STUDIES ON PREVENTION OF ENTERIC COLIBACILLOSIS IN GERMFREE NEONATAL PIGS

By

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CHAPTER I

INTRODUCTION

Escherichia coli is a frequent inhabitant of the small intestine and lower bowel of warm blooded animals. Additionally, E. coli has been isolated from cases of mastitis, urogenital infections, abortions, diarrheal diseases, and other pathological processes (8). E. coli is not, however, the only bacterial agent associated with the above pathological processes, but appears to be one of the more prevalent. For instance, Sojka (49) reported that necropsy of 6000 pigs in the United Kingdom revealed that 36% of these deaths could be attributed to E. coli infections while only 1.3% of these deaths could be attributed to species of Salmonallae.

Many serotypes of E. coli exist. Many of these serotypes appear to be harmless but may act as opportunists (47). Of the serotypes associated with enteric colibacillosis or an infection of the intestinal tract with E. coli, many appear to be host specific in that they produce disease in only one host specie. For instance, serotypes commonly found in pigs are very rarely encountered in cattle, sheep, poultry, or man (48).

Escherichia coli has been associated with three disease syndromes in swine as described by Neilsen (34). These syndromes are: 1) neonatal colibacillary diarrhea, 2) weanling colibacillary diarrhea, and 3) edema disease. These disease syndromes are believed to be due to an

increase in the number of certain serotypes of E. coli which are capable of producing a toxin in the small intestine of the pig which has an effective action of producing disease (34).

Neonatal colibacillary diarrhea usually occurs in the pig during the first few days after birth. In many cases of neonatal colibacillary diarrhea only a few individuals in a litter will be infected initially but once the disease is established, it spreads quickly through the litter and in some instances eventually the entire herd (3). Neonatal pigs with colibacillary diarrhea usually begin scouring around 15 hours after infection (42). Typical scours associated with neonatal colibacillary diarrhea is first creamy in color and consistency but later becomes watery and colorless. Scouring is accompanied by a roughened haircoat, arched back, and signs of dehydration (24). Death usually occurs within 30-33 hours after infection. In some instances, however, individuals recover from neonatal colibacillary diarrhea and begin to improve clinically approximately 4 days after onset of clinical signs (42).

Many of the pathogenic strains of E. coli, which have been associated with neonatal colibacillary diarrhea possess a K 88 antigen (10). Jones and Rutter (19) reported E. coli strains which possess a K 88 antigen adhere to and colonize the intestinal mucosa while K 88 negative E. coli strains did not. However, Nagy, et al. (32) found that K 88 negative E. coli also colonized the intestinal mucosa, resulting in diarrhea and weight loss in colostrum deprived pigs. Truszcrynski and Ciosek (52) additionally found that K antigens were not toxigenic and that enteropathogenic capabilities were strain dependent.

Smith and Halls (47) observed that injection of either cell free

extracts or live cultures in ligated intestinal loops basically produced the same effects. This may be due to the fact that enteropathogenic strains of E. coli are capable of producing enterotoxins which are found in cell free extracts. Thus far, two types of enterotoxins have been characterized. One of the enterotoxins is heat-labile and is inactivated after 15 minutes at 60°C (17). The other is a heat-stable enterotoxin which resists heating at 100°C for 30 minutes (9). Some strains capable of producing neonatal colibacillary diarrhea have been found to produce both of these enterotoxins but not all pathogenic strains produce both heat-labile and heat-stable enterotoxins (41). The ability of an E. coli strain to produce an enterotoxin may be controlled by a transmissible plasmid (41).

