THE RELATIONSHIP OF ESCHERICHIA COLI TO THE METRITIS-MASTITIS-AGALACTIA SYNDROME IN SWINE

by

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TABLE OF CONTENTS

INTRODUCTION

Post-parturient disorders with early lactation failure in the sow have become increasingly important in recent years in those areas where intensification of swine operations has occurred. Diseases of this type have been reported in all the intense pork producing countries of the world. Ringarp (1960) states that in Sweden these disorders are regarded as one of the greatest and most difficult problems in pig practice and are a major economic problem in the pig industry. Loveday (1964) reported that in South Africa "the occurrence of this disaster has so increased in recent years with the intensification of pig production methods that it today constitutes one of the most serious problems facing both clinician and producer". In the United States these conditions are found nationwide and stand as one of the major factors limiting increased swine production.

Owing to the complexity of the disease and the various manifestations in any one case, terminology has become quite confused. The terms agalactia or MMA complex (mastitis, metritis, agalactia), which are now popular in the lay press, have come to include any condition resulting in a cessation or decrease in milk flow. These results can be the consequence of a number of factors ranging from starvation to various infectious diseases. It therefore becomes necessary to further delineate the specific condition to be dealt with in this work.

The condition considered in this work is a specific disturbance that occurs at or soon after parturition but does not occur outside the puerperium. In this respect, it is interesting to study the classification of Ringarp (1960). On the basis of clinical examination of 1,180 cases of

agalactia in sows, he classified the various cases into the following five main groups:

Group 1: Cases of eclampsia (0.59%). Eclampsia usually occurs just before or during farrowing. The sow has difficulty getting up, staggers, and makes vigorous chewing movements which result in froth in the mouth. The sow may sit on her haunches and pant, fighting for every breath.

Group 2: Cases which are thought to be connected with deficient release or disturbance of the neuro-hormonal regulation of the milk ejection reflex (3.30%). These cases occur primarily in first litter gilts and result in complete agalactia. The sow farrows normally and appears healthy and well-developed.

Group 3: Cases showing definite hypoplasia of the udder. Milk production is insufficient because of deficient development of the glandular tissue (1.53%).

Group 4: Cases of hormonal or primary agalactia (6.02%). These show reduced milk secretion as the only outstanding symptom and do not fit into Groups 2 or 3. Ringarp states that many of these will turn into the type in Group 5 if not treated in an early stage.

Group 5: Cases of agalactia toxemia (88.56%). Ringarp describes the characteristic clinical features of these agalactia cases as follows: The sow appears to be healthy and suckles her piglets for 12-48 hours after farrowing. After that, milk secretion decreases. The sow begins to evince signs of sluggishness and weariness, reduced or vanished appetite, sustained or intermittent fever in some cases and in others subnormal temperature, often swelling of the udder which usually starts in the

hind sections and proceeds forward, occasionally increased lochial discharge which may be of abnormal appearance, coprostasis, stiffness and a tense or staggering gait. White or red marks may appear on the skin. On some occasions mental disturbances expressed as dazed, irritated, or aggressive moods may be noted.

In some cases of agalactia, the sow's maternal instincts are reduced and she shows little or no interest in the piglets. A common clinical picture is the sow reclining on the hot, swollen udder while the pigs weakly circle and root at her in an attempt to obtain milk. The litter rapidly undergoes starvation and dehydration which result in hypoglycemia and death. Often diarrhea will occur in the debilitated pigs, accelerating death. It can readily be seen that early detection and rapid response of the sow to treatments are necessary to avoid severe economic losses due to death of most or all of the litter. The condition appears to be infective and often will strike all subsequent farrowings after its initial appearance.

The clinical syndrome just described appears to be the same as that described repeatedly in the literature under various names such as enzootic metritis, puerperal septicemia, post-parturient fever, mastitis, metritis, agalactia, MMA complex, and puerperal fever. It is this condition, consisting of those cases falling into Group 5 of Ringarp's classification as well as many of those in Group 4, with which this study is concerned.

Much needs to be known about the etiology of this condition and how the disease may be influenced by management before effective prophylactic

or therapeutic programs can be developed. At present, there are no effective preventive methods. Treatment gives variable results with at best an unsatisfactory outcome due to the expense of treatment, loss of baby pigs, and slow growth of remaining pigs.

The present research was undertaken to identify and clarify etiologic factors with the expectation that such information would provide a base for development of effective control measures, either within the framework of this study or in future research.

LITERATURE REVIEW

A survey of the literature on post-parturient disorders associated with agalactia in sows reveals that there has been little research done on this problem, the one exception being the extensive and well-documented work reported by Ringarp (1960) of Sweden. There have been numerous articles published on the subject but most deal with empirical treatment of the conditions and clinical observations of the affected herds. Many speculations are advanced as to the causes of the conditions but little data is presented to support the conclusions.

There are scattered reports in the early literature of early lactational failure in sows but most of the cases described lack the severity and herd involvement cited in reports after the 1950's.

Etiology

Speculations as to the primary causes of post-parturient agalactia are many and varied.

Lynch (1914) states that a deficiency of milk is almost always traceable to too much dry feed and not enough of the soft foods, slops, and green feeds. Many others have incriminated nutritional factors as the cause. Baker (1934) felt that bacterial toxins contained in the feed led to endocrine disorders in the sow. Dykstra (1955) and Densmore (1965) both list as one of the causes of agalactia the improper feeding of the gestating sow. Loosmore and Harding (1961) and Loveday (1964) all describe instances where the inclusion of aflatoxin in the

sow's ration led to inappetance and agalactia in the sow.

Many writers reported that the syndrome is related to a lack of exercise and overfeeding of the sow prior to farrowing resulting in constipation and autointoxication (Tharp and Amstutz 1958, Ringarp 1960, Loveday 1964, Densmore 1965).

Numerous bacteriological examinations have been carried out on agalactia cases and there is much support for the theory that an infectious agent may be the primary cause of the syndrome. Adler (1951), Langham and Stockton (1953), and Helmboldt et al. (1953) isolated Aerobacter aerogenes from cases of mastitis in sows. Hogg (1952) isolated a beta-hemolytic streptococcus and suggested the possibility of venereal transfer. Blood (1957), in two samples from the uterus of cases of "enzootic metritis", obtained growths of staphylococci, streptococci, and coliform bacteria as did Brooksbank (1958) from many samples of milk from sows with mastitis. Day (1961) and Densmore (1965) described two distinct types of infectious agalactia. The first was caused by a C. pyogenes infection of the mammary gland and uterus and was characterized by a thick, creamy, purulent vaginal discharge, body temperature of 103-106°F., and a uniform induration of the udder. The second was due to an Escherichia coli gastro-intestinal toxemia characterized by a slight temperature elevation, little or no udder congestion and a small amount of clear or flocculent vaginal discharge. Smith (1965) incriminated E. coli and Salmonella sp. as causes of agalactia in the sow.

Recently, Moore et al. (1966), claim to have isolated a Mycoplasma from four cases of metritis in sows.

In addition numerous other workers have isolated Escherichia coli from cases of mastitis or metritis. Jackson (1952) isolated overwhelming numbers of E. coli from uterine discharges, intestinal contents, and blood smears from the heart of sows with post-parturient fever and agalactia. Hebeler (1954) found mastitis "invariably" associated with Bact. coli infection and also demonstrated the organism in the uterine discharges. Sumner (1957) consistently found Bact. coli infection in mastitis cases. Murphy and Ryan (1958) isolated coliform bacteria in pure or mixed cultures from vaginal swabs taken from cases of post-parturient fever.

Snoeck (1959), examining cases of puerperal septicemia in sows, reported that he recovered E. coli from the uterus of such cases three days after farrowing but not after 6, 14, or 30 days. Geurden et al. (1960) concluded that sepsis was provoked by infection, mainly E. coli, developing soon after farrowing, possibly by organisms of intestinal origin.

Ringarp (1960), culturing 127 tampon samples from the uterus and 167 milk samples from sows with "agalactia toxaemica", found Escherichia coli or coliform in 94 of the uterine samples and 124 of the milk samples.

Switzer (1965) believed that most cases were produced by bacterial infection, primarily coliform and possibly hemolytic Streptococcus sp. He also described Klebsiella mastitis characterized by an acute, localized gangrenous mastitis that is highly fatal and does not respond to treatment.

Schalm et al. (1964) described experimental coliform mastitis in the cow. Following inoculation, signs of toxemia consisting of fever, increased heart rate, muscular tremors, depression, anorexia, and swelling of the gland were noted. Remission of symptoms occurred rapidly and the

gland was usually cleared of the organism in 4 to 9 days.

These same workers in a later paper (Carroll et al. 1964) produced evidence that systemic signs of toxemia were referrable to intramammary lysis of organisms by inflammatory cells followed by release and absorption of endotoxin.

Baier et al. (1954) did not consider E. coli mastitis to be a purely local disorder. They suggest that a disturbed intestinal microflora may lead to septicemia and, under circumstances favoring the udder as a predilection site for infection, colimastitis. They were able to produce acute coli mastitis in the cow by subcutaneous injection of E. coli endotoxin. These cases yielded live coli organisms in the milk and blood.

Loveday (1964) attempts to explain the diversity of etiologic factors by suggesting that immediately following parturition the sow is precariously balanced between normality and disease. Moderate stress in the form of nutritional deficiencies, constipation, adverse environment, or other stresses might then provoke an endocrine disturbance and sufficient infection and/or intoxication to cause lactational failure. He further suggests that adrenal exhaustion due to excessive stress may result in increased invasion of the circulation by bacteria and toxins from the intestine.

Anderson and Brunson (1959) demonstrated that acute stress increased the susceptibility of rabbits to intravenous endotoxin.

Ringarp's work (1960) tends to substantiate this concept of a delicately balanced animal subject to disease should stress factors arise. He was able to produce typical symptoms in a variety of ways immediately

following parturition. These included:

1. Drastic dietary changes the last week of gestation.
2. Feeding poor quality fodder the last week of gestation.
3. Oral administration of an anti-peristaltic agent (opium) during the last week of gestation to produce constipation.
4. Feeding large quantities of skim milk the last week of gestation.
5. Oral administration of methylthiouracil during the last month of gestation to induce hypothyroidism.
6. Induction of a relative adrenal-cortical insufficiency through cessation of a long course of treatment with massive doses of corticosteroids. In a similar experiment Moll (1956a,b) demonstrated in experimental laboratory animals that susceptibility to E. coli endotoxin per os and bacterial invasion of the blood stream from the alimentary tract both increased during periods of adrenal suppression.

These same procedures failed to produce any symptoms when carried out on sows 5-12 days after farrowing.

Histological Changes

Reports of histological changes present in post-parturient agalactia are very limited and assessment is made doubly difficult because the histological condition of the reproductive tract in the normal post-parturient sow has not been studied adequately. Palmer et al. (1965a,b) studied the reproductive organs from 40 lactating sows. They reported that many of the larger follicles on the ovary were atretic during early lactation. The corpora lutea degenerated rapidly after parturition.

Uterine epithelium appeared to be degenerate during the immediate post partum period and underwent regeneration which began at 7 days post partum and was complete by the 21st day. The stratified squamous vaginal epithelium was five to eight cells thick 1 day post partum and decreased to two or three layers by 14 days post partum, then increased to 12-15 layers at 4 days postweaning.

Ringarp (1960) carried out histological examinations of uterine biopsy samples from nine clinically healthy sows and from 46 spontaneous cases of agalactia toxemia. In the clinically normal sows the findings ranged from nothing remarkable (3 cases) to an extensive edema and moderate hyperemia in one case. The endometrial epithelial cells appeared to be flat. Examination of the 46 cases of agalactic sows revealed lesions ranging from mild to extensive edema, severe hyperemia, and submucous hemorrhages. Four cases showed an acute endometritis with hyperemia and moderate leuko-lymphocytic infiltration in the submucous edema. In six cases a distinct focal necrotic endometritis was observed with leucocytic infiltration. In 19 of the cases the epithelial cells appeared to be taller, more "swollen" than in normal cases, and had vacuolization.

Histological examinations were also carried out on 29 samples taken from the udder tissue. His findings indicated that in those cases in which udder swelling and/or an increased pH of the milk was observed, pathologic changes could be demonstrated. These varied in character from mild catarrhal-purulent to extreme purulent-necrotic mastitis and edema and dilatation of the alveoli.

Liver tissues were examined in 14 spontaneous cases. In five, no

changes were observed. In the other nine cases regressive changes were observed consisting of vacuolization of the liver cells, especially centrolobular, and centrolobular reaction-free necrosis. The nuclei of the liver cells displayed pyknosis, karyorrhexis, and karyolysis. In some cases a fine droplet fatty change was seen in the Küpffer cells.

Two mild cases showed no kidney changes. Two severe, febrile cases showed fine-droplet fatty change in the tubular epithelium. No changes were demonstrated in the pituitary. The adrenals showed a small to moderate amount of lipid in the zona reticularis (3 of 4 cases).

Cross et al. (1958) studied the histological changes in the mammary gland of normal and agalactic sows. They reported that before farrowing the alveoli were small and filled with a hyaline, eosinophilic secretion which changed to basophilic at four days prepartum. In the agalactic sow, the alveoli contained no milk secretion but were packed with ghost cells and polymorphonuclear leucocytes. Biopsies collected on the second day post-farrowing in sows experiencing typical symptoms, revealed severe inflammatory changes with the alveoli packed with polymorphs and ghost cells.

Martin et al. (1967) reported an apparent increase in atretic follicles in agalactic sows over control sows. They also reported degenerative changes in the adrenal and pituitary glands as opposed to the findings of Ringarp (1960).

Treatment

Many reports have been published in the literature relative to the successful treatment of agalactia. Most are based on clinical experience and are in close agreement as to the most successful course of treatment.

The common denominator in almost all suggested treatments is the injection of antibacterial agents and oxytocin. Callaway (1960), Tharp and Amstutz (1958), and Ringarp (1960) also advise the use of oral or injectable laxatives to promote evacuation of the digestive tract.

The use of preparations that provide adrenal support has been suggested (Felgate 1960, Noble et al., 1960, Kalvelage 1963). Ringarp (1960) reported that the standard treatment plus 100 mg. prednisolone lowered the three week mortality to 23.0% as compared to 30.6% on standard treatment only. Ludvigsen (1961) demonstrated that administration of ACTH in the terminal days of pregnancy markedly decreased the incidence of mastitis, metritis, and agalactia. Rattner (1957), Altemeier and Cole (1958), and Thomas (1958), also pointed out the value of providing adrenal support in the management of E. coli endotoxemia.

Care of the piglets in all cases of agalactia is also stressed. Due to the rapidity with which hypoglycemia develops if the piglets do not receive milk, they must be supplied with glucose, either orally or intraperitoneally, and kept warm and dry.

Prophylaxis

Several different approaches have been used in the prevention of agalactia. These have yielded variable results. Efforts have been directed at reducing the predisposing stress factors. There are numerous reports that overfeeding the gestating sow, resulting in overly fat animals at parturition, contributes to the incidence and severity of the condition. Allen and Lasley (1960) reported that gilts with the thickest backfat pro-

duced the least amount of milk.

Sumner (1957) suggested a drastic reduction in feed 14 days prepartum. Ringarp (1960) also recommended reduction in feed the last 8 to 10 days of gestation and avoidance of drastic changes in the diet. Addition of laxative agents such as wheat bran or Glauber's salts is suggested if there are signs of coprostasis.

Partial sterilization of the digestive tract with antibacterial agents is recommended by some. Switzer (1965) recommended a suitable drug, especially nitrofurans, in the feed prepartum or neomycin injections in the sow just prior to farrowing, both measures designed to reduce the coliform flora of the digestive tract and the probability of bacteremia.

Ringarp (1960) reported that the oral administration of streptomycin or streptomycin plus laxatives to the sows four days before the expected farrowing date reduced the incidence of agalactia toxemia from 48 out of 216 in the controls to 7 out of 216 in the treated animals. Davis (1965) used erythromycin injections and nitrofurazone in the water and reported a dramatic reduction in the number of post-parturient disorders and a clearing of hemolytic streptococcus and E. coli from the vagina. Ludvigsen (1961) reported that 30-100 units of ACTH on the 110th day of gestation decreased parturition time by 25% and also decreased the incidence of still births, metritis, mastitis, and agalactia.

The use of biologicals in the prevention of agalactia has been reported and the use of E. coli antiserum apparently offers the most dramatic prophylactic measure. Sumner (1957) reported that 50 cc. of "Aggrecolin",[1]

[1]Bayer Products, Ltd., London, England.

a polyvalent E. coli antibacterial serum prepared from horses, administered 5 days prepartum, completely stopped the occurrence of agalactia. Murphy and Ryan (1958) gave 70 cc. E. coli antiserum one day before farrowing and 70 cc. at the time of farrowing to sows in a problem herd and reported no new cases. Ringarp (1960) treated half of 184 sows with 75-100 cc. of polyvalent coliserum obtained from horses. These injections given intramuscularly five days before expected farrowing reduced the incidence from 18 of 92 in the controls to 0 of 92 in the treated sows.

Smith (1965) and others have reported the successful use of commercial and/or autogenous bacterins. Sumner (1957) and Murphy and Ryan (1958) however, reported that bacterins gave unsatisfactory results in those herds successfully treated with antiserum. Other reports are also conflicting as to the effectiveness of this method of prophylaxis.

Kerr (1955) demonstrated that a local resistance might be elicited due to locally produced antibodies. He found high vaginal and uterine antibody titres following the instillation of dead Brucella abortus antigen. These antibodies did not pass into the circulation. Pierce (1959) also pointed out the error in assuming a distribution of antibody between the plasma and mucous surfaces and considered muco-antibodies of primary importance in the body's defense against characteristic types of infection which are non-invasive and confined at a mucous surface. He suggested that orthodox immunization procedures stimulating humoral antibody may not confer a local immunity.

Outterridge et al. (1965) presented results suggesting that much of the antibody in milk was locally produced. Carroll et al. (1964); however,

could find no evidence of an antibody response or tolerance following intramammary inoculations of endotoxins and attributed the glands' transitory resistance to be due to inflammatory exudate.

MATERIALS AND METHODS

Experimental Animals

Two experimental trials were conducted. In the first trial the animals used were ten first litter gilts of Yorkshire breeding. These gilts were purchased at a farm sale approximately one month before expected farrowing. They appeared to be in excellent condition and were not overly fat.

