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COMPARISON OF PRODUCTION, MANAGEMENT, AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA FROM ORGANIC AND CONVENTIONAL DAIRY HERDS

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COMPARISON OF PRODUCTION, MANAGEMENT, AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA FROM ORGANIC AND CONVENTIONAL DAIRY HERDS

By

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ABSTRACT

COMPARISON OF PRODUCTION, MANAGEMENT, AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA FROM ORGANIC AND CONVENTIONAL

By

Kenji Sato

The production and management, prevalence and antimicrobial susceptibility of *E. coli*, *Salmonella spp.*, *Enterococcus spp.* and *Campylobacter spp.* isolated from bovine feces, *Staphylococcus aureus* isolated from bulk tank milk were compared between organic and conventional dairy herds. Thirty organic dairy herds, where antimicrobials are rarely used for calves and never used for cows, were compared with 30 neighboring conventional dairy farms, where antimicrobials were routinely used for animals for all ages. A seven-page questionnaire was used to assess management and production during 2000-2001. The organic farms had significantly fewer cattle than did the conventional herds. The average daily milk production per cow in organic dairy herds was lower than that of conventional herds. The incidence of clinical mastitis and bulk tank somatic cell count on organic farms was not statistically different from that of on conventional farms. There was little evidence of other fundamental differences between two farm types in other major management and production parameters.

Fecal specimens from ten cows and ten calves on 120 farm visits yielded 1,120 *E. coli* isolates, 7 *Salmonella* spp, which were tested for resistance to 17 antimicrobials. A total of 332 *Campylobacter* spp. isolates were tested to four antimicrobials (ciprofloxacin,

erythromycin, gentamicin, and tetracycline). A total of 2,049 *Enterococcus* spp were tested to 3 antimicrobials (Quinupristin / dalfopristin, gentamicin, and vancomycin). Of the 118 bulk tank milk samples in Wisconsin, 71 samples (60%) yielded at least one *Staphylococcus aureus* isolate, and a total of 331 *S. aureus* were collected and tested for resistance to 15 antimicrobials. The susceptibility of *S. aureus* were also compared with Danish study.

Our study shows significantly lower prevalence rates of AR in *E. coli* for seven antimicrobials (ampicillin, streptomycin, kanamycin, gentamicin, chloramphenicol, tetracycline, and sulphamethoxazole) in organic dairy herds, as compared to conventional herds. Two *Campylobacter* isolates from conventional dairy farms were resistant to ciprofloxacin and none of the isolates were resistant to gentamicin or erythromycin. Tetracycline-resistance in *Campylobacter* was 41.5% (66/159) for organic and 47.4% (82/173) for conventional herds, which was not statistically significant. A significant lower rate of resistant *Staphylococcus aureus* was detected to only one antimicrobial on organic farms in our Wisconsin study (ciprofloxacin) and on conventional farms in the parallel study in Denmark (avilamycin). *Staphylococcus aureus* isolates from Wisconsin had higher probability of reduced susceptibility to 7 out of 14 comparable antimicrobials, whereas Danish isolates had higher probability of reduced susceptibility to only two drugs. Differences in antimicrobial susceptibility between organic and conventional farms were small relative to the differences observed between the two countries.

Although the organic farms had converted to organic farming methods at least 3 years before our study, antimicrobial resistance clearly presented long after antimicrobial selective pressure had been withdrawn.

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LIST OF ABBREVIATIONS

AR	Antimicrobial Resistance
ATCC	American Type Culture Collection
BCS	Body Condition Score
BTSCC	Bulk Tank milk Somatic Cell Count
CDC	Center for Disease Control and Prevention
CM	Clinical Mastitis
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
EASS	Environmental and Animal Sanitation Score
ELISA	Enzyme-Linked Immunosorbent Assay
ESBL	Extended-spectrum β-lactamase
GEE	Generalized Estimating Equation
MDCH	Michigan Department of Community Health
MRSA	Methicillin Resistant *Staphylococcus aureus*
MIC	Minimal Inhibitory Concentration
NAHMS	National Animal Health Monitoring System
NARMS	National Antimicrobial Resistance Monitoring System
NCCLS	National Committee for Clinical Laboratory Standards
OD	Optical Density
SBA	5% Sheep Blood Agar
SCC	Somatic Cell Count