At birth the pig appears to be very susceptible to E. coli as well as other pathogenic bacteria. This is possibly due to the fact that there is very little, if any, transfer of maternal antibody across the placenta (4,34) and the lack of sufficient acidity of the stomach of the pig to prevent proliferation of bacteria in the stomach (43). The neonatal pig, may acquire some maternal antibody through ingestion of colostrum. Colostrum has been reported to contain high levels of antibodies in which 60% of the colostral whey protein is immunoglobulins (36). The neonatal pig appears to be capable of absorbing large amounts of these immunoglobulins after ingestion of colostrum during the first 24 hours of life. However, during this time there is a gradual decline in both the rate of absorbtion (7) and in the immunoglobulin level of colostrum (5,13,55). During the first 24 hours of lactation, IgG, the primary immunoglobulin of colostrum (4,13,36), decreases 5 fold; IgA, the primary immunoglobulin of milk (13,36), decreases 3 fold; and IgM

remains relatively constant (13,38). During the first week of lactation there is a 30 fold drop in IgG, but IgA and IgM remain relatively constant (13) at levels found at the end of 24 hours of lactation.

Recently reported studies suggest that pigs ingesting colostrum or serum from sows which have been vaccinated or innoculated with a pathogenic strain of E. coli appear to be better protected from the pathogenic strain of E. coli than pigs ingesting colostrum or serum from non-vaccinated sows or pigs not ingesting colostrum or serum (6,14,35, 50,53). Nagy (31) also reported that neonatal pigs that received colostrum from vaccinated sows showed 6% mortality versus 84% mortality of neonatal pigs that received colostrum from non-vaccinated sows. Also, Dobescru, and Zygraich (15) found that local immunization of mammary glands of sows with heat-labile E. coli enterotoxin was followed by the appearance of antibodies in colostrum which were capable of protecting pigs from this E. coli strain after the first week of life. However, vaccination of sows must occur several weeks prior to farrowing in order to be of any significance in protecting the neonatal pigs against colibacillary diarrhea (6,14,35,50,53).

Neonatal pigs appear to be capable of producing antibodies at a very early age. For instance, Sharp (46) found that antibodies present in the serum of 12 day old pigs were not of maternal origin. Prokesova and Rejnek (40) reported that neonatal-colostrum-deprived pigs were capable of actively synthesizing serum IgA 100 hours after birth. Kim, et al. also (20,21) reported that neonatal germfree pigs devoid of immunoglobulines responded to intraperitoneal injection of antigen with specific 19s IgG production at 48 hours of age. Murray (30), however, suggested that serum antibody levels could not be related to the level

of protection on mucous surfaces such as the small intestine which appear to be the portal of entry for E. coli (34).

At present it is believed by some workers that cells in the small intestine are capable of producing antibodies. Porter, et al. (39) observed that immunoglobulins secreted from the small intestine of the pig included IgM and IgA. Porter (37) also reported that IgM was synthesized during the first week of life and that IgA was prominent from day 10 on. Kohler and Bohl (22) observed that antibodies were detectable in the small intestine within 8 days following oral infection with a nonpathogenic strain of E. coli, but the level of antibody remained relatively low until intravenous vaccination. Corley (11,12) found that gastrointestinal administration of live E. coli resulted in production of specific agglutinins in intestinal secretions four days post inoculation. These agglutinins were found at a greater level in serum than in intestinal secretions 12 days after inoculation. Corley (11,12) also reported that local gastrointestinal administration of a bacterin resulted in increased protection for colostrum deprived germfree neonatal pigs.

Since the neonatal pig does acquire passive immunity through ingestion of colostrum and is also capable of eliciting an immune response at a very early age, it may be possible to combine the effects of passive immunity and active immunity to better protect the neonatal pig from enteropathogenic E. coli. This study was initiated to determine if administration of colostrum with a low antibody titer and possible stimulation of the local immune system of the gut with specific antigen would render protection for the neonatal, germ-free pig against enterpathogenic E. coli 0149, K 88, a.c:H 10 (Abbottstown) and to determine

a minimum lethal dose of E. coli 0149 for 7 day old germ-free colostrum fed pigs.