The animals used in the second trial were purchased at a livestock sales barn three days before the first animal farrowed. They consisted of eight Yorkshire first litter gilts. These gilts were excessively fat, even to the point that some had difficulty in moving.

Experimental Design

On the basis of personal observations and cultures taken from many cases of metritis, vaginitis, and mastitis, and many reports in the literature incriminating Escherichia coli as an etiologic factor, it was decided to first attempt to reproduce the typical symptoms by inoculation of the parturient gilts with a culture of E. coli isolated from a herd where naturally occurring cases of postparturient agalactia were currently a problem. Two trials were carried out as follows:

Trial I: Trial I was carried out in January, February, and March of 1966. The gilts were housed and fed in a single room 40' X 30' which was partitioned into individual 5' X 12' pens along each side. The gilts were penned two or three to a pen until close to farrowing at which time

they were placed in individual pens. They were turned out twice a day for feeding and watering and were fed approximately 1½ pounds of a gestation ration at each feeding. After farrowing they were fed and watered in their pens and were given 4 to 5 pounds of feed at each feeding. The composition of the gestation and lactation rations used is given in Table 1. No attempt was made at isolation within the group but no outside swine came in contact with them.

Five of the gilts were due to farrow 2-3 weeks later than the other five. Two of the early and three of the later gilts were designated as uninoculated controls with the intention that should the disease become established in the early inoculated gilts the three later controls would serve as contact controls to give an indication of the infectiousness of the organism. However, should the early inoculates fail to develop disease, the contact controls would be inoculated with vaginal discharges from active field cases to determine if some other unidentified agent present in the discharge could reproduce the typical syndrome.

The experimental animals to be inoculated consisted of three early (numbers 2, 6, and 8) and two late (numbers 5 and 9) farrowing gilts. These gilts were inoculated with a viable culture of Escherichia coli. Number 2 was inoculated immediately following farrowing, number 6 about ten hours post-parturient, number 8 twenty-four hours pre-parturient, and 5 and 9 approximately three weeks pre-parturient.

The culture used was obtained in the following manner. A vaginal swab was taken from a sow two days prepartum. This sow was in a private herd which had been experiencing problems with metritis, vaginitis, and

Table 1. Gilt rations used in Trial I

Ingredients (lbs/ton)	Gestation	Lactation
Ground no. 2 yellow corn	1517	1417
Soybean meal (44% protein)	400	400
Dried beet pulp	---	100
Calcium carbonate (38% Ca)	10	10
Dicalcium phosphate (26% Ca, 18% P)	45	45
Iodized salt	10	10
Trace mineral premix[a]	3	3
Vitamin premix[b]	15	15

[a]Trace mineral premix furnishing the following trace elements and amounts:

iron	90-100 PPM
copper	6-8 PPM
manganese	60-90 PPM
zinc	60-100 PPM
cobalt	1-3 PPM.

[b]Vitamin premix ingredients - per 15 pounds of premix:

Vitamin A	4.5 million I.U.
Vitamin D	1.2 million I.U.
Riboflavin	6.0 grams
d-Pantothenic acid	12.0 grams
Niacin	27.0 grams
Vitamin B_{12}	30.0 milligrams
Carrier	--

hypogalactia in those sows which had previously farrowed. Culture of the swab on blood agar yielded numerous E. coli, alpha-hemolytic streptococci, and a few Proteus spp. A colony of E. coli was picked, transferred to tryptose broth[1] and incubated 18 hours. This broth culture was checked for purity, inoculated into differential media, and used for the inocula-

[1]Difco Laboratories, Inc., Detroit, Michigan.

tion of the experimental sows in Group 1. Differential media revealed the following characteristics for the organism; formation of acid and gas from dextrose, slow fermentation of both lactose and sucrose, Simmon's citrate negative, indole positive, urea negative, H_2S negative, gram negative, non-motile rod. This organism biochemically resembled an Escherichia coli previously isolated from the vaginal discharges of two sows in the herd. The dextrose culture was lyophilized for future inoculations approximately 30 hours after inoculation.

Inoculation of the gilts was accomplished through use of cotton-tipped applicators and plastic inseminating pipettes. The wooden stick of the applicator was inserted about 1½ inches into the lumen of the pipette. This portion of the pipette was then heated slightly over a Bunsen burner until a slight twisting motion could draw the plastic around the wooden stick, thus securing it firmly in the pipette when cooled. This was then wrapped and autoclaved. At the time of inoculation, the vulva of the gilt was cleansed with cotton saturated with 70 percent isopropyl alcohol and the labia of the vulva spread apart by digital traction. The applicator was then dipped in the broth culture and inserted into the vagina of the gilt, care being taken to avoid touching the lips of the vulva so that insertion began approximately two inches internally. Insertion was made as deeply as possible, usually approaching the cervix. Vaginal swabs were taken at the beginning of the experimental period. In addition, vaginal swabs were taken daily on all post-parturient gilts until all symptoms had disappeared and E. coli was no longer isolated. Swabs were also taken following inoculation to determine if the organism had established in the vagina.

Swabs from the gilts in Trial I were cultured on blood agar plates. As a result of personal communication with Rhodes Scherer of the National Animal Disease Laboratory and publication of his paper on the use of Tergitol-7 media (Scherer 1966), this additional media was used on all subsequent swabs taken beginning with Trial II. An attempt was also made to establish the colonial morphology of some of the isolates from Trial I which had been saved back on agar slants. Scherer's classification in regard to colonial morphology on Tergitol-7 is used in this paper with the simplified designation as rough(R), intermediate(I), and mucoid(M) (Fig. 1,2,3)

Cultures of the swabs taken at the beginning of the experiment disclosed that none of the gilts in Group I harbored an established E. coli flora in the vagina at that time as indicated by recovery of E. coli in two of three consecutive samples.

Trial II: Trial II was carried out in March, April, and May of 1966. The eight gilts were housed in isolation units of the Veterinary Medical Research Institute. Four were in individual units and four were housed two each in two larger units. These animals received 3 pounds of a gestation ration daily before farrowing and up to 10 pounds following farrowing. They were fed and watered in their own units. The formula for the gestation-lactation ration is presented in Table 2.

Cultures of the vaginal swabs taken at the beginning of this trial revealed that three of the eight gilts already harbored an established vaginal flora. Gilt 4N harbored an abundant flora of Proteus vulgaris in the vagina which persisted until her death three days after sampling was initiated. Death was believed to be due to a severe kidney

Table 2. Gilt ration used in Trial II

Ingredients (lbs/ton)	Gestation-lactation
Ground no. 2 yellow corn	1068
Ground oats	500
Soybean meal (44% protein)	290
Dehydrated alfalfa meal (100,000 I.U. Vit. A/lb.-17% protein)	100
Dicalcium phosphate (26% Ca, 18% P)	30
Iodized salt	10
Trace mineral[a]	2
Vitamin premix[b]	2

[a]Trace mineral premix furnishes the following trace elements and amounts:

Iron	60-70 PPM
Copper	4-5 PPM
Manganese	40-60 PPM
Zinc	50-100PPM
Cobalt	1-2 PPM

Vitamin additions per ton of feed:

Vitamin A	1.0 million I.U.
Vitamin D	400,000 I.U.
Riboflavin	4.0 grams
d-Pantothenic acid	8.0 grams
Niacin	18.0 grams
Vitamin B_{12}	20.0 milligrams

and bladder infection of *P. vulgaris*. Sow 16 had an intermediate *E. coli*, and gilt 34 a mucoid *E. coli* as part of their vaginal flora. These two were therefore excluded from the experimental design and held as positive controls. Of the remaining five animals two (numbers 4S and 9S), were designated as negative controls and three, (numbers 9N, 35,

and 36), as inoculates. Gilts 35 and 36 were inoculated one day before farrowing with a reconstituted broth culture of the lyophilized E. coli used in Trial I. Gilt 36 was reinoculated immediately after farrowing and gilt 35 was reinoculated one day after farrowing.

Gilt 9N appeared to be several weeks from farrowing when she was inoculated twice with the broth culture used to inoculate the other gilts. This E. coli failed to become established in the vagina. The inoculum was plated on Tergitol-7 media and was found to contain both M and R forms in approximately equal numbers. An inoculum prepared from a culture isolated from gilt 34 consisting entirely of the M form was then used and readily became established in gilt 9N.

Clinical Examinations

The gilts were observed twice daily during the entire experimental period and any deviations from the normal noted. The degree to which each of the major symptoms was manifested was arbitrarily designated as follows:

Depression:

slight - gilt remained recumbent when attendant entered, not alarmed unless pigs squealed.

moderate - gilt showed no interest in pigs, reluctant to arise.

severe - Gilt got up only if urged and prodded, quickly laid down again.

Anorexia:

slight - Ate some but not all of the feed.

moderate - Nosed in the feed and possibly ate a few mouthfuls.

severe - Showed no interest in the feed.

Swollen udder:

slight - Few glands swollen

moderate - Most of the udder somewhat swollen.

severe - Entire udder swollen, hard.

Vaginal discharge:

slight - Few drops, vulva moist.

moderate - Discharge evident, some dripping on floor.

Copious - Large amounts, coating tail and perineum, dripping on floor.

Hypogalactia:

slight - Pigs appeared hungry, rooting at udder constantly.

moderate - Pigs obviously hungry, some becoming thin and dehydrated.

severe - Pigs all thin and dehydrated, some deaths.

Diarrhea in baby pigs:

mild - One or two pigs scouring.

severe - Most pigs scouring, some dehydrated and thin.

Temperatures were taken once or twice daily during the post-parturient period. Symptoms other than those described above, such as hysteria and muscular tremors, were also noted.

Bacteriological Examinations

Bacteriological examinations were carried out utilizing the facilities of the Department of Veterinary Microbiology and the Veterinary Medical Research Institute. Samples were cultured on 5% bovine or equine blood agar and Tergitol-7 medium with 2, 3,5-triphenyltetrazolium chloride added.

Collection of samples

Vaginal swabs Vaginal samples were taken after first cleaning the labial surfaces with cotton soaked in 70 percent isopropyl alcohol. Sterile cotton-tipped swabs were then introduced into the vagina through the labia of the vulva which were held apart by digital traction. Care was exercised so that the swabs entered the vagina about 2 inches internally, thereby avoiding external contamination. The swabs were extracted in the same manner and placed in sterile cotton stoppered tubes.

Milk samples Milk samples were obtained by first cleaning the udder with alcohol soaked cotton. Twenty I.U. of oxytocin was then administered intravenously. The first milk expressed was discarded before the sample was collected in a sterile tube and stoppered.

Urine samples Urine samples were obtained in a few instances and were collected in a sterile tube at about the middle of spontaneous urination. This often occurred after the animal had been forced to rise.

Blood samples In two instances blood samples were taken for bacteriological examination. Samples were obtained by puncture of the anterior vena cava using a sterile needle and syringe.

Rectal swabs Rectal swabs were taken in several instances and were obtained by inserting cotton swabs approximately four inches into the rectum.

All samples reached the laboratory and were plated within two hours.

Culture media

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. Twenty-five ml. of citrated equine or bovine blood was added to the cooled agar base and dispensed into plastic petri dishes.

Tergitol-7 agar plates Tergitol-7 Agar[1] was used with 2,3,5-triphenyltetrazolium chloride added at the rate of 40 milligram per liter of medium. The media was dispensed into petri dishes, dried overnight in an incubator, and stored at 5°C.

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

Morphology

Colony characteristics of isolated E. coli on Tergitol-7 were determined with a Bausch and Lomb stereoscopic binocular microscope by use of reflected and transmitted light.

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[1]Difco Laboratories, Inc., Detroit, Michigan.

Serology

Two cultures of E. coli were serologically grouped by Paul Glantz, Department of Veterinary Science, Pennsylvania State University. These samples were isolated from gilt no. 5 in Trial I. One was isolated from the milk and one from the vagina at a time when the gilt was manifesting symptoms of mastitis and vaginitis.

Identification

E. coli organisms isolated were tested according to the schema of Edwards and Ewing (Edwards and Ewing 1962) based on action on dextrose, lactose, sucrose, Simmon's citrate, urea, H_2S, and indole.

Bacteria other than E. coli were grouped in the following broad categories: alpha-hemolytic streptococci, beta-hemolytic streptococci, micrococci, hemolytic micrococci, diphtheroids, Klebsiella spp., Proteus spp., and Pseudomonas spp. No attempt was made to further identify these organisms.

Necropsy and Histological Examinations

Gilt 34 of Trial II was necropsied on the twenty-second day after farrowing. Euthanasia was accomplished by use of an electrical current. Tissues for histological examination were collected immediately after exsanguination from the kidney, lung, liver, ovary, uterus, cervix, vagina, and bladder. The tissues were fixed in 10 per cent phosphate buffered formalin solution, dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin. The sections were cut 8 microns in thickness and stained with Delafield's hematoxylin and ethyl eosin.

RESULTS

Trial I

Clinical observations

The ten gilts used in Trial I were observed for approximately one month before the first animal farrowed. During this time they were clinically normal and in good condition for farrowing. Five farrowed approximately three weeks before the remaining five. Of the first five, gilts 1 and 10 were early contact controls. Gilts 2, 6, and 8 were inoculated and are designated early inoculates. Of the remaining five, gilts 3, 4, and 7 are referred to as late contact controls and gilts 5 and 9 as late inoculates. The clinical features of the syndrome which were observed are illustrated in Graph 1 through Graph 10. The severity of each is depicted as outlined previously. The occurrence of diarrhea in the young pigs is included since scours is often observed associated with field cases of agalactia. The isolation of *E. coli* from vaginal swabs is also included to show its correlation to the occurrence of symptoms. Constipation was not observed in any of the gilts and farrowing was completed within 10 hours in all instances.

Early contact controls - Gilts 1 and 10 No symptoms were observed in the control gilts. Both litters grew normally with no apparent disease to the time of weaning at about six weeks of age. Three deaths in the piglets were due to trauma from the sow. No other deaths occurred.

Late contact controls - Gilts 3, 4, and 7 Symptoms occurred in all three of the late control gilts in varying forms. Gilt 3 farrowed

15 pigs but experienced hysteria during farrowing and killed eight. The gilt was slightly depressed the day after farrowing and muscular tremors were noted. At this time a slight vaginal discharge developed which continued for four days. Forty-eight hours after farrowing hypogalactia was apparent from the poor and weakened condition of the piglets. Two of the remaining seven piglets died of apparent hypoglycemia in the ensuing twenty-four hours. Depression, anorexia, and vaginal discharge from the sow were most evident on the third day postpartum, after which progressive recovery occurred. No udder swelling or congestion was present.

The dominant clinical feature of gilt 4 was a mastitis which developed on the first day following farrowing concurrent with depression and anorexia. By day two postpartum a severe hypogalactia was evident by the actions and condition of the piglets. In the first three days following farrowing six pigs died from apparent hypoglycemia and two more were crushed by the sow. Severity of the symptoms diminished after the third day postpartum and recovery was essentially complete by day five. Supplemental milk was provided the pigs and the sow weaned eight pigs at four weeks of age.

Gilt 7 did not develop a vaginal discharge but did have a hypogalactia of the symptom-free or hormonal type described earlier as falling into Group 4 of Ringarp's classification. Three of the piglets died from apparent hypoglycemia and three others from trauma by the sow. The gilt weaned seven pigs at four weeks.

Early inoculates - Gilts 2, 6, and 8 Symptoms were observed in all the early inoculates. Gilt 2 did not develop symptoms until the third day postpartum. Mild to moderate depression, anorexia, and vaginal discharge were evident from day three postpartum to day five, had subsided on day six, and the sow had evidently recovered by day seven. The piglets showed some signs of hunger on day three but were content the next day and remained healthy to weaning.

Gilt 6 developed a copious grayish-white vaginal discharge accompanied by severe depression and anorexia the day after farrowing. These symptoms did not subside until the fourth day postpartum but recovery was complete by the sixth day. An elevated temperature accompanied the symptoms. The skin of the udder had a mottled appearance on day one but the udder remained pliable. In spite of the severity of symptoms, the litter did not appear to suffer greatly and only two deaths were attributed to hypoglycemia.

A slight vaginal discharge was evident in gilt 8 beginning approximately ten hours after farrowing. This persisted, becoming moderate in amount on the second day and finally subsiding on the sixth day. Other symptoms were not evident until day three postpartum when slight depression, anorexia and udder congestion were noted. The piglets appeared hungry and all were scouring. Death of three of the pigs was attributed to hypoglycemia and diarrhea. Recovery was apparently complete by the sixth day postpartum.

Late inoculates - Gilts 5 and 9 Symptoms appeared in gilt 5 beginning on the third day after farrowing and consisted of a moderate

vaginal discharge, mild depression and anorexia, and slight hypogalactia. By day four postpartum depression, anorexia, and hypogalactia had all increased in severity and severe swelling and hardening of the udder had occurred. Recovery apparently began on day five and was complete by day six postpartum. Five piglet deaths were attributed to hypoglycemia and one to trauma by the sow.

Gilt 9 had a clinical picture which was unique in that she was the only gilt to evidence symptoms before farrowing. Approximately one week prepartum depression and anorexia were observed. This was accompanied by an elevation in temperature that lasted about four days. Recovery was apparently complete and parturition was normal. The only postpartum symptom noted was a mild anorexia on the fourth and fifth day after farrowing. The litter remained healthy and no deaths were considered to be due to hypogalactia.

Bacteriological findings

Escherichia coli E. coli isolations made from vaginal swabs of the Trial I gilts are shown in Graph 1 through Graph 10. The shaded area is merely a reflection of the colony numbers isolated on blood agar plates and as such is not a true indication of the severity of the infection. E. coli was recovered from all gilts except the two early contact controls, 1 and 10. Swabs from these gilts did not yield E. coli, either prepartum or up to eight days postpartum. E. coli was recovered during the postpartum period from all eight of the other gilts. Clearance was apparently accomplished in four to six days in all gilts except gilt 3 in which an isolate was still recovered nine days

after farrowing. Gilts 7 and 9 were the only animals from which an E. coli was isolated before farrowing. Recovery was first made from gilt 7, a late contact control, on February 13, three days after the first inoculations were made in the herd and 19 days before farrowing. The organism apparently established in the vagina and was recovered at each subsequent sampling up to the time of farrowing. Gilt 9, a late inoculate, was inoculated on February 12 and recovery of the organism was made the following three days but then apparently was cleared from the vagina. However, it reappeared on February 23rd and was isolated consistently until March 8, six days postpartum. Recovery was not made on the day of parturition nor on the last day prepartum. E. coli was recovered from the milk of gilts 4 and 5 in pure culture at a time when they were showing symptoms of mastitis. In all cases the occurrence of symptoms coincided with the recovery of E. coli from the vaginal swabs.

