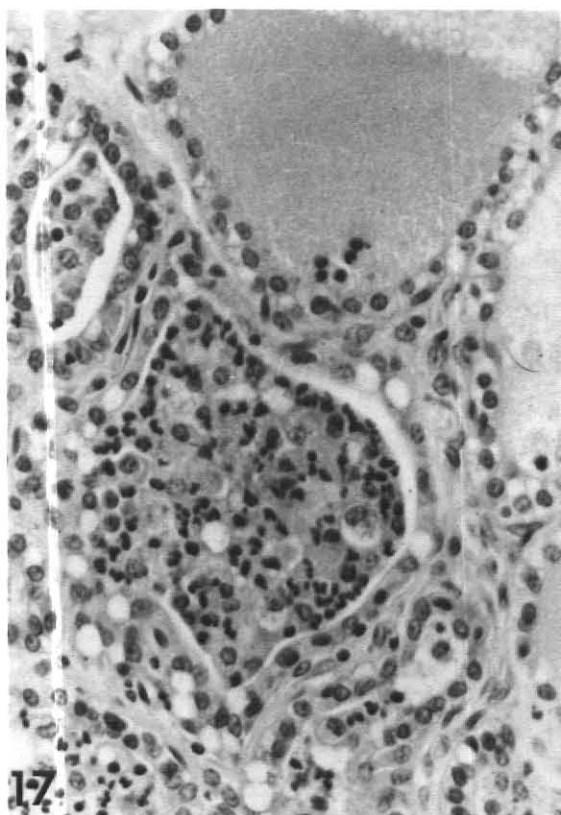


Fig 21 - Day 5 PI. Photomicrograph of acute A. seminis mastitis. Note diffuse involvement of acini, intralobular interstitial tissue and ducts, and degenerating leukocytes. H&E. X165.

Fig 22 - Day 5 PI. High magnification of an acinus with acinitis. Note dissociation and desquamation of the epithelium, lumen filled cellular exudate and debris, and epithelial and periacinar leukocytic infiltration. H&E. X660.

Fig 23 - Day 5 PI. Photomicrograph of a large lactiferous duct. Note lumen (L) with some exudate, leukocytic infiltration of epithelium (arrow), squamous metaplasia (M), and periductal fibrosis (F). H&E. X330.

Fig 24 - Day 5 PI. Section of a large lactiferous duct with an irregular surface and complete loss of epithelium (arrows), metaplasia (M), and fibrosis of the duct wall and periductal tissue. H&E. X165.

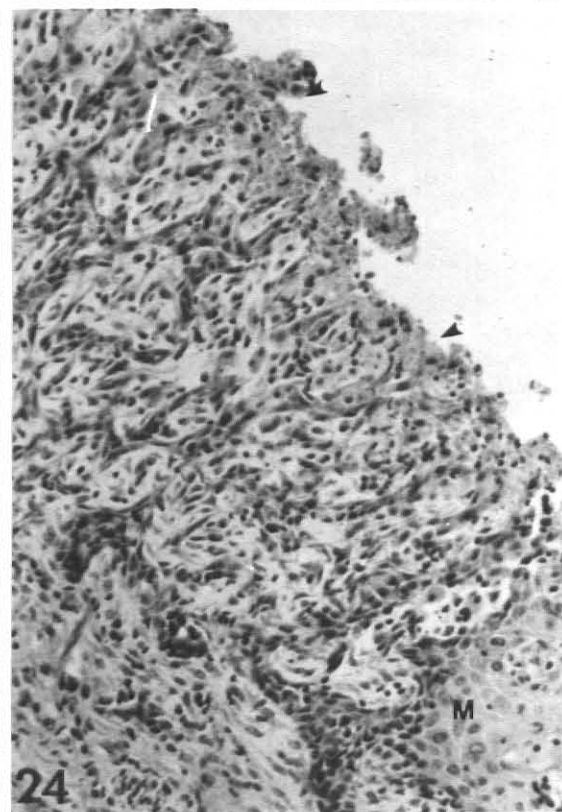
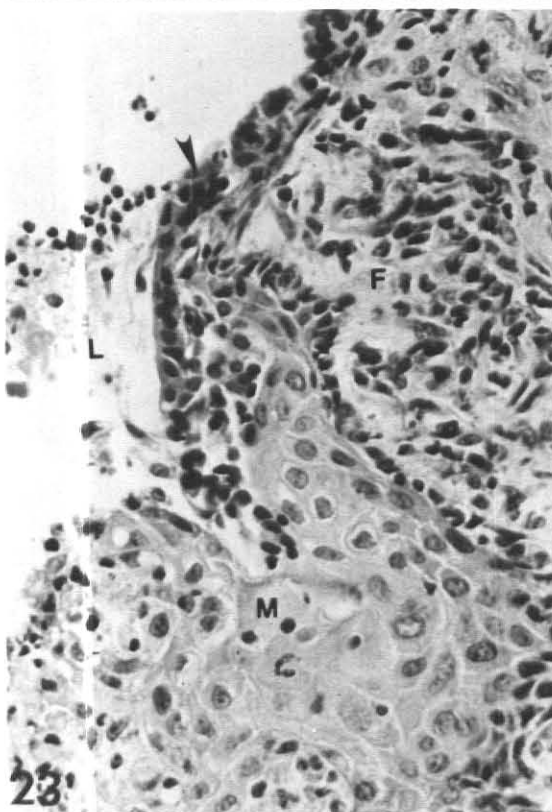
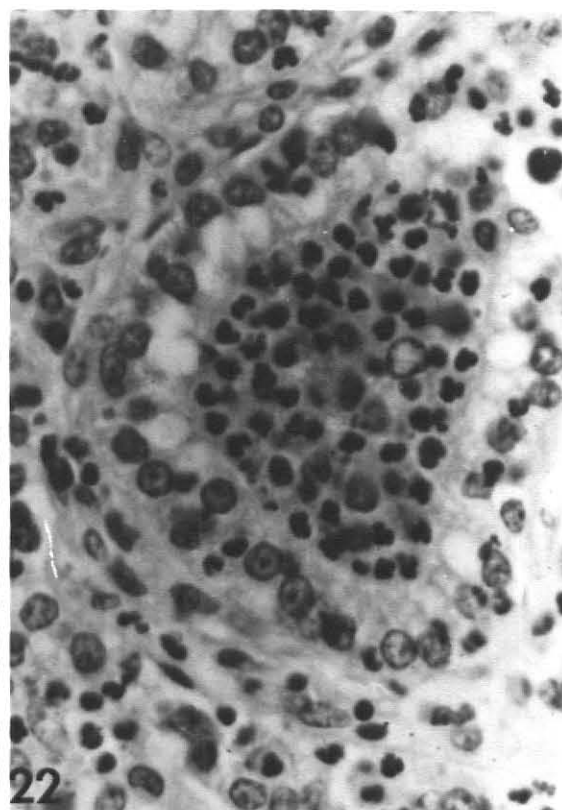
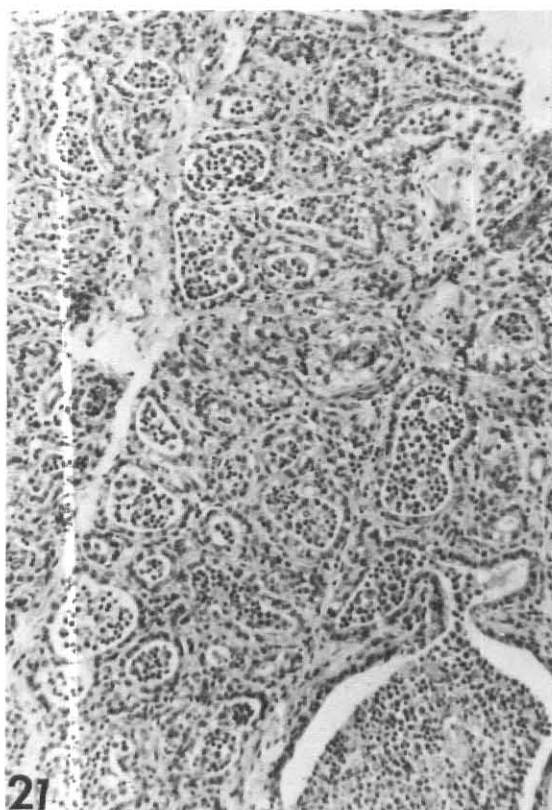


Fig 25 - Day 6 PI. Photomicrograph of acute purulent galactophoritis. Note variation in lobular involvement. The lobule in the upper right quadrant is relatively normal apart from some thickening of the intraacinar septa. H&E. X165.

Fig 26 - Day 6 PI. Acute galactophoritis with marked epithelial and diffuse interstitial infiltration by leukocytes. Note duct lumen blocked by inflammatory exudate and desquamated epithelium, and interstitial edema and fibrosis. H&E. X165.

Fig 27 - Day 6 PI. Section of mammary gland with almost complete replacement by granulation tissue. Note focus of inflammatory cells. H&E. X165.

Fig 28 - Day 6 PI. Area of more chronic involvement of lactiferous duct by granulation tissue and diffuse periductal fibrosis. Note scattered leukocytic infiltration and some metaplasia of the ductal epithelium. H&E. X165.

