RESEARCH REPORT
PORK SAFETY

Title: A Survey of Patterns and Persistence of Antimicrobial Resistance on Swine Farms Using Three Different Antimicrobial Use Strategies
NPB# 98-236

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Abstract

This study characterizes antimicrobial resistance patterns on three farms with varying antimicrobial use strategies. Farm A uses antimicrobics extensively in the feed and as needed for individual animal therapy. Farm B uses antimicrobics in the feed on a limited basis and as needed for individual animal therapy. Farm C has not used any antimicrobics in the past 28 years. Salmonella, campylobacter, and E. coli isolates are characterized.

Salmonella and campylobacter prevalence between farms are not consistent. The highest Salmonella prevalence is on Farm A and the lowest Salmonella prevalence occurs on Farm C. There are no common serotypes on any of the farms suggesting that there is geographic grouping of isolates which may be influenced by production type, facilities, presence of other livestock in the area, and other factors. The prevalence of Campylobacter was high on all farms. Of the Campylobacter isolated, all were Campylobacter coli as determined by PCR.

Characterization of azithromycin, clindamycin, erythromycin, and tetracycline resistance patterns in campylobacter suggests an association with stage of production. The same pattern occurs with and flofenicol in salmonella. Sows, both gestating and lactating (farrowing), have the least detectable resistance. Suckling, finisher, and nursery pigs, respectively demonstrate higher levels of resistance. This finding is consistent across farms and suggests an age or stage of production associated effect on phenotypic resistance.

Significant antimicrobial resistance variation exists between farms. Farm C tended to have less resistance overall than did Farms A and B. However, resistance testing results on Farm C Campylobacter, Salmonella and E. coli isolates suggest that antimicrobial resistance can persist on farms for long periods of time after discontinuation of antimicrobial use. It is likely that factors other than antimicrobial use influence persistence of resistance.

For pork producers considering restricted use of antimicrobics to improve pork safety, results of this study suggest that resistance will decrease with withdrawal. However, resistance is likely to persist. Further, the degree of resistance reduction is inherently linked to the type of bacteria surveyed, the antimicrobics for which resistance is monitored, and the age of the animals surveyed. Antimicrobial use is an apparent contributor to resistance outcomes, however, factors other than antimicrobial use affect antimicrobial resistance outcomes.

Introduction

Bacterial resistance to antimicrobial agents has emerged as a global concern. Thus, antimicrobial use in both human and animal populations is being scrutinized. Of particular concern is resistance seen in nosocomial, commensal and zoonotic pathogens. As zoonotic agents, resistant bacteria within livestock are proposed to be a significant risk to human health. Fueling this concern is the widespread detection of multi-resistant enteric pathogens, such as Salmonella typhimurium DT104. Use of antimicrobials is contraindicated in most cases of human foodborne illness, however, immune status, septicemia, and other factors may warrant their use. Increased morbidity and mortality in these cases may result if targeted pathogens are resistance to the prescribed treatment. Because of these concerns, there is a need to understand factors affecting antimicrobial resistance determinants as detected in livestock populations.

Benefits of Antimicrobial Use in Livestock

There are three justifications put forth for the use of antimicrobials in livestock; for improved productivity, for improved human health, and for relief of animal pain and suffering. In pig production, antimicrobials are valuable tools [1, 2], and are widely used. Results of the 1995 National Animal Health Monitoring System survey of swine farms indicate that 92.7% use feed additive antimicrobials (USDA-APHIS-VS-CEAH 1995). Thus use restrictions have significant implications for pork producers. Beran [3] suggests that antimicrobial use in livestock benefits human health by decreasing the incidence of zoonotic diseases such as leptospirosis, anthrax, and ornithosis. The most obvious benefit of livestock antimicrobial use is relief of animal pain and suffering. Proponents of use restrictions suggest that with proper husbandry, similar productivity, health and welfare can be accomplished. Livestock groups agree that good husbandry is essential for health, but are concerned that good husbandry alone is insufficient, particularly when dealing with endemic diseases or acute outbreaks. Further, foregone feed efficiency and rate of gain in pig production are not likely to be recovered through good husbandry alone [4]. The benefits of antimicrobial use must be weighed against potential human health risk, however.

The Status of Public Policy On Agricultural Antimicrobial Use

In the U.S., multiple reviews have been conducted by both government and private agencies into the effect of livestock antimicrobial use on human health [4-15]. Each suggests that there is justification for concern though there is no concrete data which suggests that harm has resulted from antimicrobial use in animals. All agree that the matter needs further study.

In Europe, a very different scenario has unfolded. In 1971, the British limited animal antibiotic use, particularly feed additive antibiotics, to prescription use only (the Swann Report) [16]. Sweden, in 1971 also limited the use of feed additive antimicrobials [17]. In 1999, the European Union banned the used of virginiamycin, spiramycin, tylan, and bacitracin as feed additives. Two recent World Health Organization plenary conferences called for greater monitoring and restricted use if warranted [18, 19]. In both the U.S. and foreign countries the greatest concern is the use of feed additive antimicrobials, particularly those used for growth promotion and feed efficiency.

In response to these concerns, the Food and Drug Administration Center for Veterinary Medicine has proposed a new framework for drug approvals [20]. The framework calls for restricted use if 1) the antimicrobial is considered important in human medicine: 2) post approval monitoring indicates increasing resistance development; 3) consequential pathogen load increases; and/or 4) the antimicrobial is used for mass treatment of herds i.e, feed additives or water medication. First application of these principles is evident in FDA/CVM's November 2000 published intent to withdraw all approvals for use of fluoroquinoles in poultry.

The Issue

Proponents of livestock antimicrobial use restrictions believe that high level antimicrobial use in livestock creates human health risk. There are five fundamental premises for this position:

- livestock antimicrobial use selects for resistant bacteria;
- the more extensive the use, the greater the potential for resistance development and/or expression;
- discontinued use will result in loss of resistance determinants within populations resulting in less human health risk
- resistant bacteria are easily and frequently passed through the food chain; and,
- once passed to humans, resistant bacteria frequently pass resistance determinants to bacteria residing in the human host, some of which are pathogens.

In reverse order, the following reviews what is known about each of these premises.

Resistance transfer

While the etiologic fraction has not been determined, transmission of resistance factors [21, 22] as well as transmission of the bacteria through food consumption has been documented. New molecular analytic techniques have improved our understanding of resistance mechanisms and resistance transfer. Resistant genetic material is not only mobile within the bacteria [23], but is also transferrable between bacteria [24].

Resistant genes can be mobilized and transferred between bacteria by plasmids, phage, conjugation, and accumulation of unbound or free DNA. Because of the ease of

gene transfer, it is postulated that clinically significant resistance is primarily acquired from other bacteria. Little is known about the presence of resistance genes in relatively innocuous bacteria such as commensals and Campylobacter coli, however, it is very possible that these organisms are significant and may even be the primary reservoirs of resistance. Samples from soil, water, and feed suggest that resistance resides not only within enteric flora, but also within environmental flora [25]. The degree that resistance within environmental flora contributes to resistance within animal flora has not determined. However, genetic sharing is suggested to be frequent.

Mobile genetic elements containing resistance genes may be transposed within and between bacteria. Discovery of resistance transfer mechanisms, such as transposons and integrons, [23, 24, 26] have greatly improved our understanding of bacteria-to-bacteria resistance transfer. Interspecie transfer of nucleic acids occurs through free, plasmid, and chromosomal units. In some circumstances these units bear resistance determinants. Transposons or "jumping genes" have the potential to move depending upon environmental pressures. Additionally, resistance genes relocated within gene cassettes. The mobile genetic elements, known as integrons, may be relocated within plasmids or chromosomes based again on environmental pressures. Integrons also play a role in dissemination of genes between bacteria. Even though these mechanisms are not well understood, it is likely that they impact phenotypic resistance variance and thus prevalence.

There is considerable debate within the scientific community as to how frequently genetic acquiescence occurs [27]. In fact, this is one of the key elements of the debate over agricultural antimicrobial use. It is suggested that the odds of resistance transfer increases the more closely related the bacteria. The possibility of resistance transfer is particularly alarming to public health officials. They are concerned that genes encoded for resistance may be transferred to multiple species of bacteria resulting in significant risk to human health.

Zoonotic Potential

The incidence of foodborne disease is reported (CDC Morbidity and Mortality Report, however, the range of occurrence is highly variable and is significantly confounded by storage and handling. Further, the degree that farm level, abattoir, and processing contribute to passage of resistance through the food chain is unknown. Thus, the real rate of resistant determinant transfer through the food chain can only be postulated.

Effect of Discontinued Use

There few reports on the effect of discontinued antimicrobial use on resistance prevalence. In 1972 the University of Kentucky discontinued both therapeutic and subtherapeutic use of antibiotics at the Kentucky Agricultural Experiment Station Farm in Princeton, KY. Change in the antimicrobial resistance pattern was monitored for the next 126 months. Tetracycline resistance in fecal coliforms isolated from swine decreased nearly 50% from 82 to 42% after antimicrobial use was discontinued [28]. Although the farm has continued to be operated free of antimicrobials since 1983, patterns of antimicrobial resistance have not been reassessed (Cromwell, personal communication). Further, over the long term, prevalence of resistant organisms appears to be antimicrobic specific.