CHAPTER ONE

INTRODUCTION, STUDY OBJECTIVES AND STUDY METHOD

INTRODUCTION

The wide use of penicillin began in 1942 and by 1945 the first report were made of antibiotic resistance bacteria. Resistant *Staphylococcus aureus* was described in a London hospital, and by 1949, approximately 59% of the *S. aureus* isolated in this hospital were reportedly resistant to penicillin. Some bacteria naturally developed resistance to antibiotics, long before the development of commercial antibiotics. Bacteria can develop resistance to certain drugs through mutation or can acquire resistant genes from other bacteria (plasmid, transposon, and transformation). The genetic traits for resistance has been shown to spread from one species of bacteria to other species. Antimicrobial resistance was not recognized as a major threat to human medicine until 1970's, when new antimicrobials developed during 1950~1960's were used to combat these resistant bacteria. For example, nosocomial infections with penicillinase producing *S. aureus* were already observed in 1950's, but were controlled by methicillin, and cephalosporins. One of the most important challenges was recognized in 1980's when MRSA (Methicillin Resistant *Staphylococcus aureus*) and other multiple-drug resistant bacterial infections were recognized in hospitals all over the world. Several strains of *S. aureus* had even acquired resistance to heavy metals and disinfectants used in hospitals. Advances in medical technology and an increased population of immunocompromised people, (cancer treatments, transplantations, diabetes, old age, and HIV) has further

exacerbated the problem of antimicrobial resistance in the hospital and nursing home environment.

The effects of antimicrobials as growth promoters for food animals were recognized soon after the initiation of antibiotic mass production, when higher growth rate were observed if the residue of tetracycline production were fed to mouse, poultry and swine. Also, the prophylactic use of antimicrobials for food animals became a common practice and allowed large scale livestock production systems. Soon thereafter, large scale livestock productions were accused of using antibiotics as a substitute for good hygiene and husbandry, however, consumers undoubtedly benefited from this mass productions which resulted in a high quality product at a relatively inexpensive price.

Reports came from Norway (Klare, 1995) and Denmark (Aarestrup, 1995) on the possible association between avoparcin, used as a growth promoter, and the occurrence of vancomycin resistant *enterococci* in livestock. Such reports reversed the European trend toward antibiotic use as feed additives in animal production. Currently, the European Union has banned feed grade antimicrobials as growth promoters. Although the DANMAP 2002 data showed that feed-grade antimicrobials was decreased, therapeutic antibiotic use for livestock did increase. Other research data from Europe, however, showed mixed results and it still remains to be seen what benefits to human society might be realized by curtailing the use of antimicrobials for food animal growth promotion.

The assessment of human health risk and benefits associated with the uses of antimicrobials in livestock industry had been the main issue for many years. There is still disagreement regarding the impact on public health caused by use of antimicrobials for food animals. Some have suggested that antibiotics should be banned from animal

agriculture and be reserved exclusively for human clinical treatments (Mudd, 1996). Others contend that the prescribing practices in human medicine [not animal agriculture] are the major contributing factor to the current antimicrobial resistance problem (Cook, 1997; Schwartz et al., 1997). Assessments are needed regarding the degree to which use of antibiotics in animal agriculture is contributing to the proliferation of antibiotic resistant bacteria, and the extent to which animal agriculture can function with reduced dependence on antibiotics (Dowling, 1997; Wiedemann and Grimm, 1996). It needs to be determined if a reduction in antimicrobial use in animal agriculture could eventually lead to a reduction, or at least a deceleration, in developing antibiotic resistance. Also, it is important to estimate the effect of policy changes on animal welfare and productivity. Some people insist the possibility of our food coming from diseased animal would increase or human food supplies would be reduced due to impact of death and disease. Population-based field data on antimicrobial resistance should be used as the basis for all policy changes regarding antibiotic usage.

Organic dairy production may provide us the opportunity to evaluate one of possible consequences of the reduction in antibiotics usage on dairy farms. Organic dairy operations, which have long histories without the use of antimicrobials, allow us to simultaneously observe the effect of this management change on the prevalence of antimicrobial resistant bacteria and disease incidence in the farm animals. Intervention studies with antibiotic withdrawal for only a year or two may result in premature conclusions, since such studies make the assumption that the selective pressure will rapidly act upon the micro flora of the farm. The resistance to tetracycline in *Escherichia*

coli was known to have been remained 126 months after disuse of antimicrobials (Langlois et al. 1988).

Although antibiotic usage for food animals increased in the 1960s ~ 1990s, most attempts to institute the judicious use of antibiotics were aimed at the human medical community, and hospitals in particular. As consumers and physicians learned of the extensive use of antibiotics for animal agriculture, they began to pressure the agricultural community to abandon the use of some antibiotics and to limit the use of antibiotics. It was, and still is, unclear how much of the human medical problem with antimicrobial resistance is caused by antibiotics fed to our food animals.