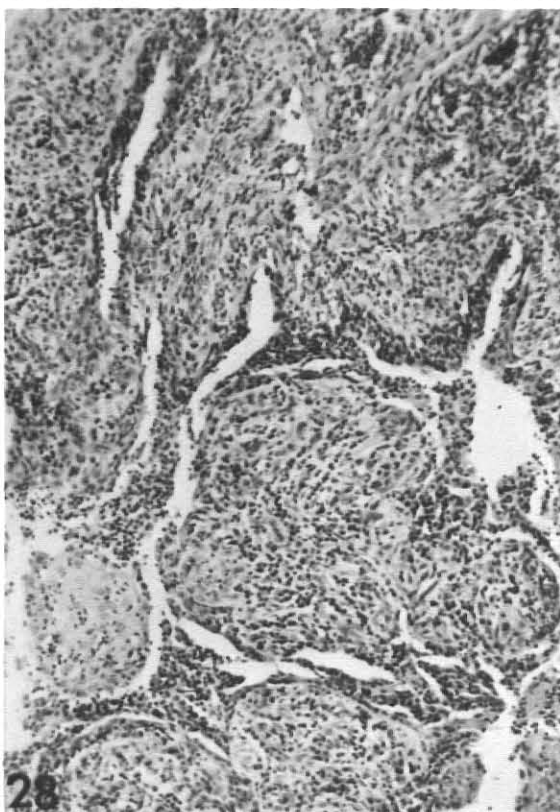
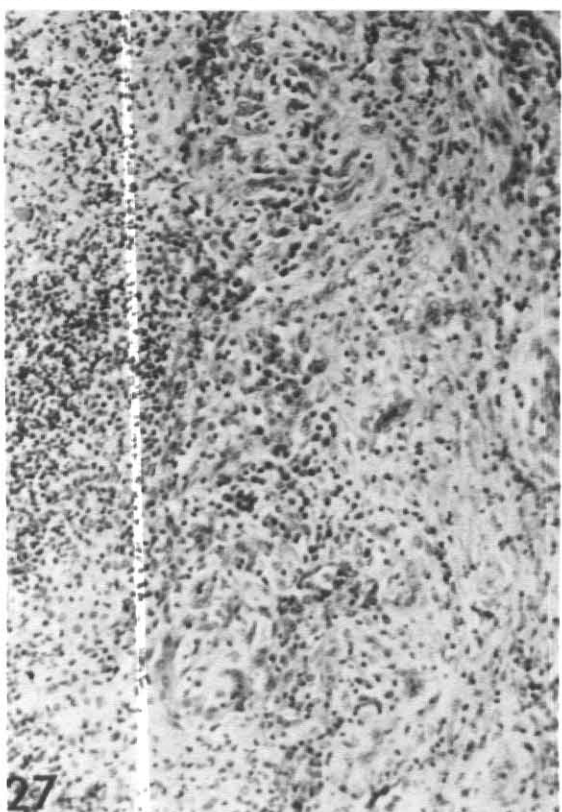
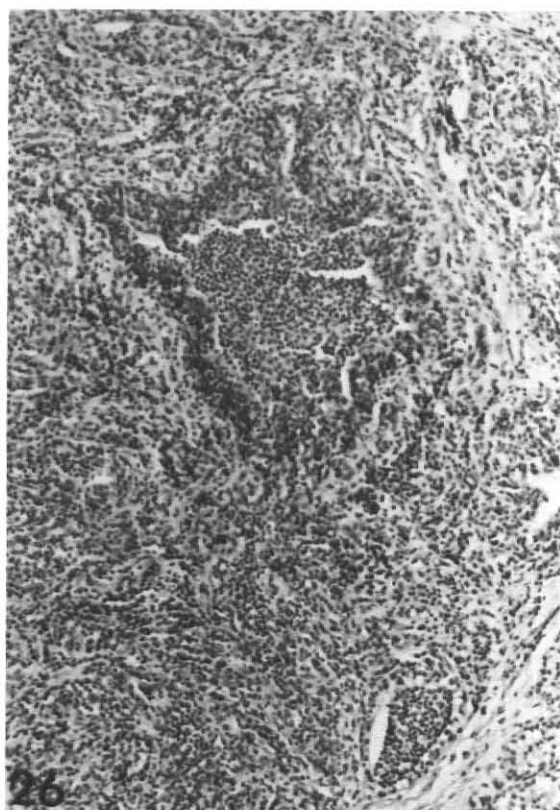
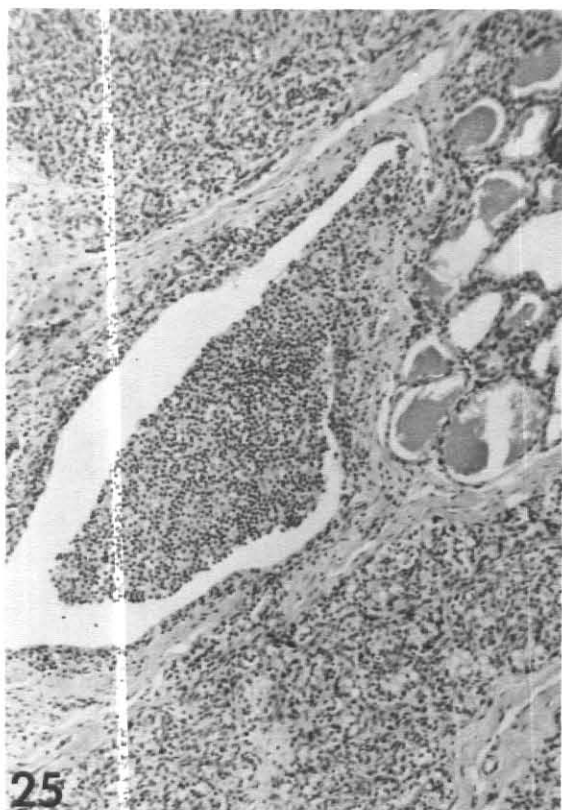
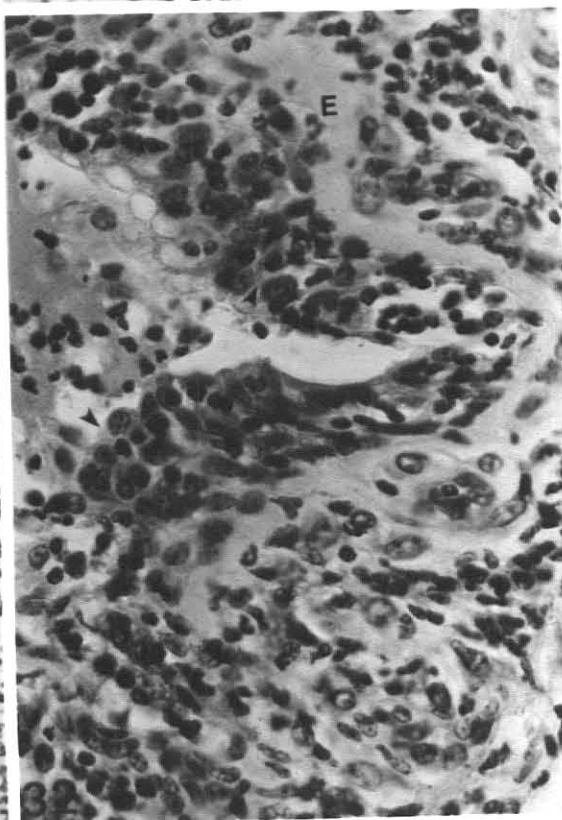
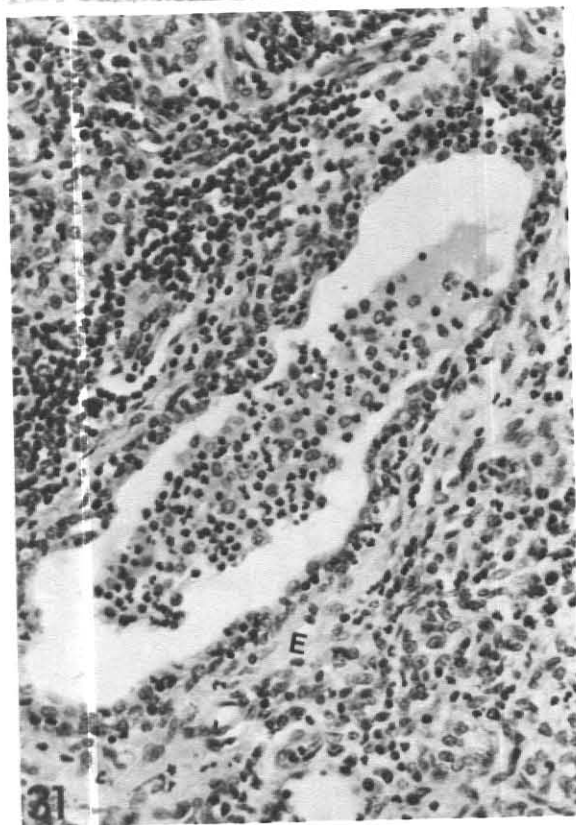
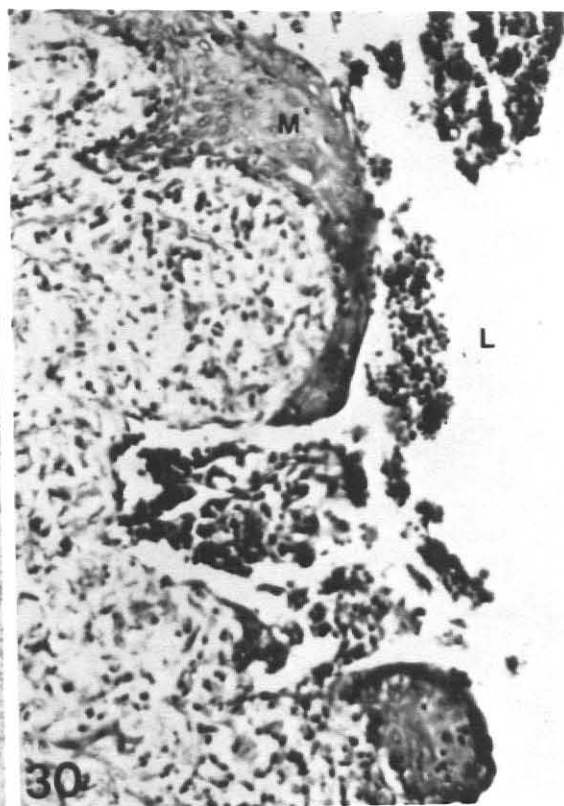


Fig 29 - Day 6 PI. Galactophoritis of a large duct. Note the lumen is almost blocked by granulation tissue, epithelial metaplasia, and extensive periductal fibrosis. H&E. X66.

Fig 30 - Day 6 PI. High power view of wall of a large duct with galactophoritis. Note lumen (L) containing disintegrating leukocytes and desquamating epithelium, metaplastic epithelium (M), and replacement of the wall and periductal tissue by granulation tissue. Trichrome. X165.

Fig 31 - Day 6 PI. Acute galactophoritis involving a lobar duct. Note inflammatory exudate in the lumen, primarily neutrophils and desquamated epithelial cells, leukocytic infiltration of the wall, and the serofibrinous exudate (E) and leukocytes in the interstitium. H&E. X330.

Fig 32 - Day 6 PI. Acute galactophoritis of a large duct. Compare with Fig 30. Note the protein and cellular rich exudate in the lumen, marked leukocytic infiltration of the ductal epithelium (arrows), thickening of the duct wall, and serofibrinous exudation (E) and leukocytic infiltration of the periductal interstitium. H&E. X660.



severely affected lobules had necrosis with loss of architecture and fibrosis (Fig 27). Significant changes involved the larger lactiferous ducts: leukocytic infiltration of the epithelium, epithelial hyperplasia and metaplasia, and fibroblastic and myoepithelial cell reaction of the wall (Fig 28, 30, 32). Fibroblasts began to form papilliform projections into the duct lumens or to invade the inflammatory exudate in some ducts forming granulation tissue cores (Fig 28 and 29). The intraacinar and intralobular interstitial tissue was thickened by fibroplasia and leukocytic infiltration, mainly mononuclear cells and neutrophils (Fig 31). Changes in the supramammary lymph node was the same as the earlier periods PI.

Day 7 PI - The lesions in ewe #7810 varied in severity even in the same lobules. Some areas had ductal, acinar and interstitial changes similar to those in the earlier ewes. Some areas were necrotic and others were replaced by fibrous tissue (Fig 33 and 36). The reaction in the interstitium was mononuclear and not neutrophilic. The changes in the larger ducts were similar to those at day 6 PI but more severe with polypoid projections (Fig 34), squamous metaplasia (Fig 35), and fibrosis (Fig 36). The supramammary lymph node was reactive and contained many plasma cells in the sinuses.

Fig 33 - Day 7 PI. Fibrosed area of mammary gland. Note diffuse involvement and obliterated ducts (arrows) with leukocytic infiltration of the epithelium. H&E. X165.

Fig 34 - Day 7 PI. Cross section of a polypoid thickening of the wall of a lactiferous duct with desquamation of the epithelial cells. Note hydropic degeneration of the lamina propria cells and some infiltration with lymphocytes and macrophages. H&E. X330.

Fig 35 - Day 7 PI. Photomicrograph of part of a large lactiferous duct with marked squamous metaplasia of epithelium with underlying granulation tissue. Note degenerating inflammatory exudate in lumen (L). H&E. X165.

Fig 36 - Day 7 PI. Fibrosed area of mammary gland with a lactiferous duct in the center blocked by a polyp of granulation tissue. Note foci of leukocytes scattered throughout the section and a few acini in the upper right corner. H&E. X66.

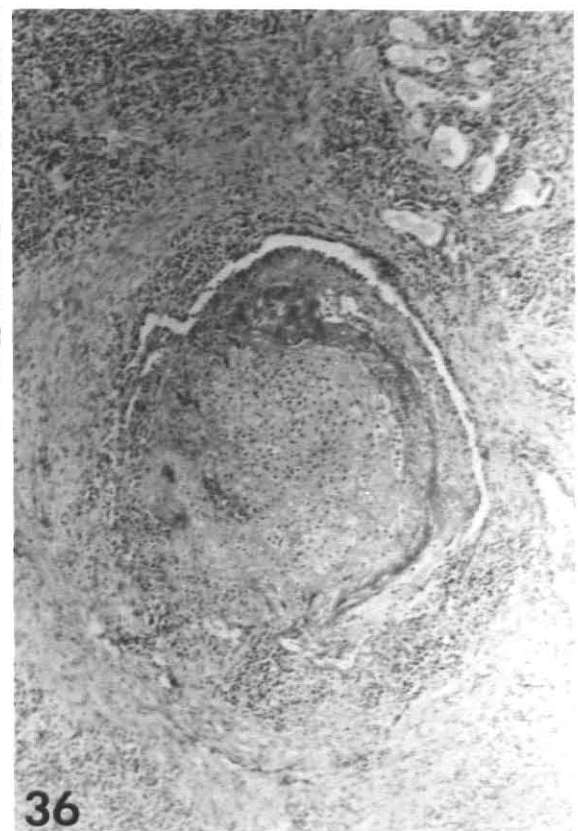
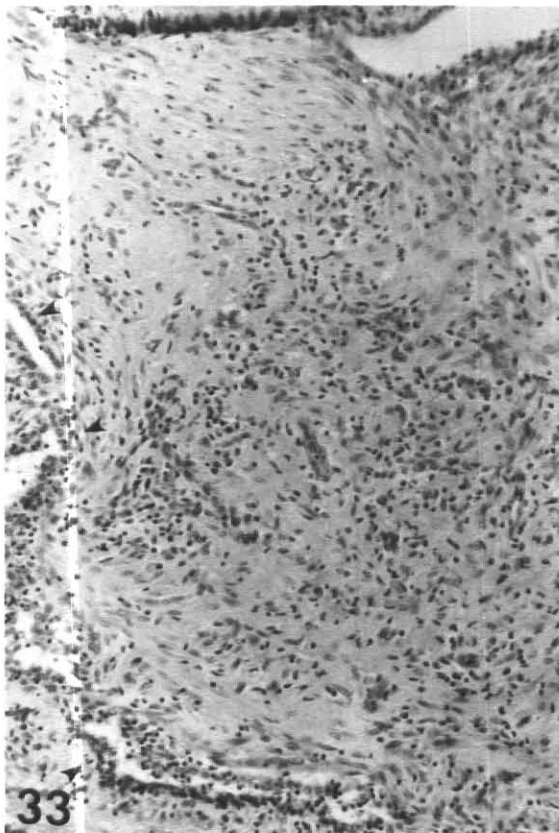


Fig 37 - Day 8 PI. Photomicrograph of a less affected area of the mammary gland. Note thickened intraacinar septa by leukocytes, mainly mononuclear cells. H&E. X165.

Fig 38 - Day 8 PI. Photomicrograph of a large lactiferous duct with squamous metaplasia of the epithelium. Note the irregular surface (L), penetrating stratified squamous epithelium, underlying granulation tissue, and disintegrating leukocytes in lumen, within the metaplastic epithelium, and periductal granulation tissue. H&E. X165.

Fig 39 - Day 8 PI. Photomicrograph of a large lactiferous duct almost completely blocked by a polyp covered by squamous epithelium that is penetrating the underlying granulation tissue. Trichrome. X165.

Fig 40 - Day 8 PI. Photomicrograph of another area of the same mammary gland depicted in Fig 37-39. Note infiltration of interstitium and acini by leukocytes, mainly mononuclear cells, and serofibrinous exudate (E). H&E. X660.