Effect of Extensive Use

There are no farm level studies examining the effect of extensive antimicrobial use on resistance prevalence, though empirical data suggest that resistance prevalence increases.

Selective Pressures Affecting Resistance Prevalence

Studies suggest that multiple environmental and management factors are associated with phenotypic expression of resistance. For example, phenotypic resistance can vary depending on pig age, and housing [29], as well as the occurrence of stress events such as moving and transportation [30]. It is not surprising that pigs on different antimicrobics exhibit varying phenotypic patterns [31, 32]. Dosage level [33] and duration of treatment may affect phenotypic expression [34]. Even the degree of multiple resistance can be affected by antimicrobial regime [32]. Further, a bacteria X drug X therapeutic regime interaction appears to exist [34]. It is not known whether the persistence of resistance varies depending on the type of bacteria surveyed within herd settings. Nor is it known whether or not mass treatment use as feed additives or water medication affects resistance prevalence. Because of these potential confounders, it is not surprising that interpretation of phenotypic data is imprecise.

Gene Effects on Resistance Prevalence

Diversity of resistance genes within bacteria affects phenotype outcomes. In a study by Lee [35] bacterial isolates were collected from three farms with differing antimicrobial use strategies. Bacteria, phenotypically demonstrating resistance to tetracycline, were genotypically evaluated. Within those isolates with tetracycline resistance, there was a great deal of genotype variation. Multiple tetracycline resistant genotype variants were identified both within and across farms. This study demonstrates that genetic diversity affects the degree of resistance expression. Thus, singular or multiple genes expressing resistance to the same antimicrobic may be present within isolates, and this diversity profoundly affects bacterial resistance character.

Summary

There is a need to better understand 1) how extensive antimicrobial use on farms affects resistance prevalence, 2) how discontinued use affects resistance prevalence, and 3) how various bacteria respond to differing antimicrobial use strategies. Guidance on these matters will be particularly beneficial to pork producers considering antimicrobial free production. Further, without this characterization, there will likely be greater political pressure calling for restrictions based upon the “imminent hazard provision of the Food, Drug, and Cosmetic Act”.

Objectives

Our goal is to execute an epidemiologic study which characterizes the relationship between various antimicrobial use strategies and antimicrobial resistance. Our first specific objective is to determine if the level of antimicrobial use impacts the development of antimicrobial resistance as evidenced in E. coli, Salmonella sp., and Campylobacter sp. isolated from swine farms. Our second specific objective is to determine the prevalence of *E. coli, Salmonella* sp., and Campylobacter sp. on farms employing different antimicrobial use strategies. Our third objective is to determine what effect does prolonged discontinued use of antimicrobials have on resistance.

Procedures

Sampling Procedures

Farms were sampled once every three months. Approximately 150 samples (30/group) were collected from farrowing, gestating, suckling, nursery, and finishing pigs. Samples were transported overnight to the laboratory and cultured. From Farm A, 750 samples were collected, from Farm B 600 samples were collected, and from Farm C 509 samples were collected. A herd history was obtained from each farm.

Bacteriology

Salmonella spp. were isolated from fecal samples using primary (GN Hajna and tetrathionate, Difco®) and secondary selective enrichment (Rappaport R-10, Difco®) followed by isolation on selective plating media (BGSulfa and XLT4, Difco®). Presumptive positive colonies were inoculated into triple sugar iron and lysine iron agar for biochemical conformation. Presumptive positive isolates were then serogrouped by slide agglutination using serogroup specific antisera (Difco®) and were sent to National Veterinary Services Laboratory for serotyping.

For isolation of Campylobacter, fecal dilutions (1:4 and 1:40, feces/PBS) were direct plated (100ul) onto Campy-Cefex agar and incubated under micraerobic (5% O2, 10% CO2 and 85% N2) conditions 48 h at 42 C. All campylobacter isolates were speciated by PCR.

Generic E.coli were isolated using Petrifilm EC (3M company) as per manufactures directions.

Antimicrobial Resistance Testing

Salmonella and E. coli isolates were tested for resistance to antimicrobials using a semi-automated system (Sensititre®) as per the manufacturer's directions (Trek Diagnostics, Westlake, OH). Representative campylobacter isolates were selected across stages of production and tested for resistance to antimicrobials using the E-Test (ABBiodisk).

Statistical Analysis

Because most of the outcome variables have a binary response (SUSC or RESIS), the logistic regression model was fitted. All the data analysis were carried out by SAS procedure PROC GENMOD. Whenever there are significant effect(s), the corresponding probability estimates and their confidence intervals are used for graphic presentation.

Results

Prevalence of Salmonella and Campylobacter (Tables 1 2, and 3)

Of the 750 samples from Farm A, seventy-five (10%) of the samples were positive for *Salmonella* with the order of recovery frequency as follows: nursery (23.3%), farrowing (10.7%), gestation (7.3%), suckling (7.3%) and finishing (1.3%). Six serogroups have been identified with serogroup B identified most often (76%). Fifteen serotypes have been identified, an untypable 4,12:1-monophasic has been identified most often (62.7%) followed by *S. infantis* (9.3%) and *S. typhimurium* (4%). From three of the five visits, samples were cultured for *Campylobacter* and 129/450 (28.7%) of the samples were positive. *Campylobacter* was most often recovered from nursery (38.9%) followed by suckling (30.0%), gestation (26.7%), finishing (24.4%) and farrowing (23.3%) pigs.

For Farm B, 600 samples have been cultured for *Salmonella*. Thrity-eight (6.3%) of samples were positive for *Salmonella* with the order of frequency of recovery as follows: farrowing (17.5%), gestation (10%), suckling (2.5%) and nursery (1.7%). No *Salmonella* was recovered from finishing pigs. Only two serogroups (B [76.3%] and E [23.7%]) and three serotypes *S. derby* (76.3%), *S. london* (21.1%) and *S. anatum* (2.6%) were identified. Of the 600 samples, 450 were cultured for *Campylobacter*. Fifty-five were positive for *Campylobacter* (12.2%) which was recovered most often from suckling (18.9%), farrowing (12.2%), nursery (11.1%), finishing (10%), and gestation (8.9%) pigs.

For Farm C five visits have been completed and a total of 509 samples have been cultured for *Salmonella*. Eight (1.6%) of samples were positive for *Salmonella* with the order of frequency of recovery as follows: gestation (4.7%), farrowing (2.9%) and nursery(0.6%). No *Salmonella* was recovered from suckling or finishing pigs. Four serogroups were identified with serogroups D and C2 occurring most often (37.5% each). Of the five serotypes identified, *S. newport* was the most common serotype (n=3; 37.5%). Of the 509 samples, 405 were cultured for *Campylobacter*. One hundred-eight (26.7%) were positive for *Campylobacter* which was recovered most often from the nursery (48%), followed by suckling (25%), farrowing (21.4%), gestating (18.2%), and finishing (12.4%), pigs.

Salmonella and *Campylobacter* prevalence between farms is not consistent and that the highest *Salmonella* prevalence occurs on Farm A which uses antimicrobials both therapeutically and subtherapeutically. Conversely, the lowest prevalence of both *Salmonella* and *Campylobacter* occurs on Farm B which uses minimal antimicrobials. However, the lowest *Salmonella* prevalence, but highest *Campylobacter* prevalence, occurred on Farm C which has not used antimicrobials in the recent past. Campylobacter isolates from all farms were determined by PCR to be Campylobacter coli.

Antimicrobial Resistance in Salmonella Isolates (Figure 1 and 2)

From Farm A, 10.6% (n=75) of the Salmonella isolates demonstrated resistance to any of the antimicrobials tested. One isolate (1.3%) was resistant to one antimicrobial and seven isolates (9.3%) were resistant to two or more (range 2-4) antimicrobials. For Farm B, 76% of the Salmonella isolates demonstrated resistance to two or more antimicrobials. Farm C Salmonella isolates were sensitive to all antimicrobials tested. The vast majority of the resistance in Farms A and B was to tetracycline, streptomycin, and sulfamethoxazole.

Antimicrobial Resistance in Campylobacter Isolates (Figures 3, 4 and 5)

Fifty eight Campylobacter isolates from Farm A were evaluated for antimicrobial resistance. Isolates expressing resistance to a single antimicrobial were resistant to tetracycline. Of all the campylobacter surveyed, 41.4% were resistant to two or more antimicrobials.

Forty-four Campylobacter isolates from Farm B were evaluated for antimicrobial resistance. Forty percent of isolates examined for resistance from Farm B were resistant to a single antimicrobial, while 27.3% of the isolates tested were resistant to two or more antimicrobials.

Forty-three Campylobacter isolates from Farm C were evaluated for antimicrobial resistance. Of these, 11.6% expressed resistance to a single antimicrobial, while one isolate (2.3% of those examined) was resistant to two or more antimicrobials. This finding suggests that antimicrobial resistance can persist on farms for long periods of time after discontinuation of antimicrobial use, albeit at low levels.

The majority of resistance detected in campylobacter was to azithromycin, clindamycin, erythromycin, and tetracycline. This is particularly true for Farms A and B.

Statistical Analysis

Where significant differences are detectable (Tables 4,5, and 6), graphic comparisons of resistance are shown (Figures 6-19). Confidence intervals (vertical lines) are shown for each farm. Overall, there is less resistance in Farm C as compared to the other two farms. Farm B demonstrates less resistance than Farm A, however, the difference is less evident.

