THE AEROBIC BACTERIAL FLORA OF THE VAGINA AND ITS RELATIONSHIP TO FERTILITY IN SWINE: A CLINICAL STUDY

by

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INTRODUCTION

The aerobic bacterial flora of the vagina of swine was determined and its relationship to subsequent infertility was evaluated in this study. Normal flora or non-specific infections are often mentioned in the literature as a cause of infertility but are usually indefinitely defined.

The importance of infertility in swine is exemplified by the fact that only about one-half of the ova produced are represented by viable young at farrowing. It is widely accepted by swine producers that four sows must be maintained for every three litters produced. Early pregnancy is the most crucial period for survival of the conceptus; approximately 30 per cent of the ova are not represented by live conceptus on the 25th day of gestation. Bacterial infections of the reproductive tract may be a cause of impaired fertility and early embryonic death. Leptospirosis and brucellosis are well defined causes of reproductive failure in swine but the significance of common aerobic bacterial flora of the swine vagina on reproduction is not established.

REVIEW OF LITERATURE

Microorganisms of the Genital Tract

A review of the literature reveals that little has been done in establishing the normal flora of the porcine genital tract. The predominant veterinary literature concerns studies of the bovine genital tract. Considerable controversy exists about the role of bacteria in abortion and repeat breeding problems in cattle. No definite agreement exists in the literature about what constitutes pathogenic bacteria in the genital tract of cattle and other species.

Day (1918) identified Streptococcus sp., Staphylococcus sp., and a short bacilli as the predominant organisms isolated from bovine uteri obtained from a slaughterhouse. Forty-four per cent of the non-gravid and 68 per cent of the gravid uteri were sterile.

Conklin et al. (1931) examined 80 gravid bovine uteri of which only seven were sterile while 71 had a bacterial flora. Micrococci, bacilli, escherichia, streptococci, and staphylococci were isolated in 35, 26.2, 23.7, 21.2, and 10 per cent respectively. In addition, 19 of the 80 specimens were found to have gross pathological lesions of either the uterus or the placenta. Eighteen of the pathological tracts were shown to contain a bacterial flora. Those uteri having an amniotic fluid of high acidity or high density were shown to contain large numbers of bacteria. All bacteriologic-

ally sterile uteri were shown by chemical analysis to have a normal amniotic fluid. It was the opinion of the authors that this microflora accounted for a high percentage of those abortions observed in the first three months of gestation in cattle.

Fitch and Bishop (1932) randomly selected 126 bovine uteri from an abattoir. Eighty-one gravid and 45 non-gravid uteri were examined soon after slaughter. Only nine per cent of the uteri were classified as containing bacteria. Bacteria such as alcaligenes, escherichia, micrococcus, staphylococcus, and corynebacterium were isolated.

Staphylococcic abortion in cattle was reported by Pounden and Krauss (1947). The staphylococci were isolated from uterine exudate and the internal organs of one calf. This preliminary report was followed with a more complete report by Pounden et al. (1947) on staphylococcic abortion in a dairy herd. Five abortions occurred in a herd of 50 cows. Necrosis of the cotyledons was noted in all cases with large quantities of purulent exudate. All had been inseminated with semen obtained from the same bull. During a seven month period, six (37.5 per cent) of the 15 pregnancies resulting from use of this bull terminated in abortion. Staphylococci were isolated from the bull semen, aborted fetuses, necrosed cotyledons, and uterine exudates.

Bartlett (1949), in a review of reproductive infertility caused by disease, incriminated streptococci, staphylococci, corynebacteria, and pseudomona as possible pathogenic organisms. Clark and Stevenson (1949)

found that 12 of 13 non-gravid bovine uteri were bacteria free while nine gravid uteri were all free of bacteria. Rabbit blood agar and meat infusion broth were used for initial isolates. The authors expressed the idea that the healthy non-gravid or gravid bovine uterus is bacteria free.

Easley et al. (1951) isolated Staphylococcus pyogenes from the uteri of 43 per cent of 39 slaughter cows, most of which had been repeat breeding animals. Lindley and Hatfield (1952) cultured uterine washings from infertile dairy cows. Twenty-six separate species of bacteria were isolated. The number of cows cultured and the per cent positive to bacteria were not given. The authors concluded that low grade bacterial infections appear to be a cause of infertility. Micrococci, neisseria, and corynebacteria were most often found.

Herrick (1952) examined four cows at slaughter that had been serviced three or more times without conception. No gross lesions were evident, but micrococci, streptococci, and pseudomonus were isolated. After sampling normal bovine uteri by sterile irrigation, Wulf and Dracy (1952) concluded that the normal bovine uterus is not sterile. Olds (1953) reviewing the literature on infertility in cattle concluded that generalized infections are frequently found in the uterus and cervix.

Hardenbrook (1958) obtained uterine biopsies for bacteriological sampling with a Nielson type instrument. Staphylococci, streptococci, corynebacteria, proteus, and Escherichia coli were commonly found. The

authors felt that 80 per cent of breeding failures in Illinois were due to non-specific infections and that streptococci, staphylococci, and corynebacteria were responsible for two-thirds of these cases.

Hawk et al. (1958) disagreed with many of the former workers, feeling that little evidence existed that infectious agents play a major role in lower fertility of cows. This concept was based on sampling of 62 uteri 16 days post breeding.

Gavrilets (1959) cultured the uterus and vagina of 36 clinically healthy cows and 28 species of bacteria were isolated. Staphylococcus aureus was predominant, occurring in 16 cows, while Staphylococcus citreus was isolated from 15 cows.

Trotter (1961) examined 74 bovine uteri from Hereford cows having a history of infertility. Fifty-four per cent of the uteri yielded various combinations of organisms of which Staphylococcus pyogenes (albus) was most prevalent. Trotter expressed the opinion that infertility is probably not related to bacterial involvement of the uterus.

Another approach to determine the effect of infection in repeat breeders and infertility cases among animals has been the bacterial sampling of the cervix and anterior vagina. Such culturing practices have been conducted for a number of years in the equine and bovine species.

Weitz (1947) surveyed the flora of the vagina of 100 apparently normal cows and 180 infertile cows. Alpha hemolytic streptococci, non-

hemolytic staphylococci, Corynebacterium renale, and Bacterium coli were isolated in 96, 52, 44, and 42 per cent respectively of the 100 normal cows. The list of the more prevalent bacteria from the vagina of the 180 infertile cows included some bacteria that may be more pathogenic. Bacterium coli, alpha hemolytic streptococci, Corynebacterium pyogenes, and staphylococci were isolated in 48.6, 35.0, 27.6, and 26.6 per cent of the cases.

A series of bacteriological examinations of the cervix of 53 infertile cows was conducted by Hatch et al. in 1949. Twenty-seven were positive with the predominant organisms being gram-positive cocci and diphtheroids. Micrococci were the most numerous of the gram-positive cocci. A preliminary conclusion was that bacterial infections, whether clinically apparent or not, may be responsible for many cases of infertility.

Easley et al. (1951) obtained sterile bacteriological samples from 56 (39 per cent) of 146 normal cows in contrast to eight (20 per cent) of the repeat breeding cows sampled. The predominant organism, Micrococcus (Staphylococcus) pyogenes was found in 26 per cent of the normal cows and in 43 per cent of the 39 slaughtered cows.

Gunter et al. (1955) undertook an investigation to determine the microbial flora of the reproductive tract of normal cows at the time of mid-estrus. A total of 260 cervical and uterine samples were taken from 106 animals at successive heat periods. Of the animals classified as normal 38 per cent were sterile. Only five per cent of the animals classified as

repeat breeders were sterile. Most of the diphtheroids, streptococci, and micrococci found in the normal breeding animals were saprophytic types. The diphtheroids, micrococci,and streptococci isolated from difficult breeders were primarily pathogenic strains. Of the streptococci, only two pathogenic species were identified from normal cows, whereas 23 of 27 isolates from difficult breeders were found to be pathogenic. These isolates were identified as Streptococcus pyogenes. Corynebacterium pyogenes was the pathogenic species of diphtheroid isolated. Micrococcus (Staphylococcus) pyogenes was isolated from 33 per cent of the animals sampled. Only 15 per cent of the micrococci from normal breeders were coagulase positive. This was one of the first investigations reviewed that attempted to correlate the presence of pathogenic bacteria with difficult breeding. These investigators readily acknowledged that bacterial infection was one of the causes of difficult breeding.

Contrary to the conclusions advanced by Gunter et al. (1955) that bacteria play a role in some difficult breeder cows, Gibbons et al. (1959) concluded that the presence of bacteria in the cervical mucus had no effect on subsequent breeding performance. Gibbons et al. based this conclusion on the results of intrauterine therapy and cervical culturing before and after calving. Of the 207 cows cultured at 30 days before and 30 to 60 days postpartum 83 cows (40.1 per cent) showed bacteria in the cervical mucus. Pathogenic types were evident in 26.6 per cent of the cows while 13.5

per cent harbored non-pathogenic bacteria. Streptococci were the most predominant organisms while staphylococci were next in frequency.

Clinical studies by Johanns (1967) of the prepartum and postpartum vaginal-cervical bacterial flora of 68 cows agreed with other recent reports that bacteria probably do not influence conception intervals and services per conception. Escherichia, micrococci, bacilli, and streptococci were the four main genera isolated in this study. It was evident that escherichia and streptococci were prominent following parturition and that micrococci and bacilli became more prominant at the 30 day postpartum samplings.