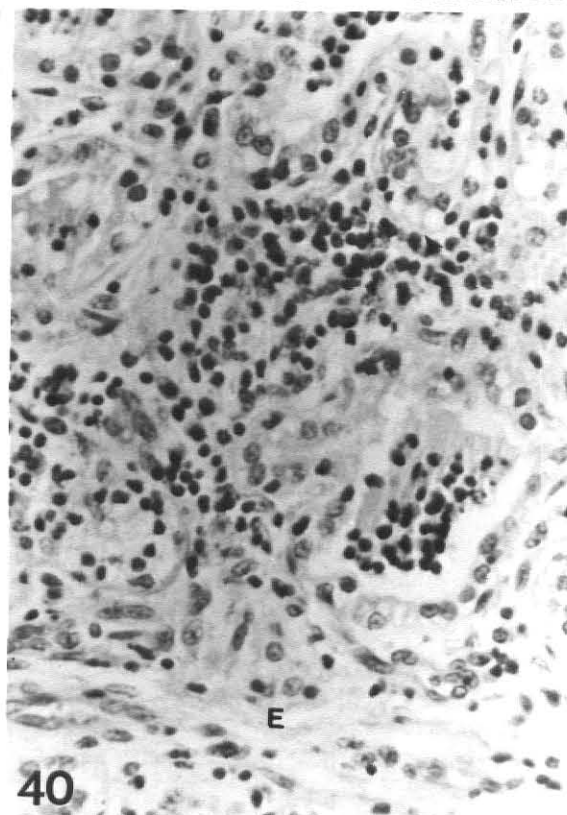
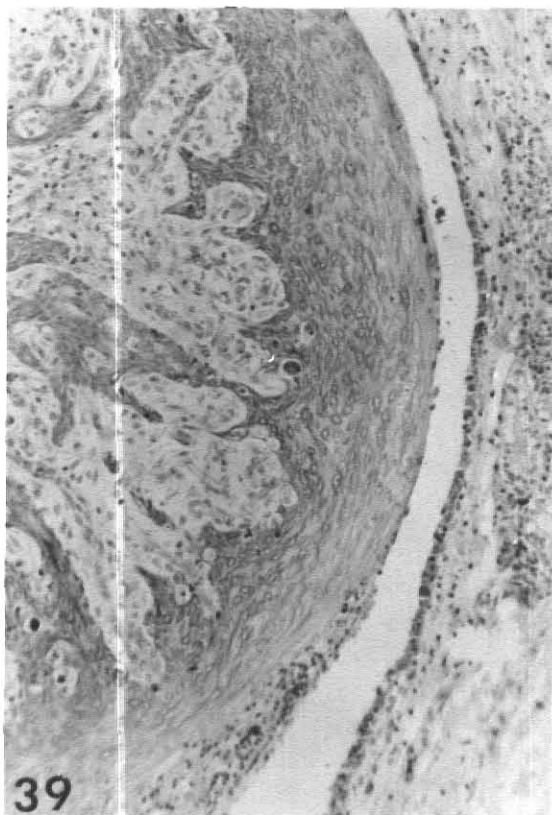
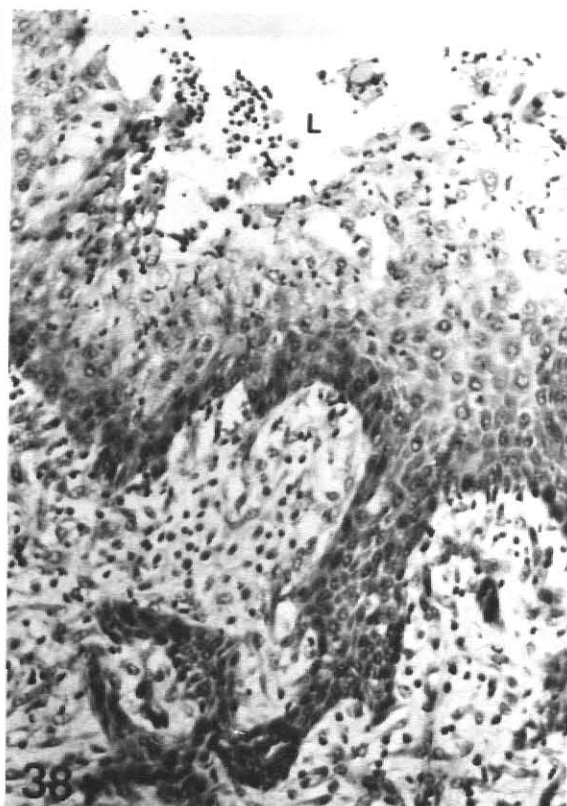
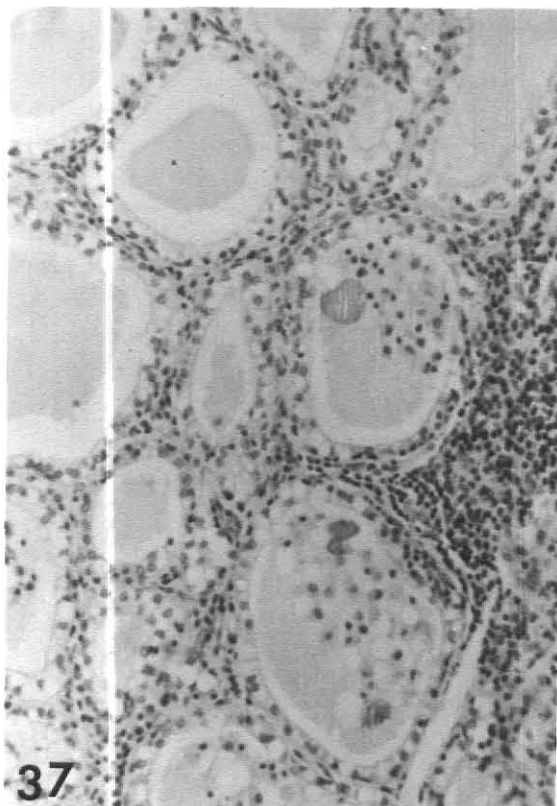
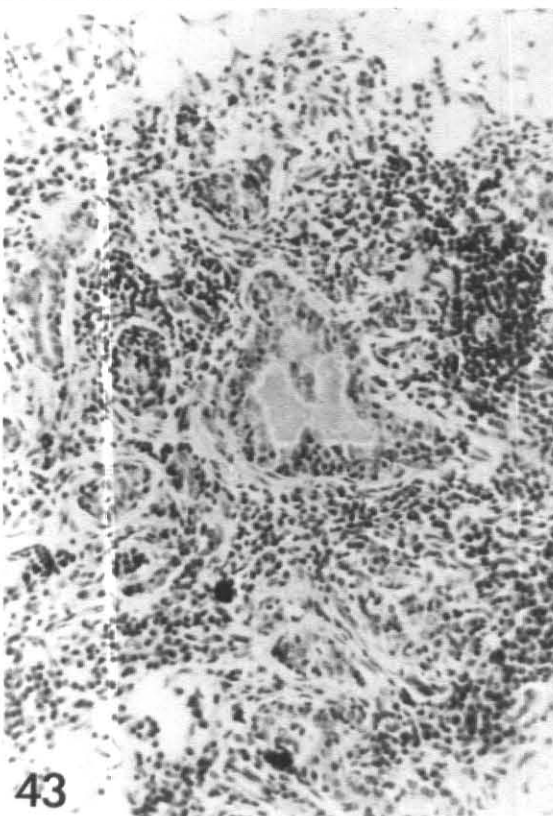
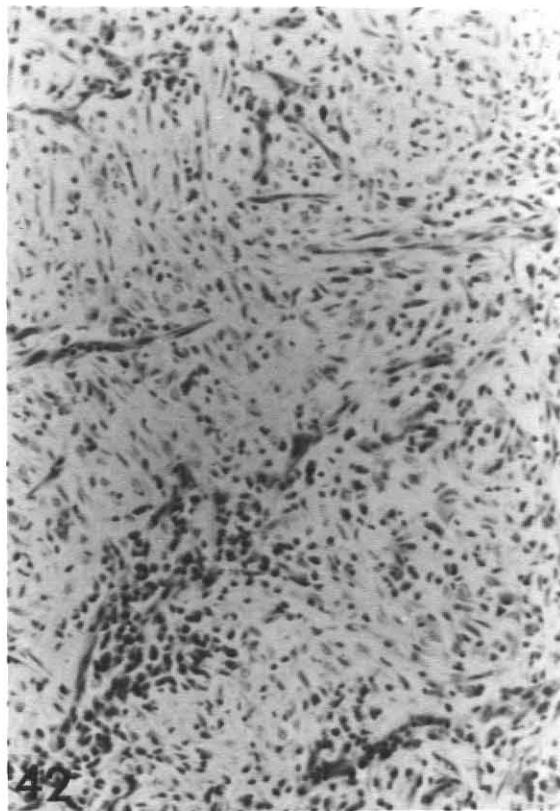
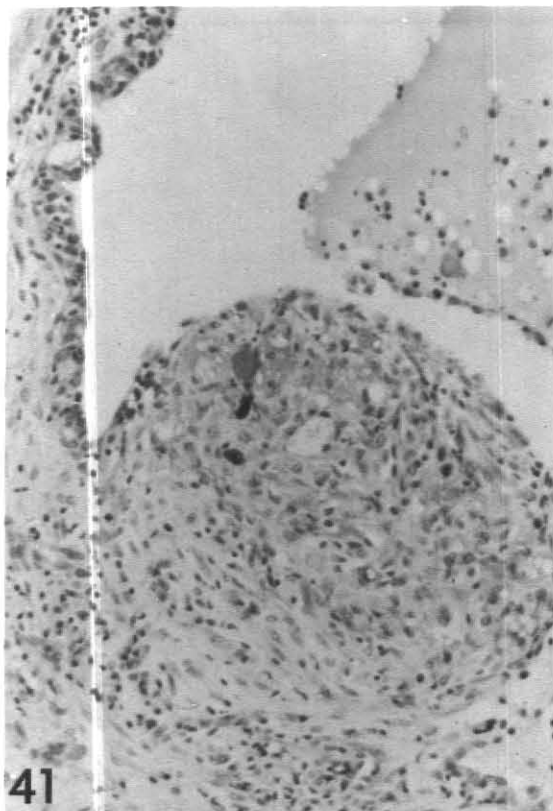


Fig 41 - Day 9 PI. Photomicrograph of a large lactiferous duct with a polyp projecting from a fibrosed wall. H&E. X165.

Fig 42 - Day 9 PI. Note diffuse granulation tissue and some mononuclear cell infiltration. H&E. X165.

Fig 43 - Day 9 PI. Photomicrograph of another section of the same gland in Fig 42. Note diffuse involvement with mononuclear cell infiltration, some fibroplasia, and two small calcified foci. H&E. X165.

Fig 44 - Day 9 PI. Photomicrograph of a large lactiferous duct with tumor-like projections. Note desquamated epithelium, small necrotic foci (N), and small calcified foci resulting from blocked acini. H&E. X165.



Day 8 PI - The lesions observed in ewe #7736 were essentially the same as those at day 7 PI except they were more advanced in some areas. There were areas of minimal change, mainly focal intralobular interstitial thickening by leukocytic infiltration (Fig 37). The inflammatory cell response was primarily mononuclear (lymphocytes, macrophages and plasma cells) and not neutrophilic (Fig 40). Neutrophilic infiltration and degenerative changes, indicative of recent involvement was observed in some lobules. Squamous metaplasia of ductal epithelium, granulation polypoid formation, and ductal and periductal fibrosis were prominent findings at day 8 PI (Fig 38 and 39). The supramammary lymph node changes were similar to those at day 7 PI.

Day 9 PI - The findings in this nonlactating ewe (#7745) were similar to those at day 7 and 8 PI. The inflammatory response was primarily mononuclear, mainly lymphocytes and plasma cells, but some neutrophils and eosinophils were present (Fig 43). Ductal epithelial metaplasia was less severe but polypoid thickening of the walls was still prominent (Fig 41 and 44). Necrosis and desquamation of ductal epithelium were common findings (Fig 41 and 44). Lobular fibrosis varied from moderate to replacement of glandular tissue (Fig 42 and 43). Eosinophilic, spherical, intracytoplasmic bodies of varying size were observed within acinar epithelium and macrophages; some were found