It was also unclear how difficult it would be for animal agriculture to function efficiently without antibiotics, or how quickly antibiotic sensitivity might return to bacterial populations after the selective pressure for antibiotic resistance was removed. It was also clear that one answer to these questions could not be given for all antibiotic resistance mechanisms found in all species of bacteria. Rather, the answers would have to specifically address each "bug – drug" combination for the various uses in livestock production. For example, it may be that the use of fluoroquinolone is not very essential to poultry production, resistance genes are readily transferred to human pathogens such as *Campylobacter*, but that the genes that enable this resistance rapidly disappear in the population when the use of fluoroquinolone is ceased. A very different set of answers may exist for the use of tetracycline in swine operations.

This dissertation attempts to answer some of the above-stated questions regarding the role of agricultural antibiotics in the epidemiology of antimicrobial traits.

STUDY OBJECTIVES

The overall objective of this study is to determine if dairy farms with at least a three year history of zero or minimal use of antibiotics have lower rates of antimicrobial resistant bacteria than do region-matched conventional dairy farms which make frequent use of antibiotics. Production and management parameters were also collected to estimate production, disease and management factors which enables organic operation feasible. The study was intended to provide scientific base to assess a possible outcomes of reduced use of antimicrobials on dairy farms.

STUDY METHOD

Thirty organic dairy farms and thirty neighboring conventional dairy farms in Wisconsin was selected for the study. As such, each organic farm was matched with a conventional farm based on region of the country. All farms were administered a seven-page questionnaire to measure overall frequency of disease and other production and management factors. From each farm, fecal samples were obtained from 10 calves and 10 cows, and a milk sample will be obtained from bulk tank. *Escherichia coli*, *Salmonella* spp., *Enterococcus* spp., *Campylobacter* spp. from fecal samples and *Staphylococcus aureus* from milk samples were selected for this study. Since only a few isolates of *Salmonella* and a few resistant *Enterococcus* were obtained, the result of *Salmonella* and *Enterococcus* were not included in this dissertation.

Because of the need to detect changes in degrees of susceptibility rather than dichotomized resistance, micro broth dilution methods (Sensititre®) was used for *E. coli*, *Salmonella*, *Staphylococcus* and *Enterococcus*, and gradient disk diffusion minimal

inhibitor concentration method (Etest®) was used for *Campylobacter*. By having a full range dilution scheme, it became possible to detect minor differences in MICs for a number of different bacteria. Analysis of the data was multi-factorial including susceptibility profiles, distribution of MICs, MIC_{50}s, MIC_{90}s, and model MICs in addition to categories of susceptibility of specific bacteria.

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CHAPTER TWO

LITERATURE REVIEW

Antimicrobial resistant bacteria from animals

Food animal agriculture has been suspected of fostering antimicrobial resistant (AR) bacteria, which can then be transmitted to people through direct contact, food of animal origin, and environment contamination (McEwen, 2002). Some resistant bacteria isolated from human infections have reportedly been traced back to farm animals (Lyons, 1980; O'Brien, 1982; Spika, 1987; Molbak, 1999; Fey, 2000; Van Den Bogaard, 2001). There is increased interest in research that addresses food animal agriculture's relative contribution to the antimicrobial resistant problem (Sorum et al., 2001; Torrence, 2001).

Farm-level studies have shown an association between antimicrobial usage in food animals and rates of antimicrobial resistant in animals (Mathew, 2001; Van Den Bogaard, 2001). Generally, farms with higher antimicrobial usage have a higher proportion of resistant bacteria, as well as the presence of multi-drug resistance strains. However, the magnitude of the contribution of livestock production practices to the growing antimicrobial resistant problem is unclear.

The studies based on diagnostic submissions from ill animals that have recently undergone antimicrobial therapy may have resulted in higher measure of antimicrobial resistance than what might be present in the normal population (Schroeder et al., 2002). Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP, 1996 - 2001) reported higher prevalence of resistant bacteria in diagnostic submission samples and lower resistance rates in samples from animals at slaughter. For

example, a high proportion (80-86%) of *E. coli* isolates from Denmark were resistant to ampicillin in diagnostic submission, whereas only 0-8 % of *E. coli* from slaughter cattle were resistant to ampicillin when measured on presumably health cattle (Bager, 1997).