Similar studies of cervical-uterine infection in the equine species have been conducted. A total of 3,853 Thoroughbred mares in Ireland were sampled during the years 1957 to 1963. Collins (1964) reported that bacteria play a significant role in mare infertility. Of 3,853 mares cultured, 494 had beta hemolytic streptococci, 327 had coliform organisms and 120 had Staphylococcus aureus. Farrelly and Mullaney (1964) countered with the assertion that most mares harbor bacteria and fungi in the reproductive tract and yet produce normal foals. They felt that a feature of the normal mare is that the micro-flora, while a permanent resident in the posterior part of the tract, is only temporarily present in the anterior regions.

During one breeding season a total of 3,705 Thoroughbred mares were cultured via the cervix. Brooksby et al. (1965) reported 19 per cent of these samples were positive for bacteria. Beta hemolytic streptococci were

found in 49 per cent of all infections. Escherichia coli constituted 16 per cent of the isolates. Mixed infections were reported for 11 per cent and eight per cent yielded Klebsiella pneumoniae. Bain (1966) sampled 570 cervical swabs from mares. Beta hemolytic streptococci was recovered 175 times while Escherichia coli and coagulase positive Staphylococcus aureus were isolated 53 and 28 times respectively. The gravity of the presence of beta hemolytic streptococci in uterine infections was borne out when only 39 per cent of the mares affected produced a live foal in the same year. An equally grave prognosis was indicated when Escherichia coli, Staphylococcus aureus, or Klebsiella pneumoniae were encountered.

Similar organisms have been isolated from the cervix of human cases of infertility. Matthews and Braxton (1951) isolated Escherichia coli, Streptococcus viridans, hemolytic streptococci, diphtheroids, and hemolytic and non-hemolytic staphylococci from women.

During this review of the literature no information was found concerning the bacterial flora of normal porcine uteri, or cervical-vaginal areas. A few cases of specific bacterial infections in swine associated with abortion and infertility were reported.

Fennestad et al. (1955) reported Staphylococcus aureus to be a cause of metritis, return to heat, and abortion in 18 female swine. Staphylococcus aureus was recovered from aborted fetuses, placental tissues, and internal genital organs of several affected females. The infection source

was traced to a boar with preputial infection and an abscess in the anterior portion of the bladder. Only four of the 18 females mated with this sire terminated in normal pregnancy. Two types of staphylococci were isolated. Those colonies that were flatter and had a rather wide alpha hemolysin zone with an even transition to a wider beta hemolysin zone were called type A. Type B colonies were distinctly convex, drier, and had a narrow alpha zone and a wide beta hemolysin zone. Both types showed pigmentation and coagulase production. All were typed by the same undiluted phage 3A.

An analysis of 67 outbreaks of abortion and stillbirths in pigs, Saunders (1958), listed bacterial pathogens as being recovered from aborted fetuses in 11 out of 34 problem herds. Staphylococci, Escherichia coli, streptococci, and Corynebacterium pyogenes were the most common isolates.

Thorne and Nilsson (1961) reported abortion in a sow 87 days pregnant. The number of dead expelled fetuses was 13. A purulent discharge from the vulva continued for two weeks. A total of 48 isolates of staphylococci were made from the fetuses, placenta, amniotic fluid, and uterine secretions from the sow. A narrow zone of complete hemolysis and a wide beta toxin zone were observed around the colonies on bovine blood agar plates. Rabbit plasma was coagulated in 2.5 hours and all isolates gave an identical phage pattern: 29/52/52A/79/80/81/K_S6/6/7/77+.

Studies by Treqier and Homburger (1961) indicated that microorganisms are present in the uterine cavity of 17 per cent of the mice examined.

Surgical examination and routine histologic study revealed no uterine disorders. Five mice strains were examined and each strain varied somewhat as to the type and frequency of bacteria types. A total of 1,080 uteri of both mature and immature mice were examined by direct histological examination and culturing of uterine washings. Escherichia coli, Bacterium aerogenes, Proteus vulgaris, and Streptococcus fecalis were the most prevalent. Bacteria were observed adhering to the endometrium and the mucoid material in histological sections.

Uterine Infection

Relatively few studies have been conducted concerning the ability of various bacteria to infect the uterus of domestic animals. Rowson et al. (1953) determined that the anterior vagina and cervix of cows is relatively sterile, but the introduction of pathogenic bacteria during the luteal phase resulted in cervicitis and pyometria. Fennestad et al. (1955), after isolating Staphylococcus aureus from aborting sows, conducted infectivity studies in gilts, rabbits and mice. Only the pathogenic (type A) Staphylococcus aureus produced lesions in these test animals.

Wohanka and Hubrig (1962) infused the uterus or vagina of cows with 20 ml. of virulent Corynebacterium pyogenes cultures shortly after parturition. The procedure had little or no effect on subsequent fertility.

Singh (1965) infused Escherichia coli, Staphylococcus epidermidis,

Staphylococcus aureus, Streptococcus fecalis, or Corynebacterium bovis into the uterus of five healthy heifers 24 hours after insemination. At a subsequent return to estrus the five heifers were inseminated only. At slaughter nine to 56 days later, none were pregnant or had uterine infections. Infiltration of lymphocytes and mast cells was noted in the stratum compactum of the five uteri.

Antibiotic Response

Various attempts at using antibiotics in cows during puerperium to improve conception at subsequent breedings have been reported. Likewise, intrauterine therapy of difficult breeders has been reported with variable results. Herrick (1952) treated 78 hard to settle cows with one gram streptomycin and 400,000 units of penicillin in 25 ml. of three per cent sodium citrate. This was administered during early heat. These animals responded with 67 per cent conception at the first service. Similar success was reported by Easley (1951) and Lindley (1954). Sacchi et al. (1958) infused the uterus of 163 repeat breeder cows in five groups with 100 mg. of oxytetracycline hydrochloride. Significantly higher rates of conception ($P<.05$) occurred in all groups.

Less success with intrauterine antibiotics was reported by Ulberg et al. (1952). A total of 48 cows that had been inseminated four or more times without conception were involved. In the study, 16 were treated at

the beginning of heat and inseminated 12 hours later and seven were treated 17 days after estrus and inseminated at the subsequent heat period. The remaining 25 cows served as controls. Those animals that failed to return to heat were slaughtered at 34 days post breeding. Live embryos were found in 34 per cent of the treated animals and in 56 per cent of the control animals. Thus it appears that antibiotic treatment inhibited conception, but the difference was not statistically significant. Another important finding was that the mixture of antibiotics placed in the lumen of the uterus during the luteal phase of the estrus cycle caused some endometrial inflammation. This was evident both grossly and histologically. No endometrial inflammation was observed when antibiotics were used at estrus. A similar adverse effect on conception was reported by Hinze (1958), when antibiotics were used intrauterine on approximately 150 post parturient cows.

Calaprice (1959) reported on sensitivity tests of pathogenic staphylococci isolated from the genital tract of cows. These staphylococci were found to be only slightly sensitive to penicillin, streptomycin, and sulfathiazole.

Antibiotic additives in the feed of sows and gilts have been tested for their ability to reduce early embryonic mortality and increase conception rates. Early studies suggested little advantage in use of antibiotics in sow rations. Most early research on the use of antibiotics in swine has been with low levels administered throughout gestation. Carpender (1951) and

Carpender and Larson (1953) used this approach and could not show any beneficial effect of the antibiotics on reproduction.

More recently Dean and Tribble (1962) analyzed the effect of therapeutic levels of aureomycin at breeding on reproductive performance of swine. A total of 54 gilts and sows were fed a therapeutic level (0.54 gram per head per day) for 10 days to three weeks beginning 0 to seven days prior to the breeding season. Evaluation of the results by analysis of variance indicated that sows and gilts farrowed significantly larger litters than the controls ($P<.05$) not fed antibiotics at breeding. Sosa et al. (1963) had less conclusive results using zinc bacitracin or tylocine at 200 grams per ton of feed.

Ruiz et al. (1966) used the combination of 0.5 grams chlorotetracycline, 0.5 grams sulfamethazine and 0.25 grams penicillin per head daily to test the effect of antibiotics on gilt reproduction. Ninety-six, Yorkshire Landrace cross, gilts were tested on a two by two factorial of treatments of two feed levels with or without antibiotics and sulfa. The total number of pigs farrowed by gilts receiving antibiotics was significantly higher ($P<.05$) than the number farrowed by gilts receiving no antibiotics (11.1 versus 9.6 pigs per litter).

A further report by Ruiz (1967) contained more results of this test. Conception rates were significantly increased ($P<.025$) in those gilts receiving antibiotics. A bacteriological survey was included in this study. Table 1

Table 1. Summary of antibiotic effect on bacterial numbers[a]

	Average number of organisms/gilt/sample	
	No Antibiotics	Antibiotics
Non beta hemolytic Micrococcaceae	195	144
Beta hemolytic Micrococcaceae	35.5	21
Hemolytic streptococci or corynebacteria	18.5	8
Non hemolytic streptococci or corynebacteria	132.5	65

[a]From Ruiz 1967.

is a summary of the bacteria isolated. Non beta hemolytic Micrococcaceae, beta hemolytic Micrococcaceae, hemolytic streptococci or corynebacteria, and non hemolytic streptococci or corynebacteria were the primary bacterial groups isolated. The latter three groups were found to be significantly reduced ($P < .05$ or less) in the vagina of gilts on antibiotics. The non hemolytic Micrococcaceae were reduced in gilts on antibiotics but not as significantly.

This study points out a relationship between bacteria in the genital tract and reproductive capacity. However, no direct evidence was advanced that the reduction of vaginal bacteria accounted for the increased conception rate or litter size in gilts fed antibiotics.

Resistance of the Genital Tract to Infection

Colonization of bacteria in the vestibule and vagina appears to be a common occurrence. Bacteria are found less commonly in the anterior cervix or uterus. This retarding effect on bacterial growth is related to body defense mechanisms. The formation of specific antibodies in a local immune response and the influence of the uterine tissue and its secretions are two very important local defense mechanisms.