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Table 1

Salmonella and Campylobacter Prevalence and Percent Resistance				
Farm	Salmonella		Campylobacter	
	Prevalence	Resistant (%)	Prevalence	Resistant (%)
A	10.0	10.7	28.7	62.1
B	6.3	73.6	12.2	67.4
C	1.6	0	26.7	13.9

Table 2

Prevalence of Salmonella by Stage of Production (%)			
Farm	A	B	C
n	75	38	8
Gestation	7.3	10.0	4.7
Farrowing	10.7	17.5	2.7
Suckling	7.3	2.5	0.0
Nursery	23.3	1.7	0.6
Finishing	1.3	0.0	0.0

Table 3

Prevalence of Salmonella serotypes						
Farm	A		B		C	
Serotype	n	%	n	%	n	%
S. untypable 4,12 i-monophasic	47	62.7				
S. infantis	7	9.3				
S. typhimurium	3	4.0				
S. derby	3	4.0	29	76.3		
S. london			8	21.1		
S. anatum			1	2.6	1	12.5
S. newport					3	37.5
S. fresno					2	25.0
s. mbandaka					1	12.5
S. javiana					1	12.5

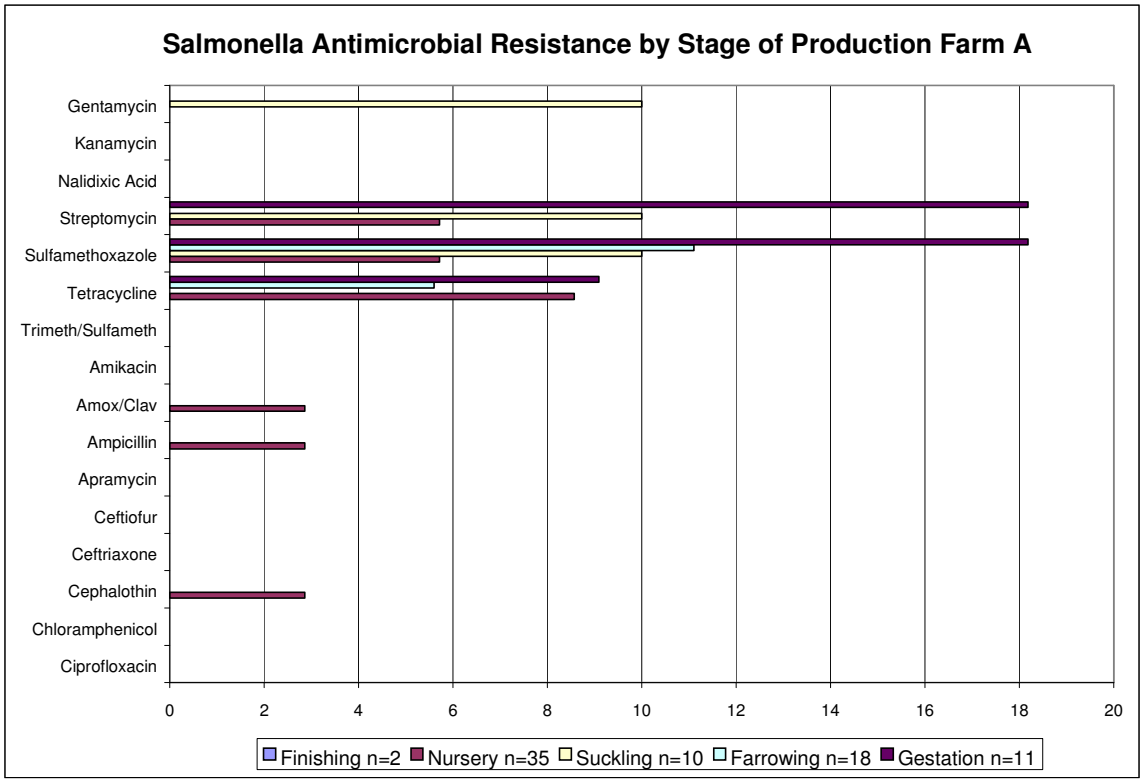

Salmonella Antimicrobial Resistance by Stage of Production Farm A
Gentamycin
Kanamycin
Nalidixic Acid
Streptomycin
Sulfamethoxazole
Tetracycline
Trimeth/Sulfameth
Amikacin
Amox/Clav
Ampicillin
Apramycin
Ceftiofur
Ceftriaxone
Cephalothin
Chloramphenicol
Ciprofloxacin
0
2
4
6
8
10
12
14
16
18
20
Finishing n=2
Nursery n=35
Suckling n=10
Farrowing n=18
Gestation n=11

Figure 1

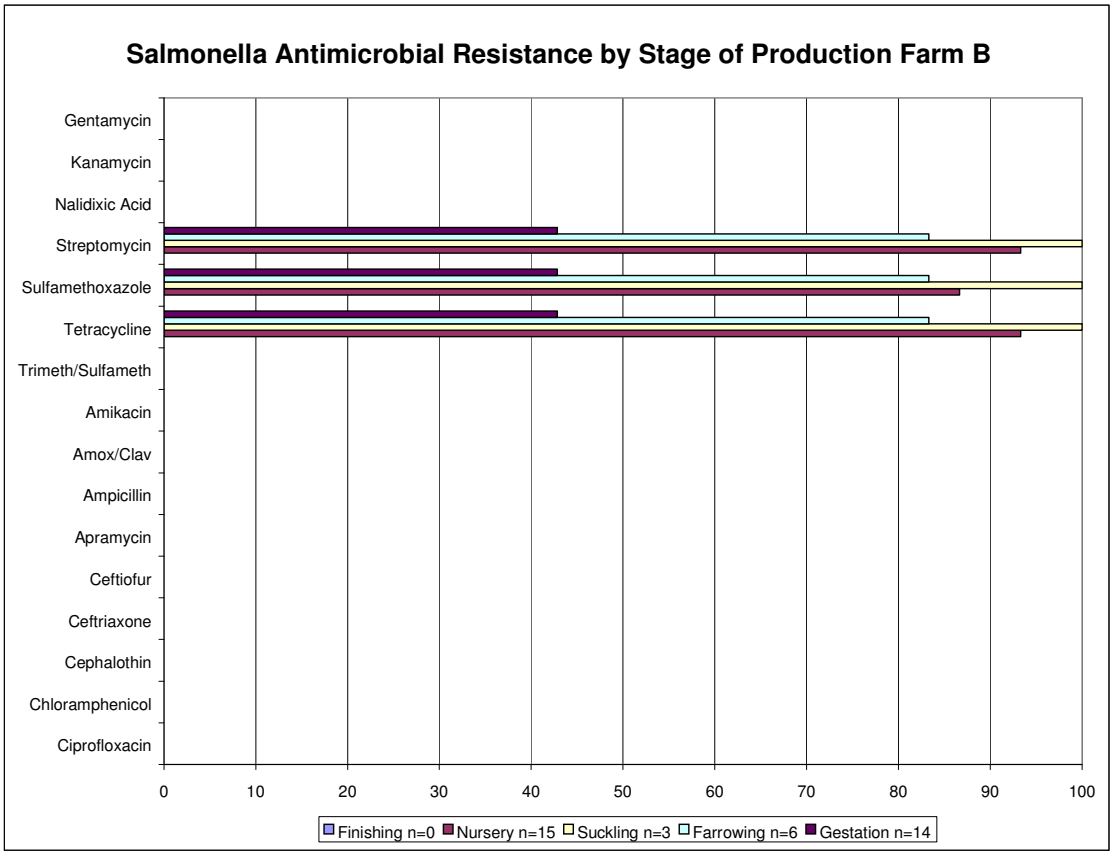

Salmonella Antimicrobial Resistance by Stage of Production Farm B
Gentamycin
Kanamycin
Nalidixic Acid
Streptomycin
Sulfamethoxazole
Tetracycline
Trimeth/Sulfameth
Amikacin
Amox/Clav
Ampicillin
Apramycin
Ceftiofur
Ceftriaxone
Cephalothin
Chloramphenicol
Ciprofloxacin
0
10
20
30
40
50
60
70
80
90
100
Finishing n=0
Nursery n=15
Suckling n=3
Farrowing n=6
Gestation n=14

