SURVEY FOR MYCOPLASMAS AND CHLAMYDIAE IN UROGENITAL TRACTS OF AGALACTIC SOWS

by

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INTRODUCTION

Mastitis, metritis, agalactia (MMA), also known as sow agalactia, is an acute disease complex of the postpartum sow, characterized by partial or complete lactation failure. Complexity is evidenced by the many names applied to the syndrome and the wide variation in clinical signs. Although the predominant sign is hypogalactia to complete agalactia, metritis is suspected because of a frequent accompanying vaginal discharge, and mastitis because of the varying amount of mammary gland swelling.

Economic loss results from baby pig death due to starvation and starvation-related diseases, such as hypoglycemia and scours. Loss is also incurred by the swine producer in time and money spent artificially feeding and medicating the weak and hungry pigs. If the pigs survive, they are often unthrifty. The mortality rate of affected sows is extremely low.

A review of the literature indicates that sow agalactia is either of recent origin or only recently recognized. The first recorded clinical descriptions of the disease were made in the late 1940's and early 1950's. Research regarding its etiology was pursued in the late 1950's and 1960's. Many non-infectious factors and infectious agents have been studied, but the primary etiology still remains to be elucidated.

Circumstantial evidence points to an infectious origin of MMA. Clinical signs such as mastitis, pyrexia, and excess vaginal discharge seem to indicate a possible infectious agent. Other evidence supporting this contention is the response obtained to antibiotics in some cases, apparent prophylactic benefit obtained by using bacterins, and reports of therapeutic and prophylactic responses to certain antiserums.

Escherichia coli is the most often incriminated infectious agent. After a study of the relationship of this organism to MMA, Ross et al. (1969) concluded that it is probably an ever-present opportunist, and at most, is merely a secondary invader. They further stated that it is possible that some other, as yet undetected, agent is the cause.

Moore et al. (1966) and Karbe (1967) have reported isolation of a mycoplasma and subsequent reproduction of clinical symptoms in susceptible swine. There is a need for further research in this area.

The primary purpose of this study was to search for mycoplasmas and/or chlamydia agents in mammary glands and urogenital tracts of sows affected with agalactia (MMA). Secondarily, the study was designed to further characterize the microbial flora of the reproductive tract of the sow and the clinical features of the disease.

LITERATURE REVIEW

A survey of the literature reveals scattered early reports of lactational failure in sows, but most cases described lack the severity and herd involvement cited in reports published since 1950. Comprehensive reviews of the literature in this area have been made by Kopf (1967), Martin (1967), and Thurman (1967) in their Master of Science theses, and by Ringarp (1960).

The early reports deal with empirical treatment and clinical observation of affected herds. Therefore, the literature reviewed here will deal with reports subsequent to 1950. Literature dealing with clinical characterization and investigation into the etiology of sow agalactia will be emphasized. The role of certain infectious agents associated with reproductive failures in both man and animals will also be discussed.

Terminology

The variability and apparent complexity of sow agalactia has resulted in use of many different names. The term mastitis-metritis-agalactia (MMA) has probably been the most popular, but sow agalactia may prove to be more accurate. Names used in published reports on postparturient disorders of sows which included agalactia are listed as follows:

Agalactia in Sows - Schooley (1953)

Agalactia Syndrome in Sows -	Armstrong et al. (1968)
Agalactia Toxaemica -	Ringarp (1960)
Enzootic Metritis -	Blood (1957)
Lactational Failure -	Loveday (1964) Cross et al. (1958)
Mastitis, Metritis, and Diarrhea in Swine -	Smith (1965)
Metritis in Sows -	Kernkamp (1958)
Metritis, Mastitis, Agalactia (MMA) -	Tharp and Amstutz (1958) Nachreiner and Ginther (1969) Thurman and Simon (1970) Ross et al. (1969) Martin et al. (1967)
Mycoplasmal Mastitis and Endometritis of Swine -	Karbe (1967)
Porcine Agalactia Syndrome -	Swarbrick (1958)
Postparturient Fever Syndrome of Sows -	Murphy and Ryan (1958)
Puerperal Fever -	Hebeler (1954)
Sow Agalactia -	Noble et al. (1960) Hogg (1952) Cross (1957)

Importance of Sow Agalactia

The significance of sow agalactia or MMA in the U. S. is emphasized by results of a poll of members of the National Pork Producers Association reported in the National Hog Farmer (October, 1968). The survey was compiled from 1,520 returned questionnaires. Conclusions drawn were that swine health problems outranked carcass, reproductive, and housing

problems, and that MMA was the most troublesome of the diseases listed. It outranked transmissible gastroenteritis and bacterial enteritis of baby pigs.

Sow agalactia appears to have world-wide distribution. Even though it may have several differing names, clinical descriptions indicate it occurs in England (Hogg 1952; Hebeler 1954); Australia (Blood 1957); Ireland (Murphy and Ryan 1958); South Africa (Loveday 1964); Sweden (Ringarp 1960); Germany (Gebauer 1952); and the United States (Tharp and Amstutz 1958; Smith 1965; Martin et al. 1967).

Economic losses are difficult to assess since the incidence and severity of the disease varies widely in any given area or farm. Ringarp (1960) reported that agalactia occurred in 3.7% of all farrowings in Sweden. Blood (1957) stated that the incidence varied from sporadic in most piggeries to 25% in one large piggery. Smith (1965) reported that the number of cases of agalactia varied from a few swine in a herd up to the entire sow population, with the severity being extremely variable. Morbidity in herds studied by Martin et al. (1967) ranged from 2 to 75%. Sow mortality was extremely low, but death or unthriftiness of baby pigs was a major economic loss. Loveday (1964) reported a seasonal change in the morbidity rate of 1% in July to 44% in October in one large pig herd in South Africa. In 1954, Hebeler stated that Puerperal Fever was one of the most

common diseases affecting the post-parturient sow. It seemed to affect all sows irrespective of age. He further stated that in large swine establishments, it often affected a number of sows and appeared to spread as an infectious disease.

A survey conducted by Nachreiner and Ginther (1969) indicated that, in the opinion of the hog producers of Wisconsin, swine diseases characterized by mastitis, metritis, or agalactia ranked second only to diseases characterized by diarrhea in economic importance.

Thurman and Simon (1970) summed up the significance of the disease by stating that results of surveys of veterinarians, swine growers, and swine researchers, suggest that the MMA syndrome reaches epidemic proportions and causes severe economic losses in many herds.

Clinical Description of Sow Agalactia Complex

In 1952, Hogg described what is now called sow agalactia (MMA). He introduced the subject by stating that it seemed to be one of those conditions which was familiar enough to veterinary practitioners, but lacked recognition in the literature. He also stated that it was doubtful if anything strictly comparable was encountered in other domestic animals, and that if it occurred, it must be but rarely. He stated that sow agalactia appeared to be widespread, and it was seen

most often in gilts or second litter sows.

Hogg further stated that the disease complex occurred shortly after farrowing. Usually there was no history of difficult parturition and the litter was strong and healthy for a short time. The sow had an adequate-to-scanty milk supply for a few hours after parturition. Several hours later, the sow assumed a ventral recumbency and refused to lay on her side to allow nursing. The pigs grew progressively weaker as they circled her, rooting at her sides. The sow showed inappetence, agalactia, and some degree of pyrexia. Often the latter was only slight. The pressing problem, of course, was the failure of the milk supply. An abnormal vaginal (uterine) discharge was sometimes present the second to third day after parturition. The discharge was white to yellow, muco-purulent, and did not resemble the foul-smelling, sero-sanguineous material normally associated with acute metritis caused by a retained, decomposing fetus, and/or placenta. A degree of mastitis was sometimes present, but this was not a constant feature. The fate of the litter was dependent on successful treatment of the sow, and early return to lactation or supplemental bottle feeding of the baby pigs.

Hogg further suggested that there were two main agalactia syndromes that fit the aforementioned clinical picture, and they could be distinguished by the response to pituitrin.

Some sows would respond to the hormone injection with a lasting substantial increase in milk flow. Other sows would not respond, or responded only slightly for a short duration.

Hebeler (1954) reported that even though some sows with agalactia showed a slight vaginal discharge, there were no definite signs of metritis. He felt that because affected sows usually adopt a position of ventral recumbency and resist suckling, the infection may originate in the mammary gland, rather than in the uterus. He separated agalactic sows into three groups:

1. Agalactia that was due to a failure of the milk let-down mechanism, and responded dramatically and with lasting effect to an injection of posterior pituitary hormone. If the sow was treated early, the pigs suffered only a slight set-back.

2. This type appeared to be due to a hormone disturbance affecting milk secretion. The sow appeared to be quite normal, and piglets obtained some milk, although their appearance obviously indicated an insufficiency within 36 hours of birth. The mammae were reasonably well developed, but did not appear to be actively producing milk. Pituitrin had no lasting effect, but Hebeler reported an intramuscular injection of 500 I.U. of chorionic gonadotropin

frequently resulted in a lasting and satisfactory milk flow.

3. The third type of agalactia was due to failure of mammary gland development, which was apparently caused by an inadequate development of secretory tissue. The condition was most frequently seen in gilts, but could occur in sows that had previously lactated normally. Hebeler theorized that this type was probably due to insufficient prolactin production by the anterior pituitary gland.

Blood (1957) described clinical findings on one farm where the incidence of agalactia was high. The affected sows became ill within 17 to 36 hours after farrowing. They showed anorexia, agalactia, and assumed ventral recumbency. Agalactia was assumed because of continual suckling attempts by the pigs, and their failure to fill out. All sows showed pyrexia, with temperatures ranging from 105° to 107° F. Usually, a purulent discharge was visible about the vulva. In some cases, an appreciable amount of thin, white, opaque, odorless material containing some mucous lay in a pool behind the sow. This discharge was not apparent after the third or fourth day. Udders contained very little milk, but otherwise appeared normal. Blood reported the mortality rate in affected sows was extremely low, but because of the marked decrease in milk flow, pig mortality was about 50% in each litter, in spite of fostering and hand-

rearing.

Tharp and Amstutz (1958) concurred with Blood's description of MMA, and added that affected sows usually showed some degree of constipation and mammary gland congestion. No milk, or only a few drops, could be expressed from the teats when hand milking was attempted. The sow showed some trembling, which they thought was due to intoxication and fever. They also stated that "agalactia post partum" of the sow has come to mean a very heterogeneous disease complex. They postulated that diseases of different etiology and type may belong to this complex. Each may have received its name from the most important and obvious symptom, which is, more or less, complete cessation of milk production.

Ringarp (1960) made a very intensive study of more than 2,000 agalactic sows, and grouped cases into five different categories:

Group 1: Some sows showed eclampsia immediately before or during farrowing. The sow staggered, the whole body shook, and she made vigorous chewing movements with froth in the mouth. Treatment with intravenous injections of calcium and magnesium preparations usually gave good results.

Group 2: Some gilts were affected with faulty regulation of the milk ejection reflex, but a few second and third-litter sows were similarly affected. They usually showed complete

agalactia immediately after farrowing, but responded readily to an injection of oxytocin. One injection was usually all that was required to maintain milk flow.

Group 3: Some fat, poorly developed gilts showed definite hypoplasia of the udder. Prognosis was poor because they did not respond to any type of treatment.

Group 4: Some cases were labeled hormonal. Sows showed a decreased milk flow as the only symptom, and developed into a group 5 category if not treated early. This group had adequate udder development, and there was only reduced milk flow. Hence, they did not fit into group 2 or 3.