Kerr (1955) showed that intrauterine inoculation with dead Brucella abortus strain 19 induced formation of muco-antibodies in high titers in the mucosa of the uterus, cervix, and vagina. No circulating antibodies were produced by this route of inoculation. Similarly, Pierce (1959) and Singh's (1965) experiments demonstrate that muco-antibodies may be produced at the surface of the bovine uterus and vagina, frequently in the absence of circulating antibodies. Singh infused escherichia, staphylococci, streptococci and corynebacteria.

The differential response of estrus and pseudopregnant rabbit uteri to induced infection was investigated by Black et al. (1953). Suspensions of Staphylococcus aureus and Escherichia coli were injected into the ligated uterine horns of rabbits in the two different hormonal states. These horns were examined at 24 hours after injection for packed cell volume. No appreciable reaction was observed in the uteri during the follicular phase, but pyometria was demonstrated at 24 hours in the horns during the

luteal phase. Hawk (1958) and Winter et al. (1960) showed the difference of bactericidal action in rabbits' uteri during estrus and pseudopregnancy was due to differences in leucocytic activity in the two types of uteri. Similar conclusions were reached by Ochi and Uchida (1963).

Hawk et al. (1960a) induced leucopenia in rabbits with N-N'-N''-triethylenethiophosphoramide (TSPA). Tests were made of the bactericidal activity of rabbit uteri and uterine exudates after induced leucopenia. Cell free uterine exudates obtained four hours after experimental inoculation of lucopenic rabbits in estrus had little or no bactericidal activity. Thus non-cellular antibacterial factors, independent of leucocytes, were not demonstrated in uteri of estrus rabbits. Winter et al. (1960) found the bacterial recoveries four hours after uterine inoculations were significantly higher from the uterine horns of leucopenic rabbits in both the follicular and luteal ovarian phases than from corresponding horns of normal rabbits in the two states. Bactericidal activity in the uteri was greater in the follicular phase than the luteal phase in both leucopenic and normal rabbits. This suggests that a non-cellular bactericidal factor may be active in the uterus of estrus rabbits.

Hawk et al. (1960b) demonstrated non-cellular bactericidal factors in inflammatory exudates from the uterus and pleural cavities of both estrus and pseudo-pregnant rabbits. The bactericidal activity of cell-free uterine exudates was proportional to the number of leucocytes in the uterine lumen,

regardless of the endocrine state of the rabbits. This suggests that the leucocytes contribute bactericidal substances to the uterine secretions. The difference in bactericidal activity within the uterus under different hormonal conditions has been shown to correspond, in the early stages of infectivity, to different rates of leucocyte infiltration into the uterine lumen. Previous experiments have shown that the uterus of the rabbit is less resistant to experimental infection with Escherichia coli when under the influence of progesterone. In utero and in vitro tests in rabbits, studying phagocytosis of starch particles by leucocytes, indicates that there is some factor in the uterine secretions, under the influence of progesterone, which inhibits the phagocytic activity of leucocytes in vivo and in vitro. Reports by Killingbeck and Lamming (1963) and Heap and Lamming (1961) suggest that the major difference in composition of the uterine secretions of rabbits under different hormonal conditions lies in the presence of an acid-soluble seromucoid component. This seromucoid component appears to be under the influence of progesterone. Later work identified polysaccharides as the active agents of this seromucoid component.

Fractionation of the uterine secretions of rabbits by Killingbeck et al. (1963) indicated that polysaccharides are responsible for inhibitory effects of phagocytosis by leucocytes. These polysaccharides appear to coat the receptor surfaces of the polymorphonuclear leucocytes.

Lamming and Haynes (1964) compared the uterine flushings from the

cow, rabbit, and rat. These flushings were collected at stages in the reproductive cycle when the uterus is known to be susceptible to infection. All contained polysaccharide material. The material is not present at other stages in the cycle when the animals are relatively more resistant to uterine infections. In the uterine flushings from the cow and rabbit, these polysaccharides are most predominant during the luteal phase. Uterine flushings from rats are somewhat different since the polysaccharides are highest during estrus. This is significant because the rat is a species in which the uterus is relatively more susceptible to infection at estrus.

Hayes and Lamming (1964) found that the uterine secretion of the pig parallels that found in the rat. Polysaccharide levels are highest during the estrus period in the rat and pig while the rabbit and cow are highest in polysaccharides in the uterine flushings during the luteal period.

It is worthy of note that the rat and pig are both cycling polytoccous animals with a similar degree of embryonic mortality. It may be that both the rat and pig are more susceptible to uterine infections at estrus.

MATERIALS AND METHODS

Experimental Animals

The experimental animals that were sampled in the bacteriological survey and that were used for infectivity studies were from the Iowa State University-Swine Breeding research herd. Emphasis on disease control has been a major part of the management program. Parent stock had been derived by caesarean section during the early 1960's. Initial conception rates approached the 90-95 percentile but with each succeeding year the conception rate dropped approximately five per cent until in 1965 the conception rate was 73 per cent with an average of 1.32 services per gilt. Table 2 is a record of reproductive performance in this herd for the years 1965 and 1966. Brucellosis and leptospirosis serological tests are conducted yearly on all breeding stock.

A bacteriological survey was conducted on virgin gilts that were part of the normal breeding program of this research herd. These gilts were in excess of 200 days of age and weighed approximately 250 pounds. Purebred Yorkshire, Hampshire, Duroc, Poland China, and Landrace were represented with the former two breeds being present in greatest number.

The initial survey was conducted during the 1965 fall breeding period. This breeding period extended from mid-November until the last of January. Ninety-one mature cycling gilts were in this group. These gilts

Table 2. I.S.U. Swine Breeding Research Herd - Breeding Records

	1965 Spring Farrowing	1965 Fall Farrowing	1966 Spring[a] Farrowing	1966 Fall[a] Farrowing
No. gilts bred	136	91	71	76
No. bred twice	21	20	33	7
No. bred three times	3	0	3	1
No. gilts farrowed	108	64	61	64
Per cent conception	79.4	70.1	85.9	84.2
No. gilts returning to heat	20	2	6	3
No. gilts not pregnant but failed to cycle	4	18	3	6
Dead or crippled	4	4	1	2
Aborted		3		1

[a]Animals used in research project.

were mated with thirteen mature purebred boars of like breeds. The boars were subjected to semen evaluation to determine sperm viability and concentration. All the males were rated good or better in this respect. A second survey group of seventy-six gilts was available for bacteriological sampling during the spring breeding period from April 12, 1966 until June 1, 1966. Proven sires were mated with these gilts. Since these two groups of gilts were used to evaluate the relationship between bacteria in the vagina and

conception, those gilts mated to boars that had less than 50 per cent conception were not included in the test analysis. Thus the chance of sudden infertility or infection in the boars was less likely to be reflected in the total analysis. During the breeding season, the gilts were observed twice daily. Gilts observed in estrus were moved to the breeding pen and bacteriological samples were collected just prior to mating.

Thirty gilts weighing approximately 300 pounds and approaching one year of age were used for infectivity studies. Purebred stock consisting of thirteen Hampshires, twelve Durocs, and five Yorkshires were divided into three groups of 11, 10, and nine animals each. Group one was composed of gilts found to be free of hemolytic staphylococci in the vaginal tract. To be classified as hemolytic staphylococci free a gilt must have had negative cultures on four occasions prior to breeding. These samples were taken every third day with the last one just preceding insemination.

Originally 11 animals qualified for group one, but one animal was later found to be infected at the time of breeding. This gilt was not included in the test results. Groups two and three had 10 and nine animals respectively. They were animals that failed to meet the criteria for being in group one. Three proven sires were used as semen donors. The three consisted of two Poland China boars and one Duroc boar weighing approximately 400 pounds and averaging 12 months of age.

All breeding stock were housed in open sheds the year around. Am-

Table 3. Ingredients of the supplement

	Pounds	Per cent crude protein
Meat and bone meal	300	50
Soybean oil meal	850	44
Dehydrated alfalfa	250	17
Dicalcium phosphate	94	
Salt	30	
Trace minerals[a]	8	
Profactor B[b]	3	
Aurofac (Aureomycin 2.5 gms./lb.	6	
Choline chloride	2.90	
Yeast	.75	
2-4-12 Fortafeed[c]	.80	

[a]4.4% Mn, 6.6% Fe, 1.3% Cu, .2% Co, .3% I, 12% Zn, 20% Mg, and 4.25% Cu.

[b]Vit. B12 supplement (20 mg./lb.).

[c]Riboflavin 2,000 mg./lb., Niacin 12,000 mg./lb., Calcium Pantothenate 4,000 mg./lb.

ple bedding and water were supplied at all times. The ration for breeding and gestation remained nearly the same throughout the test although it was increased in amount during breeding and late pregnancy. The gilts were hand fed approximately one pound of oats and four to five pounds of a 16 per cent protein (corn-supplement) ration per head daily. Table 3 lists the ingredients of the supplement.

Laboratory Techniques

Sample collection

The collection of samples for the survey of vaginal microflora of swine was conducted during estrus, just prior to mating. The period of standing heat was most suitable for collection for three reasons. First, little restraint was necessary during sample collection. Secondly, the bacterial growth in the anterior vaginal vault was reduced in quantity and would more likely represent the more resistant bacteria. Thirdly, this period served to synchronize testing with a known phase of the estrus cycle.

Cotton, saturated with 70 per cent isopropyl alcohol, was used to cleanse the labial surfaces. The labia were separated with digital pressure such that insertion of sterile swabs began approximately one and one-half inches internally. A speculum was not generally used because of the small size of the genital tracts and the resulting struggle. Two sterile swabs were inserted four to five inches into the vagina. These swabs were removed and placed in sterile cotton stoppered tubes for transport to the laboratory. Nearly all samples arrived at the laboratory within six hours after they were collected.