An attempt was made to determine the colonial morphology of the isolated E. coli on Tergitol-7 media. Streaking was done from agar slants which had been saved back from the isolates. On this basis, isolates from gilts 4, 6, 7, 8, and 9 were judged to be of the intermediate-smooth type. Other isolates from 3, 5, and 9 were of the mucoid type.

Hemolysis was not a characteristic of any of the E. coli isolates. Serotyping was done on two cultures, both of the mucoid type and both isolated on March 3rd from gilt 5. One was obtained from a vaginal swab and one from the milk. K and H serotype for both was :K28:H19, but both were negative to all 145 standard E. coli "O" group serum.

Other isolates A summary of the vaginal bacteria isolated from gilts in Trial I is given in Table 3. Organisms falling into the categories of alpha-hemolytic streptococci, micrococci, and diphtheroids were recovered at some time from all of the gilts. Proteus sp. were recovered from all but gilts 6 and 10. Beta-hemolytic streptococci were not isolated from gilts 3, 4, and 5 but were isolated from the other seven gilts. The two isolates of Pseudomonas sp. were made on consecutive days from gilt 9. Hemolytic staphylococci were recovered from gilts 6 and 9.

Trial II

Clinical observations

Eight gilts were obtained three days before the first animal farrowed. These animals, with the exception of gilt 16, were all excessively fat but otherwise appeared to be normal. Gilt 4N, from which a pure Proteus vulgaris was recovered on the first three days of sampling, nonetheless retained her appetite but was found dead on the fourth day. Post mortem examination revealed a cystitis and pyelitis with an abundant growth of Proteus from the bladder and kidney. Autolysis had progressed to the point that satisfactory tissue sections could not be obtained. Clinical symptoms which were observed in the Trial II gilts are recorded in Graph 11 through Graph 17.

Gilt 4S Gilt 4S was a negative control gilt and farrowed normally. A severe hematoma of the vulva developed during farrowing but no disease symptoms appeared and appetite and appearance of the litter remained good during the entire lactation period. No deaths were recorded

Table 3. Summary of vaginal isolates from gilts - Trial I

	Isolates from 100 samples		Isolates - 35 pre-partum samples		Isolates - 65 post-partum samples		Isolates - 22 samples sows with symptoms		Isolates - 67 samples - sows asymptomatic	
		%		%		%		%		%
Alpha-hemolytic strept.	52	52	26	74.3	26	40.0	12	36.4	40	59.7
Beta-hemolytic strept.	17	17	6	17.2	11	16.9	8	24.2	9	13.4
Micrococci	53	53	22	62.9	31	47.7	16	48.5	37	55.2
Diphtheroids	38	38	13	37.1	25	38.5	11	33.3	27	40.3
Proteus sp.	20	20	1	2.9	19	29.2	10	30.3	10	14.9
Pseudomonas sp.	2	2	0	0.0	2	3.1	0	0.0	2	3.0
Hemolytic staphylococci	2	2	1	2.9	1	1.5	0	0.0	2	3.0
E. coli	50	50	16	45.7	34	52.3	25	75.8	25	36.8*

*$P < .01$.

in the litter.

Gilt 9 S Gilt 9S was the second negative control gilt. No symptoms of disease of any kind were noted in this animal during the lactation period. The sow farrowed only two pigs but raised them to weaning at five weeks of age.

Gilt 16 Gilt 16 was considered a positive control since cultures of the pre-farrowing swabs revealed that an intermediate E. coli was established in the vagina. Parturition occurred normally and no symptoms

appeared in either the sow or litter during the lactation period. All eleven pigs in the litter reached weaning age at five weeks.

Gilt 34 Gilt 34 was also a positive control. A mucoid E. coli was determined to be established in the vagina on the basis of the initial cultures. Symptoms appeared in this gilt during the parturient period which was extended over a period of approximately 12 hours. Depression was evident during the last half of this period and diarrhea was observed in the sow near the end of parturition. Seven live pigs and one dead pig were farrowed. By the next day a vaginal discharge was beginning. Deep depression and complete anorexia accompanied by an elevated temperature were noted. Two pigs died from apparent hypoglycemia and the others were obviously hungry. On the second day postpartum a copious vaginal discharge was observed. Severe depression and anorexia were still in evidence. One more pig had died and the other four were rough haired and depressed. The third day postpartum the clinical appearance of the sow had not changed appreciably. One additional pig had died and the remaining three were very thin and weak. All three were dead by the morning of the fourth day.

Symptoms in the sow in the form of vaginal discharge, depression, and anorexia did not lessen to any extent until the seventh day after farrowing when the amount of vaginal discharge was somewhat decreased and the sow began to be more alert and show some interest in the feed. Improvement was noted for the next several days but recovery was never complete. The sow was euthanized and necropsied on the twenty-second day after farrowing.

Gilt 35 Gilt 35 was inoculated approximately twenty-four hours after a normal parturition. No symptoms of disease were noted in the sow or pigs during the lactation period.

Gilt 36 Gilt 36 was inoculated soon after a normal parturition. A slight congestion of the mammary gland was noted the next day but had cleared by the second day postpartum. A slight to moderate vaginal discharge was observed on the second through the fifth days after farrowing but the sows appetite remained good and no other symptoms were observed. All six of the pigs farrowed were weaned.

Gilt 9N Gilt 9N was inoculated twice with the lyophilized culture used in gilts 35 and 36. This inoculum failed to become established. Inoculation was then made with a mucoid E. coli culture prepared from an isolate from gilt 34.

Gilt 9N farrowed approximately one month after the other gilts. No symptoms were noted except for a slight brownish, serous discharge on the 3rd, 4th, and 5th days after farrowing. One runt pig died out of the litter of eleven up to the age of four weeks.

Bacteriological findings

Escherichia coli E. coli isolates from vaginal swabs of the Trial II gilts are shown in Graph 11 through Graph 17. E. coli was isolated from each of the gilts at least once. The mucoid type, however, was isolated consistently only from gilts 9N and 34. The only other mucoid isolate was obtained from gilt 36 on the third day postpartum. Gilt 16, which had an intermediate E. coli established in the vagina prior to farrowing, cleared at parturition and no further recoveries were made.

In gilt 4S, which was negative to E. coli before farrowing, the opposite was the case. Recovery of a rough E. coli was made on the day of farrowing and for the following five days.

E. coli isolates from gilt 36 were of the rough form on the day of inoculation. Isolation of the E. coli was made for five days following farrowing. The mucoid form was isolated on the third day after farrowing.

E. coli was isolated from every vaginal swab taken from gilt 34 with the exception of two of the last three taken about three weeks after parturition. All early isolates were of the mucoid type. A shift to the intermediate form began about the ninth day postpartum and was complete by the eleventh day. All subsequent isolates were of the intermediate type. A rectal swab taken shortly after parturition revealed that about 40% of the coliform flora were of the mucoid type and 60% rough. A urine sample taken on the first day postpartum yielded a heavy growth of apparently pure culture mucoid E. coli. Two cc. of a blood sample taken at the same time were plated directly onto blood agar and Tergitol-7. Two mucoid colonies grew on the Tergitol-7 and one E. coli colony on blood agar.

Heart blood, uterus, kidney, bladder, and colon were cultured at necropsy from gilt 34. No organisms were recovered from the heart blood. The uterus yielded a diphtheroid but was negative for E. coli. A pure, profuse E. coli of the intermediate type was recovered from both the kidney pelvis and bladder. Isolates from the colon were intermediate-rough to rough.

The isolate from the kidney pelvis of sow 34 was slightly hemolytic. Otherwise, all isolates were non-hemolytic.

Other isolates A summary of the vaginal isolates from gilts in Trial II is given in Table 4. Micrococci, alpha- and beta-hemolytic streptococci and diphtheroids were isolated from all seven gilts. Proteus sp. isolates came from gilts 4S, 9S, 9N, and 34. The Pseudomonas sp. was recovered from gilt 34 and all Klebsiella sp. isolates were from gilt 35.

Necropsy findings - gilt 34

The primary gross abnormalities noted at necropsy were in the urinary system and lungs. A slight cystitis was noted and a marked fatty degeneration of the kidney. A chronic pneumonia was found in the lungs. Some slight fatty changes were present in the liver. The genital tract did not appear to be grossly affected (Figure 4). Many developing follicles were detectible on the ovaries. The uterus, while atonic and flaccid, had undergone involution and was of normal size. The cervix was grossly normal (Figure 5). The vagina appeared normal except for the area from the urethral opening externally. A marked inflammation with some necrosis of the epithelium was noted in this area (Figure 6). No other abnormalities were noted.

Histological findings

Kidney A marked fatty degeneration of the tubular epithelium was present. Several foci of mononuclear cell accumulations were found in the cortex (Figure 8).

Table 4. Summary of vaginal isolates from gilts - Trial II

	Isolates 92 samples		Isolates 42 prepartum samples		Isolates 50 postpartum samples		Isolates 20 samples--sows with symptoms		Isolates 72 samples--sows asymptomatic	
	no.	%	no.	%	no.	%	no.	%	no.	%
Alpha-hemolytic strept.	12	13.0	5	11.9	7	14.0	2	10.0	10	13.9
Beta-hemolytic strept.	27	29.3	12	28.6	15	30.0	4	20.0	23	31.9
Micrococci	31	33.7	17	40.5	14	28.0	4	20.0	27	37.5
Diphtheroids	31	33.7	12	28.6	19	38.0	10	50.0	21	29.2
Proteus sp.	12	13.0	3	7.1	9	18.0	3	15.0	9	12.5
Klebsiella sp.	3	3.3	1	2.4	2	4.0	0	0.0	3	4.2
Pseudomonas sp.	1	1.1	1	2.4	0	0.0	0	0.0	1	1.4
E. coli	65	70.7	29	69.0	36	72.0	19	95.0	46	63.9*

*$P < .01$.

Bladder Numerous mixed inflammatory cells were present in the subepithelial area.

Uterus There was an infiltration of the submucous area with mixed inflammatory cells. Focal concentrations were also present. The epithelial cells were increased in height and showed vacuolization (Figures 9 and 10).

Vagina Section of the vagina from the area of the urethral opening demonstrated a marked infiltration of the subepithelial regions with neutrophils and mononuclear cells. Many siderophages were present, often in large, discrete foci. Some necrosis of the epithelium could be observed (Figure 7).

Liver The liver showed congestion with some fatty change present at the periphery of the lobules.

Lung A broncho-pneumonia was present with fibrosis and some abscess formation.

DISCUSSION

Etiology

The results of these trials strongly indicate that at least certain strains of Escherichia coli are capable of causing any or all of the symptoms of the metritis-mastitis-agalactia complex. This is best illustrated by the close relationship between the occurrence of symptoms and isolation of E. coli from vaginal swabs (Graph 1 through Graph 17). Remission of symptoms was closely associated with a clearing of vaginal E. coli. Of 53 samples taken from sows with clinical symptoms in the two trials (Tables 3 and 4), all but nine yielded E. coli when cultured. In all nine of these instances, E. coli had either been isolated on the previous day, was isolated the succeeding day, or was recovered on both days. Application of the chi-square test for contingency indicated a highly significant difference ($P < .01$) between the occurrence of vaginal E. coli in sows experiencing symptoms and in sows without symptoms. This was true in both Trial I and Trial II. No statistical differences were evident at $P < .05$ for any of the other bacteria isolated which would indicate a relationship to the occurrence of symptoms.

Four gilts, all in Trial II, yielded E. coli during the postpartum period but failed to develop symptoms. Three of these, (9S, 16, and 35), yielded the organism only sporadically and in small numbers (less than 5 colonies), indicating that the organism did not become established and multiply in the vagina. The fourth, (4S), yielded a rough E. coli in large numbers the first two days postpartum and in smaller numbers for an

additional three days. It is possible that a large hematoma of the vulva, which became lacerated and contaminated with fecal material, served as a locus of infection from which relatively avirulent and non-toxic fecal coliforms invaded the vagina.

The symptoms of the disease syndrome produced are parallel to those seen in other animals and man suffering from coliform infections. In man, Weil and Spink (1958) describe an elevation in temperature and signs of sepsis in the form of fever, anorexia, and malaise as symptoms associated with bacteremia due to gram negative bacilli. The post parturient symptoms seen in the sow bear a striking resemblance to the clinical picture previously described for experimental coliform mastitis in the cow.

It appears logical that release and absorption of E. coli endotoxin is responsible for the systemic signs observed in the mastitis-metritis complex of sows. Such a conclusion is supported by the fact that corticosteroids and ACTH are valuable in the management of post parturient disorders in swine as well as E. coli endotoxemia.

Predisposing Factors

The success achieved in introducing the disease into the trial gilts through introduction of live cultures of E. coli into the vagina is in opposition to results reported previously. The predisposing factors necessary for the disease to develop are not known. An attempt was made to eliminate as many of the commonly accepted predisposing factors as possible in both trials. Since symptoms were more severe in the Trial I than the Trial II gilts, a discussion of the possible factors involved is in order.

Farrowing quarters

The farrowing quarters in both instances were thoroughly cleaned and disinfected before the gilts were moved in. Temperature was maintained at approximately 70° in both cases.

Constipation

Constipation was not noted in either trial.

Condition

Trial I gilts were in good condition for farrowing. Trial II gilts were overweight.

Changes in feed or environment

The Trial I gilts were moved to the farrowing quarters well ahead of farrowing and had no change in feed or feeding arrangements. Over a period of three days the Trial II gilts were moved from farm to sale barn to research facilities when the first gilts to farrow were less than one week from farrowing. Changes in the feed and ration probably resulted in a reduction of feed intake.

Size of litter The Trial I gilts farrowed extremely large litters. The ten animals farrowed a total of 133 pigs. It is possible that the large litters placed an additional stress on the sows. The influence of stress and adrenal hormones on the occurrence of symptoms had been discussed previously. That a disturbance in the hormone balance occurred is suggested by the fact that 5 of 10 sows experienced hysteria at farrowing time. However, parturition occurred normally and none of the animals had an extended farrowing period. Seven Trial II gilts farrowed 52 pigs.

Vaginal coliform flora The Trial I gilts were apparently free of any vaginal coliform flora prior to the introduction of the experimental inoculum since no E. coli isolates were obtained from any of the initial vaginal swabs. In Trial II the picture was much less clear as two gilts were determined to have an established coliform flora and E. coli was recovered at least once from three of the others on the initial swabs. That a lack of prior exposure to the organism before farrowing would render the sow more susceptible to infection has not been established. The mild and transient nature of symptoms experienced by gilts 7 and 9 in Trial I and gilt 9N in Trial II suggest that such is the case, however. These gilts were the only ones in which it was known that E. coli became established in the vagina well in advance of parturition. Gilts 16 and 34 also had an established E. coli of unknown duration at farrowing. Of these five, symptoms did not appear or were of a mild nature in all but gilt 34. Gilts in which E. coli was first isolated during the postparturient period experienced more severe symptoms and presented a clinical picture identical to that commonly seen in field cases. The possible role of locally produced muco-antibodies needs further investigation.

Virulence and toxicity of E. coli The inoculum of E. coli used in the Trial I gilts was not plated on Tergitol-7 so that colony morphology is not known. It is probable that it was of the mucoid type based on the fact that it was non-motile and the majority of retained agar slant cultures from sows showing symptoms proved to be of the mucoid type. The association of mucoid properties with pathogenicity has not been clearly demonstrated. That the mucoid property is an essential characteristic of

the more highly virulent and toxic E. coli is supported by two observations made in Trial II. Gilt 9N was inoculated twice with an inoculum which apparently was reverting to the R form and contained both M and R forms. This inoculum failed to establish in the vagina. A fresh inoculum consisting entirely of the M form then readily became established following inoculation. Gilt 34 harbored a mucoid E. coli during the entire postparturient period until approximately eight days postpartum when a gradual shift to the intermediate form began and was apparently complete in two days. The disappearance of the mucoid form from the vaginal flora was closely associated with improvement in the clinical condition of the sow.

The inoculum used on the Trial II gilts was a reconstituted lyophilized culture retained from the Trial I inoculum. This inoculum was passaged through two additional media not used in the Trial I inoculum; the lyophilized dextrose culture and the tryptose broth media used for reconstitution. Mutation to a non-mucoid form was apparent by this time based on growth on Tergitol-7 agar plates. Sojka (1965) states that mucoid strains can change rapidly to non-capsulated forms. Mutation was observed in this trial on Tergitol-7 plates which had been left at room temperature for several days. "Buds" appearing at the periphery of mucoid colonies yielded entirely rough colonies on replating. Thjøtta and Waaler (1932) demonstrated that the "S" form was very unstable but was much less susceptible to the complement of active guinea-pig serum than was the "R" form. This may be an important factor explaining the increased virulence of the "M" forms.

Isolation of animals The Trial I gilts farrowed in a single large room and no isolation was attempted. Trial II gilts were farrowed in isolation units and strict isolation precautions were observed. The enhancement of bacterial virulence through animal passage is a recognized phenomena. The greater severity of symptoms experienced by the late farrowing gilts in Trial I suggests that virulence was enhanced. Passage was demonstrated by the fact that two late farrowing uninoculated controls developed an E. coli vaginal flora and concurrent symptoms.

Other disease entities The chronic bronchopneumonia found at necropsy in gilt 34 undoubtedly was an important predisposing factor influencing the severity of the symptoms. No complicating disease was observed in any of the other experimental animals.

Urinary Tract Involvement

Necropsy of gilt 34 revealed that the primary seat of E. coli infection at that time was the urinary tract. This was demonstrated by recovery of the organism in heavy and seemingly pure culture from the bladder and kidney pelvis and failure to recover it from the uterus. The marked inflammation and erosions of the vaginal epithelium from the urethral opening externally suggest that the vagina was subject to constant reinfection from the urinary tract (Figures 5, 6, and 10). Histological examination of the kidney revealed a mild pyelonephritis (Figure 9). Association of urinary tract infections with the metritis-mastitis-agalactia syndrome has not been previously reported and it is difficult to assess the significance of these findings. Hewitt et al. (1965) state that in man the

urinary tract is the portal of entry for 2/3 of the gram negative septicemias. Kass (1960) found that 6-7% of pregnant women had an asymptomatic bacteriuria compared to .5% in non-pregnant women. Of these, 42% developed pyelonephritis following parturition. Stasis and increased pressure on the urinary bladder during pregnancy are apparently predisposing factors responsible for the increased incidence in pregnancy. The difficulty in diagnosing pyelonephritis was pointed out by Marchant and Mitchell (1965). Of those cases of pyelonephritis in man demonstrated at autopsy 70% had been missed clinically. The possibility that urinary tract infections play an important role in the pathogenesis of the metritis-mastitis-agalactia syndrome cannot be dismissed and future investigations should consider that possibility.