CHAPTER II

MATERIALS AND METHODS

Maintenance of Bacterial Cultures

A lyophilized culture of Escherichia coli 0149, K 88, a.c:H 10 (Abbottstown) was obtained from Dr. D. A. Barnum and Dr. M. R. Wilson at the University of Guelph; Ontario, Canada. This particular serotype of E. coli has been associated with neonatal colibacillary diarrhea and is known to be pathogenic for neonatal pigs. This lyophilized culture was rehydrated with sterile distilled water. A loop of the rehydrated culture was then inoculated into a sterile tube of tryptic soy broth (TSB).[1] The TSB was then incubated at 37°C for 24 hours at which time 5-7 day old fertile chicken eggs were inoculated with the culture. The eggs were incubated for 24 hours and the contents of the yolk sacs were collected and dispensed into sterile vaccine vials in one-half milliliter alloquots. The vaccine vials were then frozen over dry ice, lypholized, and stored at -15°C. These lyophilized cultures were then used as stock cultures in Experiments I and II.

Preparation of Bacterin to be Used in Vaccination

Thirty-five milliliters of TSB were inoculated with the lyophilized

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[1] Difco Laboratories, Detroit, Michigan.

stock E. coli culture and incubated for eight hours at 37°C. Formalin was then added to provide a final concentration of 0.5% (v/v). The cultures were then incubated at 37°C for an additional 15-18 hours. Prior to the addition of formalin, a sample of the culture was streaked on a blood agar plate[2] and an endo agar plate[3] to check for contamination by other organisms and growth of E. coli 0149. The bacterin was then administered by stomach tube.

Colostrum

Colostrum was collected from sows or gilts within 24 hours of parturition. Colostrum from sows which had hysterotomies was collected within 24 hours after passage of placental membranes.

The colostrum was collected by manually milking the sow or gilt. Prior to milking, the sow or gilt was given 50 units of oxytocin[4] for stimulization of colostrum let down. After collection, the colostrum was filtered through gauze, frozen and stored at -15°C. Each colostrum collection was checked for antibody titers against the "O" and "K" antigens of E. coli 0149. These antibody titers checks were determined by Dr. M. R. Wilson of Syntex Laboratories, Palo Alto, California. Ten fold agglutination tests were conducted to determine the titers. Any colostrum collection with a titer above 40 was eliminated. When enough

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[2] Ibid.

[3] Ibid.

[4] P.O.P. oxytocin injection; Armon-Baldwin Laboratories, Omaha, Nebraska.

colostrum with titers less than 40 had been collected, a colostrum pool was formed and the colostrum was sterilized by using beta propriolactone as described by Amtouer and Calhound (2). Following sterilization, the colostrum was dispensed into sterile bottles in quantities sufficient for one individual pig (125 milliliters).

Challenge Cultures

Tubes containing 10 milliliters of TSB were inoculated with lyophilized stock E. coli culture. The tubes were incubated for 24 hours at 37°C. Plate counts revealed a final concentration of approximately 1.0×10^9 E. coli 0149 per milliliter. Tube dilutions were made of the 24 hour cultures in order to obtain the desired concentration of E. coli organisms per milliliter.

Clinical Observations

Following administration of the challenge dose of live E. coli 0149 the pigs were fed and observed every 6 hours for 144 consecutive hours. Each pig was checked for feces consistency, signs of dehydration, appetite, and mortality (approximate time of death when applicable).

Experimental Animals

Germfree neonatal pigs served as experimental animals. A total of 46 pigs were used. The pigs were delivered from pregnant sows by germfree hysterotomy at 112 days gestation. Anesthesia was induced with intravenous sodium thiopental[5] and maintained with halothane in nitrous

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[5]Penothal, Abbot Laboratories, North Chicago, Illinois.

oxide oxygen as described by Anderson (3). Following anesthesia, germfree pig procurement was carried out following the recommendation of Landy, et al. (25), Meyer, et al. (26), and Trexler (51) and also through use of a germicidal trap. After the pigs were awake and active, they were transferred to a germfree tub isolator where they were housed for the remainder of the experiment. This isolator was maintained at a temperature of approximately 35°C for the length of the experiment.