Identification of organisms

Identification of organisms in this survey was confined to the common aerobic bacteria and did not include anaerobic bacteria, mycoplasma, rickettsia, virus, or mycotic agents. Sample swabs were streaked directly

on five per cent horse blood agar plates to determine the organisms present. These plates were then incubated aerobically for 48 hours at 37° C. before identification was attempted. Identification was limited to genera in most cases with the exception of Staphylococcus aureus, Escherichia coli, and Corynebacterium pyogenes. Bergey's Manual of Determinative Bacteriology, by Breed et al. (1957) served as a guide for identification of bacteria. The primary basis for identification of isolated bacteria was colonial morphology, action on blood agar, gram-staining characteristics, and bacterial morphology.

Gram-positive rods were observed for pleomorphism and palisading arrangement. Cultures of gram-negative rods were checked for motility and streaked on selective media.

After the initial examination, each group of organisms was subjected to the indicated biochemical tests. Beta hemolytic streptococci were tested for carbohydrate fermentation and action in litmus milk, but alpha hemolytic streptococci were not. Gram-negative isolates were streaked on Tergital-7 medium and tested for fermentation of selected carbohydrates and indol formation.

Suspected isolates of Corynebacterium pyogenes were tested in litmus milk. Staphylococci were tested for the reduction of dextrose under anaerobic conditions. Mannitol fermentation and coagulase production were the criteria for identifying Staphylococcus aureus. Thirty-two selected

strains of hemolytic staphylococci were submitted for phage typing.

Culture media, reagents, and tests employed

Blood agar plates Blood agar plates were prepared by dissolving 17.5 grams Tryptose Blood Agar Base[1] in 500 ml. of distilled water and autoclaving for 15 minutes at 15 pounds pressure. Approximately 25 ml. of citrated horse blood was added to the sterile, cooled agar base and distributed into approximately 30 plastic petri dishes. Special blood agar plates utilizing bovine and sheep blood were made for studying the hemolytic activity of Staphylococcus aureus isolates.

Tergitol-7 agar plates Tergitol-7 Agar[1] was used with 2,3,5 triphenyl-tetrazolium chloride added at the level of 40 milligram per liter of medium. After pouring in petri dishes these were dried overnight in an incubator and stored at 5° C.

Tryptose phosphate broth Tryptose Phosphate Base[1] (5.9 grams) was dissolved in 200 ml. of sterile water, dispensed and autoclaved 15 minutes at 15 pounds pressure.

Carbohydrate fermentation media Stock sugar base consisted of 3.2 grams Phenol Red Broth Base[1] mixed in 200 ml. distilled water. Three grams of the selected carbohydrate was mixed with the stock solution, dispensed in tubes and autoclaved 15 minutes at 15 pounds pressure. Dex-

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[1] Difco Laboratories, Inc. (1953).

trose, lactose, salicin, mannite, raffinose, inulin, trehalose, and sorbitol were the carbohydrates used.

Litmus milk Litmus milk was prepared by dissolving 50 grams Bacto-Skim Milk[1] and 375 milligrams Bacto-Litmus[1] in 500 ml. of distilled water. After autoclaving the tubes were refrigerated.

Serum Broth Tryptose broth consisting of three grams Bacto-Beef Extract[1] dissolved in one liter of distilled water was autoclaved at 15 pounds pressure for 15 minutes. One hundred ml. of equine serum was added to the cooled medium and dispensed in sterile tubes.

Indol medium Five grams of Bacto-Tryptone[1] and 2.5 grams of sodium chloride were dissolved in 500 ml. of distilled water. This was dispensed in tubes and autoclaved for 15 minutes at 15 pounds pressure. Kovac's Reagent was used for Indol determination.

Coagulase test Rabbit plasma diluted 1:5 with sterile saline and dispensed in 0.5 ml. quantities was mixed with 0.2 ml. of test culture or a loop of the test colony. After inoculation, the tubes were incubated at 37° C. and read at hours two and 24.

P. A. Pattee, Department of Bacteriology, Iowa State University, conducted phage typing of selected staphylococci samples. Phages used were: 29, 52, 52A, 79, 80, 81, 6, 7, 83, 42B, 47C, 47, 53, 54, 70, 73,

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[1]Difco Laboratories, Inc. (1953).

75, 77, and 44A. This was conducted with the same phages as reported by Pattee and Baldwin (1962).

Preparation of inoculum

The preparation of a bacterial inoculum for infectivity studies was confined to hemolytic Staphylococcus aureus strain isolated from this experimental herd. Three representative strains were selected on the basis of hemolytic ability, coagulase reaction, pigmentation, and colonial morphology.

Isolate 616 was only slightly hemolytic on blood agar and showed evidence of golden pigmentation after setting at room temperature for 48 hours. A trace of fibrin was produced in rabbit plasma after 24 hours incubation. Morphologically this strain was classed a semi-smooth colony type. Isolate 1858 and 252 were smooth colony types and more hemolytic on blood agar plates. These two isolates produced white pigmentation. A definite coagulase positive reaction was demonstrated in these strains after chicken embryo passage.

The three strains were propagated by chick embryo passage. Four (six day-old) embryos were inoculated in the yolk sac with 0.2 ml. of one of the three isolates. Death occurred in all four embryos within 48 hours after inoculation. All inoculated eggs and eight control embryos of equal age were stored at 5° C. for 24 hours. After harvesting the egg yolk of both inoculated and control chick embryos, the yolk material was serially

diluted and titrated for bacterial counts on blood agar plates. Egg yolk samples from the control embryos were negative for bacterial growth. This egg yolk was dispensed in five ml. vials, frozen and stored to be used as control inoculum. Yolk samples of eggs inoculated with isolates 252, 616, and 1858 had respective bacterial counts of 50, 30 and 16×10^7 for each ml. of egg yolk.

After titration and confirmation of purity the strains were mixed and allocated in five ml. sterile vials and stored at -25° C. until used in infectivity studies. One frozen vial of inoculum was thawed and titrated for bacterial counts and purity on blood agar plates. This frozen-thawed sample of the combined strains of inoculum plated 23×10^7 bacteria per ml.

Experimental Infection of Swine

This experiment was designed to analyze the effect of introduction of Staphylococcus aureus into the uterus during estrus. Experimental exposure was designed to coincide with artificial breeding. Three proven sires were used as semen donors. Collection was just prior to use of the semen. Whole semen samples, including fluids of the accessory glands, were collected by the gloved-hand method. A gilt in standing heat was used for collection purposes. Semen was protected from temperature shock by a jacket containing water at approximately 37° C. After straining through 44 x 36 mesh gauze, these collections were mixed and distributed into

individual plastic bottles.

Inseminations were accomplished by means of a 45 cm. bovine inseminating pipette with a modified tip as described by Lovell (1965). The plastic squeeze bottles were connected to the insemination pipette by 15 cm. of rubber tubing, an apparatus similar to that used by Aamdal and Hogset (1957).

Gilts were observed daily for vulvar redness and swelling. All 30 experimental gilts cycled between January 30, 1967 and February 18, 1967. Six of the 10 control gilts and ten of the 19 inoculated gilts were inseminated two days in succession. The remainder were inseminated only once during the period of standing heat. Weather permitting, the gilts were inseminated in their respective lots. However, some days it was advantageous to collect and inseminate in a near-by heated swine barn.

After inseminating approximately 40 ml. of raw semen, four ml. egg yolk inoculum or control egg yolk was injected into the rubber tubing by means of a disposable 20 gauge needle and syringe. This material was forced through the inseminating pipette by approximately 10 ml. of the remaining semen. The control egg yolk and infective inoculum were kept frozen until just prior to use. Those gilts inseminated a second day were infused with two ml. of egg yolk or inoculum.

Following insemination these gilts were observed daily for return heat and for evidence of reaction to the inoculum. All 30 gilts were

inseminated during a 20 day period and no gilts were inseminated during return heats. No abnormal discharges or systemic reactions were detected during the 58 days of observation.

The reproductive performance of these gilts was evaluated at slaughter. Reproductive tracts were examined grossly for ovarian activity, pregnancy, evidence of fetal resorption, and abnormalities. L. N. Hazel, Department of Animal Science, Iowa State University, assisted in these examinations. Bacteriological samples were collected before the tracts were opened.

As the gilts approached 40 days post insemination, they were slaughtered. Fetal development at 40 days was such that viability was easily ascertained. This period of time allowed for return heats or evidence of anestrus. An average of 37.7 days elapsed between first insemination and slaughter of the gilts.

Examination of Genital Tracts

Usually four gilts were slaughtered as a group at a local abattoir. Genital tracts were removed from the hanging carcasses immediately after opening of the abdomen had been completed. Plastic bags were used to transport the uteri from the abattoir to the laboratory.

In the laboratory, bacteriological samples were collected from each uterine horn and any pathological areas. A spatula heated in the flame of a

bunsen burner was used to sear the uterine serosa. A searing Bard-Parker blade was used to penetrate the uterine wall. Sterile swabs were inserted up or down the uterine horn, removed and placed in sterile tubes for culturing. Culturing techniques as described previously were used with these samples. Swabs from abscesses, hydrosalpinx, and other pathological conditions were collected and processed in a similar manner.

Gross examination of the genital tracts began with the ovaries. The number of corpora lutea and their approximate duration were determined whenever possible. Differentiation was made between corpora lutea of anestrus, which included pregnancy, and corpora lutea of a subsequent estrus following insemination. This differentiation was based on the size, color and consistency of the corpora lutea. Follicular development of each ovary was observed and correlated with the existing corpora lutea. Cystic follicles were noted as a frequent abnormality of the ovary. These were observed to be well over 14 mm. in diameter.