Group 5: Economically, this group represented the most important cases. The characteristic clinical features of this group were as follows:

The sow appeared to be healthy and suckled her pigs for 12 to 24 hours (at most 36 to 48 hours) after farrowing. Milk secretion decreased quickly, and the sow began to show definite signs of sluggishness and tiredness, accompanied by some degree of anorexia. Fever varied. Often, swelling of the udder began in the posterior glands and moved forward. Increased lochial discharge appeared from some sows. Coprostasis occurred infrequently. Most sows exhibited a tense or staggering gait. On the skin, white or red marks sometimes developed, disappearing between examinations. Cyanotic areas were sometimes seen. Some sows showed a variable degree of depression or irritability. Maternal instincts were reduced.

and sows showed little or no interest in the piglets. Often, the sow had a hoarse, characteristic voice.

Ringarp felt that the disease shown by sows with group 5 symptoms could be considered as one syndrome. He named it agalactia toxaemica.

A recent study of agalactia by Nachreiner and Ginther (1969) indicated that there was considerable variation in clinical signs which accompanied agalactia in sows. Mastitis was associated with agalactia, but vaginal discharge was not. Furthermore, when there was a vaginal discharge, gross and histologic examination of the uterus failed to associate the discharge with metritis. Martin et al. (1967) also stated that vaginal discharge was not a reliable indicator of metritis. Vaginal discharge has been reported to occur frequently in clinically normal postpartum sows (Jones 1966).

Diarrhea in nursing pigs frequently must be treated along with the agalactic sow (Smith 1965).

Thurman and Simon (1970) made a study of 12 agalactic sows and reported that gross and histopathologic changes in the various tissues appeared to have little relationship to the severity of the clinical disease. They postulated that this may explain the reported variation in response to therapy among animals affected with this syndrome. Clinically, they found some distention of the mammary glands in all affected sows. The involved glands appeared to be devoid of milk. However, when incised at post mortem, copious quantities of milk

exuded. Presence or absence of excess vaginal exudate was not reported. They did state that consistent gross pathologic findings included uterine atony, edema of the uterine wall, edema of the mammary glands, and excess fluid within the lumen of the uterus. Also, acute endometritis was observed histologically in all but 3 of the 12 sows. Chronic mastitis was observed in 9 animals, while acute mastitis was diagnosed in 3 sows.

Swarbrick (1968) studied 42 cases of sow agalactia clinically and histologically. He considered the mastitis, metritis, agalactia syndrome to be a condition in which different symptoms may be more accentuated in individual cases. He considered the cardinal signs to be a post-parturient malaise and lethargy, anorexia, hot, congested glands, lack of interest in the surroundings and litter, and, as a consequence, hungry, thin, and usually squealing pigs. He also stated that the sows resented being moved. They frequently lay in a position of ventral recumbency, with all mammary glands pressed to the floor. He wondered if this was an effort to cool the hot, fiery mammary glands, or an attempt to prevent the piglets from nursing. The sows rectal temperatures varied from 101.5° to 106.5° F., and vaginal discharge was present in 38% of the cases.

Microflora Associated with MMA

Several different species of bacteria and a mycoplasma have been incriminated as etiologic agents of MMA. Jackson (1952) examined samples of vaginal exudate collected with sterile pipettes. Bact. coli (E. coli) was isolated in each case. Examination of piglets from affected sows revealed pure cultures of E. coli from the intestinal contents and heart blood. He felt that peracute diarrhea in the young piglet was caused by the same organism as that causing agalactia in the sow. He also felt that there was evidence of venereal spread in the herds he examined. He stated that agalactic sows could be effectively treated with Bact. coli antiserum.

E. coli was isolated by Murphy and Ryan (1958) in almost pure culture from vaginal exudate collected from 2 sows showing clinical MMA. An E. coli isolate from the rectum of a scouring pig was serologically the same as the sow isolates. Both sows and piglets responded to treatment with large doses of E. coli antiserum.

In an extensive bacteriological examination of sows showing agalactia toxemica, Ringarp (1960) found that E. coli predominated in samples of vaginal discharge collected with tampons. E. coli was isolated from 53 of 127 samples in pure culture and from 36 in mixed culture. Beta-hemolytic streptococci were isolated in pure culture from 8 of the 127 samples and in mixed culture from 15.

Bacteria were isolated from the uteri of 6 of 18 agalactia affected sows by Armstrong et al. (1968). Only 2 of the 18 had metritis as determined by gross and histologic examination. They stated that this observation indicated that, although bacteria may ascend into the uterus at parturition, bacteria usually do not cause metritis. Also, since nearly every E. coli isolate was of a different serologic type, they did not consider the organism a primary cause of the disease. They concluded that the role of bacteria in sow agalactia was questionable, because a predominant species of bacteria was not isolated. In addition, the systemic organs were bacteriologically negative, indicating that a bacterial septicemia did not occur.

Armstrong et al. (1968) also isolated several species of bacteria from mastitic mammary glands of 18 agalactic sows at necropsy. Mammary glands from 8 sows yielded E. coli, all of different serologic types. Klebsiella species was isolated from 3, Enterobacter aerogenes from 1, and Citrobacter freundii from 1. Mammary glands of 8 of the 18 sows were bacteriologically negative.

The role of E. coli in the pathogenesis of MMA was studied by Kopf (1967). Gilts close to parturition were inoculated intra-vaginally with a viable culture of E. coli that had previously been isolated from the vagina of an agalactic sow. Kopf stated that there was a highly significant correlation between sows with the organism in the vagina and those showing clinical symptoms of MMA.

Conversely, Ringarp (1960) was unable to reproduce any of the clinical signs of MMA by intrauterine infusions of bacteria 3 to 12 hours post-partum in 16 sows. Broth cultures and saline suspensions of E. coli and Lancefield group L, beta-hemolytic streptococci were injected as far as possible into the uterine horns, using a syringe and rubber tube. All 16 sows remained clinically healthy and suckled the piglets normally.

Smith (1965), in a study of mastitis, metritis, and diarrhea in swine, reported that the majority of cases of metritis were associated with mastitis. He bacteriologically examined 100 samples of vaginal fluid, and found E. coli in 82 and streptococci in 9. He reported that autogenous bacterins prepared from bacteria isolated from milk, vaginal fluids, and baby pigs (E. coli, Staph. albus, Streptococcus sp., and Salmonella sp.) prevented mastitis, metritis, and agalactia in sows and diarrhea in baby pigs.

Vaginal swabs from 15 sows showing lactational failure were examined bacteriologically by Loveday (1964) with the following results:

1 sow	No growth obtained
2 sows	E. coli pure culture
3 sows	E. coli and Staph. sp.
1 sow	E. coli, Staph. sp., and Streptococcus pyogenes

3 sows	Streptococcus pyogenes and Staph. aureus
1 sow	Streptococcus pyogenes and Staph. sp.
1 sow	Aerobacter aerogenes
2 sows	Proteus sp.

Tharp and Amstutz (1958) noted that metritis is usually the result of infection of the genital tract during farrowing, or possibly at the time of service to an infected boar. They stated that streptococci and E. coli had both been isolated in pure culture from uterine exudate from sows showing puerperal infection and agalactia. Stress associated with farrowing, uterine atony, and retention of placenta or fetuses was thought to be conducive to uterine infection and inflammation. They further stated that acute infectious mastitis, due to coliforms or staphylococci, occurs sporadically. They inferred that agalactia associated with caking, congestion, and edema of the udder was probably due to an improper diet and lack of exercise, and should not be confused with agalactia due to infectious mastitis. The following organisms were listed as possible causes of mastitis in sows: Staph. sp., Streptococcus sp., Spherophorus necrophorus, Actinomyces bovis, Corynebacterium pyogenes, and Mycobacterium tuberculosis. They stated that staphylococci, Actinomyces bovis, and Actinobacillus lignieresii have been isolated from granulomatous mastitis of sows. Aerobacter aerogenes has been isolated from mammary

glands and spleen of sows dying of acute postparturient gangrenous mastitis. Udder laceration caused by the canine teeth of piglets may serve as a route for bacterial invasion of the mammary gland or adjacent tissue (Tharp and Amstutz 1958).

Ross et al. (1969) studied the significance of certain bacteria common in the vaginal exudate of postpartum sows. Analysis of data on 50 sows in an institutional swine herd revealed E. coli isolated from vaginal samples collected 1 to 3 days postpartum was significantly associated with reduced pig liveability and total litter weight at 21 days postpartum. Biochemical and serological examination revealed many different types of E. coli were involved. Intravaginal inoculation of selected isolates of E. coli resulted in persisting infections in 13 of 16 sows, but clinical evidence of agalactia, mastitis, or profuse vaginal discharge was not observed. Micrococcus sp., beta-hemolytic streptococci, and alpha-hemolytic streptococci were not significantly associated with reduced pig growth and livability. Data on 20 sows indicated an association between an Actinobacillus sp. and lower litter weights at birth and 21 days postpartum. They concluded that, since nearly every sow had a different type of E. coli and intravaginal inoculation of selected isolates of E. coli did not produce the disease, the organism did not appear to have a primary role in MMA. Also, because many different types of E. coli were isolated in the same herd, transmission of E. coli between sows seemed unlikely. They speculated that the organ-

isms were derived from the indigenous flora of the intestines or the environment, that the vaginal and uterine exudate may develop as a result of some factor, normal or pathologic, and E. coli merely ascends. Ross et al. concluded that the possibility still exists that some other, as yet undetected, agent is the cause of the exudate.

Moore et al. (1966) reported isolation of a mycoplasma from the mucosal surface of the uterus and mammary glands of an agalactic sow slaughtered 4 days postpartum. A similar organism was isolated from the vaginal exudate of other sows showing clinical signs of MMA. These organisms were isolated in a tissue culture type medium, Mixture 199, under constant agitation. The organism was subsequently used to experimentally infect 4 SPF sows. The sows showed clinical symptoms of MMA, and the organism was recovered from the affected experimental animals. They proposed the name Mycoplasma hyogenitalium for the organism.

Karbe (1967) briefly mentioned isolating Mycoplasma hyogenitalium from the uteri of sows with "puerperal endometritis and hardened mammary glands". Sows exposed to the organism developed clinical signs of the agalactia complex.

Non-Infectious Factors Associated with MMA

Most of the variable environmental conditions that occur prior to parturition or during the puerperium have been suggested as primary etiologic or contributing factors in MMA. Stress, due to confinement, new surroundings, parturition, or change of feed, has been reported as a major contributing factor (Loveday 1964; Ringarp 1960; Tharp and Amstutz 1958; Jackson 1952; Moore et al. 1966; Noble et al. 1960). In addition, several workers (Jackson 1952; Tharp and Amstutz 1958) have emphasized that constipation with consequent autointoxication could be a causative factor.

Hastings (1955) felt that high environmental temperatures and lack of exercise due to confinement are definite causes of agalactia in sows.

The all-inclusive "faulty feeding practices" received attention by several workers (Ringarp 1960; Tharp and Amstutz 1958; Hebeler 1954; Vickers 1960). Hebeler (1954) further incriminated hereditary predisposition, because certain families of sows seemed to be more prone to develop MMA. Ringarp (1960) stated that agalactia may be caused by exacerbation of an existing disease.

Trapp et al. (1970) supplemented sow rations with vitamin E and selenium. They reported a decrease in incidence of the MMA syndrome compared with previous farrowings in some herds.

They stated that this was a subjective observation, and that further controlled research was needed.

Martin et al. (1967) recorded management factors common to herds troubled with agalactia and concluded that confinement was the single common factor found in each herd. They stated that a study of endocrine glands is needed with a current study of circulating hormone levels of affected sows. Hormone imbalance has been recorded as a common cause of agalactia in sows (Cross 1957; Hogg 1952; Martin 1967).

Nachreiner and Ginther (1969) collected endocrinologic data on 7 agalactic sows. Compared with data from normal postpartum sows, agalactic sows had lower thyroid weights, fewer ovarian follicles greater than 4 mm in diameter, greater combined width of the zona fasiculata-reticularis of the adrenal, elevated plasma cortisol, and depleted adrenal ascorbic acid. The authors concluded that these data indicated that postpartum agalactia in sows was associated with an increase in adrenal cortical function.