Figure 2

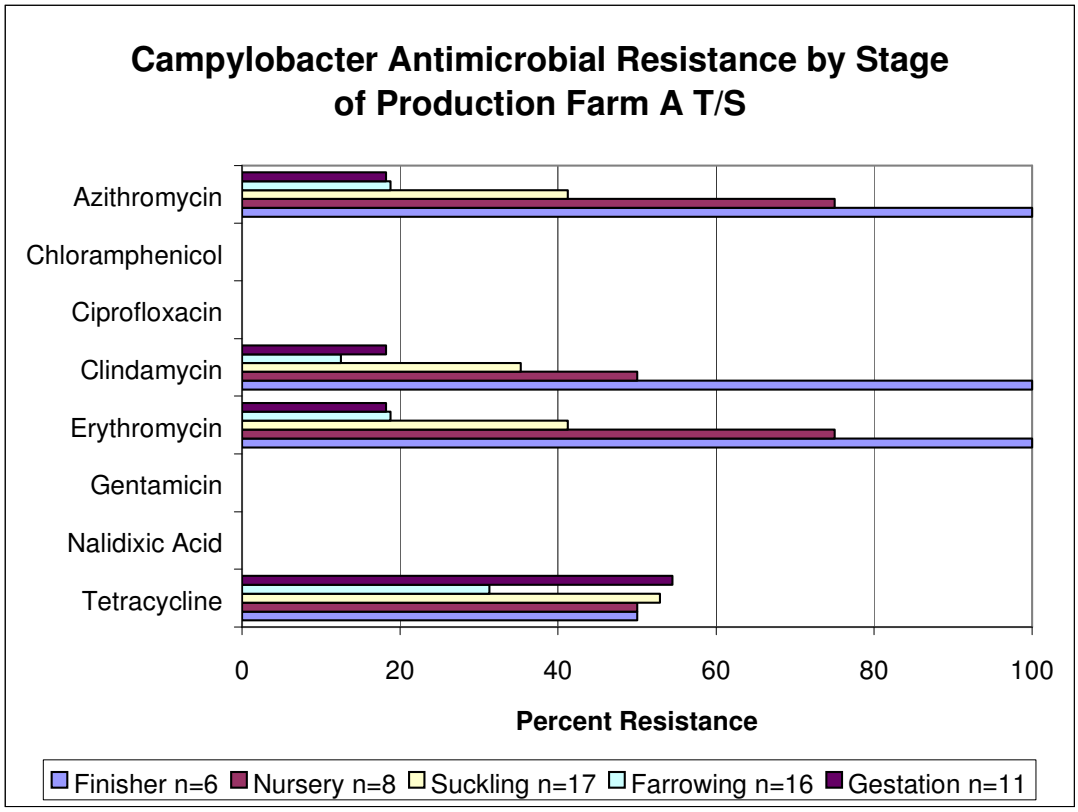

Campylobacter Antimicrobial Resistance by Stage of Production Farm A T/S
Azithromycin
Chloramphenicol
Ciprofloxacin
Clindamycin
Erythromycin
Gentamicin
Nalidixic Acid
Tetracycline
0
20
40
60
80
100
Percent Resistance
Finisher n=6
Nursery n=8
Suckling n=17
Farrowing n=16
Gestation n=11

Figure 3

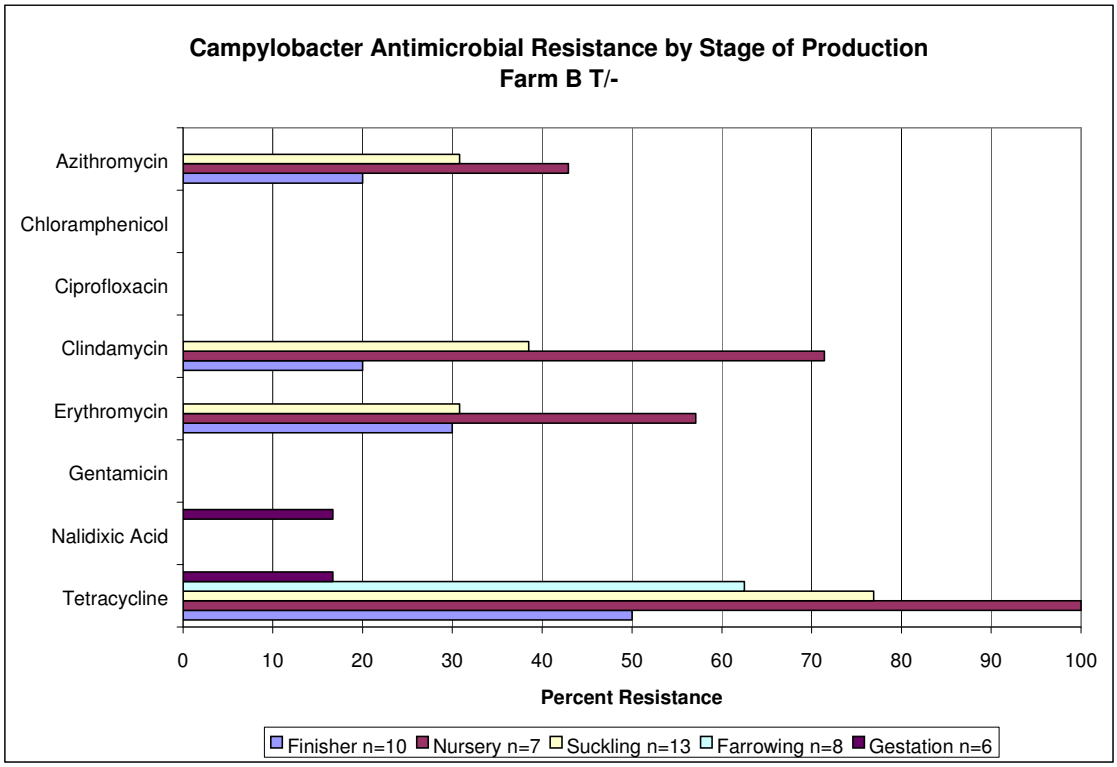

Campylobacter Antimicrobial Resistance by Stage of Production
Farm B T/-
Azithromycin
Chloramphenicol
Ciprofloxacin
Clindamycin
Erythromycin
Gentamicin
Nalidixic Acid
Tetracycline
0
10
20
30
40
50
60
70
80
90
100
Percent Resistance
Finisher n=10
Nursery n=7
Suckling n=13
Farrowing n=8
Gestation n=6

Figure 4

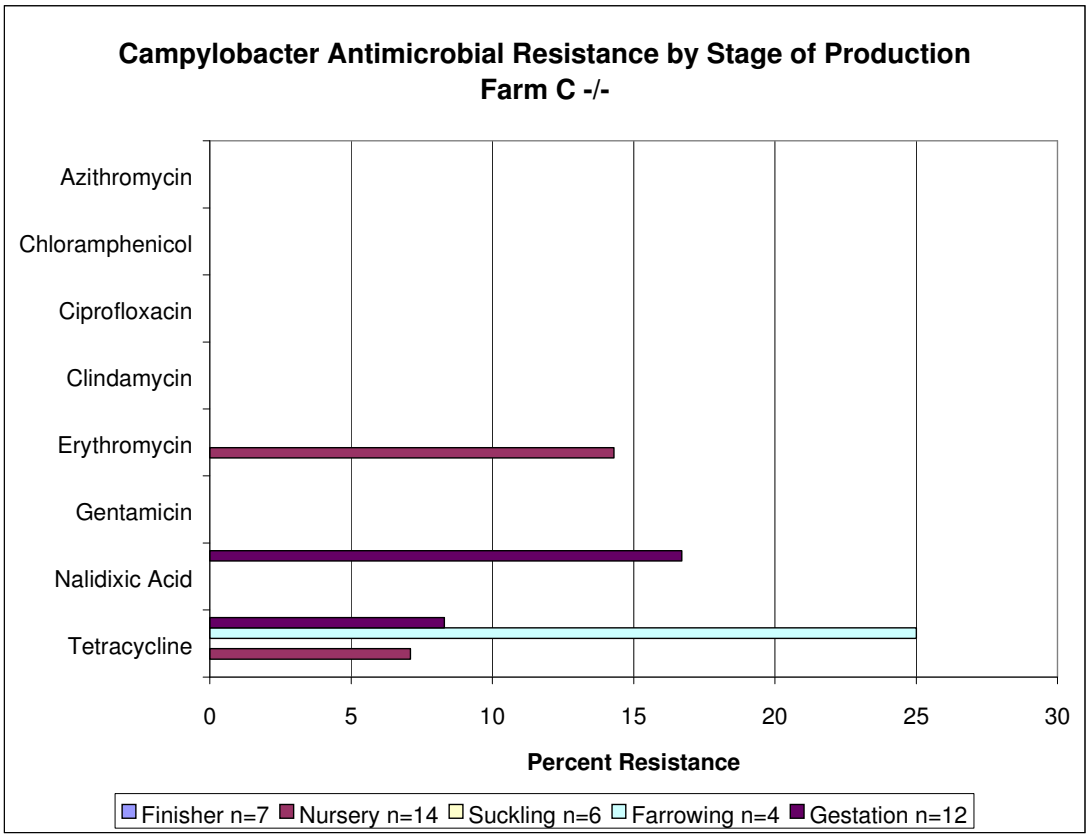

Campylobacter Antimicrobial Resistance by Stage of Production
Farm C -/-
Azithromycin
Chloramphenicol
Ciprofloxacin
Clindamycin
Erythromycin
Gentamicin
Nalidixic Acid
Tetracycline
0
5
10
15
20
25
30
Percent Resistance
Finisher n=7
Nursery n=14
Suckling n=6
Farrowing n=4
Gestation n=12

Figure 5

Figure 6. Salmonella predictive value for susceptibility.