Sterility Check of the Gnotobiotic System

During pig procurement, bacterial cultures were made of the sow at the incision site and the surface of the uterus by using sterile swabs contained in the surgical unit. Bacterial cultures of the tub isolator and contents (flooring, excreta, feces, feed bowls, etc.) were taken when materials were removed from the isolator. Each original swab was inoculated into a tube of thioglycollate medium[6] and the swab was placed in a tube of TSB. These tubes were incubated at 37°C. An endo agar plate and a blood agar plate were inoculated from the TSB tube following 24-48 hours of incubation. Each plate and tube were checked for bacterial growth daily for 3 days. If bacterial growth was noted, the isolator was considered to be contaminated and the contaminant was identified. Following introduction of the challenge dose and before decontamination of the isolators, cultures were taken of the isolator to determine if *E. coli* was present.

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[6]Difco Laboratories, Detroit, Michigan.

Experimental Procedure

Experiment I

The first experiment was performed to determine a minimum lethal dose for E. coli 0149 in 7 day old germfree pigs. Nine littermate pigs each received 10 mls of colostrum every 4-5 hours for 48 hours after birth. They were then maintained on an artificial diet[7]. At 7 days of age each pig was given a predetermined number of live E. coli organisms. Three groups of three pigs were given 10^2, 10^4, or 10^6 E. coli organisms in a one milliliter saline solution. The minimal dose which produced death of the pigs was used as the challenge dose in Experiment II.

Experiment II

The second experiment was designed to determine if colostrum and/or an oral vaccine would protect neonatal germfree pigs from enteric colibacillosis caused by E. coli 0149. Five litters consisting of 37 pigs were utilized. Four treatment groups were set up using a 2 x 2 factorial. Each pig in each litter was randomly assigned to one of these groups. The following diagram illustrates the treatment groups and the variables investigated:

	Colostrum	No Colostrum
Oral Vaccine	Group 1	Group 2
No Oral Vaccine	Group 3	Group 4

[7] SPF-lac, Borden Company, New York, New York.

Pigs assigned to Group 1 received colostrum and oral vaccine. Those assigned to Group 2 received oral vaccine but no colostrum. Pigs assigned to Group 3 received colostrum but no oral vaccine. Those assigned to Group 4 did not receive colostrum or oral vaccine. The oral vaccine was the bacterin described previously. Ten milliliters of oral vaccine was administered at birth, 2 days of age, and 4 days of age. Ten milliliters of colostrum was administered by stomach tube 4 hours after birth and every 4-5 hours thereafter for a total of 48 hours. At 7 days of age, all pigs received a dose of live E. coli 0149 by stomach tube. The pigs were then observed for clinical signs. All pigs in this experiment were maintained on SPF-lac for the length of the experiment with the exception of Groups 1 and 3. These two groups received colostrum for the first 48 hours of life and were not fed SPF-lac during this period of time.

Statistical Analysis

An analysis of variance and t-tests were performed on the data to determine statistical significance. A t value greater than 0.05 was not considered statistically significant.

CHAPTER III

RESULTS

Antibody Titer Checks of the Colostrum Samples

Antibody titer checks of the colostrum samples revealed that there was antibody activity present against the "O" (somatic) and "K" (capsular) antigens of Escherichia coli 0149 (Table I). These colostrum samples were taken from Yorkshire and Hampshire sows of varying ages. The colostrum pool which was formed contained 4920 milliliters of colostrum. The antibody titers against the "O" antigen ranged from a high titer of 20 to less than 10 (one colostrum sample had an antibody titer of 20, one had an antibody titer of 10, and five had antibody titers of less than 10 for the "O" or somatic antigen of E. coli 0149. The mean "O" antibody level of the colostrum pool was less than 12.

Antibody titers of the colostrum samples against the "K" (capsular) antigens of E. coli 0149 ranged from 40 to less than 10. There was only 1 colostrum sample that had an antibody titer of 40 and only 1 colostrum sample that had an antibody titer of 20. Two colostrum samples had antibody titers of less than 10. The colostrum pool that was formed had a mean antibody titer of less than 13 for the "K" or capsular antigens of E. coli 0149.