Fallopian tubes were examined for gross pathology and for potency. A blunt 14 gauge three inch needle was inserted into the ovarian end of the fallopian tube after the tube had been transected from the uterus and the mesosalpinx. Saline was forced into the fallopian tube by gentle pressure on a 10 ml. glass syringe. It was necessary to seal the fallopian tube to the 14 gauge needle by digital pressure.

The uterine horns were opened from the tip of each horn to the body

of the uterus. The presence and condition of embryos and placenta were noted and recorded. It was possible to correlate the condition of the uterus in most cases with the ovarian activity of the animal.

Statistical Analysis

Statistical analysis was applied to test the relationship between vaginal flora and reproductive performance. Two parameters, conception and litter size, were used in this evaluation. Conception rates of staphylococci infected and non-infected gilts were analyzed by a special approximation method for 2 x 2 tables, applying the chi-square tests for contingency as described by Ostle (1963) page 131. A significant level of five per cent was assumed. The mean litter size of hemolytic staphylococci-free and hemolytic staphylococci-infected gilts were compared, using "Student's" t test for significance of difference. Equal variance was assumed and a five per cent significance level was applied. A description of this test is found in Ostle (1963) page 119. In testing the difference in response to treatment between staphylococci inoculated and non-inoculated gilts, the chi-square test for contingency was applied.

RESULTS

Survey Results

This bacterial survey was conducted in two groups of breeding gilts. The first study consisted of 85 vaginal samples collected from 51 virgin gilts just prior to first mating, 26 second mating gilts, and eight third mating gilts. These gilts were mated in the fall for early spring farrowing. The results of the survey of the first group of gilts is recorded in Table 4. The conception rate for first service gilts was 69.6 per cent. Bacteria isolates from these gilts were identified by genera and by species whenever possible. Table 5 is a composite of vaginal cultures from gilts sampled just prior to 1st, 2nd, and 3rd services.

Organisms recovered

In the first group of gilts surveyed, the hemolytic staphylococci were isolated from 31 of the 51 virgin gilts sampled and from 64.7 per cent of all pre-breeding vaginal samples. It was noted that staphylococci was recovered in a higher percentage of repeat breeding animals.

Non-hemolytic bacteria of the family Micrococcaceae were isolated in 32 of 51 virgin gilts sampled and 60 per cent of all pre-breeding vaginal samples. These non-hemolytic bacteria failed to produce coagulase, but some anaerobically fermented dextrose. Alpha hemolytic streptococci were fairly prominent in 24 of the 51 virgin gilts. Only 29.4 per cent of the 85

Table 4. Vaginal microflora isolates from fall (1965) gilts

	No. Sampled 1st breeding		No. Sampled 2nd breeding		No. Sampled 3rd breeding	
	51		26		8	
	No. Pos. Samples	Np.[a]	Pos. Samples	Np.	Pos. Samples	Np.
Hemolytic staphylococci	31	11	16	8	8	6
Non-hemolytic Micrococcaceae	32	10	14	6	3	2
Alpha hemolytic streptococci	24	8	1	0	0	0
Beta hemolytic streptococci	19	0	4	1	0	0
Corynebacterium pyogenes	14	5	8	2	1	0
Coryebacterium spp. (other than C. pyogenes)	13	4	5	2	2	1
Bacillus spp.	9	6	4	2	0	0
Escherichia coli	4	3	3	1	0	0
Proteus spp.	2	0	1	0	1	1
Nocardia sp.	1	0	1	0	0	0

[a]Nonpregnant from this service.

Table 5. Composite of vaginal isolates from gilts--fall (1965)

	No. Isolates from 85 samples	Per Cent
Hemolytic staphylococci	55	64.7
Non-hemolytic Micrococcaceae	51	60.0
Alpha hemolytic strept.	25	29.4
Beta hemolytic strept.	23	27.1
Corynebacterium pyogenes	23	27.1
Corynebacterium spp. (other than Corynebacterium pyogenes)	20	23.5
Bacillus spp.	13	15.3
Escherichia coli	7	8.2
Proteus spp.	4	4.7
Nocardia spp.	1	1.2
Sterile samples	3	3.5

samples collected during the breeding period yielded alpha hemolytic streptococci, and they were not a common isolate of repeat breeding samples.

Beta hemolytic streptococci were found in 19 of 51 virgin gilts and 27.1 per cent of all samples taken were positive for beta hemolytic streptococci. Morphologically and on the basis of carbohydrate fermentation,

these beta hemolytic streptococci represented at least six different types. None of these streptococci types belonged to the commonly described pathogenic species, but further investigation of this genera was pursued because of its possible pathogenic nature.

Corynebacterium pyogenes and other Corynebacterium spp. were isolated in 19 and 14 of the 51 virgin gilts respectively. Of all pre-breeding samples Corynebacterium pyogenes and other Corynebacterium spp. were present in 27.1 and 23.5 per cent respectively. These isolates did not increase in incidence with repeat breeding samples. The incidence of Bacillus spp., Escherichia coli, Proteus spp., and Nocardia sp., was nine, four, two, and one isolates in the 51 gilts. These same bacteria were represented in 15.3, 8.2, 4.7, and 1.2 per cent respectively of the entire samplings.

Initial investigation indicated that hemolytic staphylococci, other members of the family Micrococcaceae, beta hemolytic streptococci, and Escherichia coli occurred frequently in the vagina and might be related to fertility. This was particularly evident in the case of hemolytic staphylococcus which was isolated from the vagina in a higher percentage of the 2nd and 3rd breeding samples as compared to 1st breeding samples. Hemolytic staphylococci were isolated in all eight gilts sampled just prior to the third mating.

A second survey was conducted during the Spring breeding period.

Table 6. Composite of vaginal isolates from gilts--spring (1965)

Number sampled at 1st breeding-62
Number sampled at 2nd and 3rd breeding-3

	1st breeding Isolates	1st breeding Np[a]	2nd and 3rd Isolates	2nd and 3rd Np	Per Cent Isolated
Hemolytic staphylococci	32	10	1	0	50.8
Non-hemolytic Micrococcoceae	31	8	1	1	49.2
Alpha hemolytic strept.	Not recorded				
Beta hemolytic strept.	2	1	0	0	4.6
Corynebacterium spp.	26	0	1	0	41.5
Bacillus spp.	11	2	0	0	20.0
Escherichia coli	2	1	1	0	3.1
Proteus spp.					
Sterile samples	2	0	0	0	3.1

[a]Np--nonpregnant from this service.

Particular attention was directed towards isolates of Escherichia coli, beta hemolytic streptococci, and hemolytic staphylococci. The results of this survey is presented in Table 6.

Bergey's Manual of Determinative Bacteriology lists two species of staphylococci, Staphylococcus aureus and Staphylococcus epidermidis. The ability to coagulate rabbit plasma and ferment mannitol distinguishes

Staphylococcus aureus from Staphylococcus epidermidis. Thirty-two isolates were tested for coagulase production and mannitol fermentation. These 32 isolates produced hemolysis of equine blood. Five of these isolates failed to show hemolysis on bovine blood agar plates. Seventeen isolates produced coagulase, although 11 of these showed only traces of fibrin rather than a definite coagulation. These same 32 isolates failed to demonstrate any susceptibility to the phage types mentioned previously in this paper. Mannitol was fermented slowly by 27 of 32 isolates. On the basis of this information it appeared that the majority of these staphylococci isolates were low virulence Staphylococcus aureus strains.

Conception rates and litter size The conception rate and mean litter size was significantly lower in those gilts harboring hemolytic staphylococci in the vagina prior to mating. A comparison of conception rates and mean litter size can be found in Table 7. Stillborn pigs were included when determining litter size. A total of 150 animals were sampled as described previously. Ninety-one samples were positive to hemolytic staphylococci at mating. Conception occurred in 56 of the 91 positive gilts while 35 infected gilts failed to conceive. Fifty-nine gilts were negative to hemolytic staphylococci at service. Of these 59 gilts, 48 became pregnant and 11 failed to conceive at that service. Conception percentages were 61.5 and 81.4 respectively for infected and non-infected gilts.

Application of the chi-square test for contingency indicated a signi-

Table 7. Summary of conception rates and litter size

	Pregnant	Non Pregnant	Per Cent Conception	Mean Litter Size
Hemolytic staphylococci infected gilts	56	35	61.5	7.75[a]
Hemolytic staphylococci free gilts	48	11	81.4	9.17[b]

[a]Sample standard deviation = 3.16.

[b]Sample standard deviation = 3.24.

ficant difference ($P<.05$) between conception in the staphylococci infected and non-infected gilts. Computation yielded $X^2=6.611$. The mean litter size was significantly lower ($P<.05$) in the staphylococci infected versus the non-infected gilts. The mean litter sizes included those pigs stillborn. Infected gilts had a mean litter size of 7.75 while non-infected gilts averaged 9.17 pigs per litter.

No statistical difference between litter size or conception rate was evident when other bacterial isolates were considered. However, those gilts yielding Escherichia coli on pre-breeding samples exhibited signs of reduced reproductive performance. Only nine Escherichia coli were isolated from the 150 samples. A total of five gilts farrowed 32 live pigs and eight dead pigs from the nine positive gilts serviced. Abortion occurred in one animal at 34 days gestation and Escherichia coli was recovered following

the abortion.

Infectivity Study

The results of the previously described survey indicate that hemolytic staphylococci significantly reduced reproductive performance in this swine herd. A continued approach to this problem was the direct inoculation of Staphylococcus aureus into the posterior cervix during artificial insemination. The results of this study can be seen in Tables 8, 9, and 10. Forty per cent of the control animals conceived with one service and 31.6 per cent of the inoculated gilts conceived. Statistically this is not a significant difference. Abnormalities of the ovaries and fallopian tubes occurred in both the control and inoculated group. In all cases the abnormalities were bilateral although the severity of the aberrations varied between the two horns. Thirty per cent of the control group exhibited abnormalities of the ovaries or fallopian tubes.