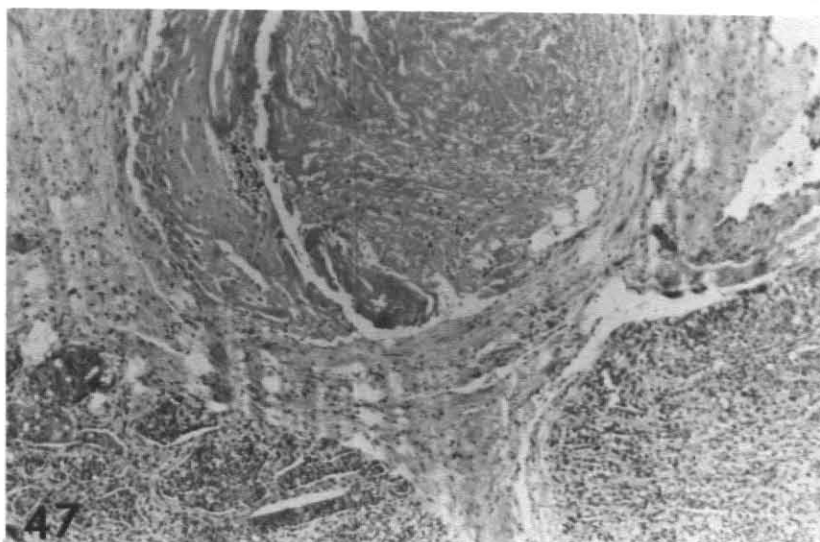
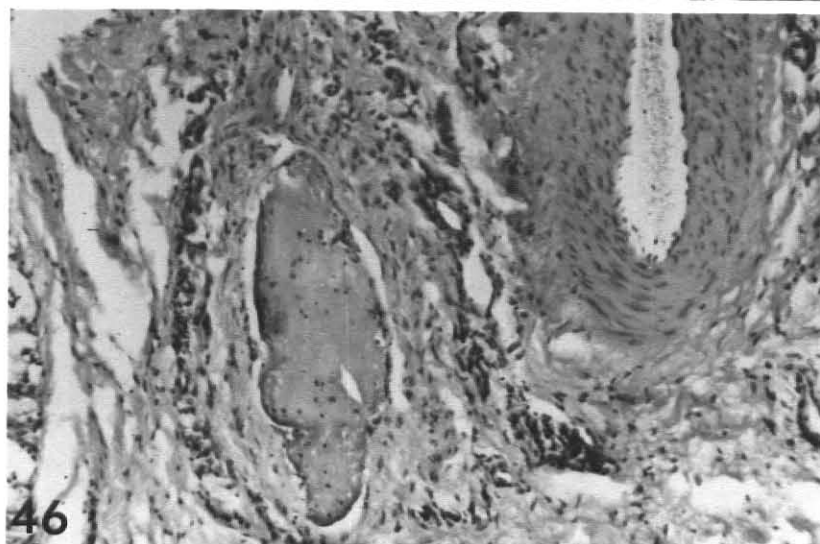
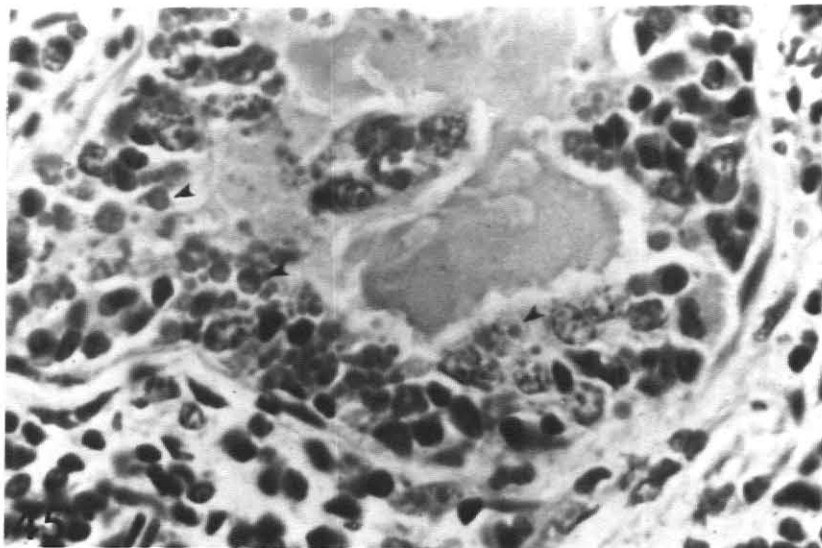
extracellularly (Fig 45). They stained by PAS and PAS diastase methods but not by von Kossa stain.

The supramammary lymph node was reactive, had many plasma cells, and contained a small abscess with fibrosis adjacent to the hilus.

Fig 45 - Day 9 PI. High magnification of Fig 43. Note the intracytoplasmic round, eosinophilic bodies of varying size (arrows) in the epithelium and mononuclear cell infiltration. H&E. X660.

Fig 46 - Day 3 PI. Thrombus in a vein in a mammary gland infected with A. seminis. H&E. X165.

Fig 47 - Day 4 PI. Thrombus in a large vein in intralobar connective tissue of an A. seminis infected mammary gland. H&E. X66.



## DISCUSSION

Infectious ovine mastitis is a complex disease because of the various causes, pathogenesis, sequelae, treatment and control, and related aspects. Most studies on ovine mastitis have involved staphylococci (Landau and Tamarin 1974).

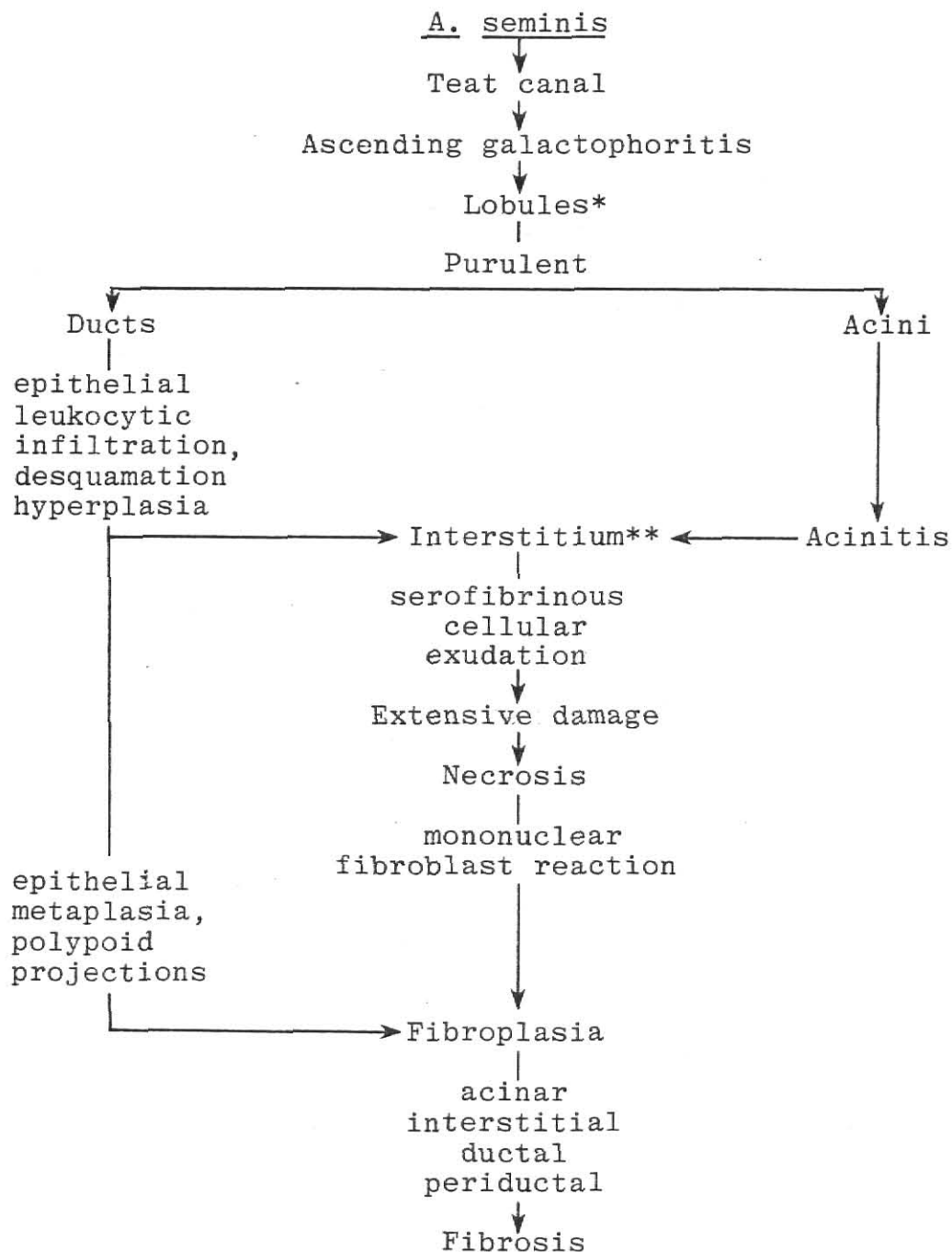
All ewes inoculated with A. seminis developed acute mastitis evident by enlargement, heat and pain in the affected gland, and transient fever. The clinical signs were apparent with 12 hours PI and peaked in 24 to 72 hours. The swelling was due mainly to the extensive inflammatory edema, a common finding with many acute bacterial mastitides (Heidrich and Renk 1967). The clinical signs were not as severe as those reported with H. ovis mastitis (Roberts 1956; Kater et al 1962) or A. seminis (Watt et al 1970); no marked systemic involvement or deaths. The difference in response could be explained by virulence of the organisms. The strains used by Roberts (1956) and Watt et al (1970) were recent virulent isolates from field cases. The organism used in this study was the original A. seminis isolated by Baynes and Simmons (1960). Susceptibility of the ewes, breed, age, and stage of lactation may also have been factors.

Histopathological changes in ovine mastitis due to H. ovis (Roberts 1956), A. lignieresii (Laws and Elder 1969) and A. seminis (Watt et al 1970) were not reported. Kater et al (1962) recorded acute catarrhal necrotizing mastitis

but gave no histopathological details. This lack of histopathological changes prevented comparisons with the microscopic findings in this study. In contrast to mastitis due to P. hemolytica, S. aureus, C. pseudotuberculosis and C. pyogenes (El-Etreby and Abdel-Hamid 1970), and H. ovis (Kater et al 1962) abscess formation was not a feature of A. seminis mastitis during the time period of this study.

The type of mastitis resulting from inoculation of A. seminis via the teat canal was similar in all test ewes and was progressive with marked heterogenicity in the degree of tissue involvement ranging from acute purulent galactophoritis to purulent acinitis, focal necrosis, epithelial metaplasia to fibrosis from one lobule to another including normal unaffected tissue (Fig 48). The lesions resembled those of streptococcal and mycoplasmal mastitis in cattle (Jubb and Kennedy 1970), streptococcal mastitis in goats (Pattison 1951), and Listeria monocytogenes mastitis in cattle (Gitter et al 1980).

The heterogenicity of A. seminis induced microscopic lesions as well as the severity of involvement of the duct system compared to the glandular and interstitial changes in the same area may be explained by ascending infection, galactophoritis to acinitis to interstitial involvement and drainage by lymphatics. There was extensive damage to acini and evidence of fibroplasia. A. seminis penetrated



\* Variable lobular involvement, some unaffected

\*\*Inter- and intralobular

Fig 48 - Sequence of events in ewes following intramammary inoculation with A. seminis.

duct and acinar epithelium by day 3 PI; after this period they were not observed microscopically as A. seminis organisms were rapidly destroyed. A. seminis, however, was isolated from the inoculated mammary gland of each test ewe at necropsy. The rapid disappearance of A. seminis from sites of inoculation was also observed in the cauda epididymidis (Al-Katib 1980), and carpal joint and lungs (Ogunjumo 1980). Similar findings were reported with experimental streptococcal mastitis in goats (Pattison 1951). He observed that streptococci briefly penetrated the duct and acinar epithelium to the lymphatic channels and were rapidly destroyed but not before initiating a marked macrophage-fibroblast reaction.

The microscopic reaction of the ovine mammary gland to inoculation of A. seminis via the teat canal may be divided into 4 overlapping phases: acute purulent, subacute purulent, necrotizing and proliferative. Acute purulent mastitis was characterized by galactophoritis and acinitis with accumulation of inflammatory exudate within the lumens consisting mainly of neutrophils and proteinaceous fluid, and little epithelial changes and some serofibrinous exudation within the interstitium. In subacute purulent mastitis, the luminal contents were highly cellular, consisting of leukocytes (neutrophils and mononuclears) and desquamated epithelial cells, moderate to marked epithelial changes,

and increased inflammatory exudation in the interstitium. Necrotizing mastitis was characterized by focal or lobular necrosis with loss of tissue architecture and little or no fibroplasia. Proliferative mastitis was characterized by granulation tissue involving the ducts, secreting tissue and interstitium, and metaplasia and polypoid outgrowths of duct epithelium.

In development of bacterial mastitis, the pathogen must gain entry into the mammary gland, survive the bacteriostatic and bactericidal factors, and then multiply in significant numbers (Heidrich and Renk 1967). Resistance to bacterial invasion is determined for the most part by the structure and function of the teat canal. Studies demonstrating how bacteria actually pass through the teat canal under normal conditions are lacking (Jain 1979). With exception of tuberculosis and brucellosis that are usually hematogenous in origin (Jubb and Kennedy 1970; Blood et al 1979), the portal of entry for mastitis causing agents is the teat orifice and canal. The pathogenesis of mastitis may, in general, be explained in 3 phases: invasion, infection and inflammation (Blood et al 1979). During invasion, the organisms pass from the exterior to milk inside the teat canal. In the infectious phase, the organisms multiply rapidly and invade the mammary tissue, and during the inflammation phase, clinical mastitis appears

or the leukocyte count in milk is greatly increased. In cattle, the teat orifice and canal may be affected by many factors such as teat lesions, teat patency, milking mechanism, growth through the teat canal, hypersensitization, intramammary factors, and intraductal factors, and strain, number of organisms present, and state of growth of the invading organism (Newbould 1964). As there are no mucus secreting cells in the mammary gland, the term catarrhal mastitis is a misnomer for purulent mastitis.

The eosinophilic, spherical, intracytoplasmic bodies observed within acinar epithelium and macrophages were determined to be proteinaceous. These are probably Russell bodies or gamma globulin granules from maturing plasmacytes.

Pseudoconcretions or so-called corpora amylacea and concretions occur in mammary glands with mastitis. The corpora amylacea are spherical, ovoid or bean-shaped structures, 30 to 250  $\mu$  in diameter, with a more or less clearly recognizable stratification. They occur in normal lactating and nonlactating glands but are more common and numerous in acini and interacinar tissue of mastitic glands. They frequently calcify, are commonly larger than the acini and may rupture the walls and pass into the lactiferous ducts. These structures frequently develop in parts of the gland where secretion is suppressed and where retained secretion is partially resorbed via the lymphatic system,

and the remnants are inspissated and calcified (Heidrich and Renk 1967).

Although A. seminis infection has been reported from Australia, New Zealand, the United States, and South Africa, there are only two reports of A. seminis or a similar organism causing mastitis in sheep (Roberts 1956; Kater et al 1962). This is surprising with the high incidence of ovine mastitis. The incidence of A. seminis mastitis in ewes may be significant but the organism missed in routine bacteriological examination because of its nutritional and gaseous requirements. The suggestion of nonvenereal transmission of A. seminis by Simmons et al (1966) was confirmed by Al-Katib (1980). Ewe-to-ram lamb transmission may clarify the current poorly understood methods of spread of actinobacillosis under natural conditions. The ewe may infect the ram fetus in utero, during birth, immediately after birth via the umbilicus, or during nursing. Simmons et al (1966) suggested the possibility of lambs being latently infected and the infection becoming clinical after sexual maturity. If this is so, then the ewe could play a greater role in the transmission of actinobacillosis than presently thought. The udder could be site of infection for carrier ewes with ram lambs being infected orally.