Geographic or temporal variations in the prevalence of resistant bacteria are believed to occur. A study conducted by Singer et al. (1998) suggested AR might be clustered in time and space, though their data were derived from a clinical diagnostic database, and so AR distributions from one region may not be applicable to other region.

Organic dairy production

Organic dairy production is drawing increasing attention because of public concerns about food safety, animal welfare and the environmental impacts of intensive livestock systems (Weller, 1996; Sundrum, 2001). Though the U. S. organic food market is approximately $6 billion, which is less than 1% of total food consumption in the USA, the organic market has been growing at 20-30 percent per year (Greene, 2000; Greene, 2001). In contrast, the organic milk market in Denmark is approximately 14% of the total milk consumption (Mann, 1999). In Denmark, national certification of organic "økologisk" farms began in 1988 and organic farms now comprise 8.4% of all Danish dairy farms and produced about 22% of the fluid (drinking) milk in 2000 (Bennedsgaard, 2003). More than 25% of total sales of dairy products in Switzerland is labeled as organic (Busato, 2000). In the UK, a 30 to 40% per annum increase of organic products was observed (Weller, 2000). Organic agriculture is being recognized by governmental bodies as a tool to improve rural income diversity and stability (FAO, 2000).

The definition of "organic" has varied from country to country and among U. S. certifying organizations, thereby causing consumer confusion. Farms in the US had been certified as organic dairies by independent certifying organizations based on their own standards. The USDA had established the federal standard, and a revised National Organic Standard was published in the Federal Register (Federal Register, 2000a; USDA, 2001) which was fully implemented in October 2002. Organic dairies must use organic feedstuffs which are grown without any chemically synthesized fertilizer, herbicides or insecticides. The use of antibiotics for therapy or prophylaxis and hormones for growth promotion or production enhancement are prohibited. Since many conventional veterinary prophylactic and therapeutic medicines are not permitted, organic dairy farmers have adopted a variety of management practices to prevent clinical disease.

Herd size

Ogini et al. (1999) studied six organic dairies in Ontario and found that organic dairy producers had comparable tillable land base and herd size (48 cows per herd) as did conventional dairies. The study in Switzerland indicated the organic herd size was equal to the national average (Busato, 2000). Large organic dairy operations can be found in other parts of the U. S. (Organic Trade Association, 1999). Average number of cows per herd and milk production per cow were 67.0 cows and 25.5kg/day in Wisconsin, but the national average were bigger than Wisconsin average (93.4 cows and 27.0 kg/day; USDA, 2002). Thirty one per cent (31%) of conventional operations used free-stall housing and 52 % of the operations used tie stall or stanchion housing (USDA, 2002). No statistical data on organic operations were available.

Milk production

Lower milk production per cow was reported in organic dairies when compared to the conventional dairies in the same region (Busato, 2000; Krutzinna, 1996; Reksen, 1999). Lower milk yield per cow is likely attributable to more pasture and less grain being used for organic dairy production (Krutzinna, 1996). Grazing farms usually produce less milk per cow than do non-grazing farms (Hanson, 1998).

Culling rate

It has been speculated that organic dairies had higher culling rates, primarily due to the development of udder infections and reproductive problems (AHI, 1998). However, Norwegian organic dairies had a 23% cull rate, whereas conventional dairies had a 35% cull rate, which resulted in more multiparous cows in organic dairies as compared with conventional dairy farms (Reksen, 1999). The U. S. average culling rate for herds of less than 100 was 24.9 (USDA, 2002). A culling rate of 20-30% is thought to optimize producer profit, however, the culling rates are difficult to compare because of different definitions of culling (Radke, 2000).

Mastitis rate

Mastitis is a major cause of economic loss in the dairy industry and the primary reason for which antibiotics are used in dairy operations (Kaneene, 1992). Field studies in Pennsylvania, Ohio, and California indicated that the average annual herd incidence of clinical mastitis was 45 to 50 cases per 100 cows (Hady, 1993). Mastitis incidence is

usually highest in July and August (Erskine, 1988). Milking hygiene and environmental sanitation are traditional ways to prevent the disease (Bartlett, 1992a).

Weller and Bowling (2000) reported that the incidence of clinical mastitis in 10 organic dairies in the UK was not significantly different from the rate of clinical mastitis in conventional dairies. Busato et al. (2000) reported the prevalence of subclinical mastitis in organic dairies in Switzerland to be lower than the national average. Barlow (2001) reported bacteriological analysis of 109 clinical mastitis quarters on 6 organic farms in Vermont, however no mastitis rate was reported.