Bilateral cystic salpingitis occurred in gilt number 1097 of the control group. Obstruction of the fallopian tube resulted but this gilt continued to return to heat. A second gilt from the control group, number 1162, exhibited mild bilateral hydrosalpinx. Figure 1 is an illustration of this condition. This abnormality could have interfered with conception, but did not interfere with return to estrus. The third abnormality in the control animals was bilateral luteinized cystic follicles as seen in Figure 2. These appar-

Table 8. Results of infectivity study, control animals lot 1

Gilt No.	Days return to heat	Slaughter Day	Preg.	No. Cl.	No. Embryos	Comments
1097	23	40	-			Cystic Fallop. tubes non patent (Bilateral)
226		33	+	12	7	
1838		34	+	10	5	
1162	22	38	-	-	-	Hydrosalpinx (Bilateral)
1475		38	+	11	7	
1354	21	37	-	-	-	A few luteinized folocles (Bilateral)
1329	19	35	-	-	-	
1521	-[b]	40	-	-	-	
304		40	+	19	13	
1705	22	42	-	-	-	
1283[a]						

[a]Dropped from control group: Hemolytic staph. at breeding.

[b]Not pregnant, has Cl of last estrus, coming into estrus.

Table 9. Results of infectivity study, lots II and III

Gilt no.	Days return to heat	Slaughter Days	Preg.	No. Cl.[a]	No. Embryos	Comments
1054	20	42	-			
1508	-	35	-			Extended anestrus
252[b]	-	33	-			Cystic ovaries-anestrus
1450	-	35	-			Cystic ovaries-anestrus
1592	20	34	-			Hydrosalpinx and abscess
1056	-	37	+	9	5	
1895	27	39	-			
1858	-	35	+	12	11	
648	-	42	+	10	7	
575	22	38	-			
1098	19	39	-			
1844	-[c]	33	-			
747	-	35	-			Cystic ovaries-anestrus
1327	-	39	-			A few cystic follicles
1424	-	39	-			Anestrus
1525	-	39	+	13	10	
616[b]	-	42	+	10	1	Bilateral pyosalpinx ovarian degeneration and cervical abscesses
1226	-[d]	42	+	8	6 (1 dead)	
1501		41	-			

[a]Number of corpora lutea.

[b]Staphylococci isolated.

[c]12-13 day old corpora lutea.

[d]Coming into heat.

Table 10. Summary of infectivity experiment

	Group I Control	Group II and III Infected
No. of animals	10	19
Abnormalities of genital tract	3	6
No. pregnant	4	6
No. animals returning to heat on normal cycle	5	5
No. animals not pregnant but not observed in heat	1	8
No. animals not pregnant but indicated cycling at slaughter	1	2
No. of animals not pregnant and anestrus at slaughter	0	6

ently developed after gilt number 1354 had returned to heat.

Six gilts or 31.6 per cent of the 19 Staphylococcus aureus inoculated animals had ovarian or fallopian tube abnormalities at slaughter. Figures 3 and 4 demonstrate luteinized cystic follicles in inoculated gilt number 1327. This gilt failed to conceive or return to estrus. Follicular measurements were approximately one cm. in diameter. Bilateral hemorrhagic hydrosalpinx occurred in infected gilt number 1592, but did not alter her return to estrus. Figures 5 and 6 illustrate a right horn hydrosalpinx and a left horn