SUMMARY

Two experiments were carried out to assess the role of Escherichia coli in the pathogenesis of the metritis-mastitis-agalactia syndrome in sows. Seventeen experimental gilts were used in the two trials. Four negative control gilts developed no symptoms nor was E. coli isolated from the vagina or milk. Five inoculated and two late contact control animals developed relatively severe symptoms concurrent with the initial isolation of E. coli from the vagina shortly after farrowing even though no E. coli had been established in the vagina prior to farrowing. Four animals had an established E. coli vaginal flora at the time of farrowing; two apparently infected from inoculation, one late contact control infected from contact with an infected gilt, and one positive control infected from an undetermined source. Symptoms observed in these cases were of a mild and transient nature. One inoculated animal did not develop symptoms nor was E. coli recovered from the vagina. One positive control animal with a mucoid E. coli established prior to farrowing developed severe symptoms of extensive duration. A chronic bronchopneumonia disclosed at necropsy was probably an important predisposing factor influencing the severity of the symptoms. Cultures taken at necropsy revealed the primary site of infection at that time was the urinary tract.

Application of the chi-square test for contingency indicated a highly significant difference ($P<.01$) between the occurrence of vaginal E. coli in sows experiencing symptoms and those without symptoms.

The mucoid property was apparently an essential characteristic of the more highly virulent and toxic E. coli. Tergitol-7 medium with tetrazolium

added proved to be an effective medium for differentiating the rough, intermediate, and mucoid forms.

On the basis of these experiments it is concluded that the variable manifestations observed clinically are all part of a single disease syndrome caused by E. coli infection and resultant endotoxin absorption that follows lysis of the organisms. The incidence and severity of infection and the symptoms evinced are dependent on the tissue resistance of the affected animal and the virulence, numbers, and toxicity of the bacteria. The process of invasion and infection may therefore be considered an interaction between the host and the coliform organisms. This balance can be altered by altered virulence of the E. coli or by changes in the resistance of the host.

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APPENDIX

Figure 1. Colony morphology of mucoid E. coli on Tergitol-7 media.

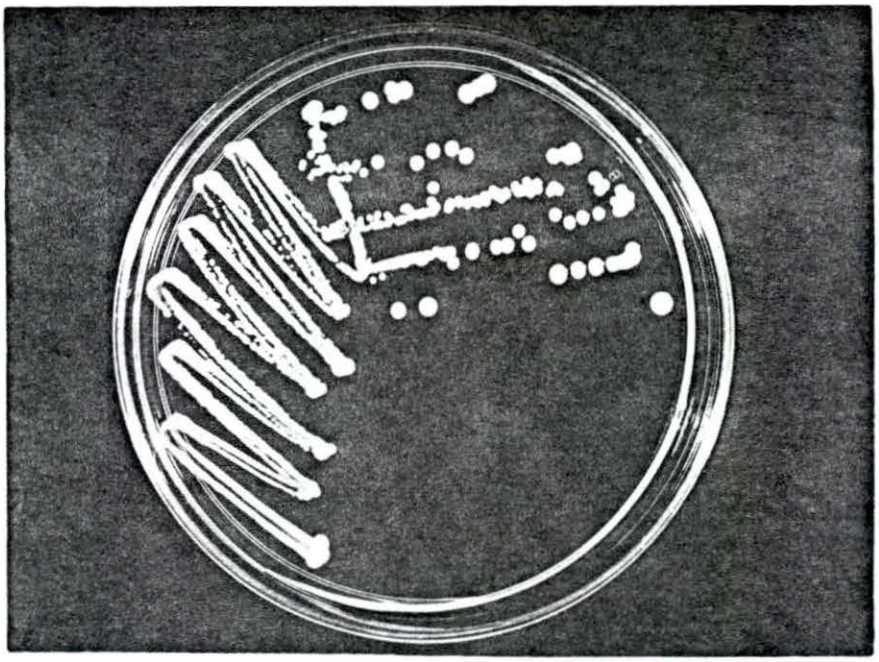
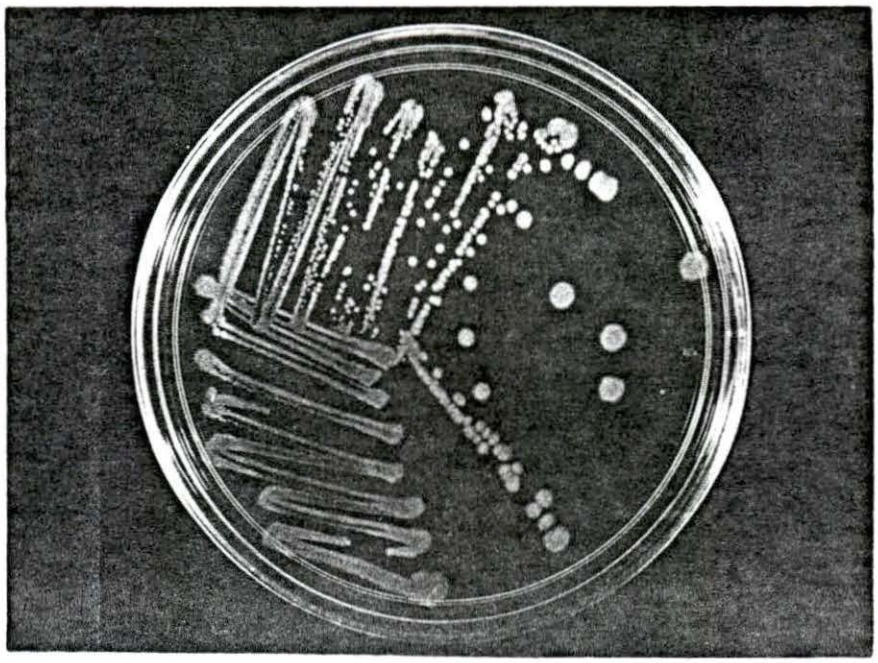
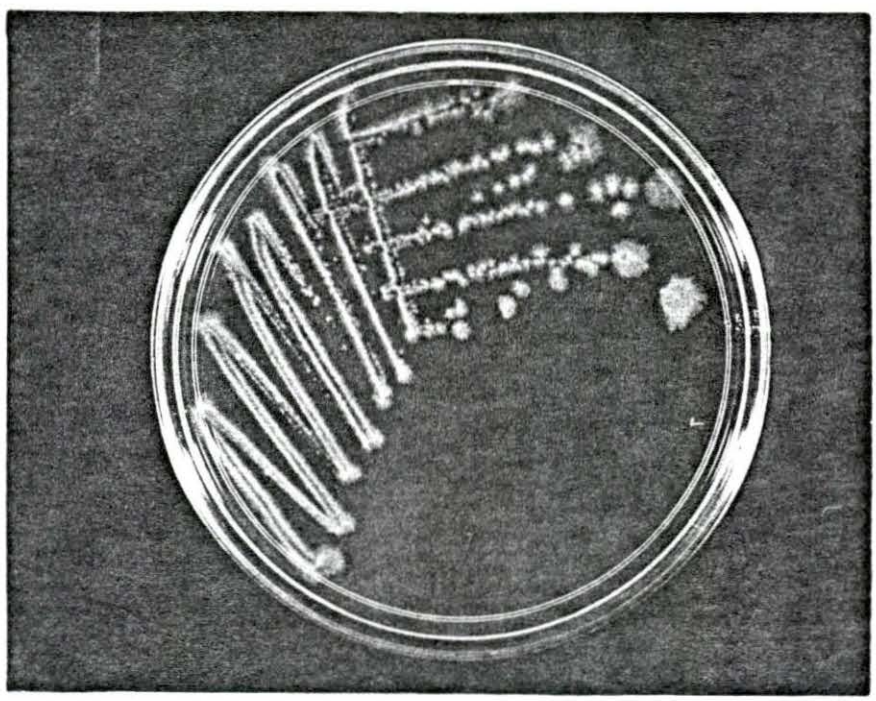


Figure 2. Colony morphology of intermediate E. coli on Tergitol-7 media.

Figure 3. Colony morphology of rough E. coli on Tergitol-7 media.





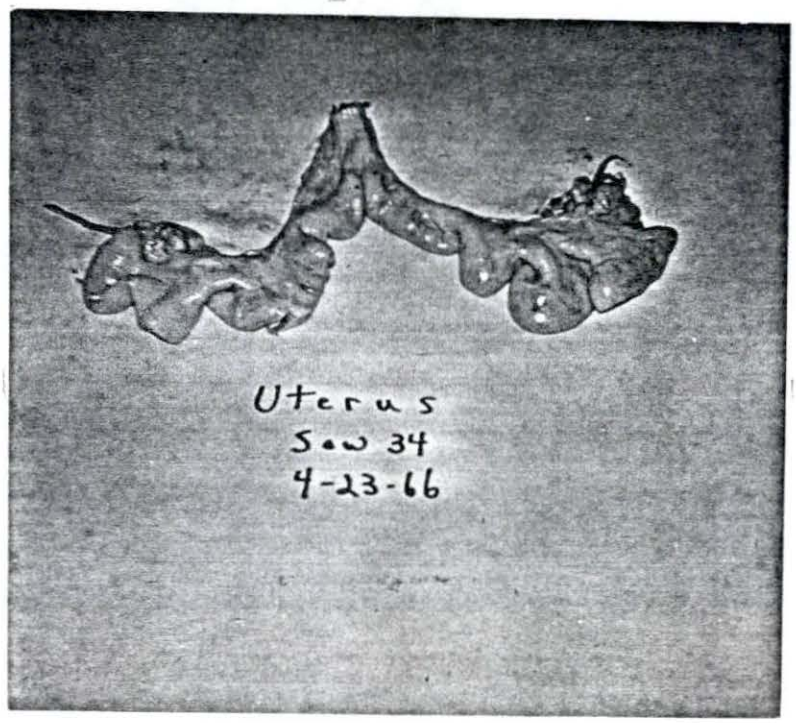


Figure 4. Gross appearance of reproductive tract of sow 34 at necropsy.

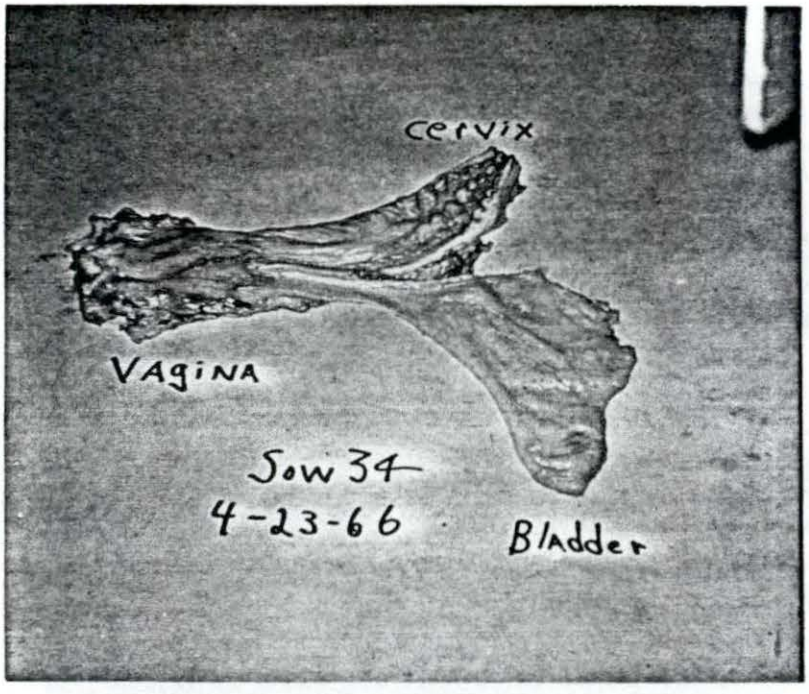


Figure 5. Gross appearance of opened bladder, cervix, and vagina of sow 34 at necropsy.

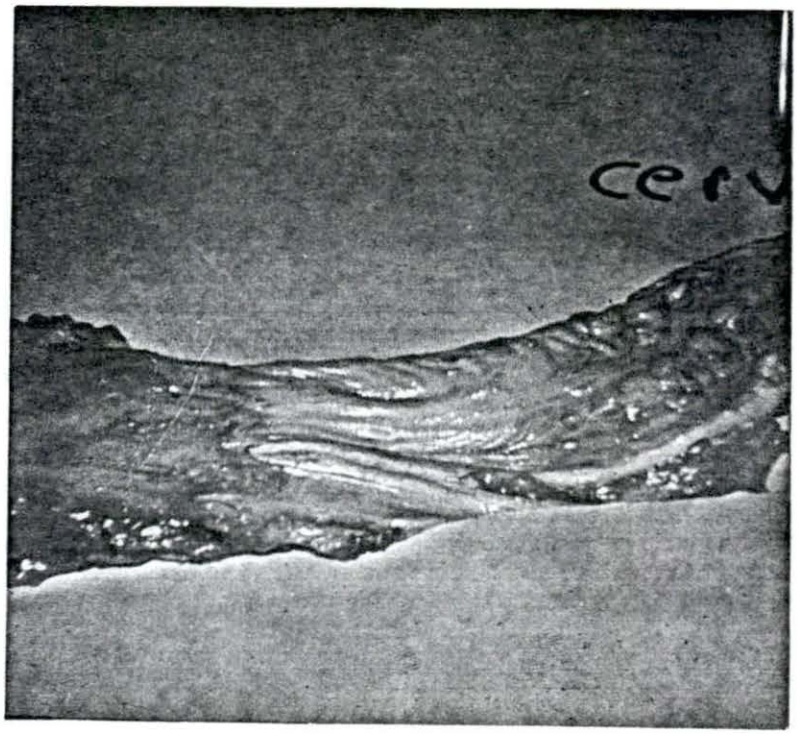


Figure 6. Gross appearance of the vagina at the site of the urethral opening. Sow 34 at necropsy.

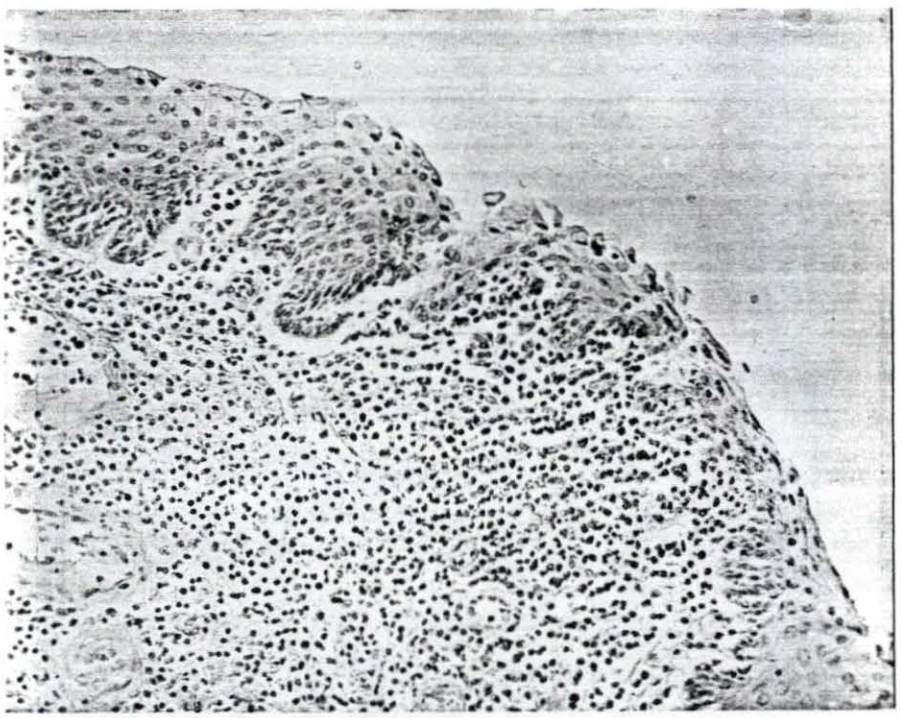


Figure 7. Microscopic appearance of vagina of sow 34. Approx. 200X. Note erosions and subepithelial infiltration of mixed inflammatory cells.

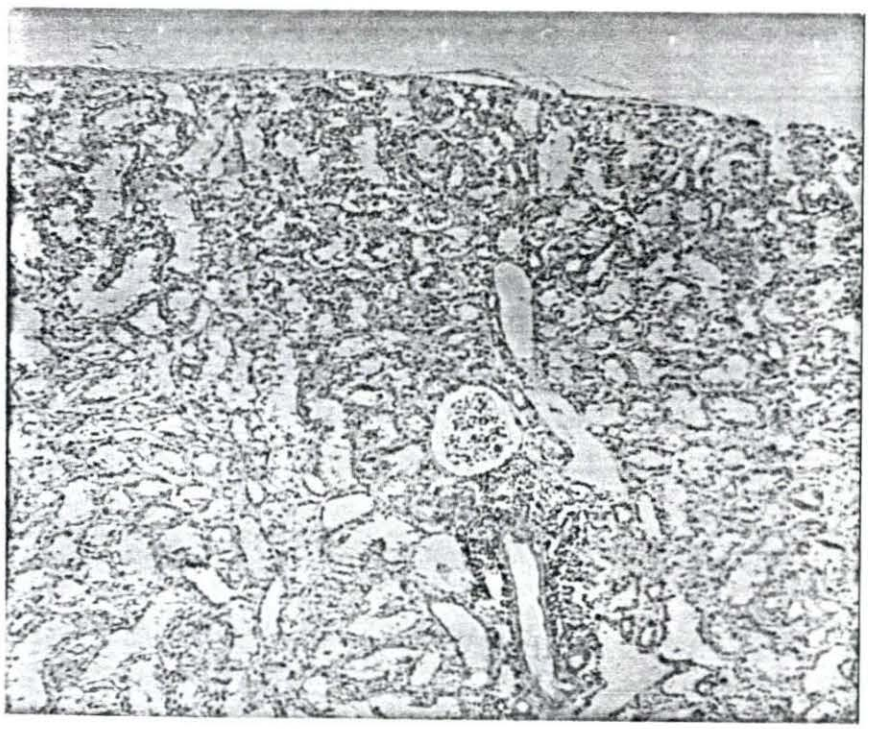


Figure 8. Microscopic appearance of kidney of sow 34. Approx. 90X. Note fatty degeneration of the tubular epithelium and foci of mononuclear cell accumulation in the cortex.