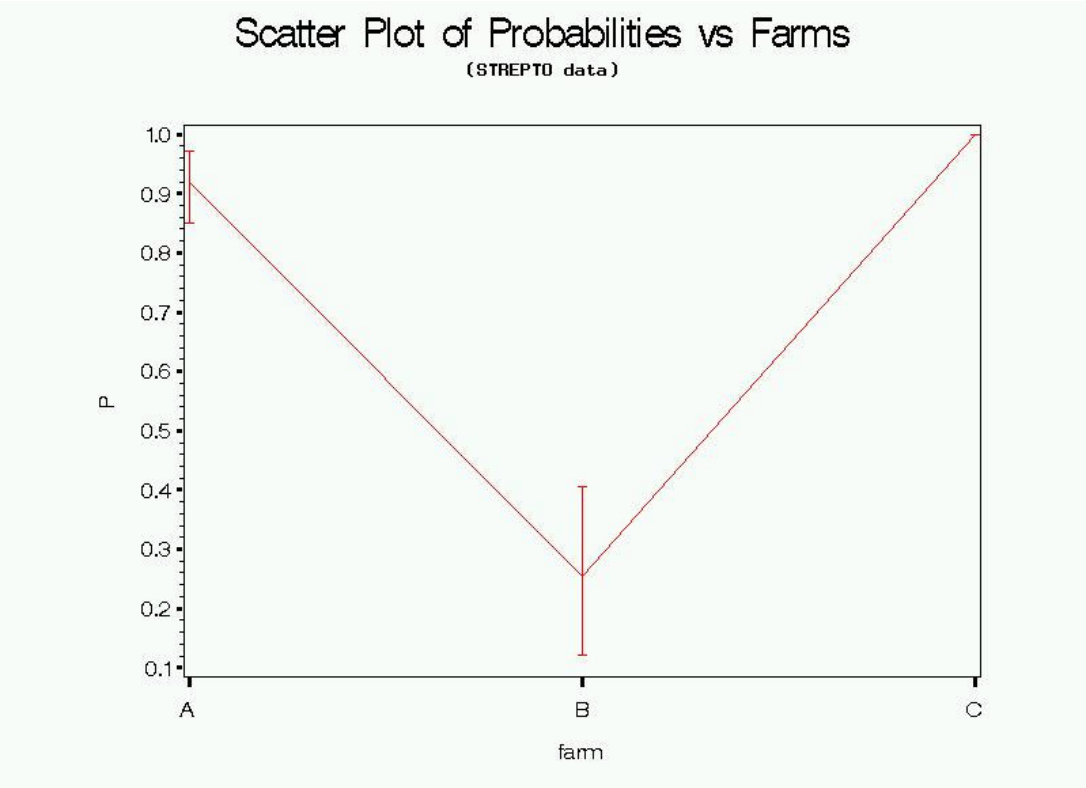


Figure 7. E. coli predictive value for susceptibility.

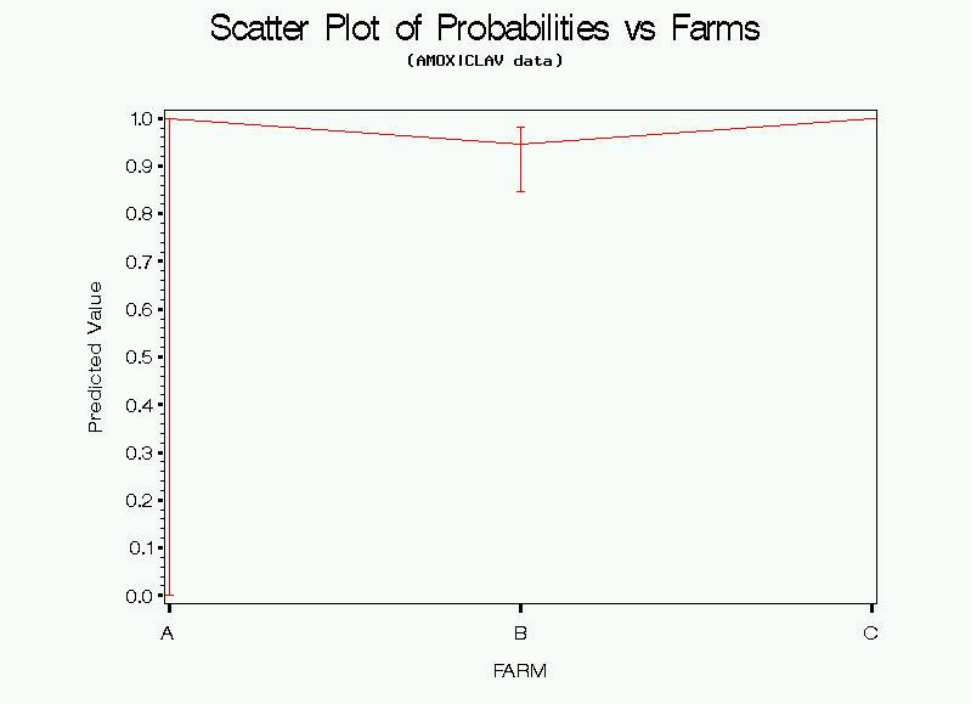


Figure 8. E. coli predictive value for susceptibility.

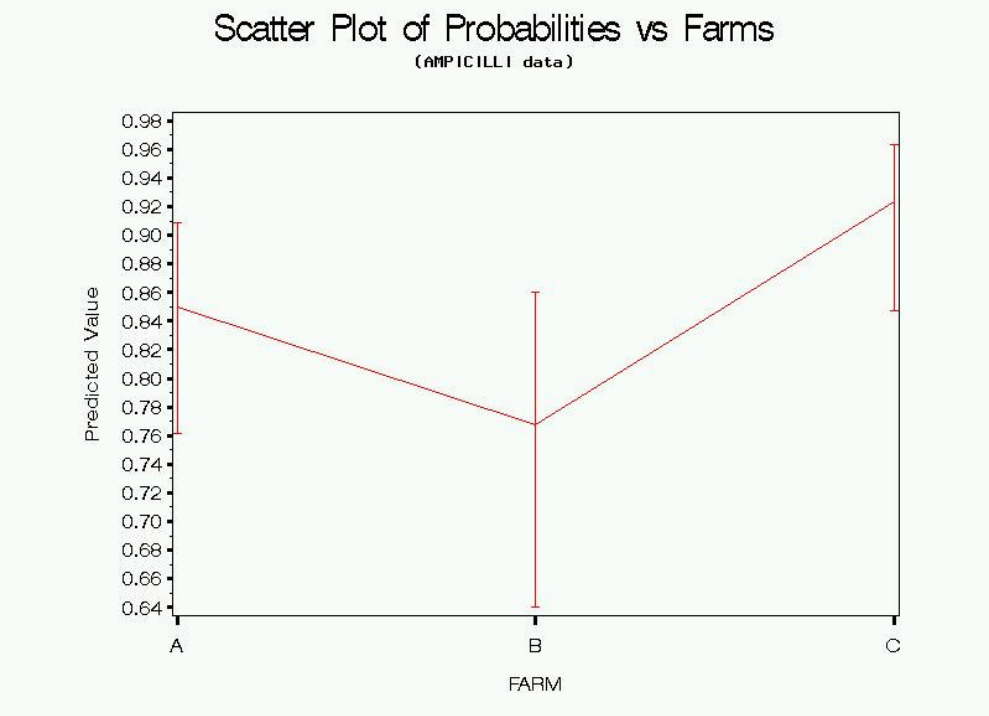


Figure 9. E. coli predictive value for susceptibility.

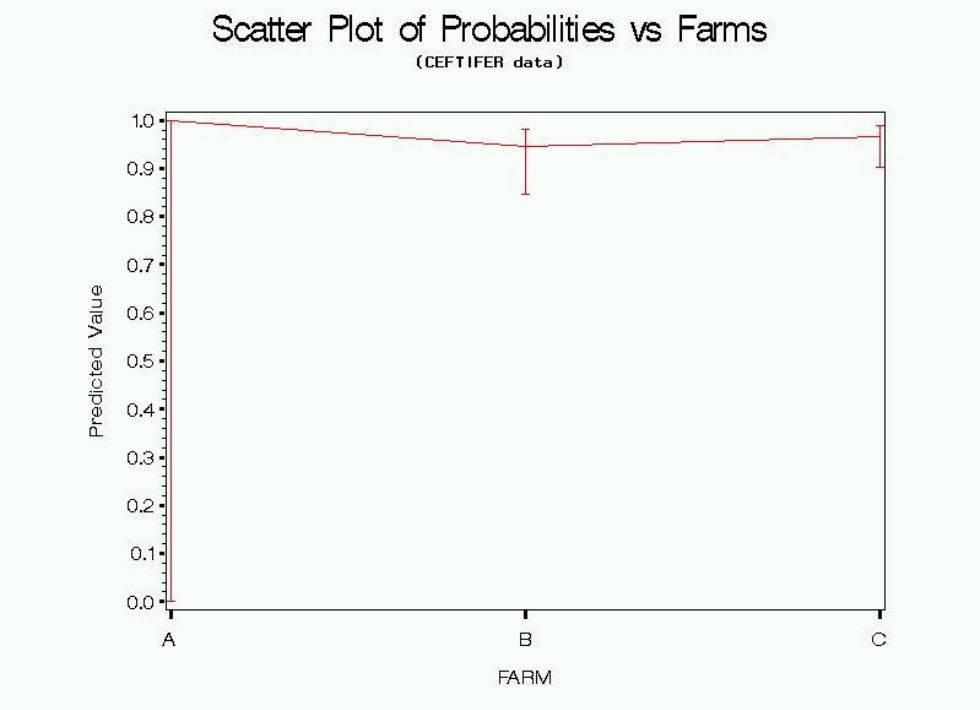


Figure 10. E. coli predictive value for susceptibility.