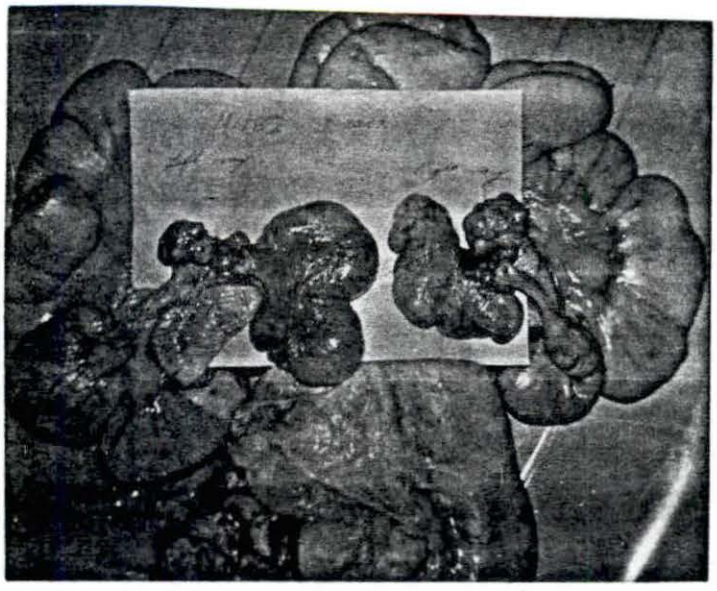

Figure 1. Control gilt No. 1162
Bilateral Hydrosalpinx

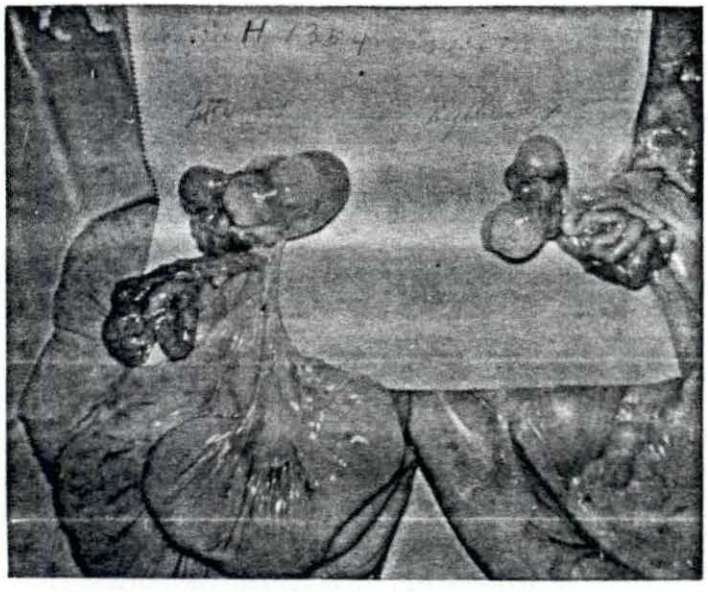

Figure 2. Control gilt No. 1354
luteinized cystic follicles

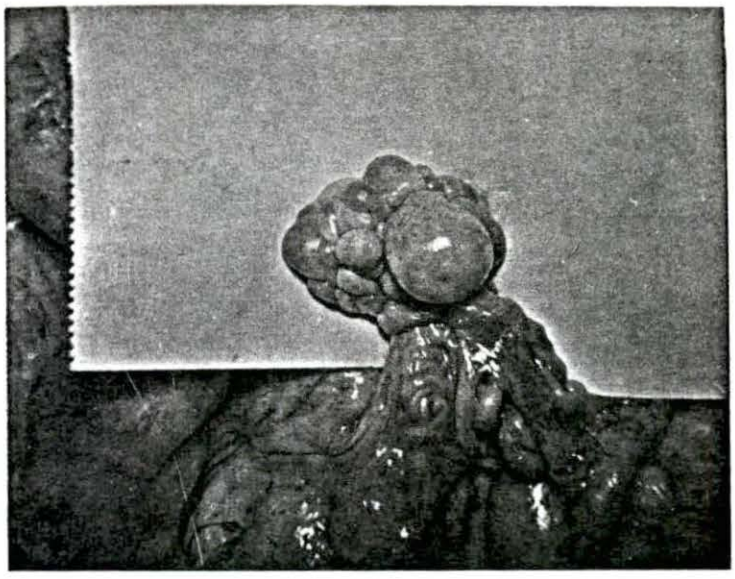

Figure 3. Infected gilt No. 1327
luteinized cystic follicles

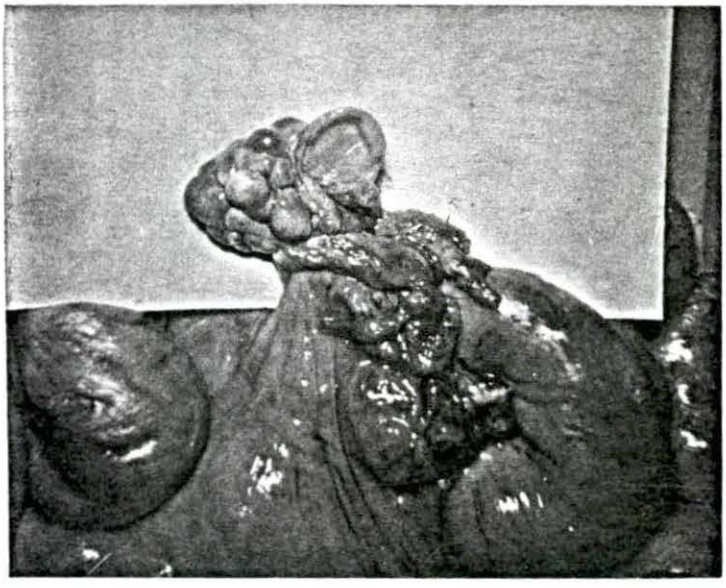

Figure 4. Infected gilt No. 1327
luteinized cystic follicle - opened

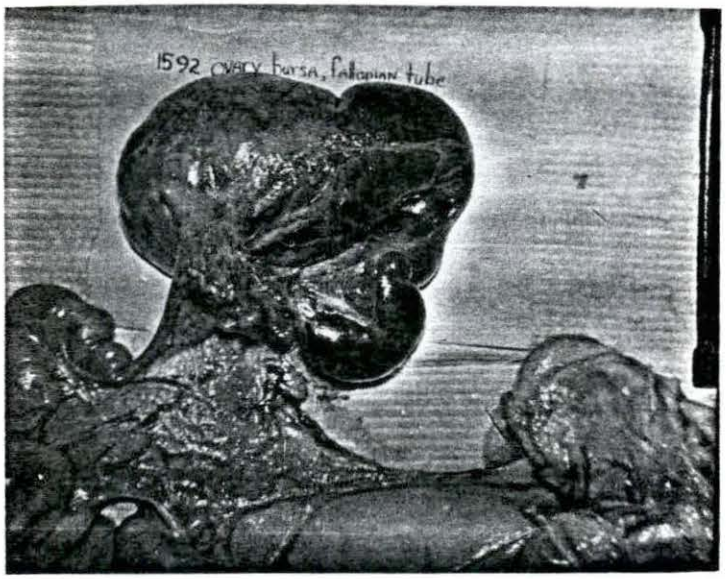

Figure 5. Infected gilt No. 1592
right horn hydrosalpinx

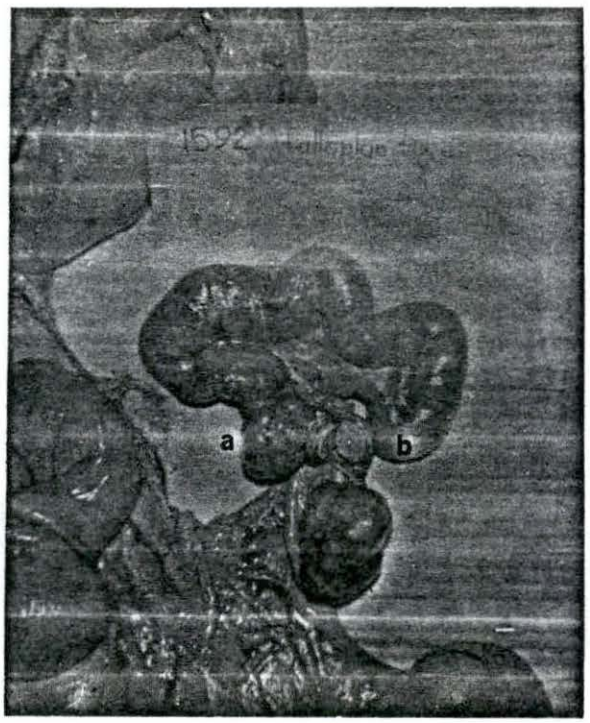

Figure 6. Infected gilt No. 1592
[a]Left horn cystic salpingitis
[b]Caseous abscess

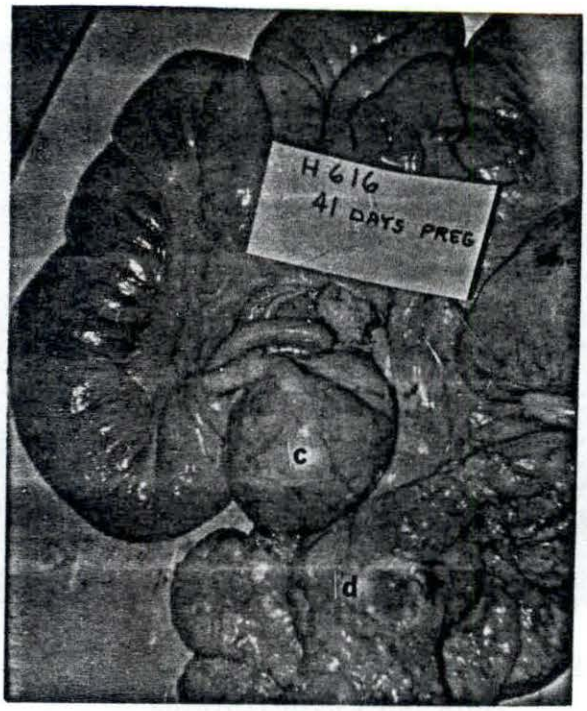

Figure 7. Infected gilts No. 616
[c]Left horn pyosalpinx
[d]Cervical abscess

Figure 8. Infected gilt No. 616
[e]Right horn pyosalpinx
[f]Fetal resorption

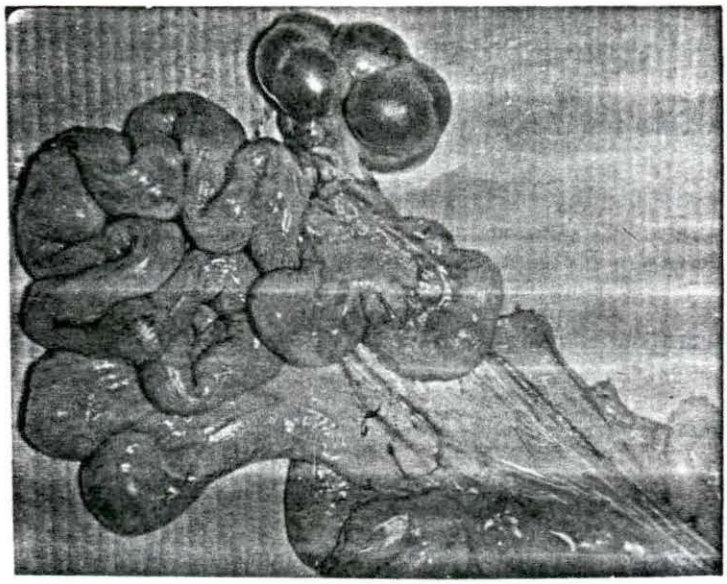

Figure 9. Infected gilt No. 747
cystic follicles

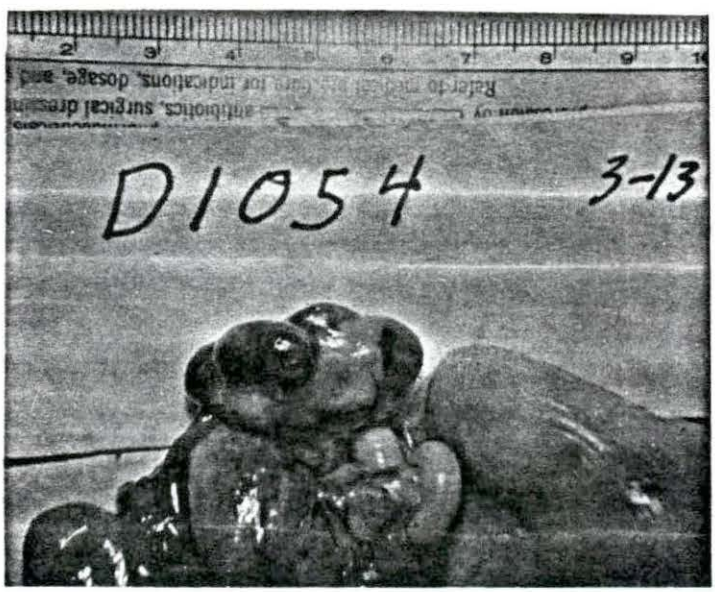

Figure 10. Infected gilt No. 1054
corpora lutea 5 days after estrus

hydrosalpinx with cystic salpingitis and a caseous abscess. Bilateral pyosalpinx with ovarian degeneration and a cervical abscess occurred in infected gilt number 616. Figures 7 and 8 illustrate these aberrations. It is interesting to note that pregnancy existed at slaughter although only one live embryo was present and fetal resorption was evident. Three Staphylococcus aureus inoculated gilts, number 1508, 252, and 747, developed bilateral cystic ovaries and failed to recycle after insemination. Figure 9 is an example of the gross appearance of these ovaries. Figure 10 shows corpora lutea on the ovary of infected gilt number 1054, five days post estrus.

The composite of reproductive performance by control and inoculated gilts is recorded in Table 10. Close observation after insemination revealed that five control animals and five infected gilts returned to estrus. At slaughter one additional control animal and two additional infected gilts were approaching estrus or were found to have cycled since insemination. Figure 10 illustrates corpora lutea of the ovary, five days post estrus.

An obvious difference between control and inoculated gilts was the per cent of nonpregnant animals that returned to estrus after insemination. Reports of observed estrus, coupled with autopsy findings indicated that 100 per cent of the nonpregnant inoculated gilts failed to show evidence of estrus. Numbers were insufficient for statistical analysis of this phenomena.

Final analysis shows that Staphylococcus aureus inoculated gilts had a slightly lower conception rate. However, the live embryo to corpora lutea ratio was similar to that recorded for control animals.

It should be noted that Staphylococcus aureus was isolated only twice from uteri collected at slaughter. Both isolates were from previously infected gilts. Corynebacterium pyogenes was recovered from gilt number 252 which also yielded Staphylococcus aureus. No other bacteria were recovered from the 29 uteri examined.

DISCUSSION

The microorganisms isolated in this study can be found in Tables 4, 5, and 6. When classified by genera, this list closely resembles those compiled in similar surveys of women, cows, mares, and laboratory animals.

This survey was confined to one herd in an attempt to limit the variables such as housing, rations, and specific disease problems. It was observed that wet lots or dry, dusty, and closely chopped bedding tended to cause contamination of the vagina with soil and bits of straw. Bacillus spp. was the common isolate under those circumstances. Frequent bedding with clean, long stemmed straw minimizes this type of contamination. Virgin gilts were sampled during estrus for two reasons. First, samples from virgin gilts would not be influenced by bacterial contamination associated with mating or parturition. Secondly, the period of estrus served to synchronize sample collection with a known period of the estrus cycle.