Chlamydiae and Mycoplasmas Associated with Genito-Urinary Disorders in Man and Animals

Various microorganisms have been reported as causes of genito-urinary disorders in man and animals. Some of these deserve investigation to determine their possible association with MMA. The organisms cited in this review have an affinity for mucous membrane lining of various parts of the host.

Chlamydial agents

Organisms of the family Chlamydiaceae are now considered to be members of a single genus, Chlamydia, and are commonly known as the psittacosis-lymphogranuloma venereum-trachoma agents. They are related in that they have a common morphology, developmental cycle, and group antigen. They are spherical, intracytoplasmic parasites, ranging in size from 200 mu to 1000 mu (Page 1966).

Chlamydiae are fundamentally distinct from viruses in that they have a cell wall similar in composition to that of gram negative bacteria, contain both RNA and DNA, are susceptible to many antibiotics, and contain an enzyme system that can catabolize glucose when separated from host cells. They are known as "energy parasites" because they are dependent upon the host cell for high energy compounds, such as ATP. All known species can be propagated in the yolk sac of embryonating chicken eggs, or in tissue cultures, although several blind passages may be necessary before death patterns or high

titers are established. They contain a lipoprotein-carbohydrate complex which resists 100° C. for 30 minutes, is serologically similar in all species, and is known as the common group antigen (Page 1967).

Human urogenital chlamydiae

Foy et al. (1967) isolated Trachoma Inclusion Conjunctivitis (TRIC) agents (chlamydiae) from the cervix of 2 of 177 women sampled during their first trimester of pregnancy. No observable pathologic condition was detected in either woman, and illness was not observed in their infants following birth.

Other workers have reported isolation of chlamydiae from human genito-urinary tracts (Dunlop et al. 1965; Jones et al. 1966).

Ford (1967) isolated Bedsonia agents (chlamydiae) from patients with non-gonococcal urethritis. He demonstrated CF antibodies to the common group antigen in the urethritis patients with Reiters syndrome.

Holt et al. (1967) also reported isolation of the trachoma agent from genito-urinary tracts of patients at a venereal disease clinic. Fourteen percent of the women cultured yielded the trachoma agent (chlamydiae) with no isolations being made from males with urethritis.

Ovine and bovine chlamydiae

Lincoln et al. (1969) studied the epizootic bovine abortion (EBA) agent. They stated that pregnant cattle were highly

susceptible to abortion when the agent was inoculated parenterally. Clinical, pathological, and cultural findings showed that following a short blood infectious phase, the EBA agent was rapidly eliminated from extragenital organs and localized in the gravid uterus, causing abortion in 12 of 23 experimental animals. The EBA agent could readily be isolated from the placenta.

The pathologic events in EBA are similar to those reported for enzootic pneumonitis and abortion of ewes (Storz et al. 1968). It has also been shown experimentally that chlamydiae recovered from diseased ovine lungs can cause abortion in pregnant ewes (Dungworth 1963).

Diseases caused by other chlamydiae

Other diseases caused by chlamydiae are trachoma in man, lymphogranuloma venereum in man, psittacosis of man and birds, murine pneumonitis, feline pneumonitis, ovine arthritis, and sporadic bovine encephalomyelitis (Page 1967). Murray (1964) isolated a chlamydia agent from conjunctivitis of hamsters. Also, certain colonies of guinea pigs have been reported to be chronically infected with similar agents causing conjunctivitis. These guinea pig agents were found to be transmitted transovarially (Storz 1961).

Page (1967) stated that agents of the genus Chlamydia are widely distributed in nature and cause a variety of pathologic effects. He further stated that it is uncertain whether the affected animals represent incidental hosts which are randomly

exposed to chlamydial agents carried by vectors or intermediate host species, or are by themselves carrier species, individuals of which develop signs of disease.

Gerloff and Watson (1970) demonstrated that certain chlamydiae may be present as a latent infection in certain strains of mice. During continuous intraperitoneal passage of liver and spleen suspension in normal stock mice, a syndrome developed which was characterized by ascites and certain other visceral changes, but seldom by clinical illness, and never by fatal illness. From these mice, a chlamydia was established in yolk sacs of chick embryos and in tissue cultures. This agent would readily infect mice when inoculated intranasally, but was without effect intracerebrally. It had a low pathogenicity for guinea pigs, and was resistant to sodium sulfadiazine. They felt these characteristics, together with the results of serum neutralization tests, indicated that the agent was not the Nigg and De Burgh strain which caused mouse pneumonitis.

Maierhofer and Storz (1969) showed that the agent of ovine polyarthritis could be established in dogs by parenteral inoculation and cause clinical signs of disease. The affected dogs had fever, anorexia, depression, pneumonia, incoordination, muscle and joint pain, and diarrhea. The chlamydial agent could be reisolated. Group specific antibodies were formed, with a maximal titer reached 21 to 28 days after inoculation. Moderate titers were detectable for one year after challenge inoculation.

Soluble, type specific antigens from chlamydiae have been produced by Fraser and Berman (1965). Purified particulate antigens from different strains were treated with deoxycholate and trypsin. Specific serotype differences were observed in complement-fixation tests of these antigens with homologous and heterologous antisera. This technique could prove valuable in immunological, epizootiological, and taxonomical studies of the group.

Preparation of an antigen cross reactive with the chlamydia group antigen from organisms other than chlamydiae has been described by Mikami et al. (1969). A Herellea-like (HL) organism was used to prepare a heat-stable antigen. This antigen reacted strongly with known ornithosis (psittacosis) antibody.

Chlamydiae have been proven to cause enteritis and death in young calves (York and Baker 1951; Storz et al. 1966) and can be isolated from fecal samples using a procedure for separation of the agent from contaminating viruses and bacteria (Storz et al. 1969). Centrifugation in 3 cycles at a force of 1800 x g is sufficient to "decontaminate", and does not eliminate the agent. Dilution of the specimens during differential centrifugation was necessary to minimize the effects of toxic substances from fecal matter that may kill 7-day old chicken embryos. Storz et al. (1969) further stated that the developing chicken embryo is the optimum medium for isolation and maintenance of chlamydial agents responsible for common intestinal infections in animals. The response of calves to intes-

tinal infection with chlamydiae is age dependent, and newborn calves can be highly susceptible (Storz et al. 1966).

Certain chlamydiae can cause acute mastitis when inoculated into the bovine mammary gland (Corner et al. 1968). They reported that intramammary infusion of the enzootic abortion agent of ewes produced an acute mastitis. Clinically, cows showed pyrexia, anorexia, decreased milk production, swelling of the mammary gland, and marked alteration in the physical quality of the milk. Histologically, the basic lesion was necrosis of alveolar and duct epithelial cells. The agent was demonstrated in all of the mammary gland 3 days after inoculation, illustrating the rapid spread of the organism.

Criteria used by Wilson and Thomson (1968) to identify an organism from pneumonic calf lung as a chlamydial agent included characteristic staining of intracellular inclusions in yolk sac entoderm cells, growth pattern in chick embryos, presence of group complement-fixation antigen in infected yolk sac suspension, and stimulation of antibody production to chlamydiae group antigen. Of these, the presence of group CF antigen in yolk sac suspension was the most reliable. An additional and useful criterion was the appearance of "inclusions" of chlamydiae grown in tissue culture systems. However, whether all chlamydiae will grow in tissue cultures was not determined, and there was some variation in the appearance of intracellular inclusions of different strains.

Mycoplasmas

Mycoplasmas are small (120-600 mu, averaging 200 mu), pleomorphic, devoid of a cell wall, and capable of growth in the presence of penicillin. Most species require highly enriched media containing sterols and, in some instances, other growth factors, such as D.P.N. They form characteristic minute, "fried egg" colonies on solid medium. T-strain mycoplasmas form smaller, more granular colonies. Because of their small colony size, they have been named T for tiny. Mycoplasmas are widely distributed in nature and have been isolated from almost all domestic and laboratory animals. Pathogenicity varies greatly between species. Some are definite pathogens; others are ordinarily commensals and probably produce overt disease only when the host resistance is lowered. Certain mycoplasmas are considered strictly saprophytes. New species are being isolated and identified as new techniques and newer, more complex, media are developed (Dierks 1966).

Several species of mycoplasmas have been isolated from the urogenital tracts of man and animals.

Human genital mycoplasmas

M. hominis has been isolated from the lungs of aborted human fetuses. Harwick et al. (1967) stated that correlation was found between these abortions and presence of complement-fixation (CF) antibodies in the maternal serum, and a rise in

antibody titer could be demonstrated in women carrying this organism in the vagina at the time of abortion. M. hominis has been isolated from amniotic fluid obtained at Caesarean section (Brunell 1969).

T-strain mycoplasmas were isolated from the genital tracts of men and women, from fetal membranes at spontaneous abortion, and from the urethral tracts of men with non-gonococcal urethritis (Kundsin et al. 1967). A higher percentage of isolations were made from the cervix of women who had experienced premature deliveries and increased fetal wastage than from "normal" control women (Shepard et al. 1964).

T-strain mycoplasmas

T-strain mycoplasmas were first isolated by Shepard (1954) from the human urogenital tract. He stated that small colony size made them particularly difficult to study. Shepard and Lunceford (1965) also observed that T-organisms of human origin metabolize urea with the production of ammonia.

With the addition of urea and phenol red, growth of the organism produced a color change. Taylor-Robinson et al. (1968) were able to detect the organism in the urogenital tract of cows by this "color change" technique. They compared bovine isolates with human isolates and found them to be similar. Isolations were made from 16 of 49 (33%) slaughtered cows. They were encountered more often in the urethra and bladder

than in the vagina. Positive isolates usually showed the color change within two days of incubation, but occasionally five days were required. T-mycoplasmas were isolated in media without inhibitors from urethral scrapings of cows and from seminal fluid of a bull. On solid media, bovine T-mycoplasmas produced colonies 5-12 μ in diameter, which were similar to those produced by human isolates. In liquid media, the growth cycle of bovine T-mycoplasmas was similar to human isolates. Growth was more rapid than that of large colony-forming (classical) mycoplasmas. The organism required serum in the growth medium. They were more sensitive to erythromycin and thallium acetate than some classical mycoplasmas. Electron microscopy of bovine and human T-mycoplasmas revealed cells which varied in size from 80 mu to 420 mu, and which were bounded by a triple-layered membrane, features similar to classical mycoplasmas.

Gourlay (1968) reported isolation of T-strain mycoplasmas from pneumonic calf lungs. Lung tissue obtained at necropsy was triturated in phosphate buffered saline (pH 7.4) and inoculated into broth with and without thallium acetate in 4 serial ten-fold dilutions. Growth produced an alkaline shift of pH due to the metabolism of urea and was indicated by a color change of phenol red in the broth. A drop of culture from the highest dilution in which this change occurred was placed on solid medium. When a similar color change was observed in the solid medium, the surface of the medium

was examined for T-strain mycoplasma colonies under a stereoscopic or conventional microscope at 100x magnification. Occasionally, the lung material was macerated directly onto solid medium, and although a color change could be seen following incubation, it was usually difficult to identify the T-strain colonies due to the debris from the inoculum. All cultures were incubated at 37° C. and solid medium plates were placed in a mixture of 5% CO_2 in nitrogen.