It is concluded from this study that:

1. A. seminis is pathogenic for the ovine mammary gland;
2. the clinical signs and pathological findings in A. seminis are nonspecific;
3. A. seminis can survive within ovine mammary tissue for at least 9 days PI;
4. it is suggested that milk from an A. seminis infected gland may result in a latent infection in ram lambs; and
5. further studies over a longer period by light and electron microscopy and immunofluorescence are required to expand our understanding of A. seminis mastitis in ewes.

## SUMMARY

Sequential pathological changes in the mammary gland were studied in 10 ewes, 8 test and 2 control, following inoculation of A. seminis via the teat canal. The left mammary gland of the test ewes was inoculated with 2 ml of a 24 hour broth culture containing  $2.0 \times 10^9$  CFU per ml. The control ewes were inoculated with 2.0 ml of uninoculated broth. The right mammary glands served as uninoculated controls. Following inoculation, the ewes were examined twice daily, rectal temperatures recorded, and the udders carefully palpated. The ewes were euthanatized and immediately necropsied as follows: 2 on day 3 PI and one each on day 4, 5, 6, 7, 8 and 9; one control on day 10 and the other on day 11.

The 8 test ewes had transient temperatures up to 3.2 F from 12 to 24 hours PI that varied from ewe to ewe and returned to normal within 36 to 60 hours PI. The inoculated glands were enlarged (3 to 5 times normal), turgid or consolidated and hot. At necropsy, the glands lacked the typical spongy structure and compartmentation was obvious. They contained whitish-creamy or greenish-yellow viscid secretions in contrast to clear milk or watery fluid from the uninoculated glands on control ewes. A. seminis was isolated from all test glands but not from the uninoculated

glands or other body organs. The supramammary lymph nodes on the inoculated side were slightly larger and edematous compared to the uninoculated side. None of the control mammary glands had any gross or microscopic changes.

Microscopically, the changes were marked throughout the inoculated gland and there was little or no difference between the lactating and nonlactating glands. The reaction to A. seminis may be divided into 4 overlapping phases: acute purulent, subacute purulent, necrotizing, and proliferative. The acute purulent phase occurred primarily within the first 5 days PI and was characterized by an ascending galactophoritis and involvement of acini in many lobules, accumulation of inflammatory exudate and neutrophils within the lumens, some epithelial degeneration and desquamation, serofibrinous exudation in the interstitium, some venous thrombi, and a few small necrotic foci. The subacute purulent phase (day 5-7 PI) was characterized by highly cellular exudate within affected lumens, leukocytes (neutrophils and mononuclears) and desquamated epithelial cells, moderate to marked epithelial changes, and increased exudation within the interstitium and signs of fibroplasia. Changes in the larger ducts became prominent: epithelial hyperplasia to squamous metaplasia and ductal and periductal fibroplasia. The necrotizing phase (day 4-7 PI) was characterized by thrombosis and focal or lobular

necrosis and early fibroplasia. The proliferative phase (day 6-9 PI) was characterized by granulation tissue involving the ducts, periductal interstitium, intralobular acini and interstitial tissue, and squamous metaplasia and polypoid outgrowths of the lactiferous duct epithelium. The above changes were variable in all inoculated glands especially from day 4 PI. It was concluded from the study that A. seminis was pathogenic for the ovine mammary gland and that the clinical and pathological findings were nonspecific.

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## APPENDICES

A. Standardized Necropsy Form

## EWE ACTINOBACILLOSIS PROJECT

Ewe # \_\_\_\_\_ Breed \_\_\_\_\_ Age \_\_\_\_\_ Date: \_\_\_\_\_ Necropsy # \_\_\_\_\_  
 Days PI \_\_\_\_\_ Body Condition - Good, Fair, Poor Died/Euthanatized \_\_\_\_\_

---

## MAMMARY GLANDS

Left/Right/Both

Normal; lactating, dry; swollen - diffuse, nodules; secretion - normal, watery, clots, fibrin; abscess - consistency, creamy, serofibrinous, fibrinous, purulent, color - creamy, grayish-white, grayish-green, grayish-yellow, grayish-greenish-yellow

## OTHER SYSTEMS

Respiratory - nasal and sinus cavities; larynx; trachea; bronchi; lungs - L/R; pleura - normal, pleuritis, hydrothorax, pyothorax, fibrin, fibrous adhesions

Cardiovascular - pericardium; epi-, myo-, and endocardium, valves, aorta, other arteries and veins

Digestive - mouth, tongue, esophagus, rumen, abomasum, small intestine, large intestine; pancreas, liver - normal, necrotic foci

Lymphatic - spleen, lymph nodes

Urinary - kidneys, ureters, urinary bladder, urethra

Genital - vulva, vagina, cervix, uterus, oviducts, ovaries

Endocrine - thyroids, parathyroid, pituitary, adrenals

Nervous - meninges, brain, spinal cord

Musculoskeletal - muscles, bones, spine, joints, costochondral junction, sternum, bone marrow

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 Laboratory Examination

Sites cultured: left and right mammary glands, uterus, vagina, heart, spleen, joints, other \_\_\_\_\_

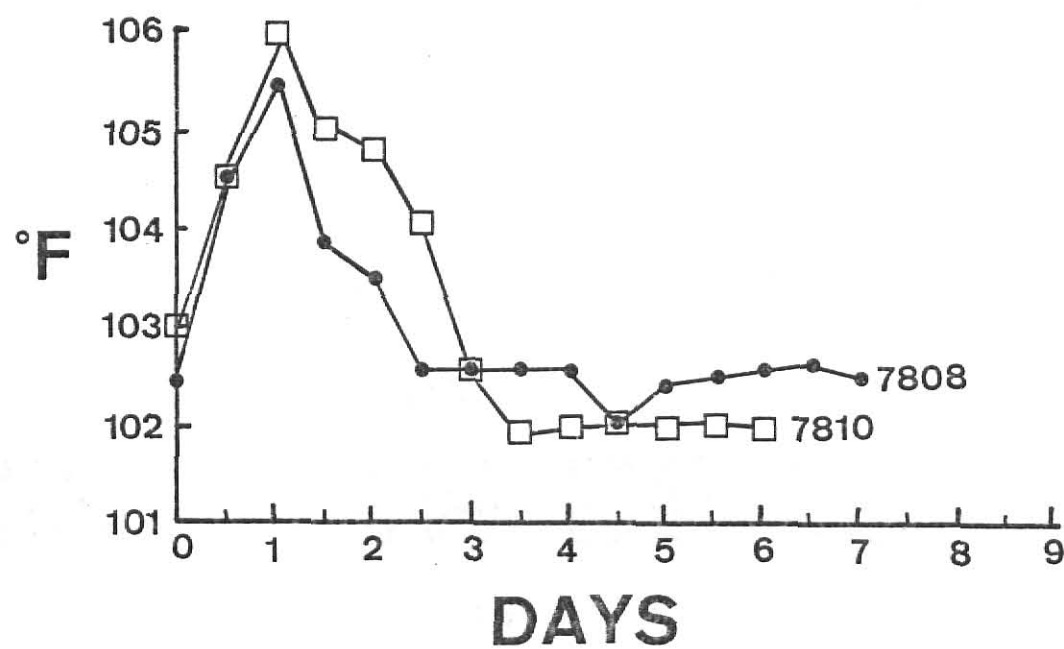
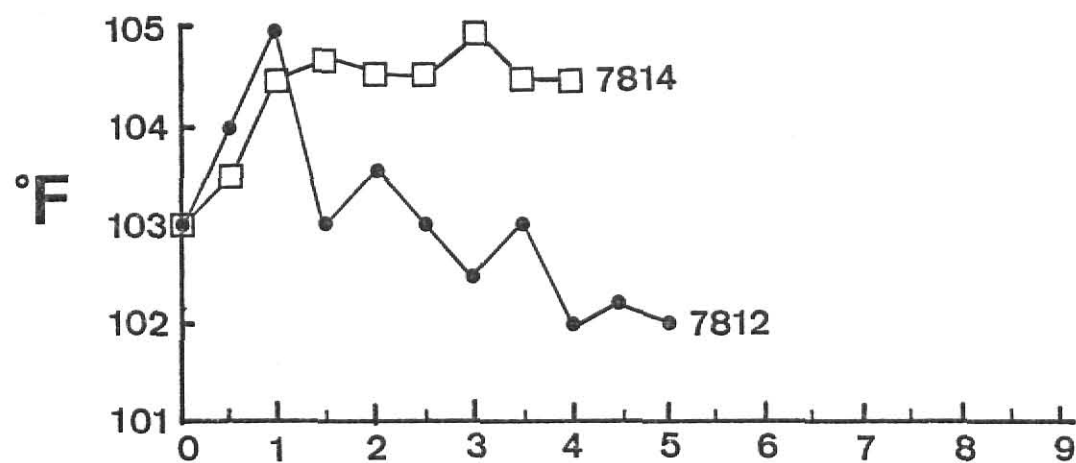
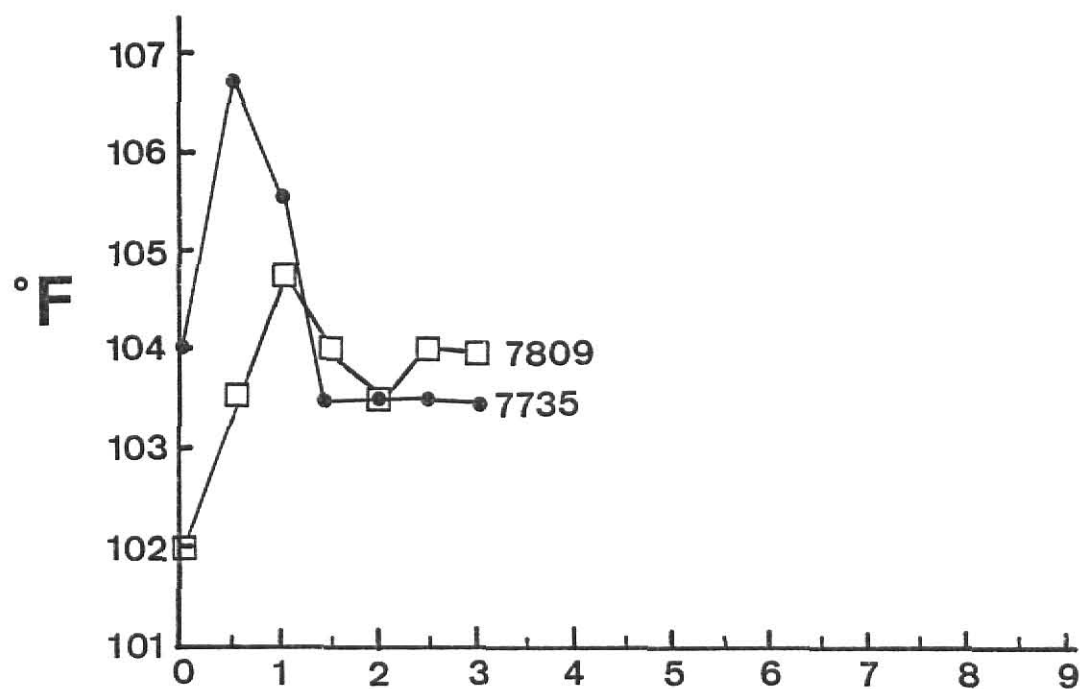
A. seminis recovered from: \_\_\_\_\_

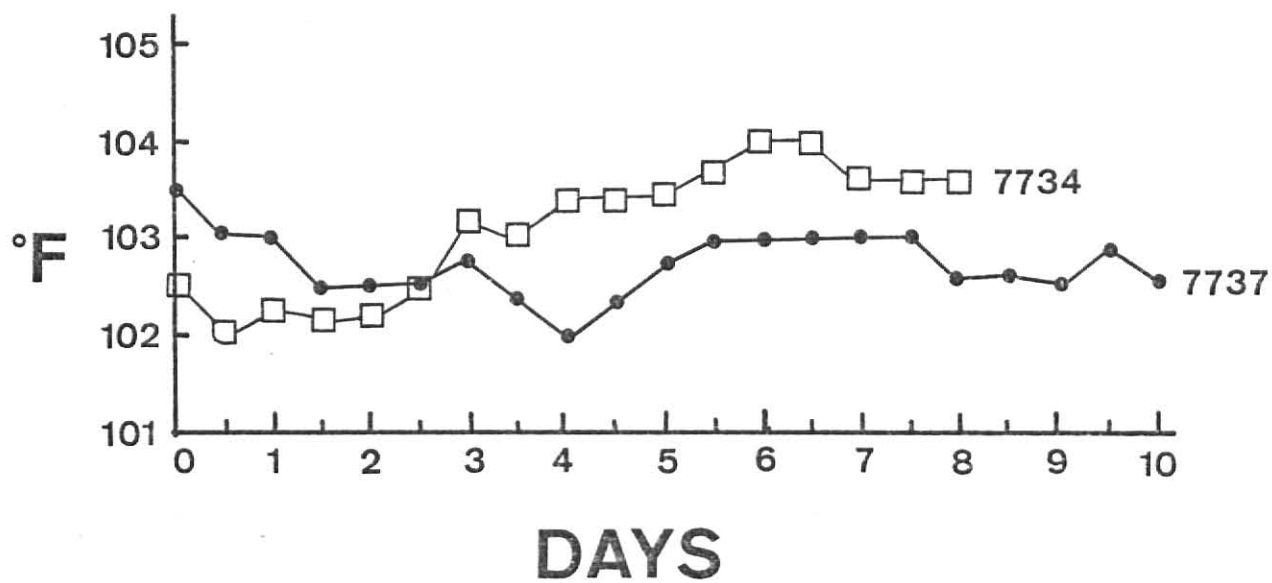
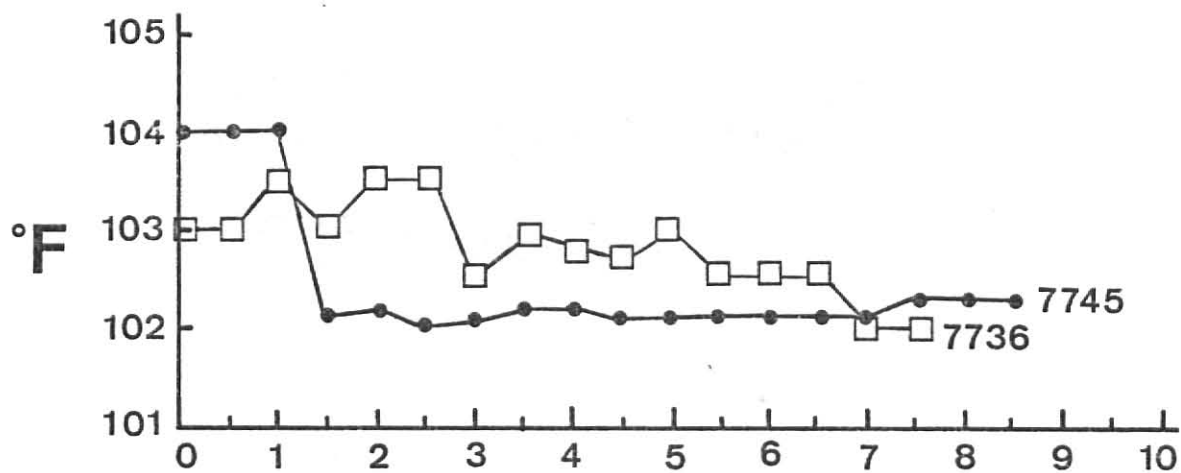
Organs photographed: \_\_\_\_\_