Conception rates and litter sizes were recorded for the gilts sampled to determine if relationships existed between specific bacterial isolates and reproductive performance. Such a relationship was evident with hemolytic staphylococci. Conception rates were approximately 20 per cent lower in gilts harboring hemolytic staphylococci. Applying the chi-square test for contingency, conception rates were found to be significantly reduced ($P<.05$). Similarly an average reduction of 1.4 pigs per litter was recorded for gilts harboring hemolytic staphylococci at breeding. The "Student's"

t test showed the average litter size was significantly reduced ($P<.05$) when this organism was isolated.

This does not conclusively indict hemolytic staphylococci as the direct cause of lowered fertility. Hemolytic staphylococci may have been an indicator of less than optimum health in the female tract. The survival of staphylococci within the less resistant tracts may depend upon multiplication and increased virulence, while in healthy tracts staphylococci may have been excluded or depressed. In the laboratory several isolates of this hemolytic staphylococci became more virulent when passed in serum broth or chicken embryos. Increased hemolysis and coagulase production were noted after these passages.

Other isolates were subjected to the same analysis. These either proved to be independent of reproductive performance or they occurred in such small numbers that analysis was not meaningful. Isolates of Escherichia coli appeared to belong to the latter category. Escherichia coli was isolated nine times, and of these nine, six gilts conceived while harboring this organism. Five gilts farrowed 32 live pigs and the other gilt aborted at 38 days pregnancy.

Further tests indicated the hemolytic staphylococci were probably Staphylococcus aureus strains of low virulence. Phage typing yielded negative results. Phages used were active against human and bovine strains, but may have lacked specificity for swine strains.

When representative strains of Staphylococcus aureus were inoculated into gilts following insemination, a slightly lower conception rate resulted. This procedure appeared to seriously impair the future fertility of several gilts. Some difference in performance was observed between control and inoculated animals, but lack of sufficient numbers in either group made statistical analysis meaningless. Conception rates were 40 per cent and 31.6 per cent for control and inoculated gilts respectively. The low conception rate in control animals may have been related to two factors. First, the inexperience of the inseminator may have affected conception rates. Secondly, the control inoculum consisted of eight day old chicken embryo egg yolk. The immune or physiochemical response to metabolites and foreign proteins in the egg yolk may have reduced conception. In the bovine the intrauterine use of certain antibiotics, in large volumes for uterine infections, tended to increase conception intervals and services per conception. A good example of this is reported by Gibbons et al. (1959). The mere presence of a sufficient volume of foreign material appears to alter the normal physiology of the uterus.

The causes of aberrations of the ovaries and fallopian tubes is not known. These abnormalities occur in approximately 50 per cent of the cases of infertility in the gilt. Three abnormalities occurred in the group of 10 control animals. A mild case of luteinized cystic follicles appeared not to affect fertility in one control animal. Bilateral cystic salpingitis and mild

hydrosalpinx occurred in two other control gilts. These two animals probably were infertile, but they continued to cycle. Four inoculated gilts had ovarian abnormalities. Bilateral multiple cystic follicles occurred in three inoculated animals resulting in anestrus. The other gilt had bilateral luteinized cystic follicles, but continued to cycle. Two inoculated gilts had abnormalities of the fallopian tubes. Bilateral pyosalpinx and hydrosalpinx occurred in these two. Future fertility was impaired in each of these animals. The bilateral pyosalpinx with ovarian degeneration occurred in a pregnant inoculated gilt. One live fetus and evidence of fetal resorption were found in this animal. Staphylococci were isolated from one of the fallopian tubes. Apparently conception occurred before an established infection extended up the fallopian tube.

Equally significant is the finding that six of the 13 nonpregnant gilts inoculated after insemination were in anestrus and had not cycled since the insemination. No gross evidence of embryo resorption was detected in these six gilts. Histopathological studies should have been conducted to determine if fetal resorption or endometritis was evident in these animals. None of the six nonpregnant control animals exhibited anestrus.

What effects massive intrauterine doses of bacteria would have on the ovarian activity of swine is not known. However, it is well documented that in the bovine certain hormonal relationships exist between the endometrium and the ovaries. An example in the bovine being the retained corpus

luteum with endometritis. Mild endometritis in swine may have a similar effect. Fennestad et al. (1955) reported that delayed return to heat was noted in Staphylococcus aureus infections in a herd of 18 female swine. The anestrus observed in the six inoculated animals may have been associated with an endometritis.

There undoubtedly exists in the reproductive tract intricate mechanisms to suppress bacterial contamination. Some of the mechanisms of immune response and physiochemical reactions have been studied and are referred to in the literature review of this thesis. The hormonal status of the individual plays a major role in the regulation of these mechanisms. Estrogenic periods enhance and progesterogenic periods usually reduce the effectiveness of these mechanisms. An exception appears to be the mucopolysaccharides found in uterine secretions of the pig and rat. Phagocytosis by leucocytes is impeded by these polysaccharides. Significantly, the rat and pig have relatively high embryo death rates as compared to the bovine or rabbit.

Clearance time studies in laboratory animals indicate that most bacteria are destroyed rapidly in the uterus and cervix during estrus. Escherichia coli and Staphylococcus aureus have been shown to be substantially reduced in the uterus of laboratory animals within four to 24 hours after inoculation. Potential pathogens may have a substantial survival rate to sustain until more favorable environments develop or they may release toxins

which influence the endometria after the bacteria are gone.

Bacteria were recovered from the uteri of two gilts approximately 38 days after inoculation. While several other gilts were apparently still under the influence of the massive inoculum, no bacteria were recovered. This finding is in agreement with the work of Singh (1965). Massive inoculation of bacteria in heifers caused non-conception for two estrus periods but bacteria were not recovered at slaughter. Histopathology showed cellular infiltration of the stratum compactum, but no serious endometritis. Fennestad et al. (1955) and Pounden et al. (1947) reported fertility problems associated with Staphylococcus aureus in boar and bull semen. These reports indicated that while most of the animals bred with infected semen had problems, only a small portion experienced abortion or clinically evident uterine reactions.

The establishment of resident bacteria in the uterus is as much a failure on the part of an individual to limit this uterine and cervical microflora as it is an expression of pathogenicity by the organism. It is almost impossible to transmit uniformly an established uterine infection to the healthy uterus. Uterine infections are not an all or none reaction, but a delicate balance between the uterine defense and the causative agent. Unfortunately the clinical parameter for evaluating uterine infection is often based on the all or none reaction. Either the animal conceives or it does not. This denotes a serious limitation of this research study in that histo-

pathological evaluation of the uteri was not integrated with clinical analysis.

The routine culturing of vaginas, cervices, or uteri from the slaughterhouse are of little value without a clinical and pathological analysis. A bacteriological survey lacking this analysis, even at its best, can only provide a general description of bacterial types and their distribution in health and disease during the survey period. It is impossible to equate disease with the simple presence of bacteria and health with their absence. Such isolates can not be considered solely in terms of pathology. The normal biology or physiology of the tract, in relation to its microflora, is of paramount significance to the understanding of disease, and to the interpretation of clinical and laboratory examinations.

The results of this survey confirm that the distribution of vaginal microflora in swine closely simulates the vaginal microflora of other animal species. Investigations of the more pathogenic Staphylococcus aureus served to demonstrate the proficiency of the uterine defense even in the face of massive inoculation. It is hoped that the results of this survey may contribute some knowledge to such conditions as post parturient metritis, repeat breeder sows, and sporadic cases of swine abortions.

Further research is needed to delineate the role of various organisms and their possible interrelationship in swine infertility problems. The defense mechanisms consisting of an interplay of hormones, local immunity,

phagocytosis, uterine secretions, and possibly other physiochemical reactions need to be considered in future infectivity studies.

SUMMARY

A survey of the vaginal flora of gilts in estrus was conducted by culturing 150 pre-breeding samples. The most significant organism, Staphylococcus aureus, was further investigated in an infectivity study involving 29 gilts. Survey findings indicate that significant infertility of gilts in this herd is associated with hemolytic staphylococci. Less conclusive evidence was advanced by the infectivity study to determine the effect on fertility of massive, intrauterine doses of Staphylococcus aureus following insemination.

The main genera isolated from this herd were staphylococci, micrococci, bacilli, streptococci, and escherichia. This list confirms that the vaginal microflora in swine closely simulates the vaginal microflora of other species. Gilts harboring hemolytic staphylococci had a significantly lower ($P < .05$) conception rate and mean litter size as compared to those gilts not harboring the organism.

These hemolytic staphylococci appeared to be primarily Staphylococcus aureus. However, considerable difference in virulence was noted between strains. None of 32 selected isolates were phage typeable. Virulence, as indicated by hemolysis, coagulase production, and mannitol fermentation was increased with chicken embryo passage.

Failure to return to heat following massive inoculation with Staphylococcus aureus was the most significant finding of the infectivity study.

Six of the 13 nonpregnant, inoculated gilts were anestrus at approximately 40 days after insemination. Bilateral pyosalpinx and fetal resorption were evident in another inoculated gilt. No definite relationship could be established between inoculation and the incidence of cystic ovaries or hydrosalpinx. Two control gilts had fallopian tube involvements that may have impaired fertility. One abnormality was bilateral cystic salpingitis and the other was bilateral hydrosalpinx. Conception rates were 40 per cent in the control gilts and 31.6 per cent in the inoculated gilts. Reaction to the control egg yolk inoculum and inexperience with insemination were considered possible causes of low conception rates in the control gilts. The embryo to corpora lutea ratio differed only slightly among the control and infected gilts. Other bacteria failed to show this relationship with fertility or occurred in such low frequency that analysis was meaningless.

Not enough information was accumulated to definitely state that Staphylococcus aureus is a significant cause of infertility in swine, but present evidence tends to suggest this. Another possibility is that the presence of hemolytic staphylococci in the anterior vagina during estrus may be an indicator of less than optimum body defense.

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