TABLE I

COLOSTRAL ANTIBODY TITERS AGAINST *E. COLI* 0149
"O" SOMATIC AND "K" CAPSULAR ANTIGENS

Sample Number	Titers Against the "O" (Somatic) Antigen[a]	Titers Against the "K" (Capsular) Antigen[b]
1	< 10	< 10
2	< 10	10
3	< 10	< 10
4	< 10	40
5	10	10
6	< 10	< 10
7	20	20

[a]"O" antigens were prepared from 18 hour broth culture; heated 2 hours in the antoclave and washed 3 times with sterile PBS.

[b]"K" antigens were prepared from 18 hour broth culture; cells were washed 3 times and suspended in 0.2% (v/v) formalin.

Minimum Lethal Dose Determination

Experiment I

The results of Experiment I which was conducted to determine a minimum lethal dose of E. coli 0149 in 7 day old germfree colostrum fed pigs are illustrated in Table II. Death was produced in all of the pigs given the live E. coli regardless of the number of live organisms given. Clinical signs consisting of diarrhea, depression, and absence of appetite were noted in all the pigs at 21 hours post challenge. In all of the pigs, dehydration and eventually death followed at 23.0 to 37.5 hours post challenge.

Two pigs in Group I survived longer after challenge than the six pigs in either Group II or Group III. The three pigs in Group I survived 37.5 hours, 31.5 hours, and 29.0 hours with a mean survival time of 32.6 hours. The three pigs in Group II survived 29.5 hours and 29.0 hours with a mean survival time of 29.2 hours. The mean survival time of Group III was the shortest (27.2 hours). The three pigs in Group III survived 29.5 hours, 23.0 hours, and 29.0 hours. The nine pigs in Experiment I had a mean survival time of 29.7 hours.

Prevention of Enteric Colibacillosis Through Use of Colostrum and an Oral Vaccine

Experiment II

The results of Experiment II are shown in Table III and Table IV. In Experiment II, 31 out of 37 (83.8%) of the pigs which received 1×10^2 live 24 hour old E. coli organisms died.

TABLE II

SURVIVAL TIME OF GERMFREE PIGS WHICH RECEIVED COLOSTRUM AND WERE CHALLENGED WITH A KNOWN NUMBER OF 24 HOUR OLD LIVE *E. COLI* 0149 ORGANISMS AT 7 DAYS OF AGE

Pig Number	Group Number	Number of Organisms Given	Survival Time
1	I	1×10^2	37.5 hours
8	I	1×10^2	31.5 hours
10	I	1×10^2	29.0 hours
3	II	1×10^4	29.5 hours
7	II	1×10^4	29.0 hours
9	II	1×10^4	29.0 hours
4	III	1×10^6	29.5 hours
6	III	1×10^6	23.0 hours
11	III	1×10^6	29.0 hours

TABLE III

SURVIVAL TIME OF EACH GERMFREE PIG IN EXPERIMENT II RECEIVING ONE OF FOUR TREATMENTS FOLLOWED BY A DOSE OF E. COLI 0149, AT SEVEN DAYS OF AGE

Group I[a]		Group II[b]		Group III[c]		Group IV[d]	
Pig[e]	Hours[f]	Pig	Hours	Pig	Hours	Pig	Hours
2-1	77	2-5	112	2-2	111	2-4	105
2-3	111	2-6	111	3-6	144(survived)[g]	3-5	60
3-2	120	3-1	48	3-9	60	4-6	41
3-8	144(survived)[g]	4-1	29.5	4-8	35	4-7	41
4-3	41	4-2	41	5-5	40.5	4-10	35
4-9	35	4-4	41	5-7	63.5	5-1	21.5
5-3	57.5	4-11	35	5-8	40.5	5-2	51.5
5-6	21.5	5-10	40.5	5-9	21.5	5-4	33.5
6-1	144(survived)[g]	6-3	144(survived)[g]			6-2	144(survived)[g]
6-4	144(survived)[g]	6-6	144(survived)[g]				

[a]Pigs received colostrum and oral vaccine.

[b]Pigs received oral vaccine only.

[c]Pigs received colostrum only.

[d]Pigs receifed neither colostrum or oral vaccine.

[e]Expressed as litter and pig number.

[f]Expressed as hours survived after challenge.