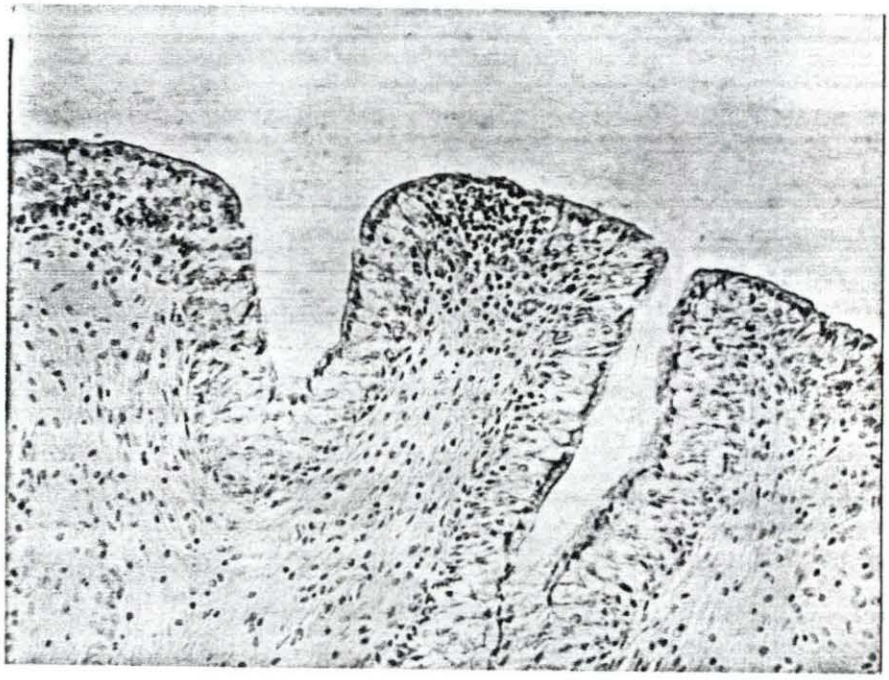
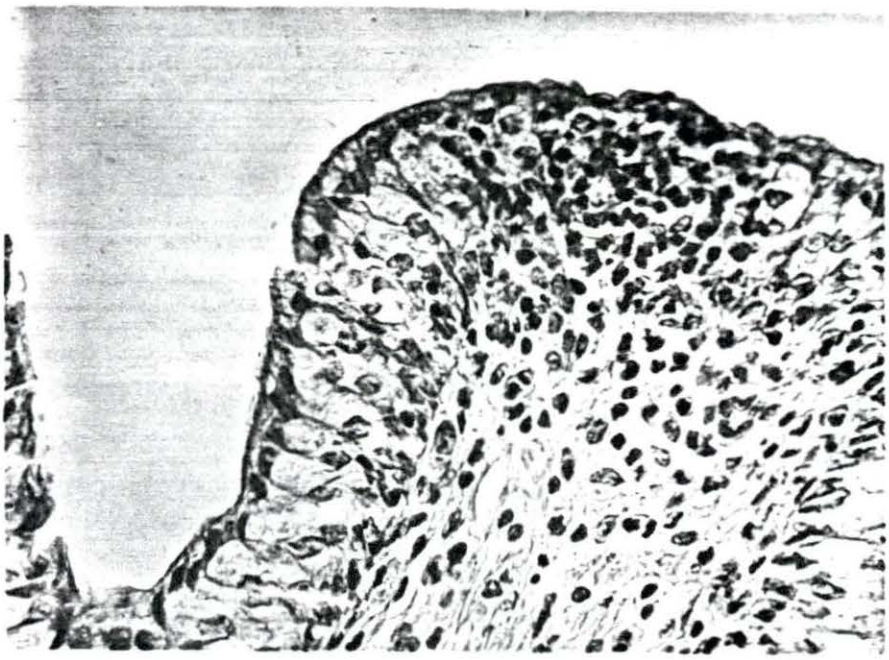


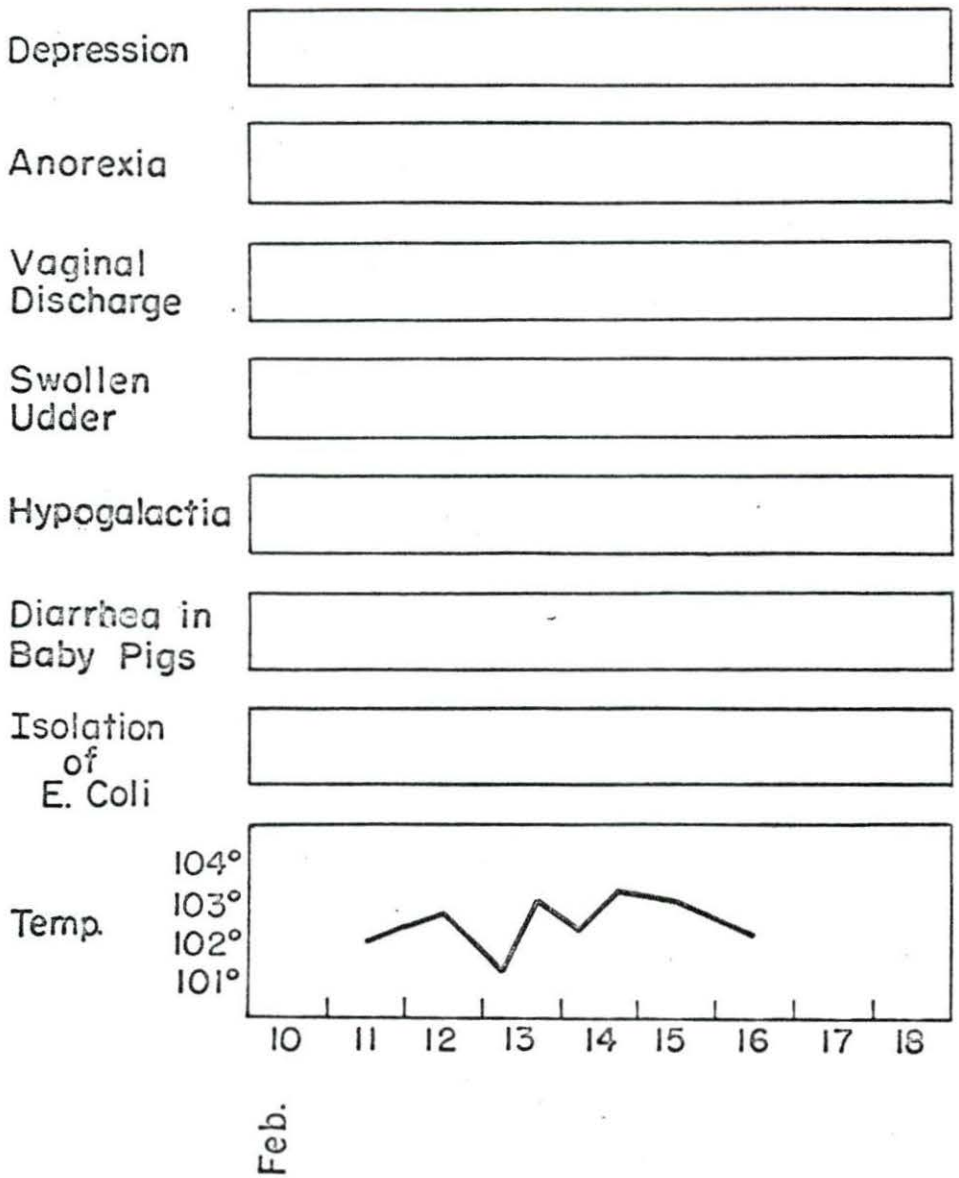
Figure 9. Microscopic appearance of the uterine epithelium of sow 34. Approx. 200X.

Figure 10. Microscopic appearance of the uterine epithelium of sow 34. Approx. 500X. Note vacuolization and subepithelial infiltration of mixed inflammatory cells.

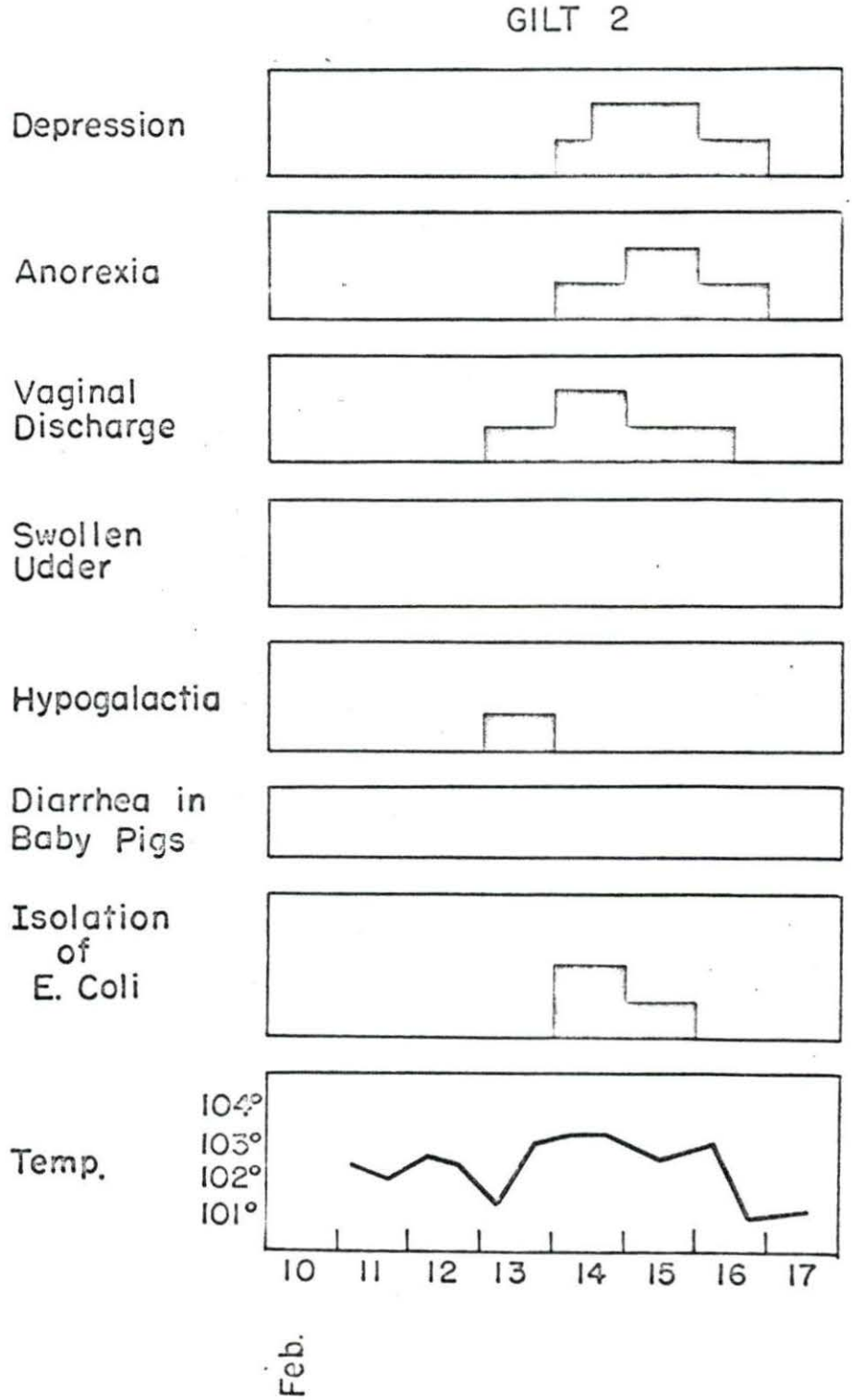


Graph 1. Gilt 1, Trial I. Early contact control; farrowed Feb. 11, 6 live, 0 dead. Lost 2 - both trauma; weaned 4.

GILT 1

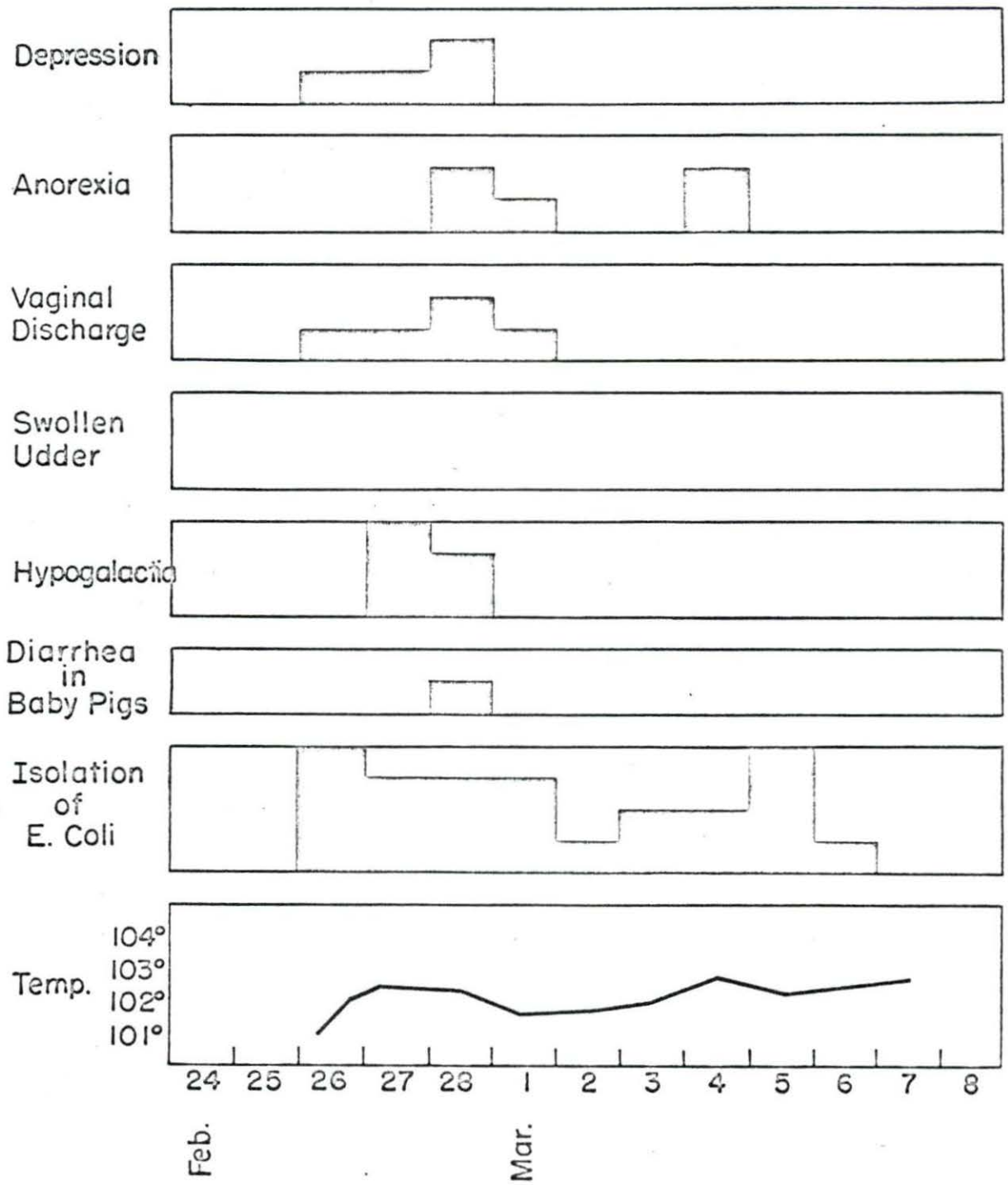

Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
10
11
12
13
14
15
16
17
18
Feb.

Graph 2. Gilt 2, Trial I. Early inoculate; farrowed Feb. 10, 11 alive, 2 dead. Inoculated Feb. 11; weaned 9.

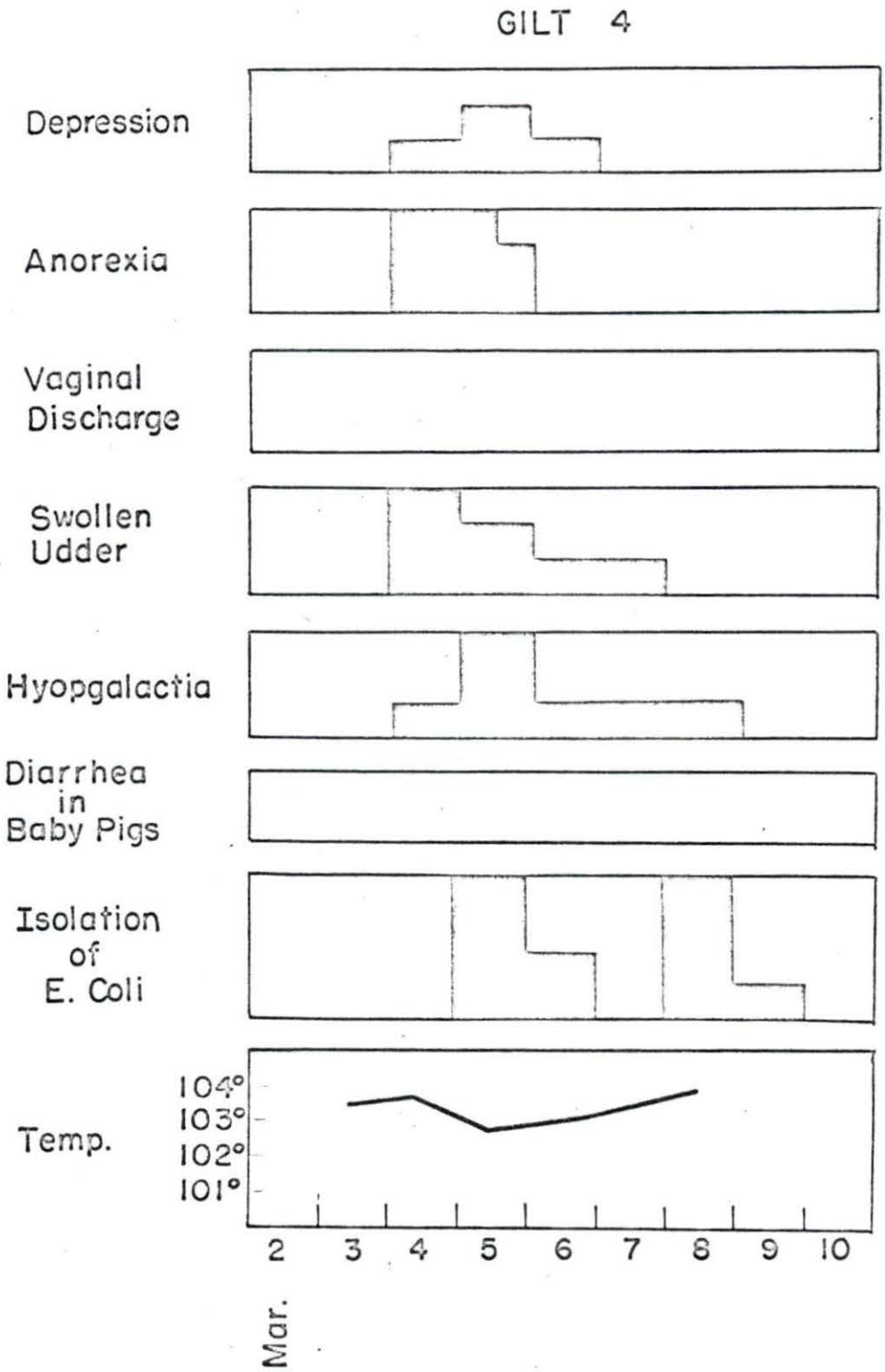

GILT 2
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
10
11
12
13
14
15
16
17
Feb.