Histopathology summary: mastitis, vaginitis, endometritis, arthritis

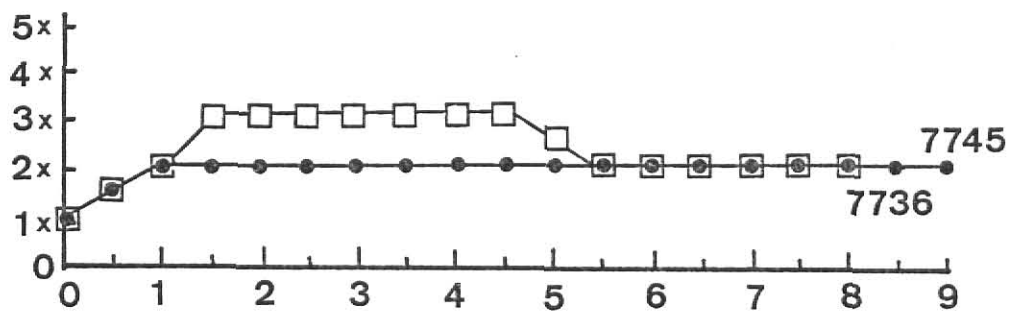
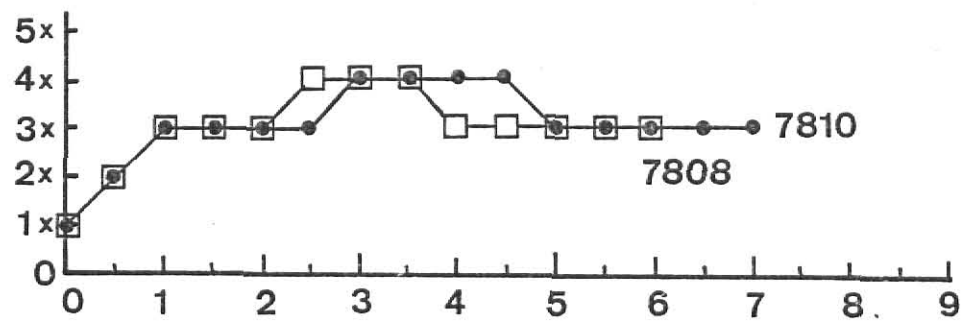
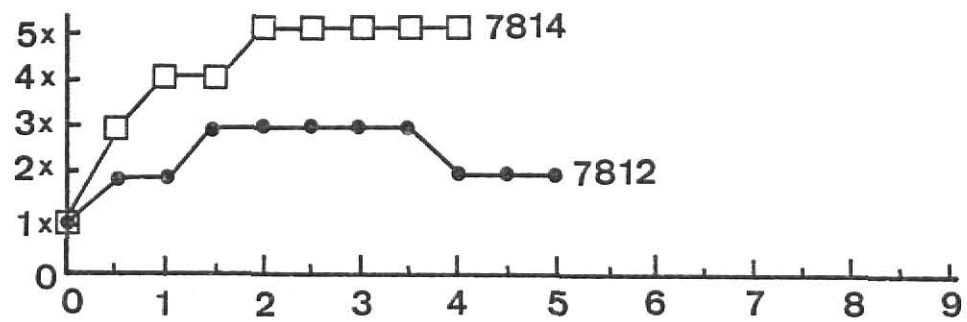
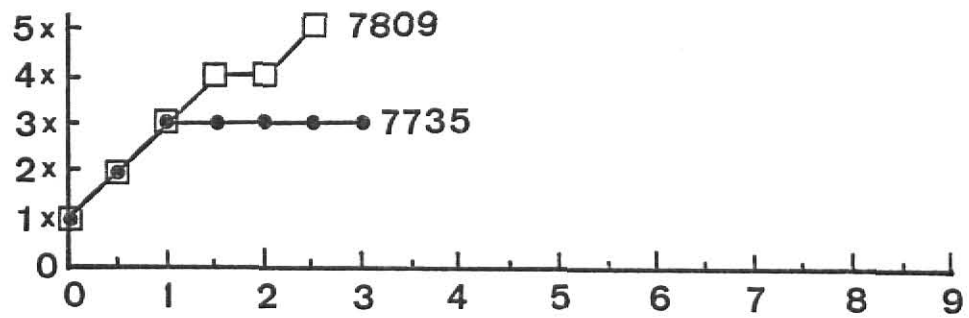
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## B. Temperature and Udder Enlargement





UDDER SIZE



DAYS

### C. Histopathological Findings

TABLE 1 - Microscopic Findings in Mammary Glands of Ewes Inoculated with A. seminis via the Teat Canal

[illegible]

TABLE 1 - continued

| Findings             | Control Ewes |    |    |   |   | Test Ewes |   |   |   |   |   |
|----------------------|--------------|----|----|---|---|-----------|---|---|---|---|---|
|                      | Days PI      | 10 | 11 | 3 | 3 | 4         | 5 | 6 | 7 | 8 | 9 |
| <u>Epithelium</u>    |              |    |    |   |   |           |   |   |   |   |   |
| Degenerative         |              | 0  | 0  | 2 | 2 | 2         | 2 | 2 | 1 | 3 | 1 |
| Necrotic             |              | 0  | 0  | 1 | 1 | 2         | 2 | 2 | 3 | 2 | 3 |
| Desquamated          |              | 0  | 0  | 1 | 3 | 3         | 3 | 3 | 3 | 3 | 2 |
| Dissociated          |              | 0  | 0  | 1 | 2 | 3         | 2 | 3 | 3 | 3 | 3 |
| Hyperplastic         |              | 0  | 0  | 0 | 0 | 0         | 0 | 0 | 0 | 0 | 0 |
| <u>Nuclei</u>        |              |    |    |   |   |           |   |   |   |   |   |
| Vacuolated           |              | 0  | 0  | 2 | 2 | 3         | 3 | 3 | 3 | 3 | 2 |
| Chromatin            |              | 0  | 0  | 0 | 0 | 2         | 2 | 2 | 2 | 3 | 0 |
| Margination pyknotic |              | 0  | 0  | 1 | 1 | 1         | 1 | 2 | 3 | 2 | 3 |
| <u>Cytoplasm</u>     |              |    |    |   |   |           |   |   |   |   |   |
| Swollen              |              | 0  | 0  | 2 | 2 | 3         | 3 | 3 | 2 | 2 | 0 |
| Granular             |              | 0  | 0  | 0 | 0 | 0         | 0 | 0 | 0 | 0 | 0 |
| Vacuolated           |              | 0  | 0  | 2 | 2 | 3         | 3 | 3 | 2 | 2 | 0 |

TABLE 1 - continued

| Findings      | Control Ewes |      |      |      |      |      |      |      |      |      | Test Ewes |     |  |  |  |  |  |  |  |  |
|---------------|--------------|------|------|------|------|------|------|------|------|------|-----------|-----|--|--|--|--|--|--|--|--|
|               | 7734         | 7737 | 7809 | 7735 | 7814 | 7812 | 7808 | 7810 | 7736 | 7745 |           |     |  |  |  |  |  |  |  |  |
|               | Days         | PI   | 10   | 11   | 3    | 3    | 4    | 5    | 6    | 7    | 8         | 9   |  |  |  |  |  |  |  |  |
| <u>Wall</u>   |              |      |      |      |      |      |      |      |      |      |           |     |  |  |  |  |  |  |  |  |
| Ruptured      | 0            | 0    | 0    | 2    | 3    | 3    | 3    | 2    | 3    | 3-4  | 3-4       | 3-4 |  |  |  |  |  |  |  |  |
| Edema         | 0            | 0    | 0    | 3    | 3    | 3    | 3    | 3    | 3    | 3    | 2         | 1   |  |  |  |  |  |  |  |  |
| Fibrin        | 0            | 0    | 0    | 2    | 1    | 1    | 1    | 1    | 0    | 0    | 0         | 0   |  |  |  |  |  |  |  |  |
| Neutrophils   | 0            | 0    | 0    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2         | 1   |  |  |  |  |  |  |  |  |
| Macrophages   | 0            | 0    | 0    | 0    | 1    | 1    | 0    | 2    | 2    | 3    | 2         | 3   |  |  |  |  |  |  |  |  |
| Lymphocytes   | 0            | 1    | 0    | 0    | 1    | 1    | 1    | 2    | 2    | 3    | 3         | 3   |  |  |  |  |  |  |  |  |
| Plasma cells  | 0            | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    | 2    | 2         | 2   |  |  |  |  |  |  |  |  |
| Eosinophils   | 0            | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0         | 0   |  |  |  |  |  |  |  |  |
| Fibroblasts   | 0            | 1    | 1    | 1    | 2    | 2    | 1    | 2    | 2    | 3    | 3         | 3   |  |  |  |  |  |  |  |  |
| SMALL DUCTS   |              |      |      |      |      |      |      |      |      |      |           |     |  |  |  |  |  |  |  |  |
| <u>Lumens</u> |              |      |      |      |      |      |      |      |      |      |           |     |  |  |  |  |  |  |  |  |
| Dilated       | 2            | 0    | 3    | 3    | 1    | 3    | 3    | 3    | 2    | 1    | 3         | 1   |  |  |  |  |  |  |  |  |
| Protein       | 3            | 1    | 3    | 3    | 1    | 1    | 2    | 1    | 1    | 1    | 2         | 1   |  |  |  |  |  |  |  |  |
| Neutrophils   | 0            | 0    | 3    | 3    | 3    | 3    | 3    | 3    | 3    | 3    | 3         | 2   |  |  |  |  |  |  |  |  |
| Lymphocytes   | 0            | 0    | 1    | 1    | 2    | 2    | 1    | 2    | 2    | 2    | 2         | 3   |  |  |  |  |  |  |  |  |
| Macrophages   | 0            | 0    | 1    | 1    | 2    | 2    | 2    | 3    | 3    | 3    | 3         | 2   |  |  |  |  |  |  |  |  |
| Plasma cells  | 0            | 0    | 0    | 0    | 1    | 1    | 0    | 1    | 1    | 1    | 1         | 1   |  |  |  |  |  |  |  |  |

TABLE 1 - continued

| Findings           | Control Ewes |    |   |   |   | Test Ewes |   |     |     |     |
|--------------------|--------------|----|---|---|---|-----------|---|-----|-----|-----|
|                    | 10           | 11 | 3 | 3 | 4 | 5         | 6 | 7   | 8   | 9   |
|                    | Days PI      |    |   |   |   |           |   |     |     |     |
| Epithelial cells   | 0            | 1  | 2 | 3 | 3 | 3         | 3 | 3   | 2   | 2   |
| Giant cells        | 0            | 0  | 0 | 0 | 0 | 0         | 0 | 0   | 0   | 0   |
| Debris             | 0            | 0  | 1 | 1 | 1 | 2         | 3 | 3   | 3   | 2   |
| Corpora amylacea   | 1            | 1  | 1 | 2 | 1 | 1         | 1 | 1   | 1   | 2   |
| Erythrocytes       | 0            | 0  | 0 | 1 | 1 | 1         | 1 | 2   | 1   | 1   |
| Granulation tissue | 0            | 0  | 0 | 0 | 1 | 2         | 2 | 3   | 3   | 3-4 |
| <u>Epithelium</u>  |              |    |   |   |   |           |   |     |     |     |
| Degenerative       | 0            | 0  | 3 | 2 | 3 | 3         | 2 | 1   | 1   | 1   |
| Necrotic           | 0            | 0  | 1 | 3 | 2 | 2         | 3 | 3-4 | 3-4 | 3-4 |
| Desquamated        | 0            | 0  | 2 | 3 | 2 | 3         | 3 | 3   | 3   | 3   |
| Hyperplastic       | 0            | 0  | 1 | 1 | 1 | 1         | 2 | 3   | 3   | 2   |
| Metaplastic        | 0            | 0  | 0 | 0 | 0 | 1         | 1 | 2   | 2   | 1   |
| Bacteria           | 0            | 0  | 0 | 0 | 0 | 0         | 0 | 0   | 0   | 0   |
| <u>Wall</u>        |              |    |   |   |   |           |   |     |     |     |
| Thickened          | 0            | 0  | 3 | 3 | 3 | 3         | 3 | 3-4 | 3-4 | 3-4 |
| Edema              | 0            | 0  | 3 | 3 | 3 | 2         | 2 | 2   | 2   | 2   |
| Neutrophils        | 0            | 0  | 1 | 1 | 2 | 3         | 3 | 3   | 3   | 2   |
| Lymphocytes        | 0            | 1  | 1 | 2 | 2 | 2         | 2 | 2   | 2   | 3   |