[g]For statistical analysis a value of 144 was assigned to those individuals which survived because the total observation period was 144 hours for each litter.

TABLE IV

MEAN SURVIVAL TIMES OF EACH GROUP OF PIGS IN EXPERIMENT II FOLLOWING ADMINISTRATION OF 1×10^2 LIVE *E. COLI* 0149 ORGANISMS

Group Number	Treatment	Mean Survival Time of all Pigs[b]
I	Colostrum plus oral bacterin	89.5 ± 15.4[a] (10)[c]
II	Oral bacterin only	74.6 ± 15.0[a] (10)[c]
III	Colostrum only	64.5 ± 14.9[a] (8)[c]
IV	No colostrum or oral vaccine	59.2 ± 13.3[a] (9)[c]

[a] Standard error.

[b] These values include a value of 144 hours for each pig that survived the challenge.

[c] Number of pigs in group.

Pigs in Group I which received colostrum and oral vaccine survived from 21.5 hours post challenge to 120 hours post challenge with three pigs surviving the challenge. Ten pigs were utilized. The mean survival time of the pigs in Group I which did not survive the challenge was 66.1 hours. The percent survival of the pigs in Group I was 30%. Two of the three pigs which survived were litter mates and the three pigs which survived were from sows out of the same herd.

Pigs in Group II received oral vaccine only. Ten pigs were utilized. Death was produced in eight of the ten pigs in 29.5 to 112 hours post challenge. The mean survival time of the pigs in Group II which did not survive the challenge was 57.3 hours. Twenty percent of the pigs challenged survived and the two pigs which survived were litter mates.

Eight pigs were used in Group III and received colostrum only. Death was produced in 87.5% of the pigs challenged and only 12.5% or 1 pig survived the challenge. The mean survival time of the pigs which did not survive in Group III was 53.2 hours with a range of 21.5 hours to 111 hours.

Pigs in Group IV were utilized as controls. Nine pigs were challenged and only 11.1% of the pigs in Group III survived. The mean survival time of the pigs which died was 48.6 hours with a range of 21.5 hours to 105 hours.

Although there was no significant difference ($P < 0.05$) between survival times of any of the groups. There was some difference in mean survival time of all the pigs within each group (Table IV). Also, there was no significant difference between the survival times of the two groups that received colostrum (Groups I and III) ($t = 1.61$, d.f. = 16)

and the two groups that did not receive colostrum (Groups II and IV) ($t = 0.77$, d.f. = 17). However, an analysis of variance indicated that there was significant variation (F ratio of 26.8 with 36 degrees of freedom) between litters but no significant variation ($P < 0.05$) between any of the 4 groups. An analysis of variance of Experiment II is illustrated in Table V.

Of the seven pigs which survived the challenge in Experiment II five were from litter 6 and the other two were from litter 3. These pigs all developed slight clinical signs consisting of varying degrees of diarrhea and inappetance but eventually their conditions improved and they recovered.

TABLE V

ANALYSIS OF VARIANCE ON Y (HOURS SURVIVED) AFTER ADMINISTRATION OF 1 x 10^2 LIVE *E. COLI* 0149 ORGANISMS TO 7 DAY OLD GERMFREE PIGS

Source	d.f.	S.S.	M.S.	F Ratio
Total	37-1 = 36	71615.2		
Between litters	4	58391.4	14597.9	26.8[a]
Between treatments	3	724.8	241.6	<1[b]
Between col. levels	1	712.1		1.3[b]
Between vaccine levels	1	10.9		<1[b]
Between col. and vaccine levels	1	1.8		<1[b]
Exp. error (litter and trt.)	11	5980.9	543.7	
Sampling error				
(Between pigs in litter and trt.)	18	6518.1	362.1	

[a] This value is statistically significant ($P < 0.001$).

[b] These values are not statistically significant ($P > 0.05$).