Graph 3. Gilt 3, Trial I. Late contact control; farrowed Feb. 25, 15 live, 0 dead. Hysteria - killed 8, lost 2 - hypoglycemia, weaned 5.

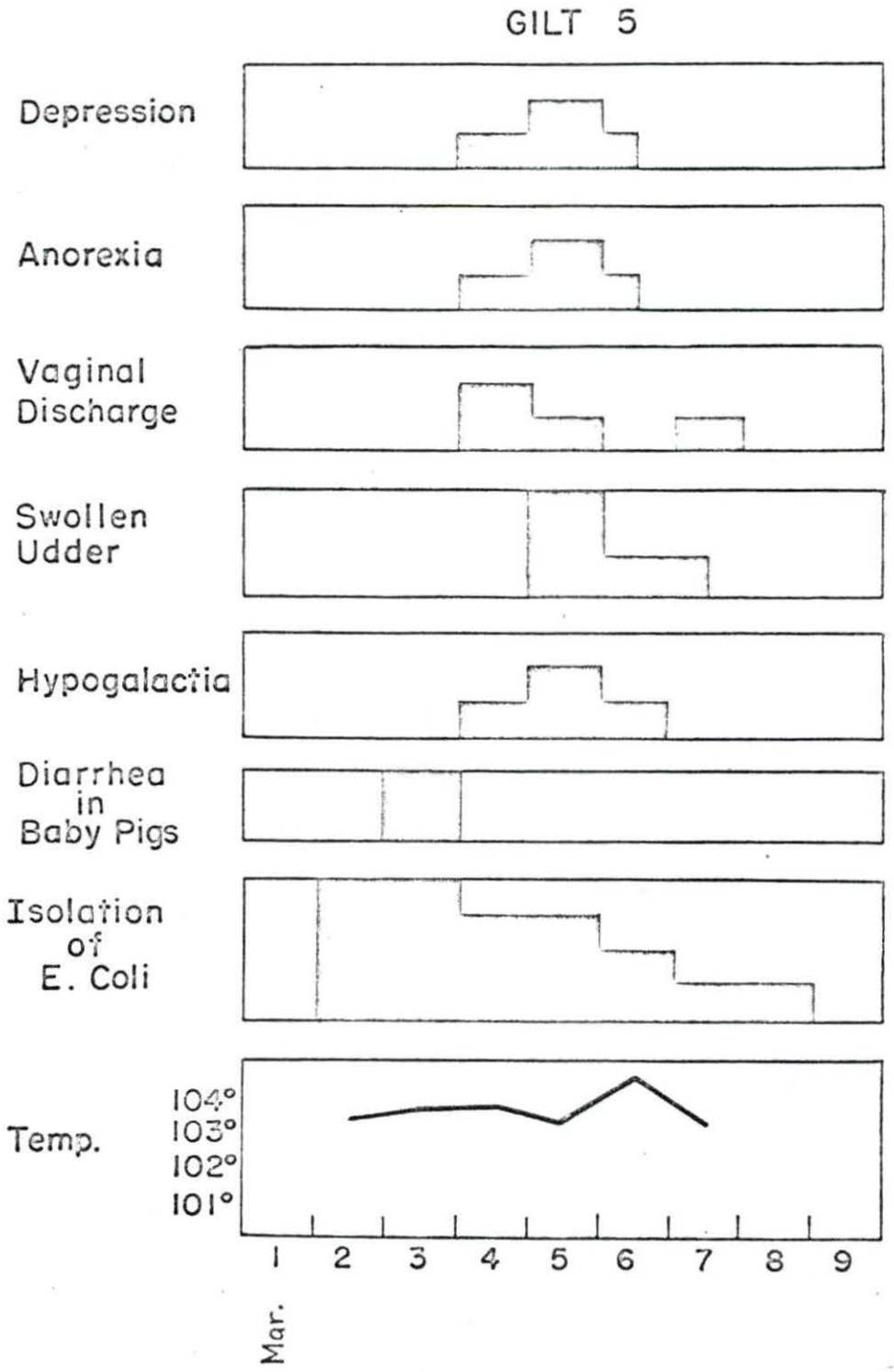
GILT 3


Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
24 25 26 27 28 1 2 3 4 5 6 7 8
Feb.
Mar.

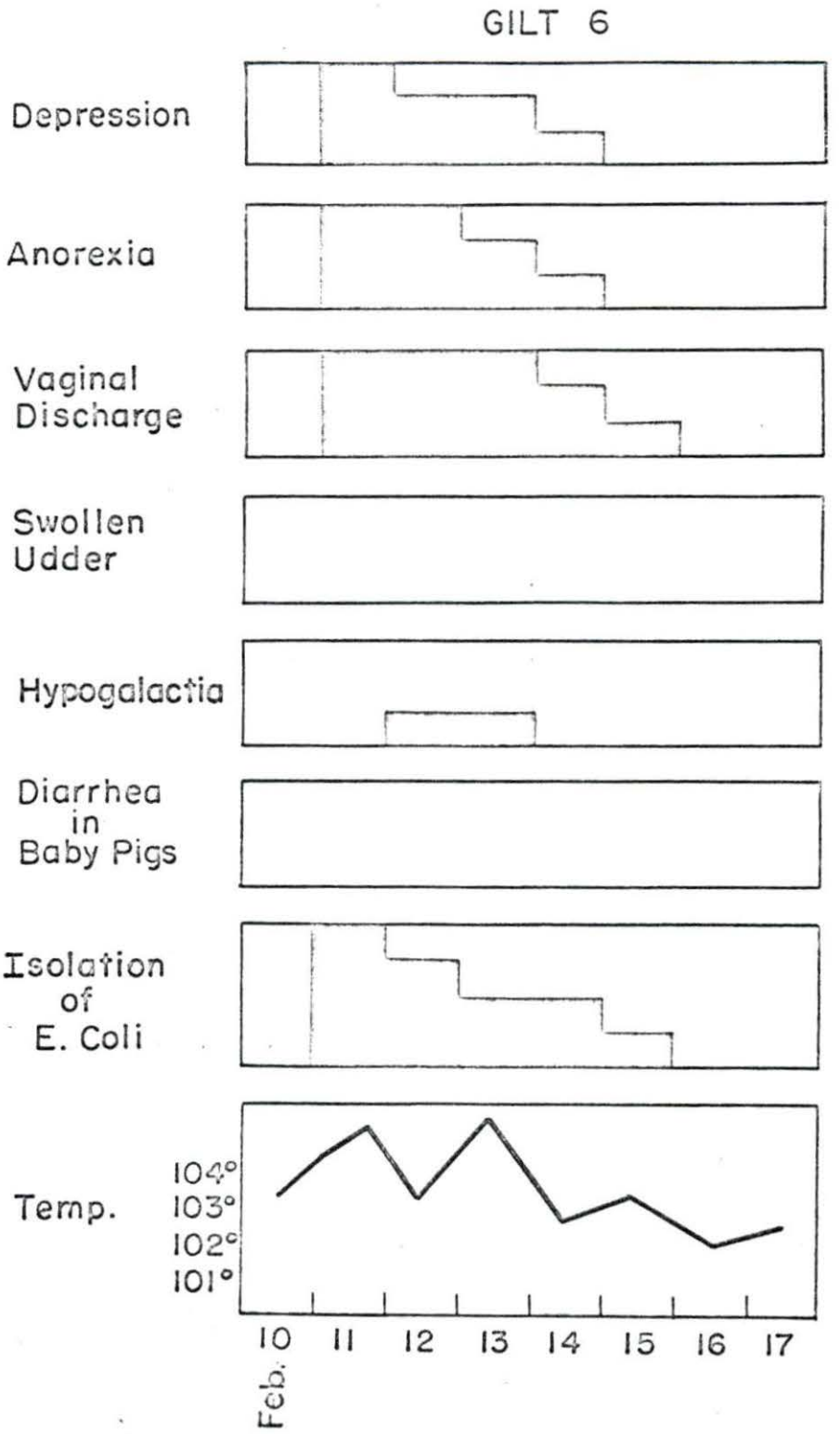
Graph 4. Gilt 4, Trial I. Late contact control; farrowed March 3, 17 live, 2 dead. Lost 6 - hypoglycemia; 2 - trauma; weaned 8.


GILT 4
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hyopgalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
2
3
4
5
6
7
8
9
10
Mar.

Graph 5. Gilt 5, Trial I. Late inoculate; inoculated Feb. 12, farrowed March 1; 10 live, 0 dead; lost 2, 1 trauma, 1 hypoglycemia. Received 4 from gilt 9. Lost 4 more, hypoglycemia; weaned 8.

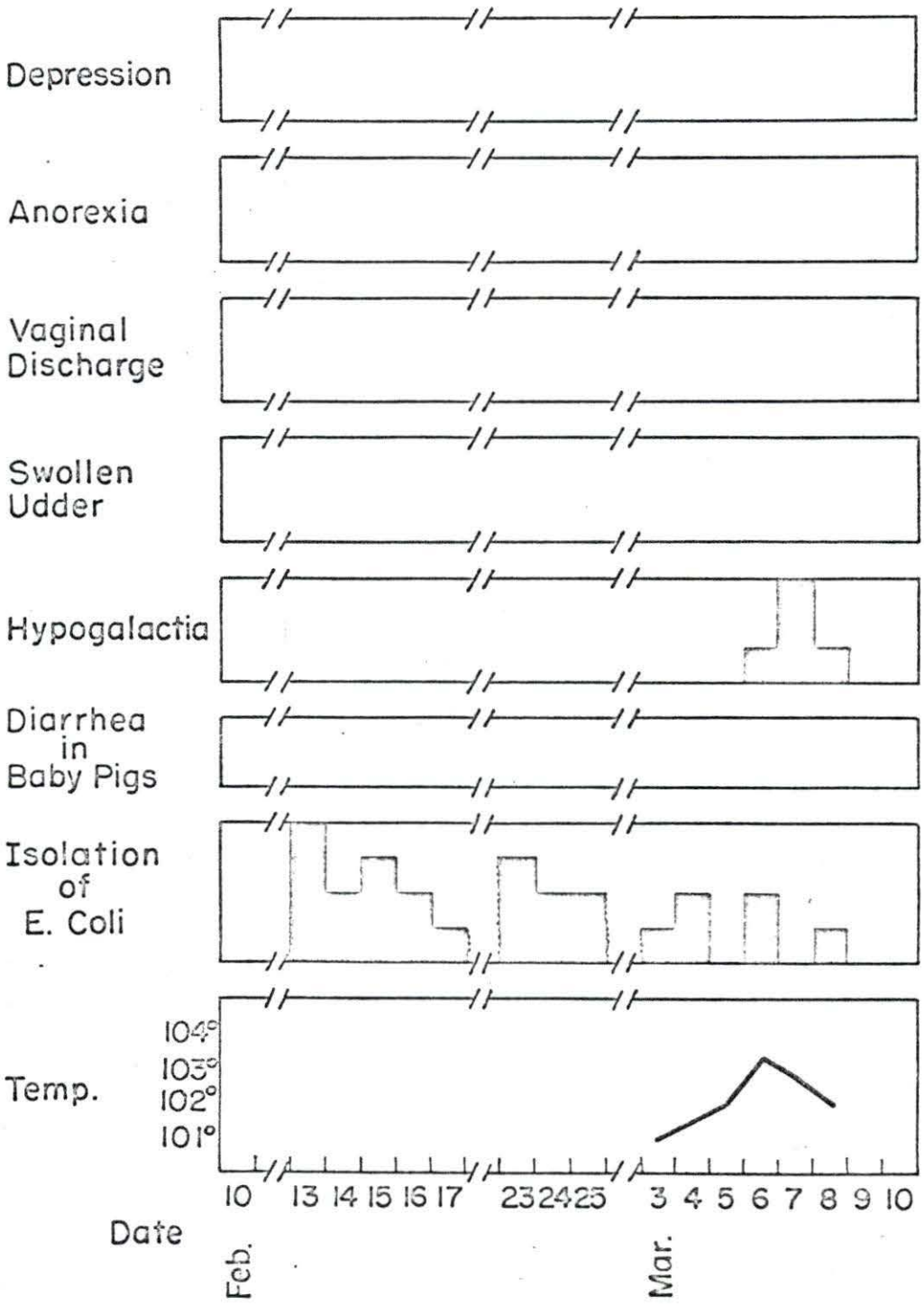

GILT 5
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
1
2
3
4
5
6
7
8
9
Mar.

Graph 6. Gilt 6, Trial I. Early inoculate; farrowed Feb. 10. 15 live, 0 dead. Inoculated Feb. 10. Two pigs given to gilt 10, 1 crushed Feb. 11; 2 hypoglycemia, Feb. 12, weaned 10.

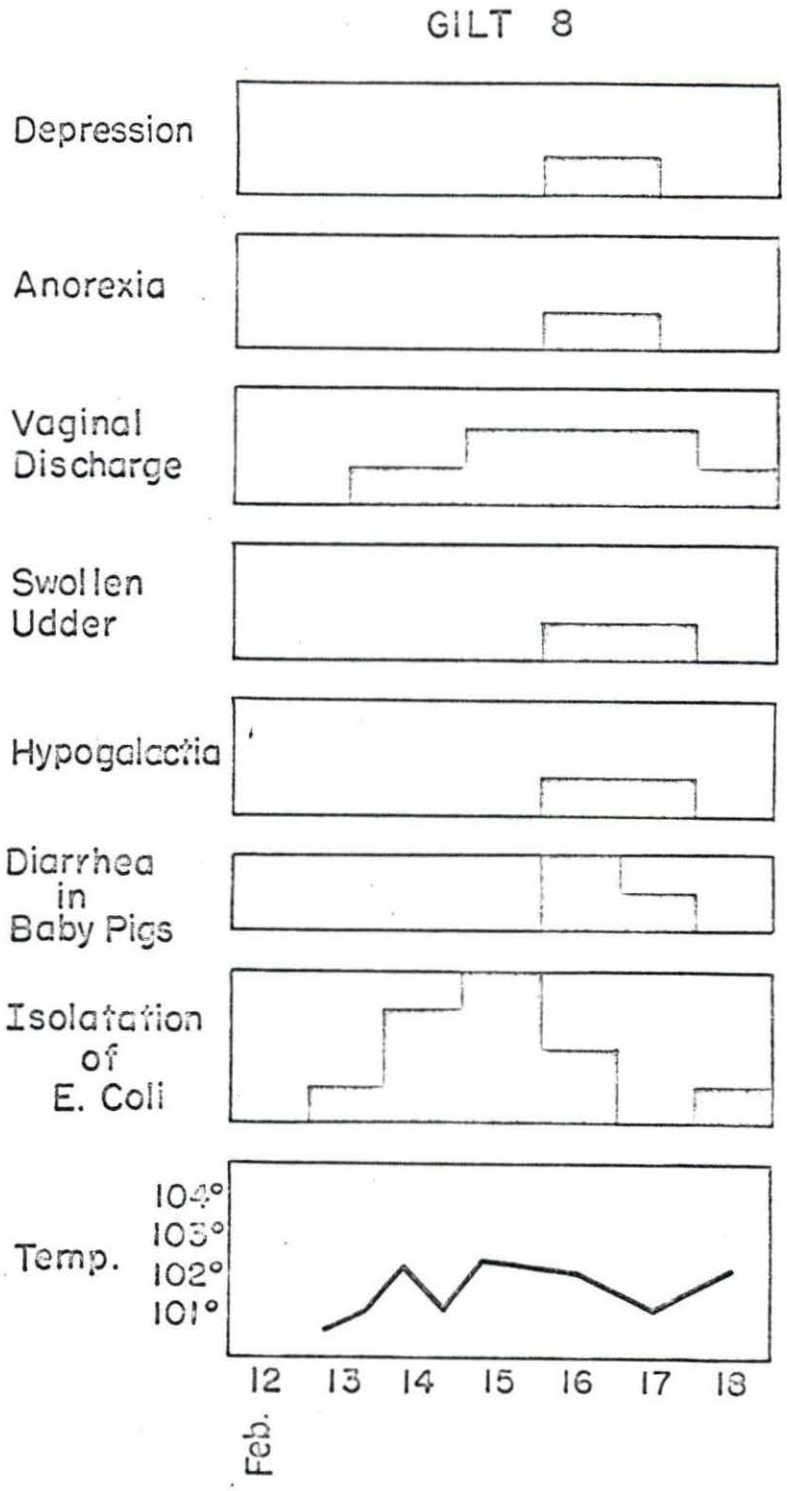

GILT 6
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
10
11
12
13
14
15
16
17
Feb.

Graph 7. Gilt 7, Trial I. Late contact control; farrowed March 4, 13 live, 0 dead. Lost 2 March 5 - crushed; lost 2 March 6 - 1 crushed, 1 hypoglycemia; lost 2 March 7, hypoglycemia. Weaned 7.