TABLE 1 - continued

| Findings                                          | Control Ewes |    |    |   |   | Test Ewes |     |     |     |     |     |
|---------------------------------------------------|--------------|----|----|---|---|-----------|-----|-----|-----|-----|-----|
|                                                   | Days PI      | 10 | 11 | 3 | 3 | 4         | 5   | 6   | 7   | 8   | 9   |
| 7734 7737 7809 7735 7814 7812 7808 7810 7736 7745 |              |    |    |   |   |           |     |     |     |     |     |
| Plasma cells                                      |              | 0  | 0  | 1 | 2 | 1         | 1   | 1   | 1   | 2   | 3   |
| Fibroblasts                                       |              | 0  | 0  | 0 | 1 | 2         | 3   | 3   | 3-4 | 3-4 | 3-4 |
| Macrophages                                       |              | 0  | 0  | 1 | 1 | 1         | 2   | 2   | 2   | 2   | 2   |
| Eosinophils                                       |              | 0  | 0  | 1 | 1 | 1         | 1   | 1   | 1   | 1   | 1   |
| LARGE DUCTS                                       |              |    |    |   |   |           |     |     |     |     |     |
| Lumen                                             |              |    |    |   |   |           |     |     |     |     |     |
| Dilated                                           |              | 0  | 0  | 0 | 0 | 0         | 0   | 0   | 0   | 0   | 0   |
| Protein material                                  |              | 3  | 1  | 2 | 1 | 2         | 2   | 2   | 2   | 2   | 1   |
| Cellular debris                                   |              | 0  | 1  | 2 | 2 | 2         | 2   | 3   | 3   | 3   | 2   |
| Neutrophils                                       |              | 0  | 0  | 2 | 3 | 3         | 3   | 3   | 3   | 3   | 2   |
| Lymphocytes                                       |              | 0  | 0  | 1 | 2 | 2         | 2   | 3   | 2   | 2   | 3   |
| Macrophages                                       |              | 0  | 0  | 1 | 1 | 2         | 2   | 3   | 3   | 3   | 2   |
| Plasma cells                                      |              | 0  | 0  | 0 | 1 | 0         | 0   | 0   | 0   | 0   | 2   |
| Epithelial cells                                  |              | 0  | 0  | 2 | 2 | 3         | 3   | 3   | 3   | 3   | 2   |
| Giant cells                                       |              | 0  | 0  | 0 | 0 | 0         | 0   | 0   | 0   | 0   | 0   |
| Granulation tissue                                |              | 0  | 0  | 0 | 2 | 3         | 3-4 | 3-4 | 3-4 | 3-4 | 3-4 |

TABLE 1 - continued

| Findings              | Control Ewes |      |      |      |      |      |      |      |      |      | Test Ewes |      |      |      |      |      |      |      |      |      |
|-----------------------|--------------|------|------|------|------|------|------|------|------|------|-----------|------|------|------|------|------|------|------|------|------|
|                       | 7734         | 7737 | 7809 | 7735 | 7814 | 7812 | 7808 | 7810 | 7736 | 7745 | 7734      | 7737 | 7809 | 7735 | 7814 | 7812 | 7808 | 7810 | 7736 | 7745 |
| Days PI               | 10           | 11   | 3    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10        | 11   | 3    | 3    | 4    | 5    | 6    | 7    | 8    | 9    |
| <u>Epithelium</u>     |              |      |      |      |      |      |      |      |      |      |           |      |      |      |      |      |      |      |      |      |
| Degenerative          | 0            | 0    | 3    | 3    | 3    | 3    | 2    | 2    | 2    | 2    | 0         | 0    | 3    | 3    | 3    | 3    | 0    | 0    | 0    | 0    |
| Necrotic              | 0            | 0    | 1    | 2    | 3    | 3    | 3    | 3    | 3    | 3    | 0         | 0    | 1    | 2    | 3    | 3    | 3    | 3    | 3    | 3    |
| Desquamated           | 0            | 1    | 2    | 3    | 3    | 3    | 3    | 3    | 3    | 3    | 0         | 1    | 2    | 3    | 3    | 3    | 3    | 3    | 3    | 3    |
| Hyperplastic          | 0            | 0    | 1    | 2    | 2    | 2    | 3    | 3    | 3    | 2    | 0         | 0    | 1    | 2    | 2    | 2    | 3    | 3    | 3    | 2    |
| Metaplastic           | 0            | 0    | 1    | 2    | 3    | 3    | 3-4  | 3-4  | 3-4  | 1    | 0         | 0    | 1    | 2    | 3    | 3    | 3-4  | 3-4  | 3-4  | 1    |
| Papillary projections | 0            | 0    | 0    | 0    | 1    | 3    | 3-4  | 3-4  | 3-4  | 3-4  | 0         | 0    | 0    | 0    | 1    | 3    | 3-4  | 3-4  | 3-4  | 3-4  |
| Bacteria              | 0            | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| <u>Wall</u>           |              |      |      |      |      |      |      |      |      |      |           |      |      |      |      |      |      |      |      |      |
| Thickened             | 0            | 0    | 2    | 3    | 3    | 3    | 3    | 3    | 3    | 3    | 0         | 0    | 2    | 3    | 3    | 3    | 3    | 3    | 3    | 3    |
| Edema                 | 0            | 0    | 3    | 3    | 3    | 3    | 2    | 2    | 1    | 1    | 0         | 0    | 3    | 3    | 3    | 3    | 2    | 1    | 1    | 1    |
| Neutrophils           | 0            | 0    | 2    | 3    | 3    | 3    | 4    | 4    | 2    | 2    | 0         | 0    | 2    | 3    | 3    | 3    | 4    | 2    | 2    | 2    |
| Lymphocytes           | 0            | 1    | 1    | 2    | 2    | 2    | 3    | 3    | 3    | 3    | 0         | 1    | 1    | 2    | 2    | 2    | 3    | 3    | 3    | 3    |
| Plasma cells          | 0            | 1    | 1    | 2    | 1    | 1    | 1    | 2    | 2    | 2    | 0         | 1    | 1    | 2    | 1    | 1    | 1    | 2    | 2    | 2    |
| Fibroblasts           | 0            | 0    | 0    | 3    | 2    | 2-3  | 2-3  | 2-3  | 3-4  | 3    | 0         | 0    | 0    | 3    | 2    | 2-3  | 2-3  | 3-4  | 3    | 3    |
| Macrophages           | 0            | 0    | 1    | 2    | 1    | 2    | 3    | 3    | 3    | 3    | 0         | 0    | 1    | 2    | 1    | 2    | 3    | 3    | 3    | 3    |
| Eosinophils           | 0            | 0    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 0         | 0    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
|                       | 0            | 0    | 0    | 0    | 1    | 3    | 3-4  | 3-4  | 3-4  | 3-4  | 0         | 0    | 0    | 0    | 1    | 3    | 3-4  | 3-4  | 3-4  | 3-4  |



TABLE 1 - continued

| Findings          | Control Ewes |      |      |      |      | Test Ewes |      |      |      |      |
|-------------------|--------------|------|------|------|------|-----------|------|------|------|------|
|                   | 10           | 11   | 3    | 3    | 4    | 5         | 6    | 7    | 8    | 9    |
|                   | 7734         | 7737 | 7809 | 7735 | 7814 | 7812      | 7808 | 7810 | 7736 | 7745 |
| Days PI           | 10           | 11   | 3    | 3    | 4    | 5         | 6    | 7    | 8    | 9    |
| INTRALOBAR STROMA |              |      |      |      |      |           |      |      |      |      |
| Amount            | 0            | 0    | 3    | 3    | 3    | 3         | 3    | 3-4  | 3    | 3-4  |
| Edema             | 0            | 0    | 3    | 3    | 3    | 3         | 3    | 3    | 2    | 1    |
| Hemorrhage        | 0            | 0    | 0    | 0    | 0    | 0         | 0    | 0    | 0    | 0    |
| Neutrophils       | 0            | 0    | 2    | 3    | 2    | 3         | 2    | 2    | 2    | 2    |
| Lymphocytes       | 0            | 1    | 1    | 2    | 1    | 2         | 2    | 3    | 3    | 3    |
| Plasma cells      | 0            | 1    | 0    | 1    | 0    | 1         | 1    | 2    | 2    | 3    |
| Macrophages       | 0            | 0    | 0    | 1    | 1    | 2         | 2    | 3    | 2    | 2    |
| Fibrin            | 0            | 0    | 2    | 1    | 1    | 2         | 2    | 2    | 2    | 2    |
| Giant cells       | 0            | 0    | 0    | 0    | 0    | 0         | 0    | 0    | 0    | 0    |
| Necrosis          | 0            | 0    | 0    | 0    | 0    | 1         | 1    | 2    | 2    | 2    |
| Fibroblasts       | 0            | 1    | 1    | 2    | 1    | 2         | 2    | 3    | 2    | 3    |
| Collagen          | 0            | 0    | 0    | 1    | 0    | 0         | 1    | 2    | 2    | 3    |
| INTRALOBULAR C.T. |              |      |      |      |      |           |      |      |      |      |
| Amount            | 0            | 0    | 3    | 3    | 2    | 2         | 3    | 3-4  | 3-4  | 3-4  |
| Edema             | 0            | 0    | 3    | 3    | 3    | 3         | 2    | 2    | 2    | 1    |
| Hemorrhage        | 0            | 0    | 0    | 0    | 0    | 0         | 0    | 0    | 0    | 0    |
| Neutrophils       | 0            | 0    | 3    | 3    | 3    | 3         | 3    | 3    | 1    | 1    |

TABLE 1 - continued

| Findings           | Control Ewes |      |      | Test Ewes |      |      |      |      |           |
|--------------------|--------------|------|------|-----------|------|------|------|------|-----------|
|                    | 7734         | 7737 | 7809 | 7735      | 7814 | 7812 | 7808 | 7810 | 7736 7745 |
|                    | Days PI      | 10   | 11   | 3         | 3    | 4    | 5    | 6    | 7         |
| Findings           |              |      |      |           |      |      |      |      |           |
| Lymphocytes        |              | 0    | 1    | 1         | 1    | 1    | 2    | 2    | 2         |
| Plasma cells       |              | 0    | 0    | 1         | 1    | 1    | 1    | 1    | 1         |
| Macrophages        |              | 0    | 0    | 0         | 0    | 0    | 0    | 1    | 3         |
| Fibrin             |              | 0    | 0    | 1         | 1    | 1    | 1    | 1    | 1         |
| Giant cells        |              | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0         |
| Necrosis           |              | 0    | 0    | 0         | 0    | 1    | 2    | 3    | 3-4       |
| Fibroblasts        |              | 0    | 1    | 0         | 2    | 1    | 2    | 3    | 3         |
| Collagen           |              | 0    | 2    | 0         | 1    | 0    | 1    | 2    | 2         |
| ARTERIES           |              |      |      |           |      |      |      |      |           |
| <u>Lumen</u>       |              |      |      |           |      |      |      |      |           |
| Congested          |              | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0         |
| Thrombi            |              | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0         |
| <u>Endothelium</u> |              |      |      |           |      |      |      |      |           |
| Swollen            |              | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0         |
| Degenerative       |              | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0         |
| Desquamated        |              | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0         |
| Necrotic           |              | 0    | 0    | 0         | 0    | 0    | 0    | 0    | 0         |



TABLE 1 - continued

| Findings                                          | Days PI | <u>Control Ewes</u> |    |   | <u>Test Ewes</u> |   |   |   |   |   |   |
|---------------------------------------------------|---------|---------------------|----|---|------------------|---|---|---|---|---|---|
|                                                   |         | 10                  | 11 | 3 | 3                | 4 | 5 | 6 | 7 | 8 | 9 |
| 7734 7737 7809 7735 7814 7812 7808 7810 7736 7745 |         |                     |    |   |                  |   |   |   |   |   |   |
| <u>Wall</u>                                       |         |                     |    |   |                  |   |   |   |   |   |   |
| Edematous                                         |         | 0                   | 0  | 0 | 0                | 0 | 0 | 0 | 0 | 0 | 0 |
| Vacuolated                                        |         | 0                   | 0  | 0 | 0                | 0 | 0 | 0 | 0 | 0 | 0 |
| Leukocytes                                        |         | 0                   | 0  | 1 | 1                | 1 | 1 | 1 | 1 | 1 | 0 |
| LYMPHATICS                                        |         |                     |    |   |                  |   |   |   |   |   |   |
| <u>Lumen</u>                                      |         |                     |    |   |                  |   |   |   |   |   |   |
| Dilated                                           |         | 0                   | 0  | 3 | 3                | 3 | 3 | 2 | 2 | 1 | 0 |
| Lymph content                                     |         | 1                   | 1  | 2 | 1                | 2 | 1 | 1 | 1 | 1 | 0 |
| Cellular content                                  |         | 1                   | 1  | 2 | 2                | 2 | 1 | 1 | 1 | 1 | 0 |
| Lymphangitis                                      |         | 0                   | 0  | 0 | 0                | 0 | 0 | 0 | 0 | 0 | 0 |

<sup>a</sup>No significant lesions found in the teat canal; exudate observed within the lumen only.