CHAPTER IV

DISCUSSION

Results of the antibody titer checks of the colostrum samples showed a small amount of antibody present in the colostrum against the "O" and "K" antigens of Escherichia coli 0149. The mean antibody titers were less than 12 for the "O" antigen and less than 13 for the "K" antigens. These antibody titers were similar to the low titers that Arbuckle (4) found in first and second litter sows which showed antibody titers of 6.6 and 7.0 respectively. These antibody titers are quite low which may be due to a lack of prior exposure of the sows to the 0149, K91, and K88 antigens. Vaccination of the sows with E. coli 0149 or several weeks prior to farrowing and colostrum collection, would have possibly resulted in higher levels of colostral antibodies. From work which has been done by Nagy (31), Dobrescru and Zyqraich (15), Wilson (53), and others, it appears possible to produce a high level of protective antibodies in the colostrum of sows and gilts through prior exposure of the animal to specific immunogens.

The results of Experiment I demonstrate the ability of E. coli 0149 to produce neonatal colibacillary diarrhea in colostrum fed 7 day old germfree pigs. This is in agreement with Ciosk and Truszczynski (10) and Sojka (49) who reported that this strain or serotype produced neonatal colibacillary diarrhea and death in pigs. Experiment I also demonstrated that neonatal colibacillary diarrhea and death can be pro-

duced with varying numbers (10^2, 10^4 or 10^6) of live E. coli organisms in germfree pigs given colostrum. Saunders, et al. (42) also found this to be the case in pathogen-free pigs. The average survival time of the three groups post challenge slightly decreased as the number of E. coli organisms given increased. This is possibly due to the fact that the larger the dose, the less time it took for proliferation of sufficient numbers of E. coli organisms to produce a sufficient amount of enterotoxin to produce neonatal colibacillary diarrhea. The average survival time after challenge with E. coli 0149 of all pigs in Experiment I was 29.7 hours. These results are similar to those of Saunders, et al. (42) who found that death occurred 30-33 hours after challenge of 12 hour old pathogen-free pigs.

Clinical signs of diarrhea, depression and inappetance were seen at 21 hours post challenge. These results are again similar to those of Saunders, et al. (42) who noted that clinical signs were manifested between 15 and 36 hours post challenge in 12 hour old pathogen-free pigs.

The results of Experiment I also suggests that a very low antibody titer in colostrum (less than 12 and 13) does not provide sufficient antibody levels to protect the neonatal pig from pathogenic E. coli. Perhaps if a larger amount of colostrum would have been administered over a greater length of time or if colostrum with a higher antibody titer would have been used, better protection of the pigs would have resulted.

Experiment I indicated that 1×10^2 live E. coli 0149 organisms would produce death in 7 day old colostrum fed germfree pigs. Experiment II was designed to determine if there was a difference in survival

time or survival rate between germfree pigs which received colostrum and an oral vaccine, colostrum only, oral vaccine only, or no oral vaccine or colostrum.

Even though there was no significant difference ($P < 0.05$) between the survival time or percent survival of any of the four groups, there was some difference between these values. Pigs given the colostrum and the oral vaccine survived the longest mean length of time (89.5 hours) and had the highest percent survival (30%). The pigs receiving oral vaccine only had the second longest mean survival time (74.6 hours) and the second largest percent survival 20%. The pigs receiving the colostrum only survived a mean time of 64.5 hours and only 12.5% of this group survived while the controls which received neither colostrum or oral vaccine survived the shortest average length of time (59.2 hours) and showed the lowest survival rate (11.1%) of the four groups. These results suggest that possibly the combined effects of the colostrum and the oral vaccine resulted in a longer average survival time and a higher percent of survival. Hoerlein (18) reported that passive transfer of antibodies through colostrum did not interfere with active antibody production in the 3 week old pig. Miniats and Ingram (28), however, found that passive transfer of antibodies through colostrum interferred with active antibody production. Some authors have suggested that prior exposure to antibodies is required before active antibody production occurs (44). This may account for the fact that the pigs which received colostrum and the oral vaccine survived the longest and a higher percent of them survived than did the pigs which received the oral vaccine only.