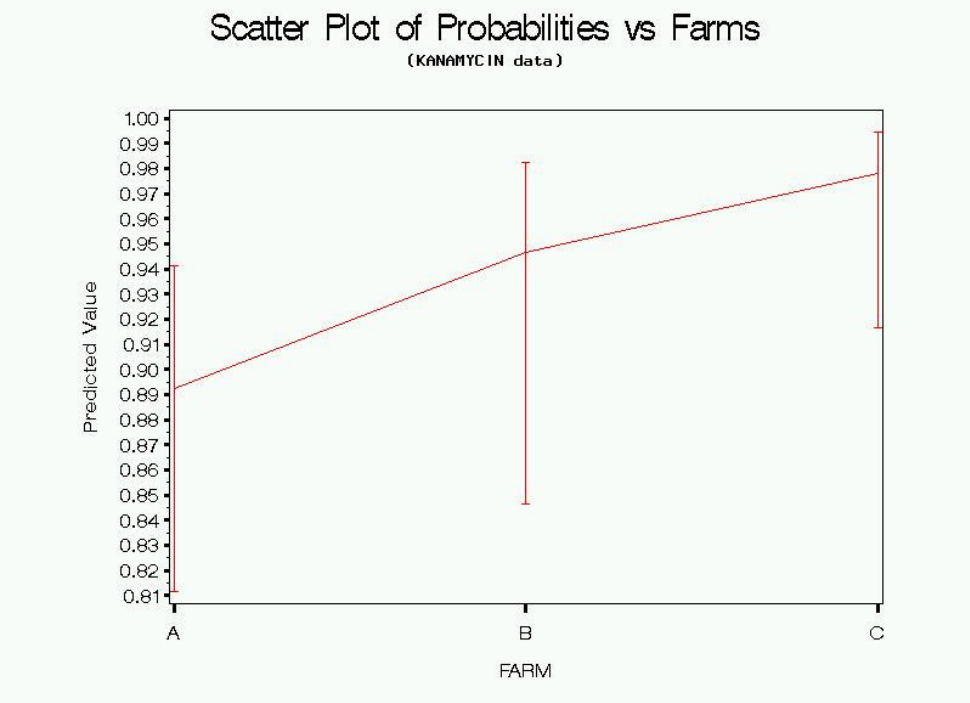


Figure 11. E. coli predictive value for susceptibility.

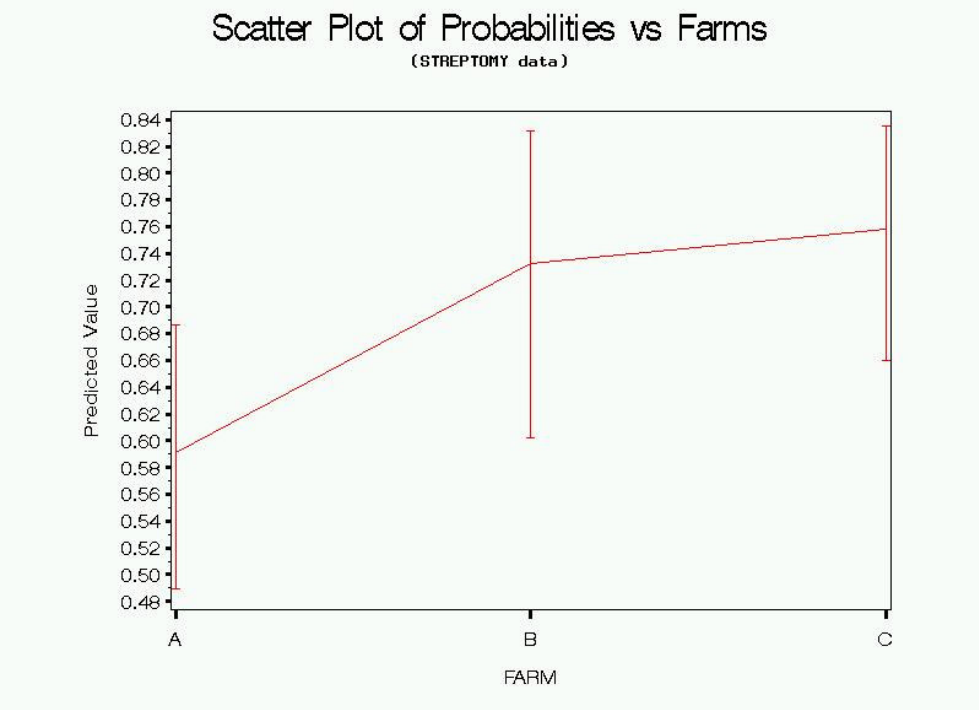


Figure 12. E. coli predictive value for susceptibility.

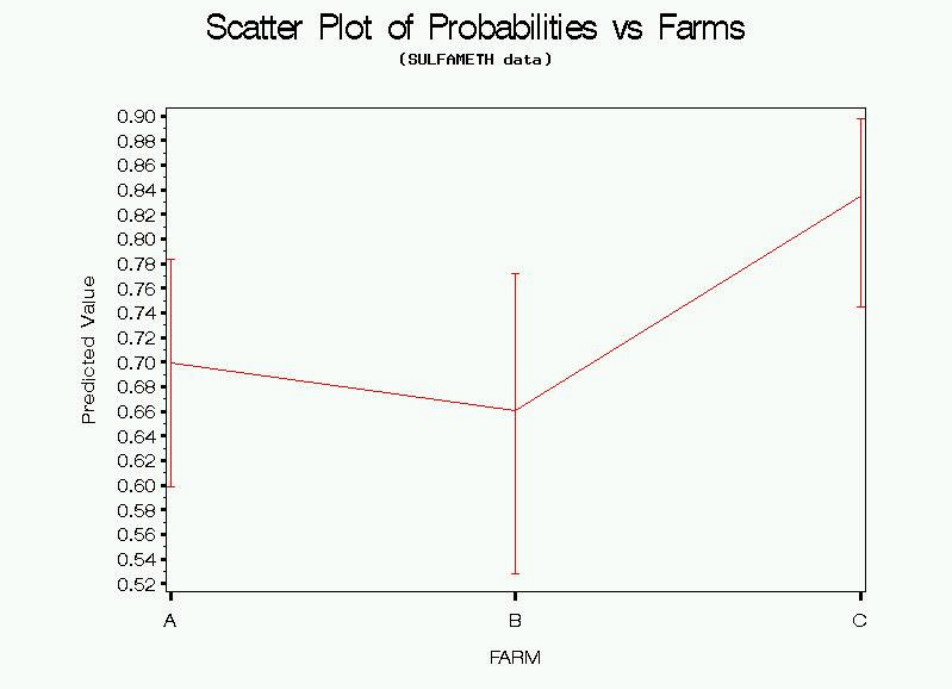


Figure 13. E. coli predictive value for susceptibility.

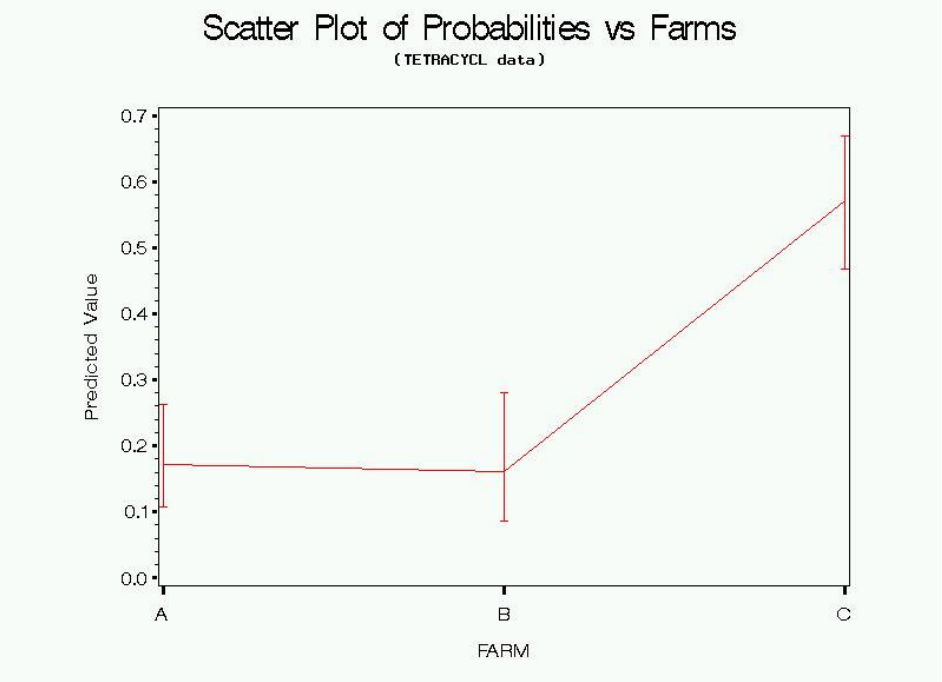


Figure 14. Campylobacter predictive value for susceptibility.