GILT 7


Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
10 13 14 15 16 17 23 24 25 3 4 5 6 7 8 9 10
Date
Feb.
Mar.

Graph 8. Gilt 8, Trial I. Early inoculate; inoculated Feb. 12. Farrowed Feb. 13, 15 live, 0 dead; lost 3 Feb. 14 - trauma; lost 3 Feb. 15-17 - hypoglycemia; weaned 9.


GILT 8
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolatation of E. Coli
Temp.
104°
103°
102°
101°
12
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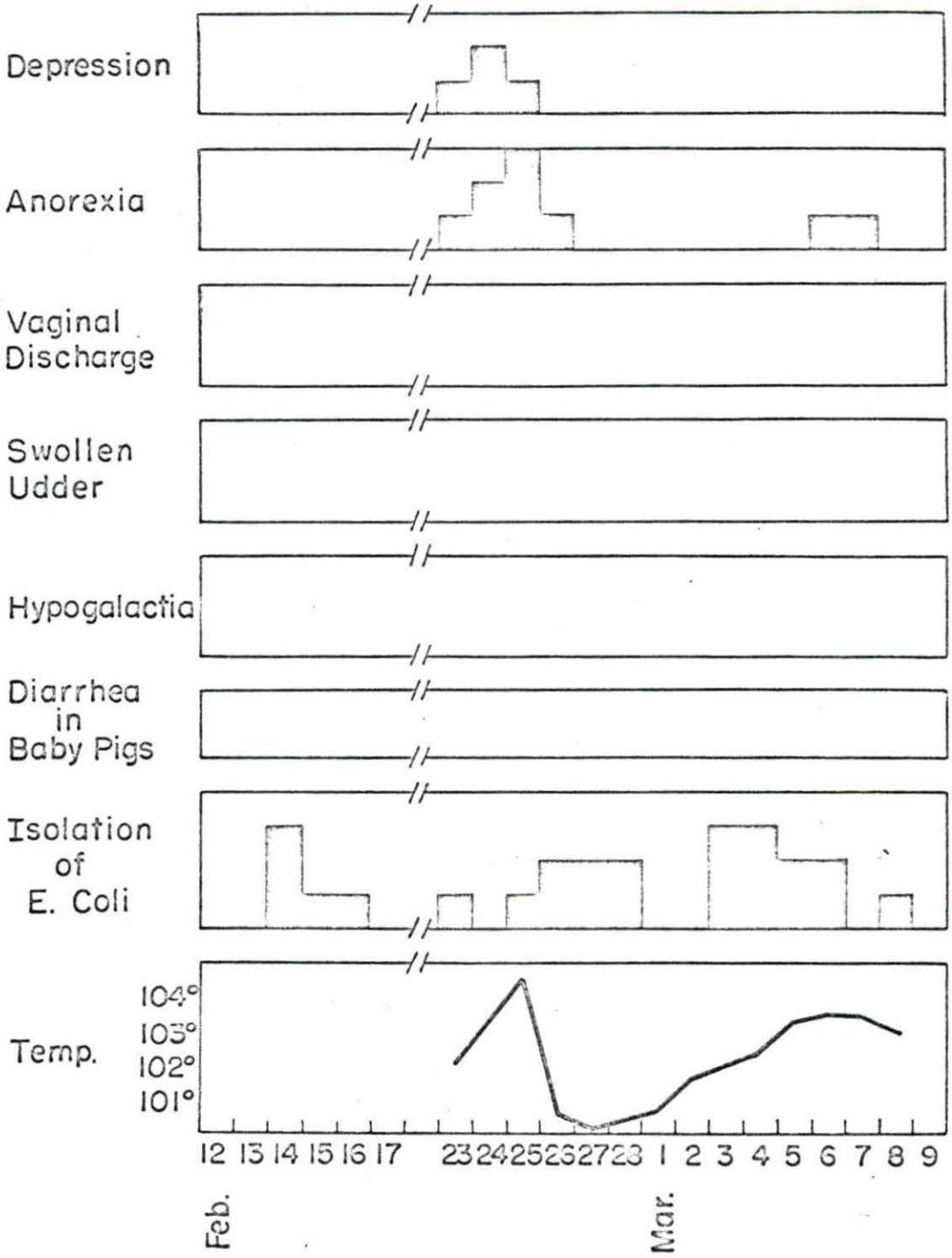
15
16
17
18
Feb.

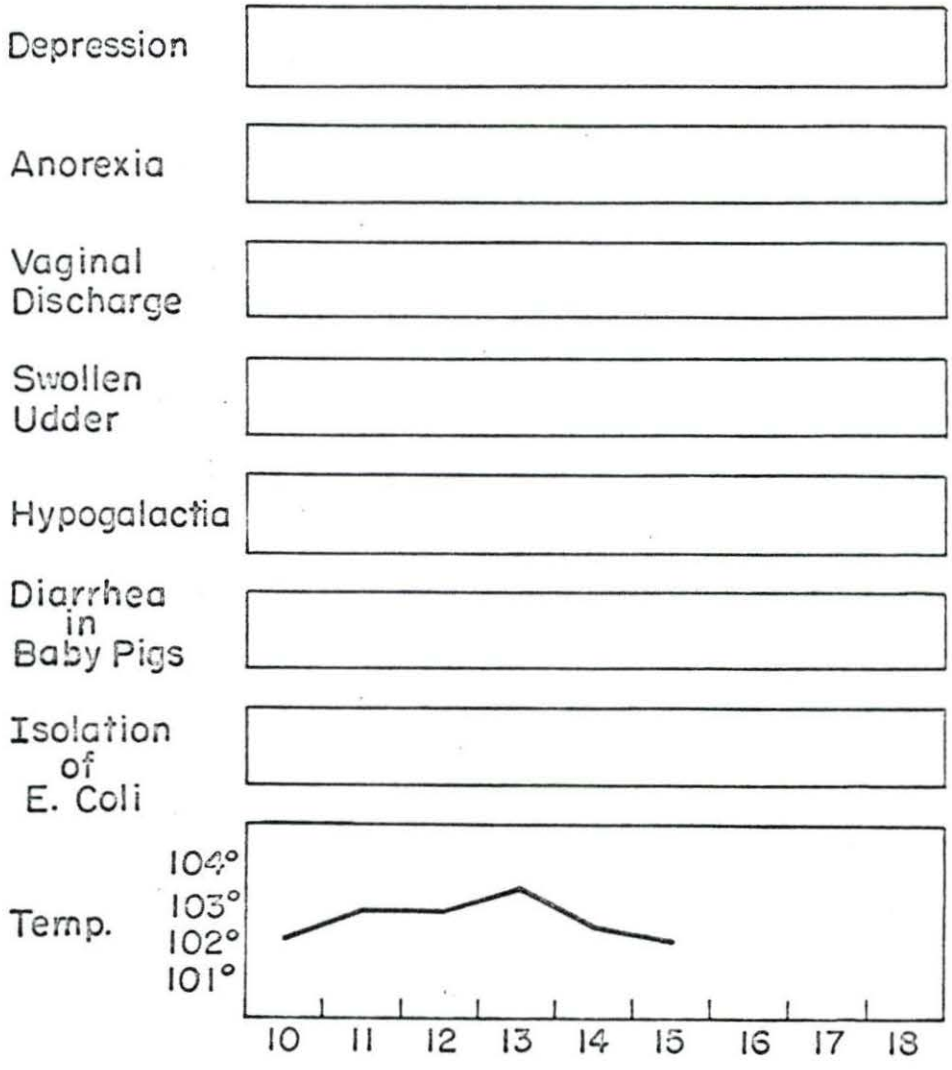
Graph 9. Gilt 9, Trial I. Late inoculate; inoculated Feb. 12. Farrowed March 2; 16 live, 1 dead. Four pigs given to gilt number 5; weaned 10.

GILT 9


Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
12 13 14 15 16 17 23 24 25 26 27 28 1 2 3 4 5 6 7 8 9
Feb.
Mar.

Graph 10. Gilt 10, Trial I. Early contact control; farrowed Feb. 9. 10 alive, 1 dead; hysteria - treated. Given 2 from gilt 6. Lost 1 Feb. 11 - trauma; weaned 11.

GILT 10


Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
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101°
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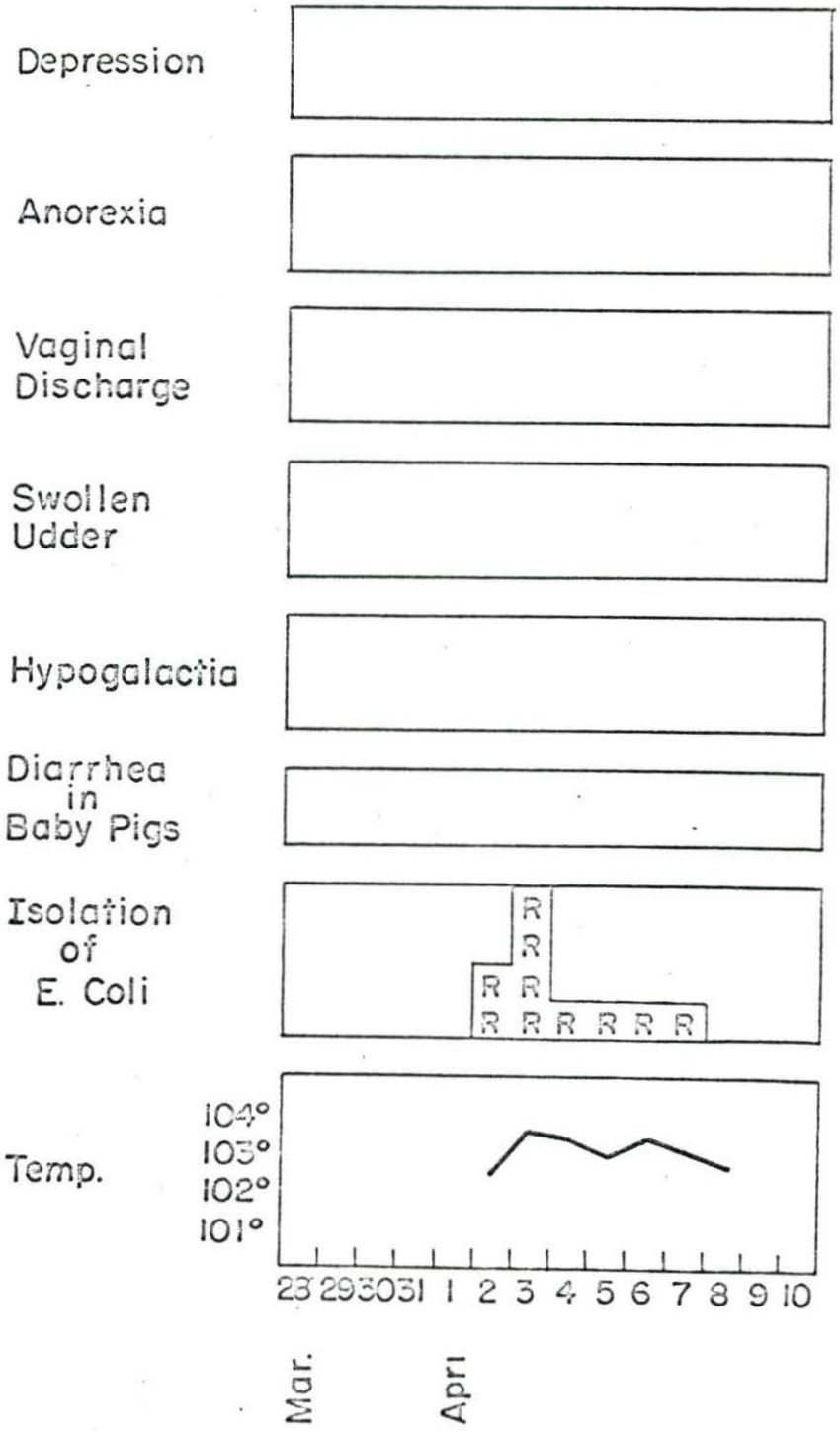
Feb.

Graph 11. Gilt 4S, Trial II. Negative control; farrowed April 2
5 live, 0 dead; weaned 5.

GILT 4S


Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
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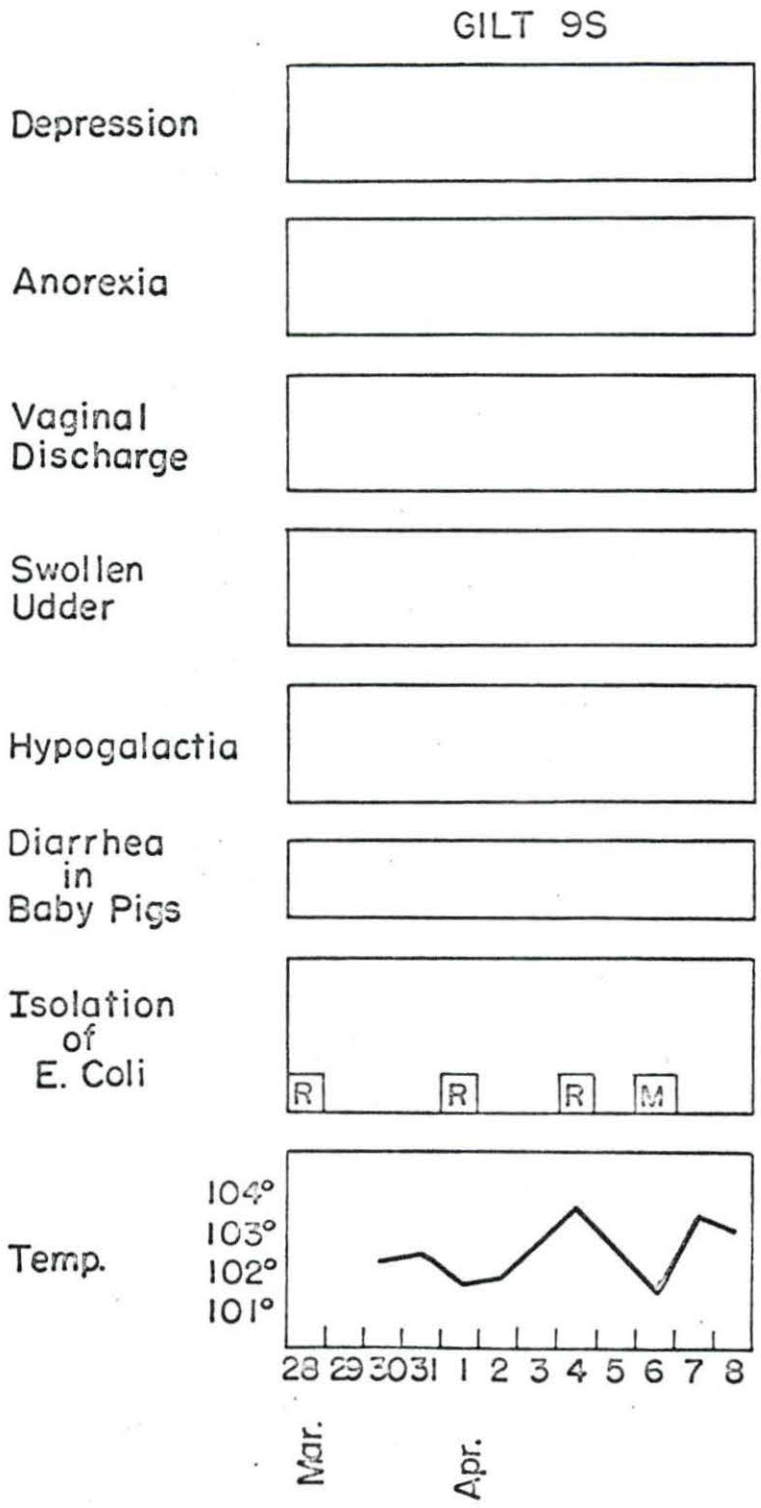
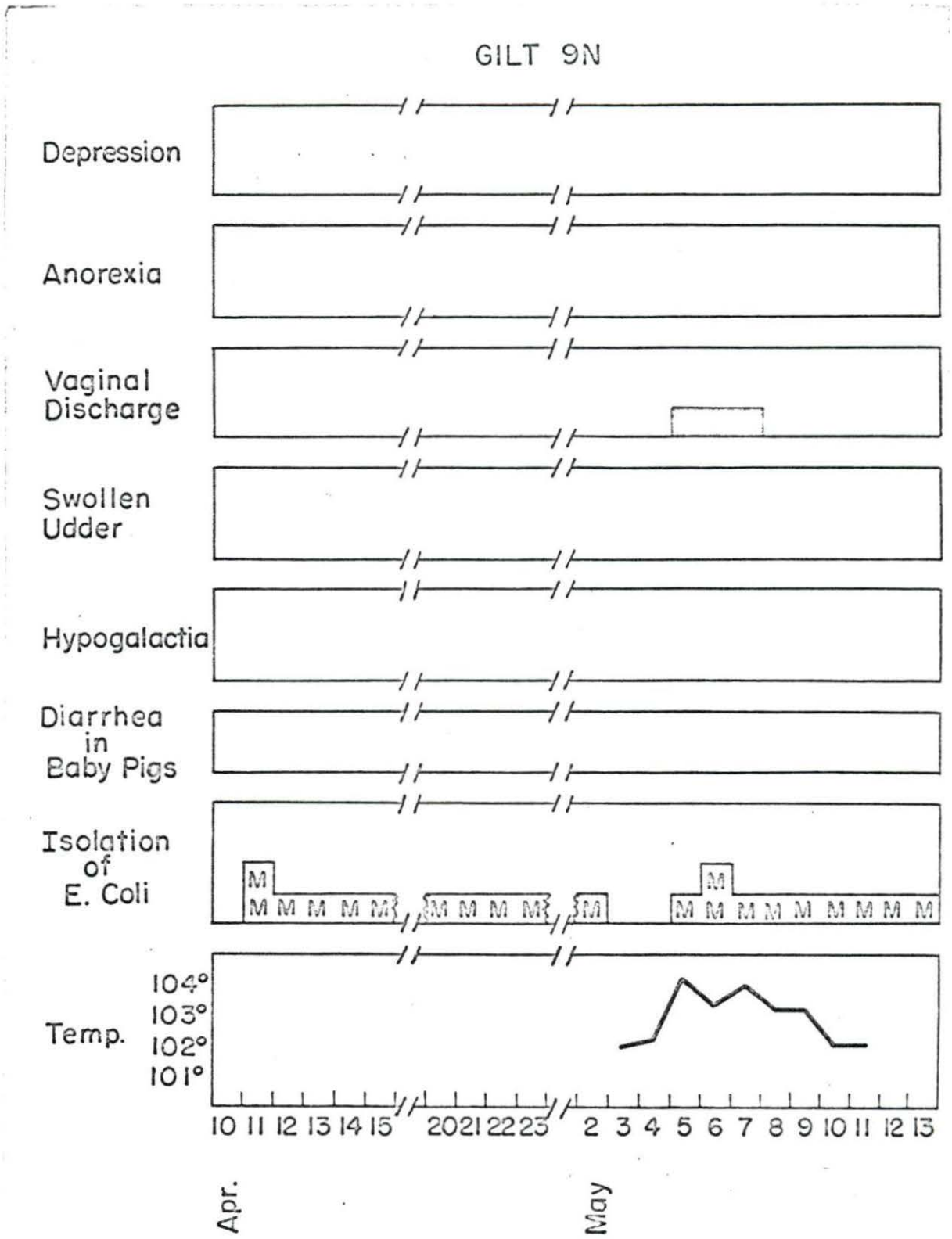
102°
101°
23 29 30 31 1 2 3 4 5 6 7 8 9 10
Mar.
Apri