In 1969, Gourlay and Thomas reported isolation of large colony and T-strain mycoplasmas from 20 of 20 cases of bovine kerato-conjunctivitis by means of the serial dilution technique. Higher titers of mycoplasmas were generally obtained from the more severe cases of pinkeye, and titers of T-mycoplasmas were generally higher than those of large colony mycoplasmas. T-strain or large colony mycoplasmas were not isolated from the eyes of six healthy calves. They concluded that there is no evidence at present that the mycoplasmas isolated cause disease in animals. However, the fact that they were frequently present in large numbers, particularly the T-mycoplasmas, in cases of kerato-conjunctivitis and not in healthy calves suggests that they play a role in this condition.

Shepard and Lunceford (1965) demonstrated that culture media at pH 6 were optimal for cultivation of T-strain mycoplasmas. The colony size averaged 50-100% larger at pH 6 than colony size obtained by cultivation at pH 8.0 with the

same medium.

Basophilic, intracytoplasmic inclusions in epithelial cells obtained from the anterior urethra in cases of non-gonococcal urethritis were thought by Shepard (1954) to be T-strain pleuropneumonia-like organisms (mycoplasmas) because these organisms could frequently be recovered from patients in which the inclusions were demonstrated. This associative relationship was interpreted as suggesting that the inclusions may be an intracellular phase of T-strains.

Black (1967) studied the influence of temperature on growth in both liquid and solid media of T-strain and classical mycoplasmas. All known serotypes of T-strains were able to grow at 25° C., and a few grew at 22° C., whereas classical mycoplasmas would not grow readily at those low temperatures. He suggested use of the temperature requirements to distinguish between the two types.

Certain normal tissue extracts were found by Kaklamanis et al. (1969) to be mycoplasmacidal during incubation at 37° C. While studying experimental infection with various mycoplasmas they observed that concentrated homogenates of infected animal tissues sometimes yielded negative cultures, while higher dilutions of the same homogenates were positive. They suggested that a thermolabile enzyme contained in fresh normal tissue may give rise to a thermostable, ether-soluble lytic agent during incubation of mycoplasma cultures, which then destroys the organisms. A similar, or identical, material

develops during prolonged storage of tissue extracts at 4° C. This observation has practical application. Isolation of mycoplasmas from tissues suspected of infection may be rendered difficult or impossible, depending on the rapidity of appearance of the lytic factor. Most tissue extracts were rendered non-mycoplasmacidal when diluted 1:128 in broth. They postulated that at least one of the active toxins was lysolecithin.

Bovine mycoplasmas

Hale et al. (1962) reported isolation of a mycoplasma from a herd of dairy cows experiencing a high incidence of mastitis. Intramammary infusions of procaine penicillin G in aqueous suspension had no therapeutic effect. The organism was isolated from abnormal milk samples on blood agar, incubated for five days in a 10% CO_2 atmosphere, and microcolonies surrounded by zones of partial hemolysis were observed at 100x magnification. They had a "fried egg" appearance, and adhered to the medium. The organism was passed into PPLO broth containing 1% PPLO serum fraction and grew after 3-5 days. They named the organism M. agalactia var. bovis.

Hartman et al. (1964) using cultures of M. agalactia var. bovis, known to cause mastitis in the bovine udder, produced varying degrees of endometritis and salpingitis in seven virgin heifers by intrauterine inoculation. Hirth et al. (1966) added this same strain of organism to bull semen,

and inseminated twelve virgin heifers. Ten of the twelve heifers required multiple inseminations before pregnancy supervened, and four failed to conceive after five inseminations. At necropsy, the four animals all showed varying degrees of chronic endometritis, salpingitis, and bursal adhesions. Well defined histopathological lesions were noted. Hoare (1969) isolated mycoplasmas from various parts of the reproductive tract of dairy cows. They were isolated most frequently from the oviducts. She noted a direct relationship of high recovery rate of mycoplasmas from reproductive tracts to infertility. Fifty-two of 73 (71%) "repeat breeder cows" yielded M. laidlawii, but only 42 of 179 (24%) cows "slaughtered for other reasons" were positive for the same organism. All cows were from two institutional dairy herds. Ordinarily, this non-sterol requiring organism is regarded as a strict saprophyte, but the investigator stated that its' presence justifies the consideration of this organism as a potential pathogen. Growth requirements as a criterion for distinguishing between pathogens and saprophytes should not be accepted unconditionally.

Ovine and caprine mycoplasmas

Agalactia of sheep and goats is caused by M. agalactia and is characterized by fibrosis and atrophy of the mammary gland. Arthritis, keratitis, and, in some cases vesiculo-

pustular skin lesions also develop. The arthritis in many cases becomes chronic, resulting in deformity. The organism may localize in the testes of males, and abortion has been observed in females. A natural infection occurs, but animals can also be infected artificially by intravenous inoculation of broth cultures or by feeding infected tissues. The disease seems to be limited geographically to the Mediterranean region (Switzer 1967).

Foggie et al. (1970) compared the immunogenicity of M. agalactia antigens prepared in a variety of ways. Killed whole-cell antigens would not completely protect lactating goats against challenge inoculation, but the severity of the disease was reduced. A live vaccine, attenuated by 40 passes on a selective agar, gave complete protection against the challenge inoculation. Goats inoculated with the live vaccine had a small residual infection with the vaccine strain when killed three to four months after vaccination. Foggie and co-workers showed that the vaccine strain could produce clinical contagious agalactia in lactating sheep.

Canine mycoplasmas

Barile et al. (1970) isolated mycoplasmas from mucosal linings of the larynx, cervix, and prostatic-urethral tissues of dogs. Isolates were identified by the plate immunofluorescence procedure. The four known serotypes of canine mycoplasmas were isolated from each tissue cultured. Each

serotype comprised approximately one-fourth of the mycoplasmas isolated. Most of the mycoplasmas were isolated from laryngeal tissues; 66 of 93 laryngeal (71%), 13 of 35 cervical (35%), and 8 of 57 prostatic-urethral (14%) tissues were positive.

Poultry urogenital mycoplasmas

Rhoades (1969) experimentally infected turkey hen reproductive tracts with M. meleagridis and reported that a high incidence (26%) of egg transmission of the organism occurred. Ascending reproductive tract infections resulted in peritonitis in 35% of the exposed hens. He stated that recovery of the organism from ovarian surfaces, but not from yolk material of mature follicles, suggests that egg transmission resulted from contamination after ovulation, rather than from transovarian shedding.

M. gallisepticum is also known to be egg transmitted. Chalquest and Fabricant (1959) significantly lowered the incidence of chicken egg transmission by dipping the embryonating eggs in oxytetracycline solution.

Mycoplasmas isolated from swine

M. hyorhinis was first described by Switzer (1953). It is common in the nasal cavities of swine, where it does not appear to cause any appreciable damage. It is a very common secondary invader in swine pneumonia, but it does not seem to enhance the severity of pneumonia. Under certain stress conditions, the organism is capable of invading the body tissues

and causes a severe polyserositis. The most severe lesion is usually pericarditis, but pleuritis, peritonitis, and arthritis may occur (Switzer 1967).

M. granularum is a common cause of arthritis in Iowa swine that weigh 75 to 225 pounds. This organism rarely invades other serous surfaces of the body (Switzer 1967).

M. hyopneumonia was described by Mare and Switzer (1965). This organism causes a chronic bronchopneumonia that was originally called "virus pneumonia of pigs" (VPP).

M. hyogenitalium was recovered by Moore et al. (1966) from uterine biopsy material obtained from an agalactic sow. They were also able to isolate the organism from mammary gland tissue and from purulent material passed from the vulva when culturing other agalactic sows obtained as field specimens. They reported reproduction of the clinical syndrome of metritis-mastitis by inoculating 4 SPF sows with the organism.

MATERIALS AND METHODS

Source of Sows and Criteria for Selection

Nineteen postparturient sows were purchased from 9 swine producers located in central Iowa. An attempt was made to select sows from herds experiencing moderate to severe morbidity rates of sow agalactia. Sows were selected which had recently farrowed, exhibited signs of acute agalactia, and were unable to adequately nourish the litter.

Clinical Examination of Sows

Sows were evaluated clinically on the farm and observations were recorded on a standard form. (Form A, p. 95 Appendix.)

Observations of Herd and Environment

Environmental and management factors were evaluated and recorded on a second form during the first visit to the herd. (Form B, p. 96 Appendix.)

Concurrent Studies on Agalactic Sows

Sows were transported from the farm to the Veterinary Medical Research Institute on the day examined. Sows were usually 2-3 days postpartum. Endocrine studies were performed on the sows over a period of 8-12 hours by Dr. William C. Wagner and co-workers. They measured adrenal response to ACTH by determining serum cortisol levels at timed intervals.

At necropsy, tissues were collected from the reproductive tract, the mammary glands, and the endocrine glands for histopathologic studies. Dr. R. F. Ross and co-workers cultured the nasal cavity, tonsils, and selected joints for M. hyosynoviae in conjunction with a survey for this organism. The results of Wagner's and Ross's work are not reported in this thesis.

Necropsy and Specimen Collection Procedures

Euthanasia of the sows was performed by injecting sodium pentobarbital I.V. Axillary vessels were severed to accomplish exsanguination and collection of whole blood. The mammary glands were removed by reflecting the ventral thoracic and abdominal wall between musculature and mammary gland tissue. Each mammary gland was incised for initial gross observation and 4 glands were selected for collection of tissue for microbiological study. Duplicate samples containing about one cubic cm of mammary tissue were collected aseptically from the 4 glands with sterile forceps and scissors. Tissues were placed in sterile 16 x 125 mm glass screw cap tubes containing 10 ml of diluent. The diluent used was a modification of M-199 tissue culture medium as described by Moore et al. (1966).

The body cavity was opened and the urogenital tract was removed and placed on the post mortem table. The surface of the tract was seared with a hot spatula at each collection site.

An incision was made into the lumen with a sterile scalpel, and the walls were reflected. About one square cm of mucous membrane and some underlying tissue was removed aseptically from the cervix, the middle section of both uterine horns, both fallopian tubes, the urethra, and the anterior portion of the vagina. Mucous membrane samples were placed in duplicate sets of sterile tubes containing diluent. The entire urogenital tract was opened and observed for gross lesions.

The remaining viscera were observed for gross lesions, and samples of liver, spleen, kidney, and heart blood were collected with cotton-tipped applicators for bacteriological examination.

Sow Urogenital Tracts from a Packing Plant

Thirty-one sow urogenital tracts were obtained from the Oscar Mayer Packing Plant, Perry, Iowa. Non-pregnant tracts were randomly selected as they were removed from the carcass while moving on the rail. Each was placed in an individual plastic bag and transported to the Veterinary Medical Research Institute in an insulated container cooled with ice. Five separate collections were made with no more than 8 tracts collected on any given day. Mucous membrane tissue samples were removed and processed as described for agalactic sow urogenital tracts.

Bovine Urogenital Tracts from Slaughter Plants

Thirty-seven bovine urogenital tracts were obtained from slaughter plants in central Iowa. Two were collected at Boone Locker, Boone, Iowa, 10 from Midwest Pack, Nevada, Iowa, and 25 from Nissen and Son Packing Company, Webster City, Iowa. Five were the most collected at any one time. The tracts were removed intact at the time of slaughter and placed in individual plastic bags. They were transported to the Veterinary Medical Research Institute in an insulated container cooled with ice. Mucous membrane samples from the cervix, both horns of the uterus, both fallopian tubes, and the anterior portion of the vagina and the urethra were removed in the same manner as described for agalactic sow urogenital tracts. The samples obtained were cultured for mycoplasmas, bacteria, and chlamydia in the same manner as described for agalactic sow tracts.

Microbiological Procedures

Applicators with samples of various tissues were streaked on 5% horse blood agar plates and incubated at 37° C. under a 5% CO_2 atmosphere in a high humidity, water jacketed incubator.[a] Plates were examined at 24 hours and again at 48 hours for bacterial growth.

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[a]National Appliance and Manufacturing Company, Portland, Oregon.