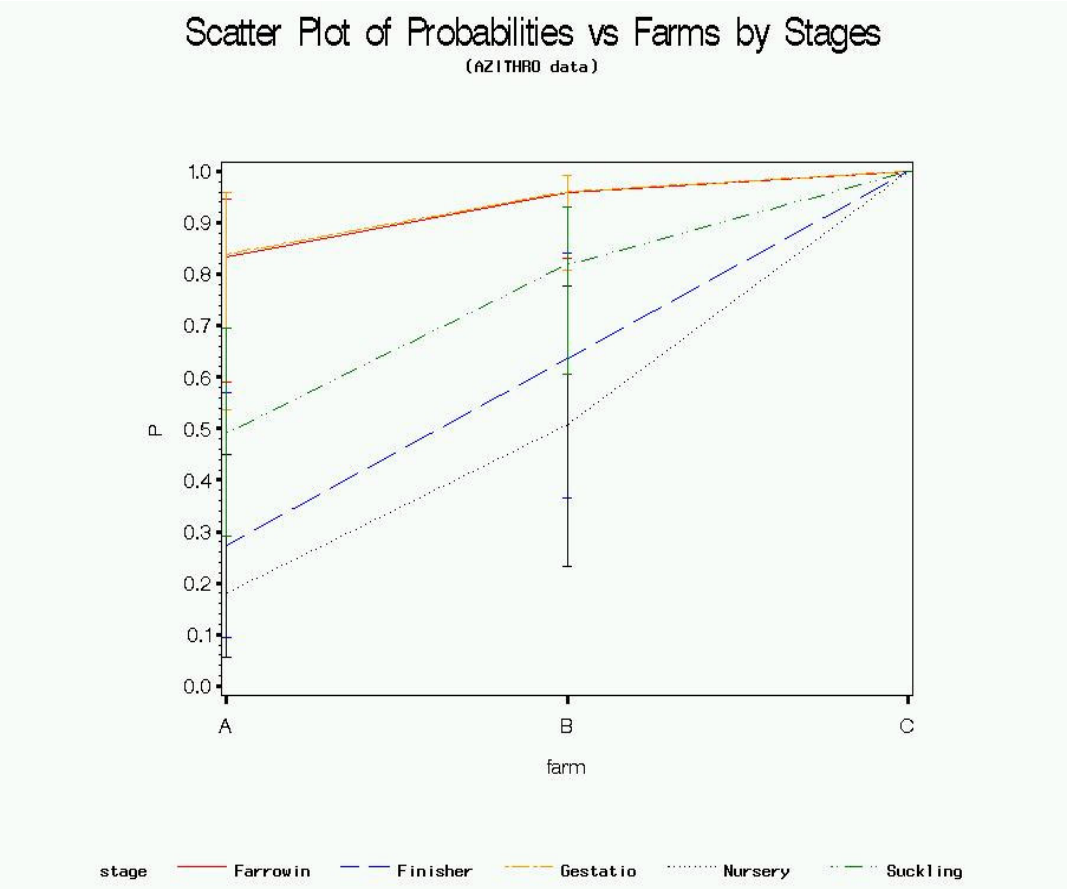
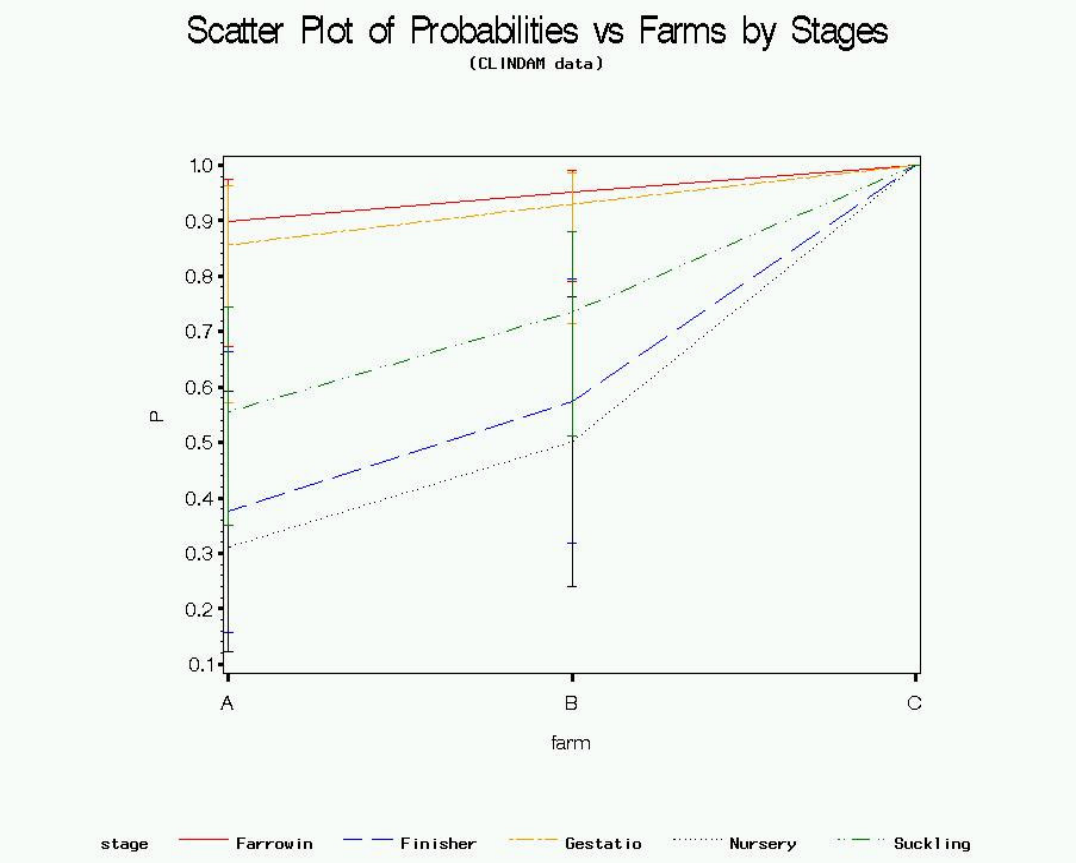


Figure 15. Campylobacter predictive value for susceptibility.



Fi

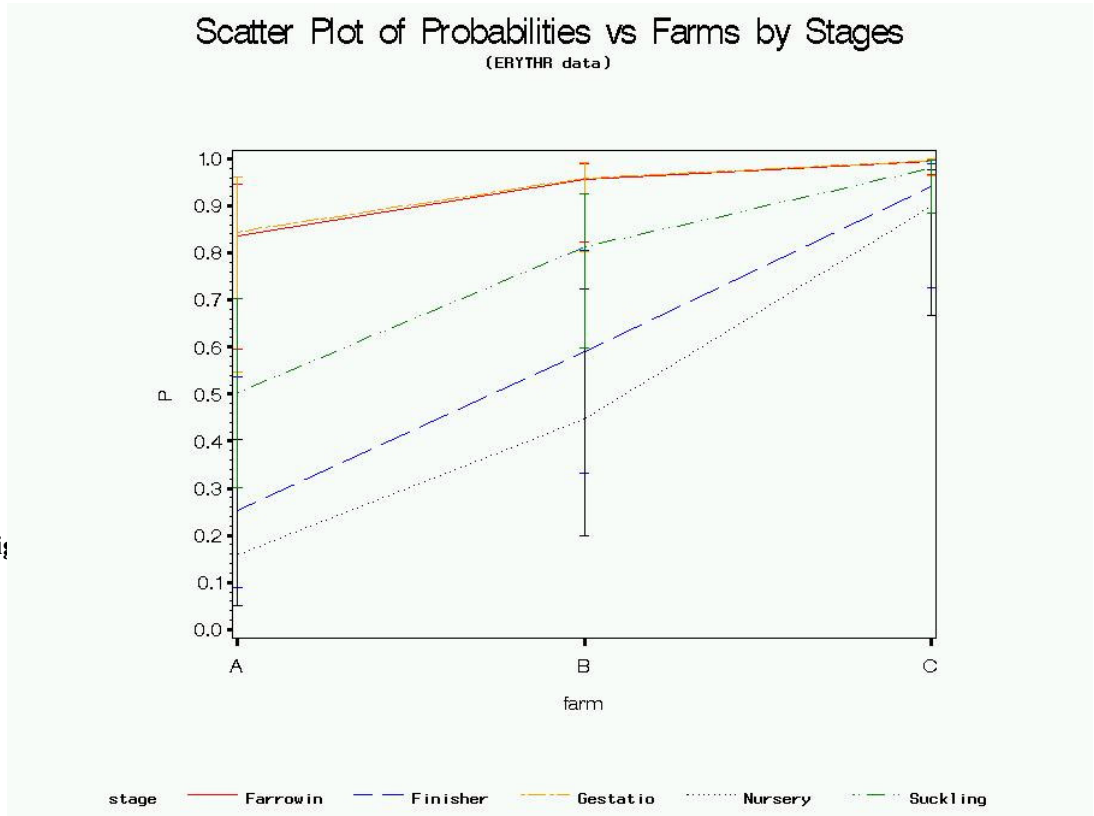


Figure 17. Campylobacter predictive value for susceptibility.