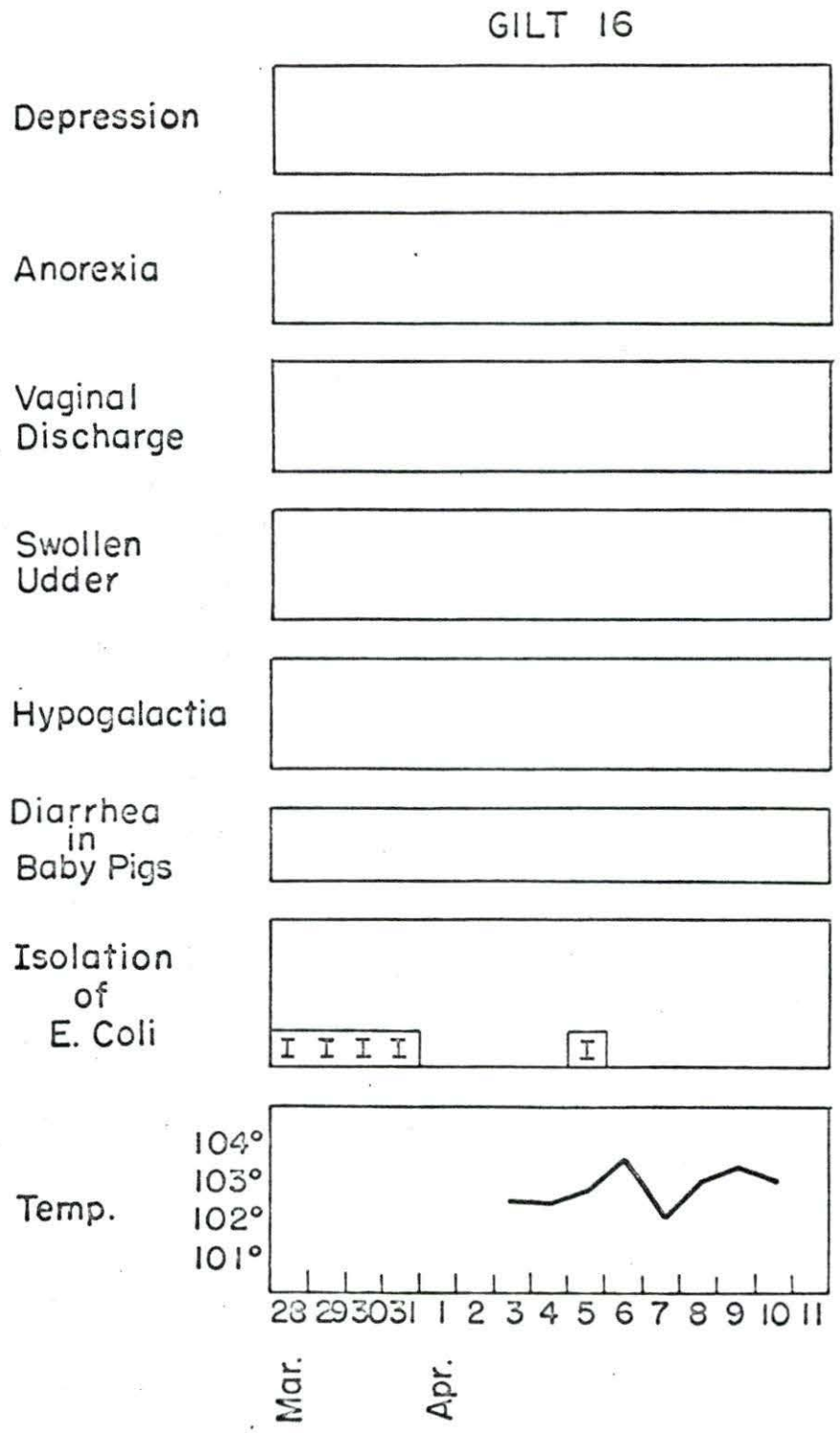
Graph 12. Gilt 9S, Trial II. Negative control; farrowed March 30
2 live, 0 dead; weaned 2.


GILT 9S
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
R
R
R
M
Temp.
104°
103°
102°
101°
28 29 30 31 1 2 3 4 5 6 7 8
Mar.
Apr.

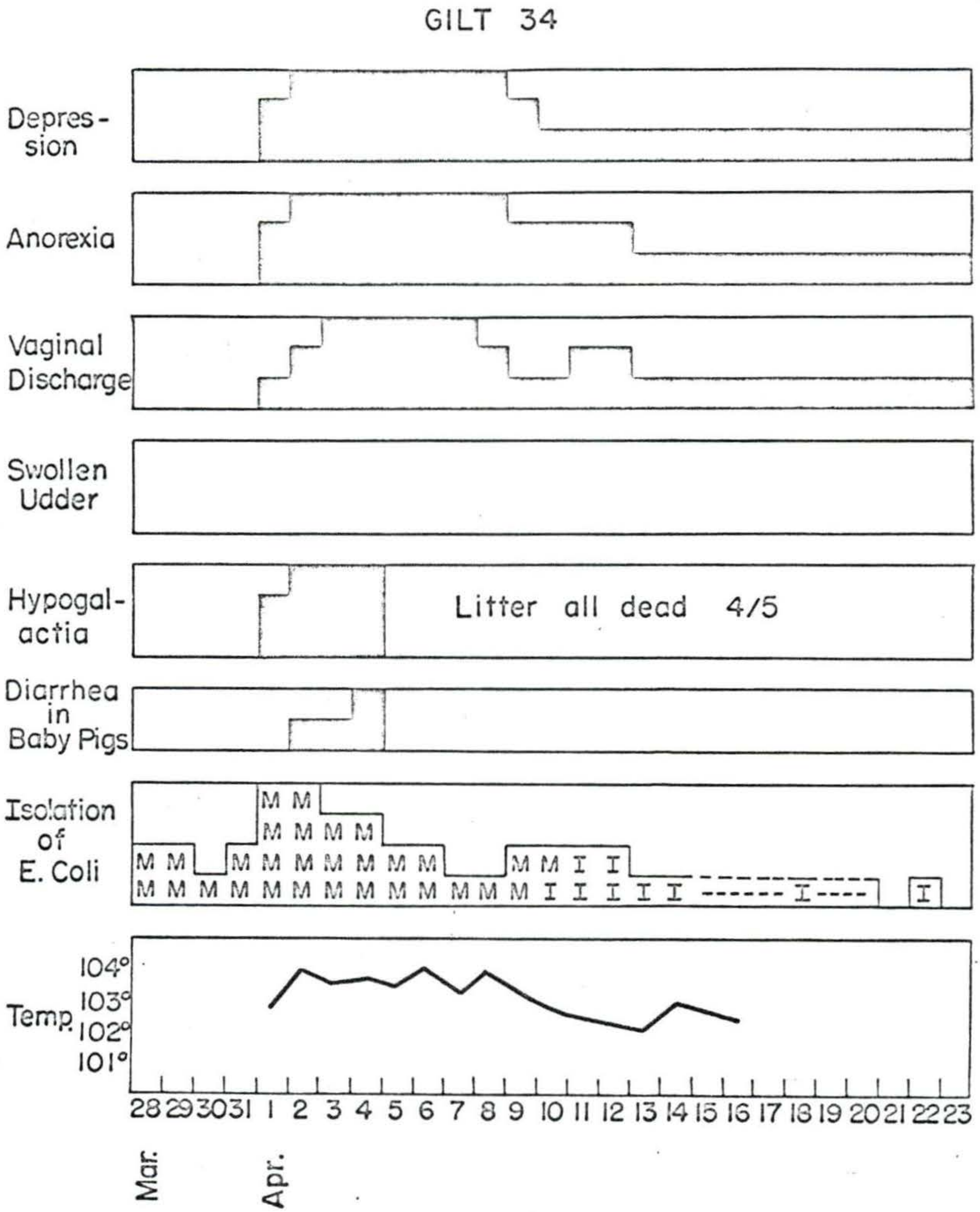
Graph 13. Gilt 9N, Trial II. Inoculate. Inoculated April 5 (I-M) Inoculated April 7 (I-M); inoculated April 11 (M). Farrowed May 2, 11 live, 0 dead; weaned 10.


GILT 9N
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
M
M M M M M M M M M M M M M M M M M M M M M M M M M M M M
Temp.
104°
103°
102°
101°
10 11 12 13 14 15 20 21 22 23 2 3 4 5 6 7 8 9 10 11 12 13
Apr.
May

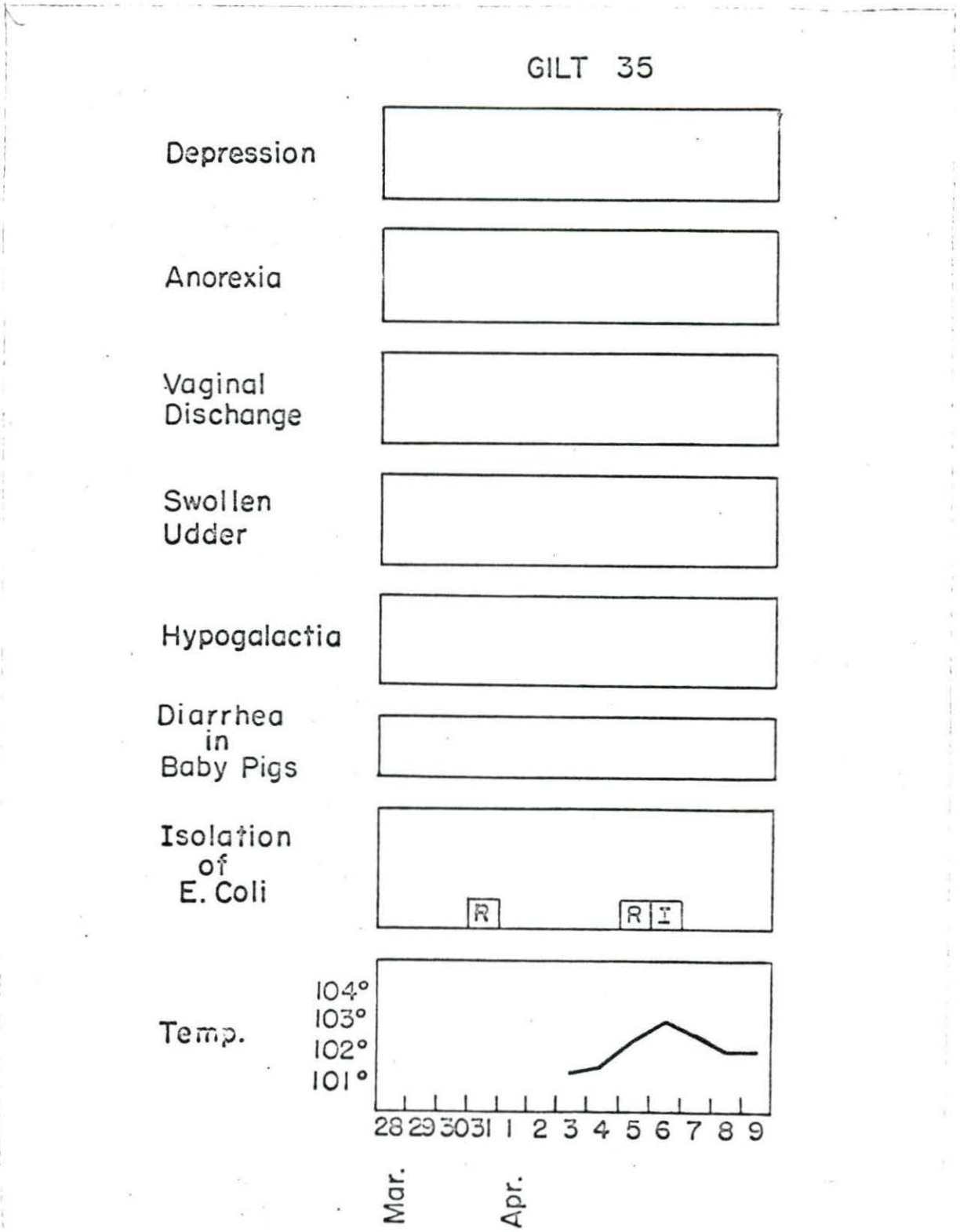
Graph 14. Gilt 16, Trial II. Positive control; farrowed April 3
11 live, 0 dead; weaned 11.


GILT 16
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
I I I I
I
Temp.
104°
103°
102°
101°
28 29 30 31 1 2 3 4 5 6 7 8 9 10 11
Mar.
Apr.

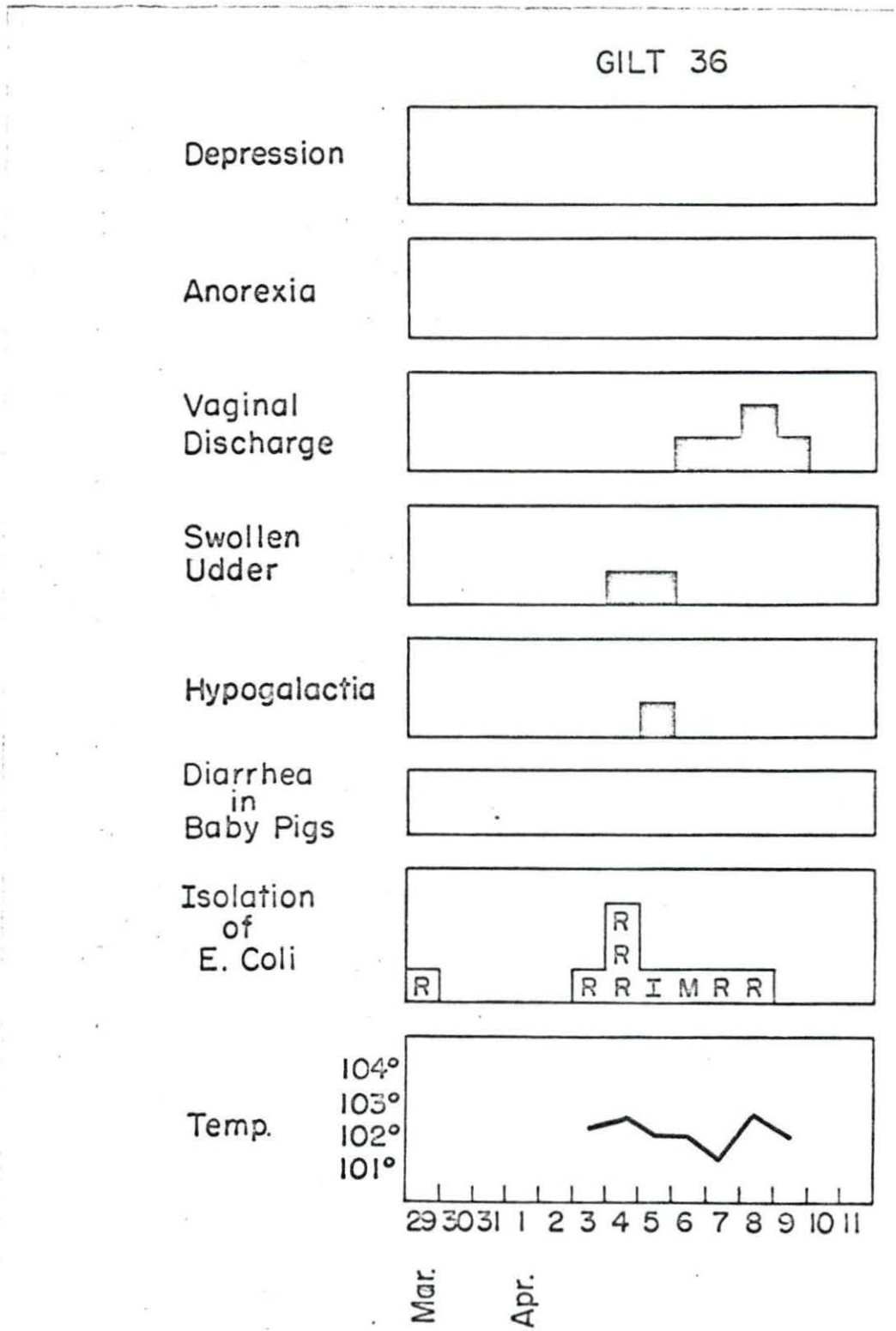
Graph 15. Gilt 34, Trial II. Positive control; farrowed April 1
7 live, 1 dead; weaned 0.


GILT 34
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Litter all dead 4/5
Diarrhea in Baby Pigs
Isolation of E. Coli
Temp.
104°
103°
102°
101°
28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Mar.
Apr.

Graph 16. Gilt 35, Trial II. Inoculate. Farrowed April 3
8 live, 1 dead; inoculated April 4. Weaned 8.


GILT 35
Depression
Anorexia
Vaginal Dischange
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
R
R
I
Temp.
104°
103°
102°
101°
28 29 30 31 1 2 3 4 5 6 7 8 9
Mar.
Apr.

Graph 17. Gilt 36, Trial II. Inoculate. Farrowed April 3
6 live, 0 dead; inoculated April 3; weaned 6.


GILT 36
Depression
Anorexia
Vaginal Discharge
Swollen Udder
Hypogalactia
Diarrhea in Baby Pigs
Isolation of E. Coli
R
R
R
R R I M R R
Temp.
104°
103°
102°
101°
29 30 31 1 2 3 4 5 6 7 8 9 10 11
Mar.
Apr.