One set of tissue samples was pooled in 10 ml of diluent and stored at -70° C. for later chlamydia isolation attempts. The other set of tissues were labeled as to original location in the urogenital tract and/or mammary gland. Each tissue sample was ground in a sterile mortar and pestle without an abrasive for about 1 minute and was designated the inoculum. A sterile pipette was used to inoculate 0.2 ml on a 5% horse blood agar plate, and 0.2 ml into each of 5 different mycoplasma media. The blood agar plate was streaked with a wire loop, incubated at 37° C. in 5% CO_2 atmosphere, and examined for colony growth at 24 and 48 hours.

Mycoplasma media:

The following formulas were used to prepare mycoplasma media.

Shepard's medium T-strain medium was prepared according to Shepard and Lunceford (1965) and designated as Shepard's medium.

Distilled H_2O	1,000 ml
Trypticase Soy Broth Powder (BBL # 01-162)	30 Gms
Horse serum (sterile, normal, unheated)	200 ml
Penicillin G potassium	1,000,000 units
Urea (sterile 10% solution)	10 ml
Phenol red (sterile 1% solution)	0.2 ml

The first 2 ingredients were mixed and heated gently until completely dissolved. The pH was adjusted to 5.5 with 1 N HCl and the mixture was sterilized in the autoclave at 121° C. for 15 minutes and cooled. The last 4 ingredients were added in an aseptic manner. The complete medium was adjusted to pH 6.5 with sterile (autoclaved) 1N HCl and tubed in 5 ml quantities in sterile 16 x 125 mm screw cap glass tubes and stored at 4° C. until used. Unused medium was discarded at the end of 3 weeks.

T-R medium T-strain medium was prepared according to Taylor-Robinson et al. (1968) and designated T-R medium.

Distilled H_2O	700 ml
PPLO broth w/o CV (Difco)	21 Gms
Dried yeast aqueous extract 25% (w/v)	100 ml
Horse serum (sterile, normal, unheated)	200 ml
Urea (sterile 10% solution)	10 ml
Phenol red (sterile 1% solution)	0.2 ml
Penicillin G potassium	1,000,000 units

The first 3 ingredients were mixed and heated gently until the solution was complete. The pH was adjusted to 6.0 with 1N HCl. Enough 1N HCl (about 10 ml) was added at this time to compensate for the 200 ml of horse serum to be added later. The mixture was sterilized in the autoclave at 121° C.

for 15 minutes and cooled. The last 4 ingredients were added in an aseptic manner and the final pH of the complete medium was adjusted to 6.0 with sterile (autoclaved) 1N HCl. The medium was tubed in sterile 16 x 125 mm glass screw cap tubes and stored at 4° C. Medium not used within 3 weeks was discarded.

M-96 medium FM-5 base medium, as described by Frey et al. (1968) was modified and prepared according to Dr. M. L. Frey (personal communication, Veterinary Medical Research Institute, Ames, Iowa, 1970) and was designated M-96 medium. This medium was supplied by Dr. M. L. Frey and associates in screw cap 13 x 100 mm plastic tubes - 5 ml per tube. They used the following ingredients in the given amounts to make up double strength solutions in 500 ml amounts.

Peptone CS (Albimi)	4 Gms
Peptone B (Albimi)	2 Gms
Peptone G (Albimi)	2 Gms
Yeast autolysate (Albimi)	2 Gms
Yeast extract (Case)	2 Gms
NaCl	5 Gms
KCl	0.4 Gm
$MgSO_4$ 7 H_2O	0.2 Gm
Catalase (General Biochemicals)	0.001 Gm
HEPES buffer (0.015 M) (Nutritional Biochemicals)	3.5 Gms

DNA (1% in tris buffer, pH 7.2) (General Biochemicals)	0.02 Gm
MEM vitamins (Grand Island Biological Company)	10 ml
Cholesterol emulsion (0.01 mg/ml)	2 ml
Glycerol	0.15 ml
L-Arginine HCl	0.06 Gm
L-Glutamine	0.09 Gm
Triple distilled H_2O q. s. to make	500 ml

The pH of the 2x medium was adjusted to 7.0 with 1% NaOH and filter sterilized using a Hormann Model 5C filter[a] with Seitz type pads. The following ingredients were added to each liter of 1x medium.

DPN 10% (General Biochemicals)	2 ml
Cysteine 10% (Matheson, Coleman and Bell)	2 ml
Swine serum (normal, unheated)	150 ml

BHI medium Beef heart infusion medium was prepared according to Ross and Karmon (1970) and designated BHI medium. The medium was supplied by Dr. R. F. Ross and associates in screw cap glass tubes - 6 ml per tube. They prepared the medium from the following formula:

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[a]F. R. Hormann and Company, Inc., Newark 4, N. J.

Deionized H_2O	800 ml
Ground Beef heart	400 Gms
NaCl	4 Gms
Mucin, bacteriological (Difco)	4 Gms
Hemoglobin (Difco)	1.6 Gms
Celite (Analytical Filteraid, Johns-Manville)	4 Gms
Turkey serum, heat inactivated	120 ml
Casamino acid	2.4 Gms
Neopeptone	2.4 Gms
Casitone	0.8 Gm
Tryptone	0.8 Gm
2,3,5-triphenyl-2H-tetrazolium chloride	40 mgs

The first 2 ingredients were combined, allowed to stand overnight at 4° C., then heated to 93° - 95° C. for 30 minutes, cooled, and filtered through gauze. NaCl, mucin, and hemoglobin were then added to the supernatant and the pH was adjusted to 7.8 with 1N HCl. The mixture was then heated again to 93° - 95° C. for 3 minutes, cooled to 45° C., clarified with Celite, and filtered through 2 layers of Whatman 934/AH filter paper. The remaining ingredients were added, and the medium was filter sterilized through a Selas 03 candle. The final pH of the medium was 7.8.

Moore's medium Mixture 199 tissue culture medium was modified according to Moore et al. (1966) and designated Moore's medium.

Basic formula:

Deionized H_2O	225 ml
Mixture 199 - 10x (Grand Island Biologics Company)	25 ml
Sodium bicarbonate mixture	2.5 ml
Lactalbumin hydrolysate mixture	31.25 ml
Mucin, bacteriological (Difco)	1.4 Gms
Horse serum (normal, heat inactivated 56° C. for 30 minutes)	10.9 ml
Dextrose 50%	7 ml
	303.25 ml

Lactalbumin hydrolysate:

Deionized H_2O	250 ml
Lactalbumin hydrolysate (enzymatic)	12.5 Gms

Sterilized twice at 121° C. for 10 minutes and

Stored at 4° C.

Sodium bicarbonate mixture:

Deionized H_2O	90 ml
Hanks balanced salt solution - 10x	10 ml
Sodium bicarbonate - ACS	10 Gms

Made fresh each time needed

The basic formula was combined as follows: The first

4 ingredients were mixed in the order given. Mucin was then added, and the mixture was stored at 4° C. overnight. Horse serum and dextrose were then added, and the pH was adjusted to 7.8 - 8.0 with additional sodium bicarbonate mixture. The medium was clarified by passage through a Whatman GF/A glass filter paper and then through a Selas 01 candle. It was sterilized with a Selas 03 candle, tubed in sterile screw capped glass 16 x 125 mm tubes, 5 ml per tube, and stored at 4° C. Potassium penicillin G was added at the level of 200 units per ml on the day the medium was to be used. Unused medium was discarded after 3 weeks storage.

Solid mycoplasma media Molten Ionagar No. 2 was poured into each of the warmed liquid mycoplasma media so that the final agar concentration was 1%. Media were poured into sterile plastic 13 x 100 mm petri plates, allowed to solidify, incubated at 37° C. for 24 - 36 hours to check for bacterial contamination, and stored at 4° C. in plastic bags to prevent dehydration.

Familiarization with Known T-Strain Mycoplasma

A culture of a known human T-strain mycoplasma was obtained from Dr. Maurice C. Shepard, U. S. Naval Medical Field Research Laboratory, Camp Lejeune, North Carolina 28542. This known T-strain was used to become familiar with

the laboratory procedures necessary to maintain the organism and to test the nutritional adequacy of each lot of T-R and Shepard's medium.

Mycoplasma Medium Inoculation Procedure

Two-tenths ml of ground mucous membrane samples in diluent was inoculated into each of 5 different liquid mycoplasma media. The final dilution of the tissue (w/v) was approximately 1:250. All cultures were incubated at 37° C. Tubes containing Moore's medium were placed on a mechanical shaker[a] to provide constant agitation. All tubes were examined daily for color change and/or turbidity. If no growth was evident by the second and fifth post-inoculation day, 0.5 ml of the original culture was transferred to fresh liquid media. Subcultures were also transferred from the original culture to solid medium after 14 days of incubation. Transfers were made with a 2 mm wire loop. Agar plates were incubated at 37° C. in a 5% CO_2 atmosphere. Plates were examined on the second and fifth day for colony formation by scanning with a SteroZoom microscope.[b]

At the first indication of a yellow to red color change

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[a]Eberbach and Son Co., Inc., Ann Arbor, Michigan.

[b]Bausch and Lomb, Rochester, N. Y.

in T-R or Shepard's media, 0.5 ml was passed to fresh liquid media, and 0.1 ml was placed on solid media. A sterile 9 inch capillary pipet, bent at right angle 2 inches from the end, was used to spread the inoculum on the agar. Plates of agar were incubated at 37° C. in 5% CO_2 and examined daily for color change and for typical T-strain colonies. When suspected T-strain colonies were observed, an attempt was made to clone a colony to inoculate liquid media. A subsequent color change in this T-strain liquid medium was considered evidence of a positive T-strain mycoplasma isolation.

Chlamydia Isolation and Propagation

Previously described composite tissues containing mucous membrane from the vagina, cervix, uterus, fallopian tubes, urethra, and tissue from the mammary gland, from each animal, were thawed in a 37° C. water bath. Each composite sample was ground in diluent in a sterilized micro blender[a] for about one minute and transferred to sterile 16 x 125 mm glass screw cap tubes. To each tube of inoculum, 500 µg per ml each of Vancomycin[b] and streptomycin[b] was added and incubated for 2 hours at 4° C. Five 6 day-old chicken embryos were inoculated with each sample via the yolk sac using 0.2 ml of inoculum. Eggs were candled daily and embryos dying before

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[a]Ivan Sorvall, Inc., Norwalk, Conn.

[b]Eli Lilly and Co., Indianapolis, Ind.

day 4 were discarded. Yolk sacs from the remaining embryos were pooled, washed free of yolk material in phosphate buffered saline (PBS) and ground in a TenBroeck tissue grinder.

PBS Formula (pH 7.4)

Solution A -	Na_2HPO_4 1.4 Gms	+ 100 ml H_2O
Solution B -	NaH_2PO_4 1.4 Gms	+ 100 ml H_2O
	Solution A	84.1 ml
	Solution B	15.9 ml
	NaCl	8.5 Gms
	Distilled H_2O q. s. to make 1 liter	

Two-tenths ml of a 20% suspension of the yolk sac tissue in PBS was again inoculated into the yolk sac of each of 5 additional 6 day-old chick embryos. Again, embryos dead in the first 4 days postinoculation were discarded. The remaining embryos were observed for gross lesions, their yolk sacs pooled, and washed in PBS to remove as much yolk material as possible. Each yolk sac was smeared on 2 glass slides. One was stained by the Gimenez technique (Gimenez 1964), and the other was stained with a fluorescent antibody conjugate supplied by Dr. M. L. Frey and associates. All yolk sacs from each sample were ground in a TenBroeck tissue grinder, and enough PBS was added to make a 20% suspension. This suspension was boiled in a water

bath for 30 minutes, cooled, and centrifuged at 750 x G for 5 minutes. The supernatant was used as antigen in the complement-fixation test using a known positive antiserum against the chlamydia group antigen.