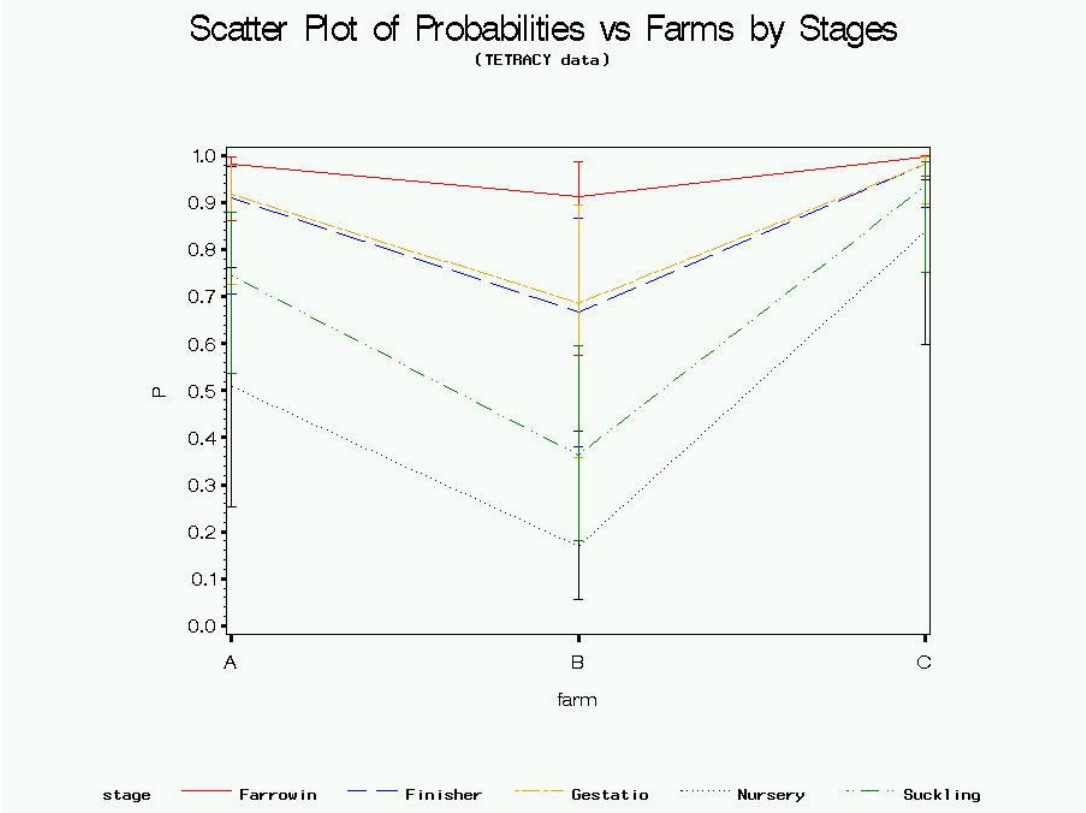


Figure 18. Salmonella predictive value for susceptibility.

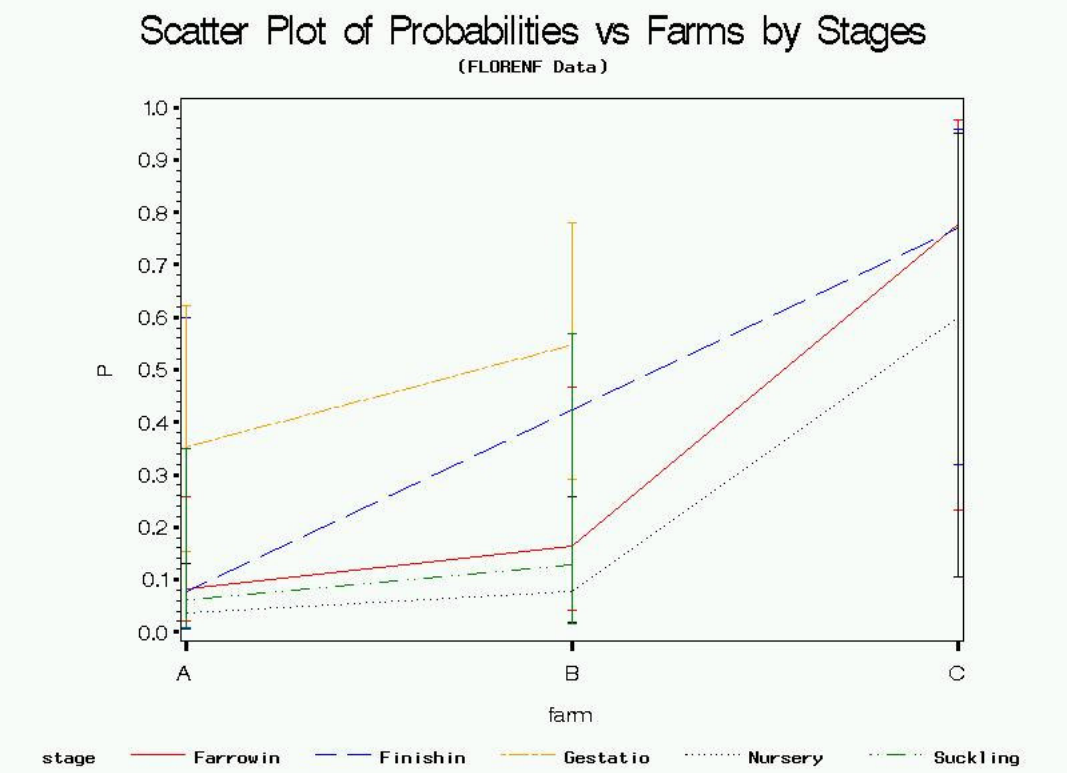


Figure 19. E. coli predictive value for susceptibility.

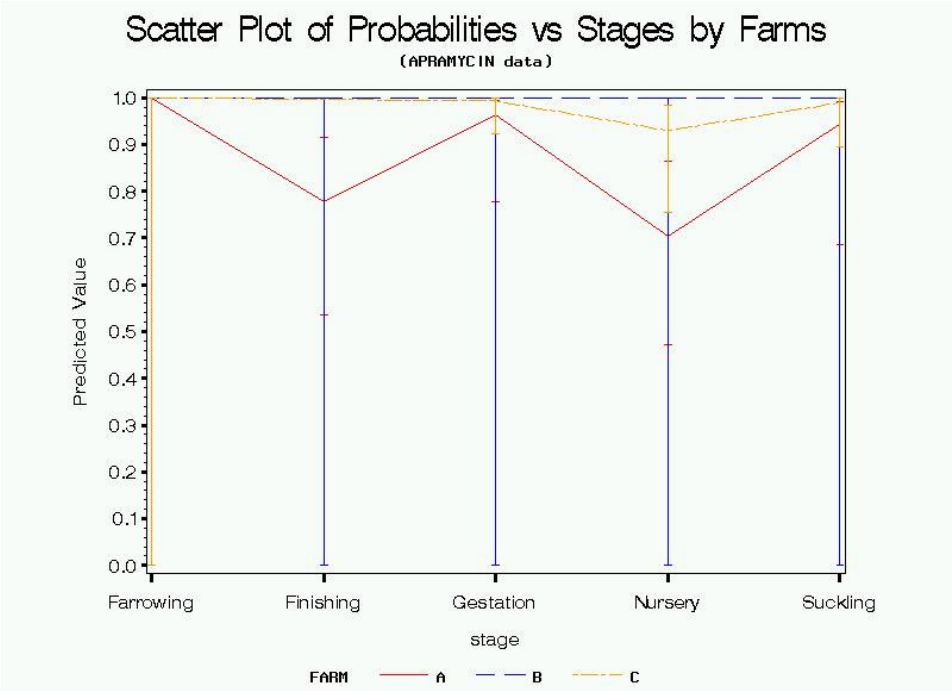

Scatter Plot of Probabilities vs Stages by Farms
(APRAMYCIN data)
Predicted Value
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Farrowing
Finishing
Gestation
Nursery
Suckling
stage
FARM
A
B
C

Table 4

Campylobacter				
Antimicrobic	Source	Degrees of Freedom	Chi-square	p-value
Azithromycin	Farm	2	42.91	<.0001
	Stage	4	23.72	<.0001
Clindamycin	Farm	2	33.66	<.0001
	Stage	4	21.03	.0003
Erythromycin	Farm	2	32.01	<.0001
	Stage	4	28.28	<.0001
Tetracycline	Farm	2	25.24	<.0001
	Stage	4	25.36	<.0001
Naladixic Acid	Farm	2	3.70	<.0001

Table 5

Salmonella				
Antimicrobic	Source	Degrees of Freedom	Chi-square	p-value
Amoxicillin	Farm	2	.89	.64
Ampicillin	Farm	2	.89	.64
Cephal	Farm	2	.89	.64
Gentamycin	Farm	2	.89	.64
Tetracycline	Farm	2	70.37	<.0001
Sulfamethazine	Farm	2	60.72	<.0001
Streptomycin	Farm	2	61.45	<.0001
Florfenicol	Farm	2	9.47	.0088
	Stage	5	16.11	.0065

Table 6

E. coli				
Antimicrobic	Source	Degrees of Freedom	Chi-square	p-value
Amoxicillin	Farm	2	8.86	.0119
Ampicillin	Farm	2	4.66	.0973
	Stage	4	6.90	.1412
Apramycin	Farm	2	13.91	.001
	Stage	4	16.42	.0025
Cefoxitin	Farm	2	5.88	.0530
Cephalothin	Farm	2	5.57	.0618
Chloramphenicol	Farm	2	1.90	.3862
Kanamycin	Farm	2	1.59	.4523
	Stage	4	2.40	.6625
Tetracycline	Farm	2	15.52	.004
	Stage	4	5.76	.2181
Ceftifur	Farm	2	2.03	.3631
Streptomycin	Farm	2	5.23	.0733
	Stage	4	3.05	.5495
Sulfamethazine	Farm	2	4.56	.1023
	Stage	4	5.36	.2525
Trisulfa	Farm	2	1.93	.3811