The results also indicate that pigs which received colostrum or

the oral vaccine may have been somewhat better protected against the effects of the E. coli. Work has been done that suggests that colostrum does contain antibodies that lead to increased resistance of the pig against neonatal colibacillary diarrhea. Svendson and Wilson (50) found that pigs fed colostrum from sows which had been vaccinated with a specific strain of E. coli resulted in better protection than pigs which were fed colostrum from non-vaccinated sows. Brandenburg and Wilson (6) also found this to be true. Corley's (11,12) work suggested that possibly administration of an oral vaccine resulted in protection of the neonatal pig against colibacillary diarrhea and that antibody production resulted. Kohler and Bohl (22) also found that oral infection of gnotobiotic pigs with E. coli resulted in slight antibody production.

The results of Experiment II also suggested that there was a significant amount of variation in survival times between the litters used in this experiment. For instance, all of the pigs in litter 6, regardless of treatment, survived. Moon, et al. (29) also found that there were differences between litters when he attempted to reproduce enteric colibacillary diarrhea in pigs. Sellwood, et al. (45) found that litters sired by certain boars possessed a non-adhesive characteristic which prevented adhesion of E. coli to brush borders of the small intestine. Wjerantne, et al. (54) also found that there was a difference in susceptibility to colibacillary diarrhea between offspring of certain sires. Wjerantne, et al. (54) and Sellwood, et al. (45) suggest that a basis of selection of sires may be based upon resistance to E. coli. Sellwood, et al. (45) go on to suggest that the non-adhesion characteristic is genetically controlled by two alleles in which case adhesion is dominant over non-adhesion. The results of the present

study and previous studies suggest that more work is warranted in the area of possible genetic effect on prevention of neonatal colibacillary diarrhea as well as other pathological processes in the pig.

The results of this study suggest that production of neonatal colibacillary diarrhea in seven day old colostrum fed germfree pigs can be achieved with as few as 1×10^2 *E. coli* 0149. Results also suggest that there was no significant difference between the survival time and percent survival of pigs which received low titer colostrum and oral vaccine, colostrum only, oral vaccine only; or neither colostrum or oral vaccine; however, there was a slight difference in the mean survival time and percent survival. Perhaps if more pigs had been used in this study, statistical significance may have been shown; however, the expense of obtaining and maintaining germfree pigs prohibited use of large numbers.

There was significant variation between litters which indicated there may have been a genetic effect. Use of the same sire and littermate sows or highly inbred sows from a single herd may have reduced the amount of variation between individuals of different litters in the same group.

Results from the present investigation and others suggest that possibly an immune response is illicited in the presence of low titer colostrum and immunogens in the germfree, neonatal pig. The present study also suggests that more work is needed to determine if indeed this does occur. Work is also needed to determine if hyperimmune colostrum and stimulization of the local immune system of the gut will result in a high level of protection for the neonatal pig. The results of this study and other studies indicate that stimulization of the local immune

system of the neonatal pig in an attempt to increase protection may be a fruitful means of protecting pigs from colibacillosis. The mechanisms by which this stimulization is achieved and the level of stimulization needed is presently a matter of speculation.

CHAPTER V

SUMMARY

This study was conducted to determine if administration of oral immunogens and/or colostrum with low levels of antibodies would render protection for the neonatal germfree pig against homologous challenge with E. coli 0149 and to determine a minimum lethal dose of E. coli 0149 in 7 day old germfree colostrum fed pigs.

Oral administration of live E. coli 0149 organisms to 7 day old germfree pigs which had received colostrum with a low level of antibodies against the "O" (somatic) and "K" (capsular) antigens of E. coli resulted in death of the pigs 30 hours after administration. Also, oral administration of 1×10^2 live E. coli 0149 organisms to 7 day old neonatal pigs which received the previously described colostrum and a homologous orally administered bacterin, or only colostrum or bacterin, or neither colostrum or bacterin resulted in no significant difference in survival time or survival rate of the pigs receiving the different treatments. However, there was significant variation between survival times in litters of pigs regardless of treatment. The results of this study suggest that more work is warranted to determine if colostrum and oral immunogens will protect the neonatal pig against E. coli and what role genetics may have in protecting the neonatal pig against pathogenic agents.

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VITA

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