The known antiserum was produced in rabbits with a chlamydial agent that had been isolated from a calf with an acute respiratory disease. The antiserum was supplied by Dr. M. L. Frey, and the test procedure was carried out by Mrs. Wendy Good.

CF Test for Chlamydia Group Antibody in Swine Serum

A modified micro technique of the CF test, as developed by the U. S. Public Health Service, Laboratory Branch, Task Force (1965), was used to determine antibody titers of swine serum to the chlamydia group antigen. Reconstitution of the complement, used in the complement fixation procedure, was modified according to a procedure developed by Michael F. Slavik, Iowa State University, Ames, Iowa, (personal communication, 1970). This modification is expected to be published in a Master of Science thesis, and should be available from the Iowa State University Library in early 1971.

Serum samples from 17 of the 19 agalactic sows, 5 normal cycling sows, and two 200 pound pigs from an institutional SPF swine herd were tested. Serum from four

200 pound pigs that had been Caesarean-derived and raised in strict isolation served as controls.

Antigen used in the CF test was supplied by Dr. M. L. Frey. It had been prepared from allantoic fluid of chlamydia infected chicken embryos, as described by Gyulai et al. (1969), and the test procedure was carried out by Mr. Steven Dale.

RESULTS

Clinical Observations on Agalactic Sows

Observations on rectal temperature, agalactia, mammary gland swelling, vaginal discharge, constipation, nursing attitude, anorexia, and depression in each sow and the general health of the pigs are listed in Table 1.

The amount of vaginal discharge ranged from profuse (rated ++++) in one sow, to none observed in 6 of the 19 sows. Three exhibited only a slight discharge (rated +) while 8 were observed to have a moderate amount (rated ++ or +++).

Rectal temperatures of 17 of 19 agalactic sows ranged from 101.2° F. to 103.5° F. with an average of 102.9° F. Temperatures of 2 sows were not recorded on the farm.

Sows showed variable degrees of agalactia as determined by lack of milk expressed during digital manipulation of the mammary glands and observation of the general nutritional state of the litter. Eight of the 19 were observed to have complete agalactia (rated ++++), 7 had severe hypoglactia (rated +++), and 4 had moderate hypoglactia (rated ++).

Three of the 19 sows showed severe swelling of the mammary glands, one of which had been observed to have complete agalactia.

Pigs in 17 of the 19 litters were weak and hungry. Pigs from one litter had all died, but pigs from another

Table 1

Clinical Observations of Sows on the Farm

Sow No.	Temp. F.	Degree of Agalactia	Mammary Swelling	Vaginal Discharge	Severity of Constipation
1	102.0	++	++	+	++++
2	103.1	++	++	++	+++
3	103.2	++	+++	++	+++
4	103.4	+++	+	++	++++
5	103.5	+++	++	+++	+++
6	102.8	++++	+	None	+
7	102.2	++++	+	None	++++
8	101.2	++++	+	None	+++
9	N.D.*	+++	+	++	+
10	103.5	+++	+++	++++	++
11	N.D.*	+++	+	+	+
12	102.5	++++	++	None	+
13	102.2	++++	++	++	+
14	102.0	++++	+++	++	+
15.	102.2	++++	++++	++	+
16	102.4	+++	++	+	+
17	102.0	++++	+++	+++	+++
18	102.5	++	++++	None	++
19	102.5	+++	++++	None	++

*N.D. - Not done

Attitude of Sow	Degree of Anorexia	Degree of Depression	Health of Pigs
Allows nursing	+	Alert	Weak, hungry
Sternal recumbency	+	Alert	Weak, hungry
Allows nursing	+	Alert	Weak, hungry
Sternal recumbency	-	Alert	Weak, hungry
Sternal recumbency	+	Depressed	Weak, hungry
Sternal recumbency	+	Depressed	Weak, hungry
Sternal recumbency	+	Depressed	Weak, hungry
Sternal recumbency	+	Alert	Weak, hungry
Sternal recumbency	+	Depressed	Weak, hungry
Sternal recumbency	+	Alert	Weak, hungry
Sternal recumbency	+	Alert	Weak, hungry
Sternal recumbency	+	Alert	Weak, hungry
Sternal recumbency	+	Alert	Weak, hungry
Sternal recumbency	+	Alert	Weak, hungry
Sternal recumbency	+++	Depressed	All dead
Sternal recumbency	+	Alert	Weak, hungry
Sternal recumbency	+	Depressed	Weak, hungry
Allows nursing	+	Alert	All healthy
Allows nursing	+	Alert	Weak, hungry

litter appeared to be strong and healthy. The sow with the healthy pigs showed extreme udder swelling and had been moderately hypogalactic. Out of 176 pigs farrowed by the 19 agalactic sows in this study, 35 pigs had died by examination time.

Three sows showed severe constipation, and 5 had been rated as being moderately constipated. Of these 8 constipated sows, 3 had been observed to be completely agalactic.

All but one owner reported that the agalactic sows did not eat as much as the owner thought they should. One sow refused all food offered, while another sow ate everything offered.

Observations on Herds of Origin of Agalactic Sows

Form B was completed on 8 of the 9 herds from which the 19 agalactic sows were derived. Observations made on the herds concerning size of sow herd, number of farrowings per year, farrowing facilities, estimated morbidity due to MMA this farrowing, estimated morbidity last farrowing, and month of year during which farrowing occurred are listed in Table 2.

Gestation rations were extremely variable between the 8 herds observed. Antimicrobial feed additives were incorporated in the gestation rations in 3 herds (Brenton, Wikland, and Boren). A different additive was used in each of these herds.

The overall health of the sows was rated good to excellent in all but one herd, and in this herd (Boren), sanitation was observed to be poor. The other 7 herds were rated good to excellent in sanitation practices. None of the owners routinely washed the sows before placing them in the farrowing pens or crates. Four of the 8 owners incorporated some kind of disinfectant in the cleaning process between farrowings (Cormany; Brenton Farms; Worthington; and Boren).

Gross Lesions Observed in Agalactic Sows at Post Mortem

The 19 affected sows were autopsied at an average of 3.2 days after parturition. The range in days postpartum was 2 - 5.

The most consistent findings in the genital tracts of all 19 sows were mild atony and slight edema of the wall of the uteri, a smooth glistening mucosal surface, and little, if any, exudate in the lumen. In addition, sow No. 1 had an extensive hemorrhagic laceration in the ventral aspect of the vagina.

Two sows, 1 and 15, were found to have an acute focal hemorrhagic mastitis, each in 1 gland only. Sow 15 showed extensive edema in the area between the mastitic gland and the abdominal musculature. The remainder of the glands in sows 1 and 15, and the glands of the other 17 sows, appeared to be relatively normal, with only slight congestion. Normal

Table 2

Observations on Herds of Origin

of Agalactic Sows

Herd of Origin	Sow Numbers	Aprox. No. in Herd	No. Farrowings Per Year
Cormany	1, 2	350	6
Brenton	3, 4, 16	700	Continuous
ISU Nutrition	5, 9, 11	100	Continuous
Ralter	6	Not recorded	--
Worthington	7, 8, 12, 13, 14	25	4
Gowitz	10	15	2
Wikland	15	10	2
Smith	17	14	2
Boren	18, 19	80	Continuous

Farrowing Facilities	Est. Morbidity This Farrowing	Est. MMA Morbidity Last Farrowing	Month this Farrowing
Crates	75%	10%	August
Crates	50%	80%	Mar.-April
Crates	10%	10%	March
---	---	---	---
Pens	90%	0%	March
Pens	10%	0%	March
Crates	30%	0%	March
Crates	10%	0%	April
Crates	90%	5%	May

appearing milk flowed freely from most glands when they were incised for initial gross observation.

The abdominal viscera in all 19 sows appeared normal. Sow No. 6 showed an acute diffuse suppurative bronchopneumonia which appeared to be due to inspiration of foreign material.

Bacteria Isolated

All samples of tissue collected from liver, kidney, spleen, and heart blood from each of the 19 agalactic sows were bacteriologically negative. Two sows yielded E. coli from the urethra, and in one sow, E. coli was isolated from the urethra and the anterior portion of the vagina. None of the mammary glands from these sows yielded a bacterial isolate. All 32 urogenital tracts from packing plant sows were bacteriologically negative.

E. coli was isolated from the anterior portion of the vagina of 3 of 37 bovine urogenital tracts, and a streptococcus was isolated from the vagina of 1.

Mycoplasma Isolations

Mycoplasmas were not isolated from any of the urogenital tracts or mammary gland tissue samples derived from agalactic sows or sow urogenital tracts obtained at the packing plant. (See Table 3.) T-strain mycoplasmas were isolated from 11 of 37 bovine urogenital tracts. The location of isolates of T-strains within the urogenital tract is listed in Table 4.

Table 3

Mycoplasma Isolation Attempts from Sows

Using Five Different Media

Agalactic Sow No.	Mammary Gland	Vagina	Cervix	Uterus	Fallopian Tubes	Urethra
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	-	-	-	-	-	-
15	-	-	-	-	-	-
16	-	-	-	-	-	-
17	-	-	-	-	-	-
18	-	-	-	-	-	-
19	-	-	-	-	-	-

Table 3 (Continued)

Packing Plant Sow Tracts	Vagina	Cervix	Uterus	Fallopian Tubes	Urethra
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-
15	-	-	-	-	-
16	-	-	-	-	-
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	-	-	-	-
22	-	-	-	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	-	-	-	-	-
26	-	-	-	-	-
27	-	-	-	-	-
28	-	-	-	-	-
29	-	-	-	-	-
30	-	-	-	-	-
31	-	-	-	-	-
32	-	-	-	-	-

Table 4

Origin of T-Strain Mycoplasma Isolates in Bovine Urogenital Tracts

Cow Tract Number	Vagina	Cervix	Uterus	Fallopian Tubes	Urethra
1	+	-	-	-	-
9	+	+	-	-	-
11	+	-	-	-	-
12	-	-	+	+	-
14	+	+	-	-	-
20	+	-	-	-	-
24	+	+	-	-	-
27	+	-	-	-	+
29	-	-	+	+	+
31	+	+	-	-	-
36	+	-	-	-	-

Chlamydia Isolations

Chlamydia isolation attempts were made on 15 of the 19 composite tissue samples collected from agalactic sows. Four samples were lost during storage, due to tube breakage.

Four of the 15 samples yielded a chlamydia-like organism. Isolation was designated positive on the basis of fluorescence in yolk sac entoderm cells using the fluorescent antibody technique, finding intracytoplasmic inclusion bodies in yolk sac entoderm cells stained with Gimenez stain, and complement-fixation titers of at least 1:8 when boiled yolk sac material was used as the antigen. Six of the 15 samples were positive with the Gimenez stain. Four of the 15 showed intracellular fluorescence, and 6 of the 15 were found to have a CF titer of 1:8 or greater when boiled yolk sac material was used as antigen. A summary of results is presented in Table 5.

Fourteen of 73 second passage chick embryos died between day 4 and day 6 post inoculation. No lesions were observed in any of the chicken embryos when yolk sacs were harvested.

Of the urogenital tracts obtained at slaughter, 2 of 32 sows and 20 of 37 cows were processed for chlamydia with no positive isolations.

Complement-Fixation Antibody Titers to the Chlamydia Group Antigen in Swine Serum

Complement-fixation test procedures using swine serum revealed antibody to the chlamydia group antigen in serum dilutions as high as 1:320. Sera from 5 of the 19 agalactic sows, 2 of the 6 normally cycling sows, and 4 of the 4 Caesarean-derived control pigs were negative at a 1:10 dilution. Four agalactic sows from which a chlamydia-like agent was isolated revealed a serum titer range of 1:80 to 1:160. Two agalactic sows, from which chlamydia-like isolations had been ruled suspicious, had serum antibody titers of 1:320 and <1:10. Complement-fixation titers of all swine sera tested are listed in Table 6.