#### D. Scanning Electron Microscopy Photomicrographs

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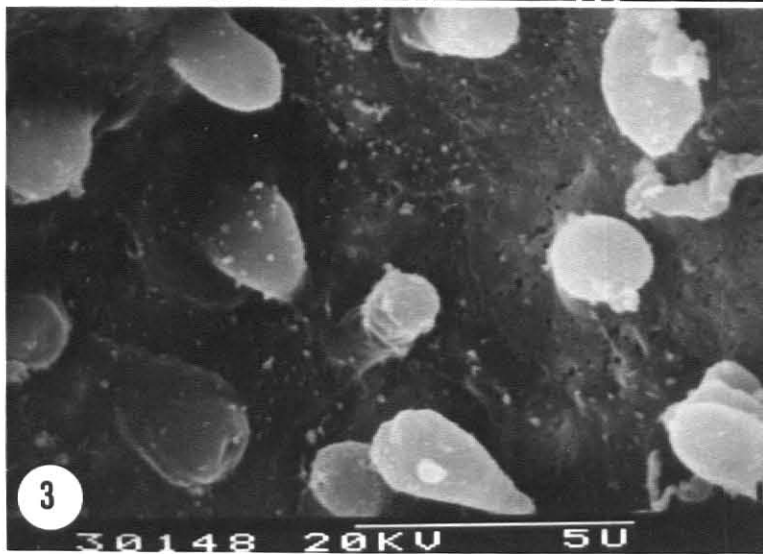
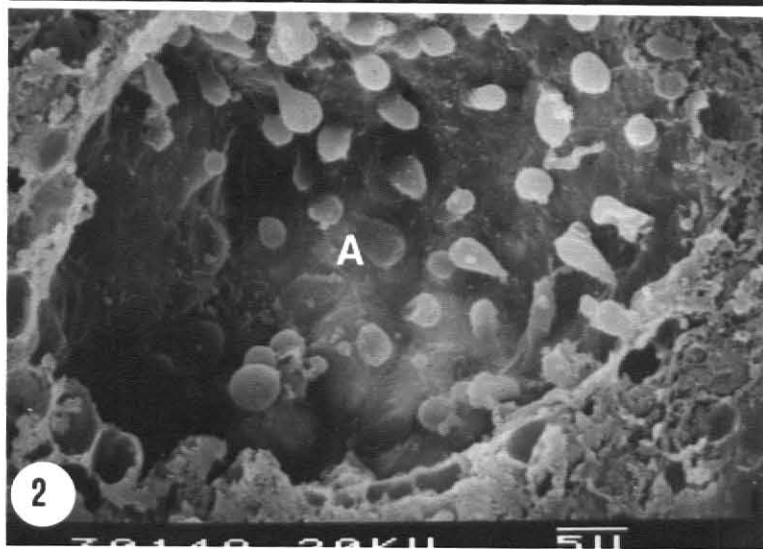
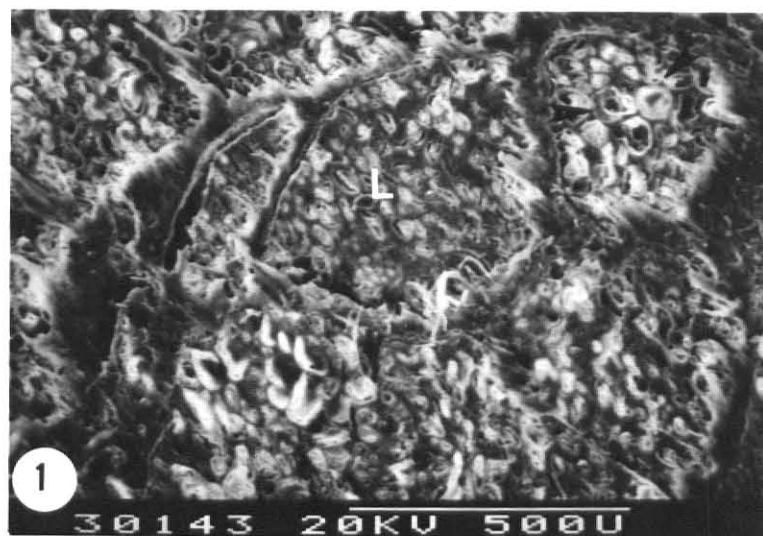
Samples used in this study were prepared from paraffin embedded mammary tissue blocks. The blocks were immersed in xylene for 3 days with the solution being changed every 12 hours. The tissue was then placed in a solution consisting of equal parts of xylene and 100% ethanol using 3 changes at hourly intervals and stored overnight in 100% ethanol. The tissues were critical point dried using CO<sub>2</sub> as an intermediate fluid. Specimens were mounted on aluminum stubs with silver paste and were sputter coated with pure gold. The tissues were evaluated with a Hitachi H-300 with a H-3010 mode in place.

Control Ewe

Fig 1 - SEM photomicrograph of uninoculated right mammary gland of test ewe at day 4 PI. Note normal lobular structure (L) and acini (arrows). 28X.

Fig 2 - Higher magnification of Fig 1 illustrating lumen of an acinus (A) and protruding secretory cells. 1,850 X.

Fig 3 - Higher magnification of Fig 2. 5,000 X.

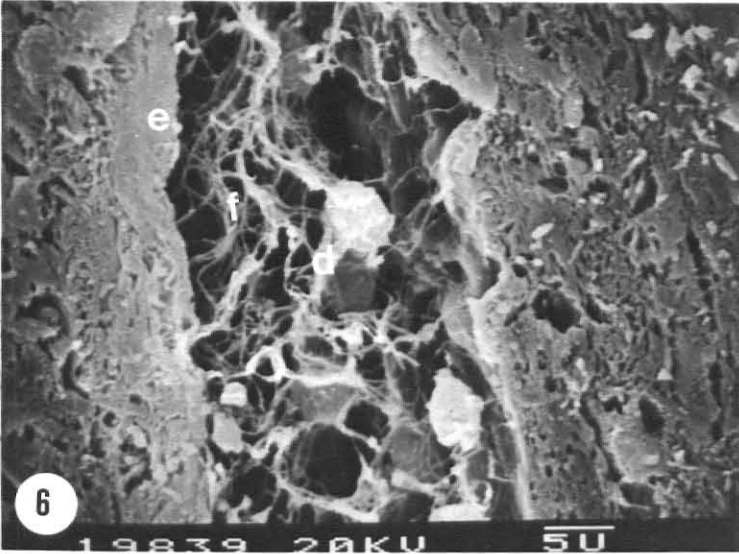
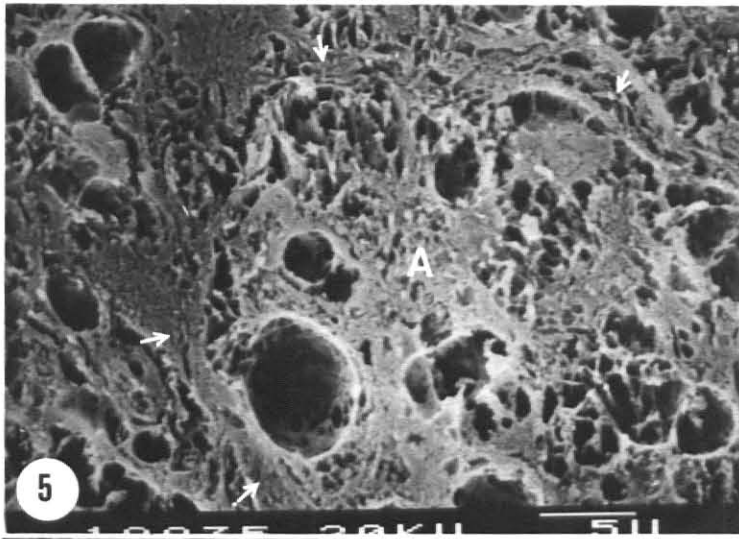
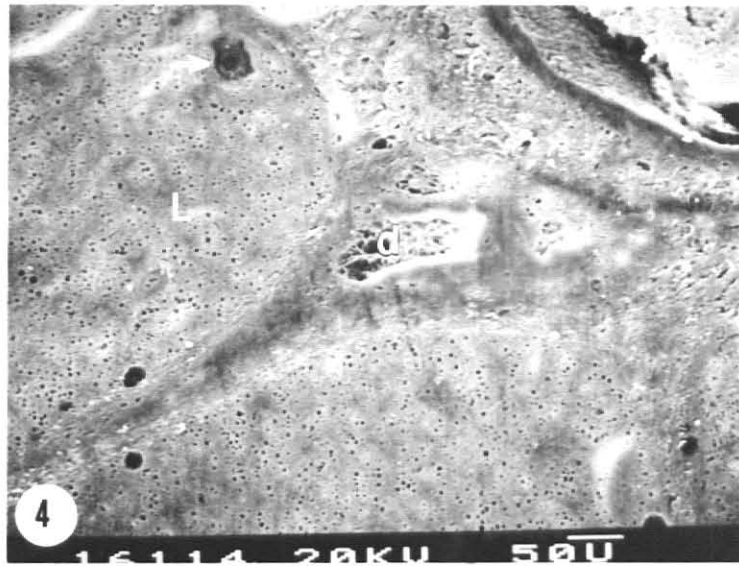


A. seminis Infected Mammary Gland

Fig 4 - SEM photomicrograph of tissue from mammary gland 4 days after inoculation with A. seminis. Note lobular structure and interlobular connective tissue: lobule (L), lactiferous duct (d), and corpora amylacea (arrow). 100X.

Fig 5 - Higher magnification of an acinus in Fig 4. Note acinus, filled with inflammatory exudate (a) and acinus borders (arrows). 3,000 X.

Fig 6 - SEM photomicrograph of a lactiferous duct within interlobular connective tissue at day 4 PI. Note ductal epithelium (e) and lumen (d) filled with inflammatory exudate rich in fibrin (f). 3,500 X.



EXPERIMENTAL ACTINOBACILLUS SEMINIS MASTITIS IN EWES

by

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B.V.M.S., Cairo University, 1973

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AN ABSTRACT OF A THESIS

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requirements for the degree

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Genital actinobacillosis is a disease with a possible high latent infection rate and little is known of the methods of transmission of A. seminis within or between sheep flocks under natural conditions. It has been suggested that ewe-to-ram lamb transmission may occur resulting in a latent infection that may become clinical with sexual maturity. As the udder may be a means of infecting ram lambs, this study was undertaken to determine the sequential pathological changes in the ovine mammary gland following A. seminis infection.

Ten Rambouillet ewes, 4 to 5 years old, were randomly divided into 2 groups, 8 test and 2 control. The left mammary gland of the test and control ewes was inoculated via the teat canal with 2 ml of a 24 hour broth culture of A. seminis containing  $2.0 \times 10^9$  CFU per ml or 2 ml of sterile broth, respectively. The right mammary glands served as uninoculated controls. Following inoculation, the ewes were examined twice daily, rectal temperatures recorded, and the udders carefully palpated. The ewes were euthanized and immediately necropsied as follows: 2 on day 3 PI, and one each on day 4, 5, 6, 7, 8 and 9; one control on day 10 and the other on day 11.

The 8 test ewes had transient temperatures up to 3.2 F from 12 to 24 hours PI that varied from ewe to ewe and returned to normal within 36 to 60 hours PI. The inoculated

glands were enlarged (3 to 5 times normal), turgid or consolidated and hot. At necropsy, the glands lacked the typical spongy structure and compartmentation was obvious. They contained whitish-creamy or greenish-yellow viscid secretions in contrast to clear milk or watery fluid from the uninoculated glands or control ewes. A. seminis was isolated from all inoculated glands and not from the uninoculated glands or other body organs. The supramammary lymph nodes on the inoculated side were slightly larger and edematous compared to the uninoculated side. No control mammary glands, inoculated or uninoculated, had any gross or microscopic changes.

Microscopically, the changes were marked throughout the infected gland and there was little or no difference between the lactating and nonlactating glands. The reaction to A. seminis may be divided into 4 overlapping phases: acute purulent, subacute purulent, necrotizing, and proliferative. The acute purulent phase occurred primarily within the first 5 days PI and was characterized by an ascending galactophoritis and involvement of acini in many lobules, accumulation of inflammatory exudate and neutrophils within the lumens, some epithelial degeneration and desquamation, serofibrinous exudation in the interstitium, some venous thrombi, and a few small necrotic foci. The subacute purulent phase (day 5-7 PI) was characterized by

highly cellular exudate within affected lumens, leukocytes (neutrophils and mononuclears) and desquamated epithelial cells, moderate to marked epithelial changes, and increased exudation within the interstitium and signs of fibroplasia. Changes in the larger ducts became prominent: epithelial hyperplasia to squamous metaplasia and ductal and periductal fibroplasia. The necrotizing phase (day 4-7 PI) was characterized by thrombosis and focal or lobular necrosis and early fibroplasia. The proliferative phase (day 6-9 PI) was characterized by granulation tissue involving the ducts, periductal interstitium, intralobular acini and interstitial tissue, and squamous metaplasia and polypoid outgrowths of the lactiferous duct epithelium. The above changes were variable in all inoculated glands, especially from day 4 PI.

It was concluded from the study that:

1. A. seminis was pathogenic for the ovine mammary gland;
2. A. seminis could be isolated from the mammary gland for at least 9 days PI;
3. the clinical signs and gross and microscopic findings observed in A. seminis induced mastitis were nonspecific; and
4. it was suggested that milk from an A. seminis infected gland may result in a latent infection in ram lambs.