Table 5

Chlamydia-like Organisms Isolated from Agalactic Sows

Sow No.	Fluorescense with FA Tech.	Gimenez Stain	CF Titer of Y.S. Material	Isolation
1	Not Done	----	-----	----
2	Not Done	----	----	----
3	+	+	1:8	Positive
4	Not Done	----	----	----
5	-	-	1:4	Negative
6	-	-	<1:2	Negative
7	+	+	1:16	Positive
8	-	-	1:2	Negative
9	+	+	1:16	Positive
10	-	-	1:2	Negative
11	-	-	1:4	Negative
12	-	+	1:16	Suspect
13	-	-	<1:2	Negative
14	-	-	<1:2	Negative
15	-	-	1:4	Negative
16	+	+	1:8	Positive
17	-	+	1:8	Suspect
18	Not Done	----	----	----
19	-	-	1:4	Negative

Table 6

Swine Serum CF Titers to Chlamydia Group Antigens

Agalactic Sow No.	Chlamydia-like Isolation	Titer
1	-	<1:10
2	-	1:80
3	+	1:80
4	-	<1:10
5	-	1:160
6	-	1:80
7	+	1:160
8	-	1:80
9	+	1:160
10	-	1:160
11	-	<1:10
12	Suspect	1:320
13	-	<1:10
14	-	1:160
15	-	1:80
16	+	1:160
17	Suspect	<1:10
18	-	1:320
19	-	1:160

Table 6 (Continued)

Sow No.	Titer
Normally Cycling Sows from SPF Institutional Herd	
4801	1:160
3801	<1:10
4251	1:320
5230	1:320
4220	1:160
5161	<1:10
200 pound hogs originating as weaned pigs from this SPF herd	
5800 G	1:160
5801 G	1:320
150-200 pound Caesarean derived, colostrum deprived pigs raised in strict isolation	
4-1B	<1:10
4-2B	<1:10
G-1	<1:10
G-2	<1:10

DISCUSSION

Evaluations of agalactic sows and the herds of origin were recorded so that the correlation between various clinical signs, herd factors, necropsy findings, and microbiologic findings could be determined. Results of these evaluations are in general agreement with observations recorded in the literature on symptoms of sow agalactia and associated herd factors.

The severity of symptoms and the morbidity rate due to sow agalactia is extremely variable. Martin et al. (1967) reported an individual herd incidence of 2-75%. The herd incidence in this study ranged from 10-90%.

In this study, confinement was the single management procedure that was consistently associated with agalactia. Martin et al. (1967) also made this observation in a study of agalactic sows from 11 swine herds. Confinement, however, is a common swine management procedure, and the association of confinement with agalactia may be because very few sows farrow unconfined in a grove of trees, or out on pasture today.

Most workers have reported that udder changes are difficult to detect and interpret. Ringarp (1960) reported diffuse swelling affecting all glands in 65.9% of 305 cases, and this was frequently more pronounced in the posterior

glands. Martin et al. (1967) reported mastitis on clinical examination in all of the 18 sows he studied, but this finding was not substantiated at necropsy. Swarbrick (1968) reported that the mammae were frequently hot as well as congested, but he observed evidence of catarrhal or necrotizing mastitis in biopsy material from only 8 of 32 sows. Because of the difficulty in accurately making a diagnosis of mastitis from clinical signs alone, the amount of mammary gland swelling was assessed clinically in this study, and a diagnosis of mastitis was not made without a histological examination. As previously stated, histology of mammary gland tissue will be done in a concurrent study, and results will not be reported in this thesis. Seven of the 19 agalactic sows exhibited clinical evidence of moderate to severe mammary gland swelling. Temperature and firmness of edematous glands is extremely difficult to evaluate. Therefore, no attempt was made to grade this nebulous clinical sign.

An increase and change in consistency of the vaginal discharge following farrowing has been reported by many workers (Hogg 1952; Hebeler 1954; Blood 1957; Ringarp 1960; Martin et al. 1967; Ross et al. 1969). Ringarp (1960) recorded that in more than 50% of the cases, the lochial discharge was more copious and sometimes changed in appearance.

The vaginal discharges were slight to very profuse. They were usually odorless, and the consistency varied from a thick, creamy pus to a muco-purulent, or even a watery, discharge. Often it contained many white to yellowish clots. Pools of this discharge could be seen behind the sow. It soiled the tail and lower leg as it escaped from the vulva, and at times increased in volume when the sow attempted to suckle (Ringarp 1960). In this study, only 1 of 19 sows exhibited a profuse vaginal discharge, and 6 of the 19 exhibited no detectable discharge.

The presence or amount of vaginal discharge does not appear to be a reliable clinical sign on which to base a diagnosis of sow agalactia. Swarbrick (1968) did not consider an increased vaginal discharge proven in this condition and mentioned that yellow, mucoid vulval discharges are of frequent occurrence in normal parturient sows. Jones (1966) stated that in most normal parturitions, a vaginal discharge could be seen for a week after farrowing. Ross et al. (1969) stated that in the 50 sows studied, there was no correlation between vaginal discharge and lower pig livability or lower total 21-day litter weights. They further stated that based on this study, it would appear that vaginal discharge was a very poor indicator of hypogalactia.

A wide range of rectal temperatures have been recorded

with this syndrome, but most workers agree that the degree of pyrexia is not great. The temperature range tends to fall between 102° and 105° F., (Hogg 1952; Jackson 1952; Ringarp 1960; Martin et al. 1967). Swarbrick (1968), on the other hand, in a series of 34 cases reported that most sows had temperatures between 104° and 106° F. Blood (1957) also reported temperatures ranged from 105° to 107° F. in a series of 11 cases of enzootic metritis. Rectal temperatures ranged from 101.2° to 103.5° F. in this series of 19 agalactic sows. It appears that the degree of pyrexia is of doubtful value in diagnosis.

In most cases of agalactia, there is some degree of anorexia, although the sow may continue to drink water or milk (Hogg 1952; Ringarp 1960). Only 1 sow of the 19 studied showed complete anorexia, 17 were observed to eat at least a portion of the feed presented, and 1 sow ate heartily. Anorexia, therefore, appears to be associated with sow agalactia, but again, is not pathognomonic for the condition. Loveday (1964) stated that the chief symptom noted was lack of appetite after farrowing: either complete anorexia for 1 or more feeds, or a much diminished appetite for the same period.

Most affected sows prefer to lie down, and although Ringarp (1960) noted no particular recumbent position, other

observers have found that many sows lie in sternal recumbency (Swarbrick 1968; Hebeler 1954). Fourteen of the 19 sows in this study assumed this position and could not be coaxed to lie on their side to allow nursing of piglets. Most animals were apathetic to their surroundings (Ringarp 1960) and often were reluctant to rise. An occasional sow appeared abnormally bad tempered (Swarbrick 1968).

The season of the year is considered to influence the incidence of agalactia. Loveday (1964) found the highest incidence in hot weather in South Africa. Ringarp (1960) recorded the lowest incidence in Sweden in January and February, and the highest incidence in April and the late summer and autumn months. Results of this study cannot be interpreted as indicating any one particular time of year, even though 17 of the 19 sows were obtained during March, April, and May. During this particular time of the year, more effort was directed toward obtaining agalactic sows for study than during the remainder of the year. Also, one would need to study the incidence of agalactia in a great number of herds over a full year in order to get a true picture of seasonal influence.

Sumner (1957) reported a higher incidence of sow agalactia in large herds than in small herds. This was not confirmed by Ringarp (1960) or Martin et al. (1967). In a large

herd, the problem could appear to be more serious. Sows selected for this study originated from both large and small herds. Again, one would need to study incidence in a great number of herds before accurate conclusions could be made.

Characteristic gross lesions have not been observed in agalactic sows. Ringarp (1960) observed signs of mastitis in some udder sections, and Martin et al. (1967) reported that the only organ consistently involved was the mammary gland, which showed edema, congestion, and nonfunctional tissue. Martin et al. (1967) also reported edema of the uterine wall, uterine atony, and focal hemorrhages of the genital mucosa. Thurman and Simon (1970) reported finding histologic changes in 12 of 12 uteri from agalactic sows studied. Lesions found in 9 of 12 uteri were classified as acute, while lesions in 2 uteri were placed in the mild, chronic category, and 1 in the advanced chronic category. Arteriolar thickening, periarteritis, and vascular necrosis were frequently observed.

In contrast, Swarbrick (1968) stated that histologic examination of portions of vagina, cervix, and uterus from 2 affected sows failed to reveal any abnormalities. He further stated that gross examination of 6 sows revealed a diffuse, mild congestion of the mucosa in the reproductive tract in all 6 cases. Small amounts of mucoid hemorrhagic material were found in the uteri, but there was no evidence of retained fetal membranes or acute metritis.

Gross lesions observed in this study were few. The hemorrhagic laceration in the ventral aspect of the vagina of sow No. 1 was probably due to trauma occurring at parturition. Only 2 of the 19 sows had gross lesions of mastitis. The reproductive tracts of all 19 sows uniformly showed slight edema of the uterine wall, a glistening mucosal surface, and lack of tone of the uterine musculature. Necropsy of normally lactating 1-3 day postparturient control sows would be necessary to properly evaluate observations on the genital tracts of agalactic sows.

A variety of bacteria have been recovered from both the milk and the vaginal discharges of affected sows. Coliform organisms seem to predominate, and these have been recovered in heavy growth and pure culture (Jackson 1952; Ringarp 1960; Noble et al. 1960; Smith 1965; Ross et al. 1969; Armstrong et al. 1968). Hemolytic streptococci of various groups seem to be the next most common organism, and these may often be found in association with E. coli. Other organisms which have been recovered include staphylococci, corynebacteria, klebsiellae, and pseudomonads. Swarbrick (1968) considered that difficulties associated with collection of satisfactory milk and uterine samples rendered the significance of any bacteria recovered antemortem as questionable. Moreover, Jones (1966) and Armstrong et al. (1968) recovered coliform organisms and

hemolytic streptococci from vaginal discharges of apparently healthy sows, but Ringarp (1960) failed to recover organisms in large numbers or pure culture from milk and uterine samples from 15 normal sows.

Bacteria were recovered from feces, milk, vaginal fluid, mammary glands, and uteri, but not from heart blood, spleen, or, with rare exceptions, selected lymph glands of agalactic sows (Armstrong et al. 1968). In that study, bacteria were recovered from the uteri of 6 and from the mammary gland of 12 of the 18 sows. In contrast, a much smaller percent of bacterial isolations were made in this study. No bacteria were isolated from mammary gland tissue or from uterine mucous membrane tissue samples. Two of the 19 sows yielded E. coli from the urethra, and in 1 other sow, E. coli was isolated from the urethra and the anterior portion of the vagina. Samples of heart blood, spleen, liver, and kidney from each of the 19 sows were bacteriologically negative.

Moore et al. (1966) incriminated a mycoplasma as the cause of sow agalactia. The organism was recovered from the mucosal surface of the uterus of an affected sow and was inoculated into 4 SPF sows. The sows were reported to have developed typical symptoms of the agalactia complex. Moore and his co-workers suggested that the organism be named M. hyogenitalium. Karbe (1967) reported isolation of

this organism from the uteri of sows with "puerperal endometritis and hardened mammary glands". Swine exposed to the organism developed clinical signs of the agalactia complex. These preliminary observations have not been confirmed.

In this study, a concerted effort was made to duplicate methods and procedures used by Moore and co-workers to make their mycoplasma isolations. To supplement this attempt, 4 additional mycoplasma media were used. These were 2 T-strain media in which known T-strain mycoplasmas had been grown, BHI medium because it supported growth of two other swine mycoplasmas, M. hyosynoviae and M. hyorhinis, and M-96 because of it's proven ability to support highly fastidious poultry mycoplasmas, i.e. M. meleagridis and M. synoviae. Cultures from all 19 agalactic sow uteri and all 32 sow uteri collected at a packing plant were judged to be mycoplasma negative. Either mycoplasmas were not present or the media and procedures used were inadequate to indicate a positive isolation.

Taylor-Robinson et al. (1968) reported isolation of T-strain mycoplasmas from 16 of 49 urogenital tracts of cows collected at a slaughter plant. T-strains were isolated more often from the urethra and bladder than from the vagina. Because T-strain mycoplasmas were not demonstrated in any of the sow urogenital tracts in this study, and because of the relatively high isolation percentage reported by Taylor-Robinson et al. (1968) from cow tracts, a decision was made to

culture cow urogenital tracts obtained at slaughter plants. The primary objective was to test presently used isolation procedures and determine the nutritional adequacy of T-strain media being used. Because, in this study, 11 of the 37 cow urogenital tracts cultured were T-strain mycoplasma positive, it was felt that isolation procedures used were adequate. In contrast to the Taylor-Robinson and co-workers report, the majority of T-strain isolations were made from the vaginas, rather than from the urethras and bladders, of cows.

Negative mycoplasma attempts from the agalactic sows are surprising, in view of the reports of Moore et al. (1966) and Karbe (1967), and the ubiquity of mycoplasmas in other locations in swine. There is considerable speculation by veterinarians that mycoplasmas could be involved in this syndrome, as these organisms are known to cause bovine and caprine mastitis. Even if mycoplasmas are not involved in MMA, it would seem that they might be involved in other reproductive disorders. They are extremely common in the human urogenital tract and the reproductive tract of cattle, although their pathogenicity is not entirely understood. T-strain mycoplasmas have been found in human and cattle respiratory tracts, but have not been reported in swine respiratory tracts.

Certain techniques in this study were especially designed to meet the nutritional requirements of T-strain mycoplasmas. Two different media were used, and toxic tissue

factors possibly present in the inoculum were diluted to supposedly non-toxic levels. Negative T-strain isolations from sow urogenital tracts and mammary gland tissue indicate they were absent or required growth factors were not supplied by the media used in this study.

Chlamydiae have been isolated from the urogenital tracts of both man and animals (Foy et al. 1967; Dunlop et al. 1965; Jones et al. 1966; Ford 1967; Holt et al. 1967; Lincoln et al. 1969; Storz et al. 1968). In sheep and cattle, chlamydial agents are known to cause abortion (Lincoln et al. 1969; Dungworth 1963). Chlamydiae isolated from the urogenital tracts of man have been considered a pathologic agent only because of their incriminating presence in patients suffering from certain urogenital diseases. Ford (1967) did, however, demonstrate CF antibodies to the chlamydia common group antigen in sera from urethritis patients and in patients with Reiters syndrome.

Chlamydia-like organisms were isolated from 4 of 15 agalactic sows in this study. An isolate was considered positive on the basis of fluorescence in yolk sac entoderm cells using the fluorescent antibody technique, finding intracytoplasmic inclusion bodies in yolk sac entoderm cells stained with Gimenez stain, and a CF titer of at least 1:8 when boiled yolk sac material was used as the group antigen. Because certain other microorganisms have been known to contain antigens cross reactive with the chlamydia group antigen (Mikami et al. 1969)

it would be necessary to carry out further test procedures in order to identify an organism as a chlamydia.

Other isolations of rickettsia-like organisms from swine have been reported. Willigan and Beamer (1955) described an organism isolated from pericardial fluid of "unthrifty" 7 week old pigs at necropsy. The organism grew in 5-7 day old chicken embryos in the presence of penicillin within 4-7 days post-inoculation. Coles (1941) reported an isolation from conjunctiva of pigs. Stoyanov (1965) and Surdan (1965) have reported on enzootic abortion in sows caused by a pararickettsial organism.

Criteria which would be helpful in identification of an unknown organism as a chlamydia include:

1. Effect of action of certain antibiotics on the organism when grown in chick embryos or in cell cultures.
2. Characteristic staining of intracellular inclusions in infected yolk sac entoderm cells.
3. Growth and death pattern in infected chick embryos.
4. Presence of group CF antigen in infected yolk sac suspension.
5. Stimulation of antibody production to chlamydia group antigen.
6. Appearance of stained "inclusions" of chlamydia grown in cell cultures.

The exact origin of the isolates could not be determined because tissue samples from each sow were combined. Isolates could have come from a single location or a combination of mammary gland tissue, mucous membrane tissue from the cervix, uterus, fallopian tubes, urethra, or the anterior portion of the vagina. Research directed toward determining the exact location of the chlamydia-like isolates now needs to be done.

There did not seem to be a direct correlation between sows yielding chlamydia-like isolates and severity of symptoms shown by these sows. (See Table 7.) A survey of normal post-parturient sow tracts for the chlamydia-like organism is needed. One cannot overlook the possibility that this organism may be a cause of other sow reproductive disorders, such as abortion or poor conception rates.

There appears to be no correlation between titer of antibodies to the chlamydia group antigen in agalactic sow sera and sows yielding chlamydia-like organisms. (See Table 6.) Results of a serological survey of swine sera by Wilson and Plummer (1966) for antibodies to the chlamydia group antigen indicated that these specific antibodies are quite common. Sera from 23% of 624 pigs were positive in an agglutination test, whereas only 8% were positive when a CF test was used.

In this study, 20 of 31 (68%) swine sera tested had a titer of 1:10 or greater. Of this group, 19 were agalactic sows, and 14 of the 19 (73%) had a titer of 1:10 or greater.

Table 7

Clinical Signs of Agalactia and Chlamydia-like Isolations

Sow No.	Chlamydia-like Isolation	Degree of Agalactia	Degree of Mammary Gland Swelling	Amount of Vaginal Discharge
1	-	++	++	+
2	-	++	++	++
3	+	++	+++	++
4	-	+++	+	++
5	-	+++	++	+++
6	-	++++	+	None
7	+	++++	+	None
8	-	++++	+	None
9	+	+++	+	++
10	-	+++	+++	++++
11	-	+++	+	+
12	Suspect	++++	++	None
13	-	++++	++	++
14	-	++++	+++	++
15	-	++++	++++	++
16	+	+++	++	+
17	Suspect	++++	+++	+++
18	-	++	++++	None
19	-	+++	++++	None

Specific antibodies to the chlamydia group antigen appear to be very common. Additional work is needed to determine the significance of these antibodies.

SUMMARY

Mycoplasmas were not isolated from mammary gland tissue or the urogenital tract of any of 19 agalactic sows. Thirty-two sow urogenital tracts obtained at slaughter, likewise, did not yield mycoplasmas. In contrast, T-strain mycoplasmas were isolated from 11 of 37 cow urogenital tracts. T-strains were found most often in the anterior portion of the bovine vagina. Five different mycoplasma media were used in all isolation attempts.

Chlamydia-like organisms were isolated from 4 of 15 agalactic sows. The exact origin of the isolates could not be determined because several tissues from each sow were pooled. The isolates originated from one or a combination of the following tissues: mammary, fallopian tubes, uterus, cervix, urethra, and/or the anterior portion of the vagina. The pathogenic significance of the chlamydia-like organisms isolated from sows was not determined.

Morbidity rate due to sow agalactia ranged from 10-90% in this study. Confinement at farrowing time was the only herd factor consistently associated with agalactia. Mammary gland swelling varied greatly between affected sows. A correlation between sows showing severe mammary gland swelling and those with complete agalactia was not apparent. Also, there was no correlation between sows with severe

mammary gland swelling and sows yielding chlamydia-like isolations.

One sow of the 19 studied showed a profuse amount of vaginal discharge. Again, there was no correlation between the amount of discharge and the degree of agalactia shown.

The origin of an excess vaginal discharge still remains an enigma. The exudate apparently does not originate in the uterus, since gross lesions of metritis were absent or were extremely mild when observed at necropsy. Even though the name mastitis, metritis, agalactia is frequently used in the literature, a varying degree of agalactia was the only clinical symptom associated consistently with the syndrome in this study. The name "sow agalactia" may prove to be a more accurate term.

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APPENDIX

MMA ETIOLOGIC AGENT RESEARCH VMRI Form A

95

Veterinarian _____ Date _____

Address _____ Sow No. _____

Phone _____ Owners Name _____

Address _____

Physical Exam of Sow on Farm

1. Temperature [ ] F
2. Degree of agalactia [+] __ [++] __ [+++] __ [++++] __
3. Rate edema of glands [+] __ [++] __ [+++] __ [++++] __
4. Number of glands involved [ ] out of [ ]
5. Discharge from vulva [None] __ [Mucus] __ [Mucopurulent] __ [Purulent] __
6. Amount of discharge [None] __ [+] __ [++] __ [+++] __ [++++] __
7. Attitude of sow [Allows pigs to Nurse] __ [Lays on Sternum] __
8. Degree of anorexia [eats well] __ [eats some] __ [eats none] __
9. Degree of depression [Alert] __ [depressed] __ [extremely depressed] __
10. Treatment of Sow -
11. [No. pigs farrowed] _____ [No. left] _____ Condition of pigs at birth [No. Strong] __

    [Willing to Nurse] __ [Weak] __ [Born Dead] __ [Mummie] __
12. Age of Sow _____
13. Weight _____
14. Health of pigs [Look good] __ [Weak and hungry] __ [Comatose to dead] __
15. Pigs [Have scours] __ [No scours] __ [Had scours and were treated] __
16. Litter No. _____
17. Degree of Sow condition [Thin] __ [good condition] __ [fat] __ [way too fat] __
18. Treatment used on rest of herd -
19. Was a mixed bactern used as preventative [Yes] __ [No] __
20. Name of Bacterin [ ] No injections [1] __ [2] __ [3] __
21. Degree of constipation None [+] __ [++] __ [+++] __ [++++] __ [Marbles] __
22. Did sow need help farrowing [Yes] __ [No] __
23. Per cent dystocia in rest of herd [ ]%
24. Date farrowed _____

MMA ETIOLOGIC AGENT RESEARCH VMRI Form B

Veterinarian _____ Date _____

Address _____ Sow No. _____

Phone _____ Owners Name _____

Address _____

Environment

1. Type of farrowing facilities [Stalls] __ [Pens] __ [Crates] __ [Tied] __ [Outside] __
2. Average time placed in farrowing facility before farrowing [_____]
3. Number of farrowings per year _____ Number in facility each farrowing _____

   [No. Gilts] _____ [No. Sows] _____
4. Sow Ration -
5. Gestation Ration [Self Fed] __ [Hand Fed] __ [Other] _____

   _____
6. Additives -
7. Amount of exercise during gestation [+] __ [++] __ [+++] __ [++++] __
8. Amount of excercise during lactation [0] __ [+] __ [++] __ [+++] __ [++++] __
9. Morbidity due to MMA this farrowing [_____]% Last farrowing [_____]%
10. Average death loss of pigs due to MMA [_____]%
11. Total size of herd on farm [_____]
12. SPF [Yes] __ [No] __
13. Breed -
14. Overall health of herd [+] __ [++] __ [+++] __ [++++] __
15. Sanitation [+] __ [++] __ [+++] __ [++++] __
16. Disinfectant used -
17. Estimation of outside temp. extremes - 7 days [_____]→[_____]
18. Estimation of inside temp. extremes - 7 days [_____]→[_____]
19. Rate of fly population [0] __ [+] __ [++] __ [+++] __ [++++] __
20. Were sows washed before going into farrowing quarters [Yes] __ [No] __

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