The mastitis rates from different studies are difficult to compare because of the regional differences, poor standardization of case definition and high variance in diagnostic acumen among the studies (Bartlett, 2001). Berry (2002) reported that farmers converting to organic status in the UK were less likely to report cases of clinical mastitis, which implies that a possible information bias may exist in comparison between organic and conventional dairy farms.

Somatic Cell Count

The mean bulk tank somatic cell counts (SCC) in Wisconsin for 1995 to 1998 was 335,000 cells/*ml* for grade A herd and 480,000 cells/*ml* for grade B herd (Ruegg, 2000). Highest SCC were observed in the month of July (USDA, 1999; Erskine, 1988). Although Weller et al. (1998) reported a higher SCC in one organic herd in a six-year longitudinal study as compared to conventional herds, other studies reported lower SCC in organic herds than what was found in conventional herds (Busato, 2000; Bennedsgaard, 2003). Busato (2000) studied 152 certified organic farms in Switzerland and found the

geometric mean of 85,600 cells/ml, which was 15% lower than the Swiss average of 100,000 cells/ml.

Antimicrobial use in dairy production

Dairy cattle rarely receive antimicrobials in the feed over long periods of time, except for medicated milk replacer which is often fed to calves. Antimicrobial use as feed additive is probably a principal reason why the total amount of antimicrobial use in swine and poultry production is much greater than what is seen in the dairy industry. Consequently, a higher proportion of resistant bacteria are found in swine and poultry isolates as compared with isolates from dairy cattle (Salmon, 1995; Bager, 1997; Mathew, 1998; Schroeder, 2002).

In the United States, five antibiotics (i.e., bacitracin methylene disalicylate, chlortetracycline, oxytetracycline, tylosin, and virginiamycin) are approved and commonly used for beef cattle or calves for prevention of liver abscesses in feedlot cattle (Nagaraja, 1998). Most antibiotic use in adult dairy cows is for treatment and prevention of clinical mastitis (Hady, 1993; Kaneene, 1992) and in dairy calves, for treatment and prophylaxis of diarrhea/pneumonia, and as a medicated milk replacer (McEwen, 2002; USDA, 2002). Calf milk replacers, which may contain antimicrobials such as chlortetracycline, oxytetracycline and neomycin, are widely used by dairy producers in the United States (Heinrichs, 1995; USDA, 2002a). Milk replacer with antimicrobials have been prohibited in Denmark since the early 1970's (Larsen, 1972). Routine intramammary treatment for all cows with long-acting antimicrobials after the end of lactation (dry-cow-treatment) is widely adopted in the US as a preventive method

(Jayarao 1999; Hardeng 2001). According to the NAHMS study (USDA, 1996), 88 % of dairy operations use udder infusion for all four quarters on almost all cows at the end of lactation.

Antibiotics are often ineffective for some mastitis pathogens such as *S. aureus* and non-severe coliform. (Kirk, 1994). Dry cow therapy infusions are effective against contagious mastitis pathogen, but are ineffective against environmental coliforms (Berry, 1997). A retrospective study of 9,007 cases of subclinical mastitis in New York and northern Pennsylvania showed the overall spontaneous bacteriological cure rate was 65% and the cure rate with antimicrobial treatment was not much better at 75% (Wilson, 1999). Recent work in Denmark showed that organic (økologisk) farmer had less frequently asked veterinarians to treat their mastitis cows, however treatment frequency and antibiotic selection is not substantially different among two type of Danish herds (Bennedsgaard, 2003).

Organic dairy operations are also prohibited to use anthelmintics. According to a recent NAHM study, over 60% of dairy operations in the U. S. normally use dewormers for at least some lactating cattle (USDA, 2002).

The optical density value of ELISA has been found to be a reasonable overall measure of parasite burden, and bulk tank milk is useful for testing whole-worm antigen (Guitian, 2000; Sanchez, 2002). The worm burden were significantly associated with seasons, highest in late summer and lowest in spring, on the study of serum antibody (Borgsteede, 2000).

Antimicrobial resistance

Avilamycin

Avilamycin a mixture of oligosaccharides of the orthosomycin group, and had been used for growth promoter primarily of broilers and some for pigs, but not for dairy cattle in Denmark (Aarestrup, 1998; Aarestrup, 2001). Avilamycin was used as growth promoter for broilers and pigs, but its use was voluntarily abandoned in Denmark by the end of 1999, to avoid the possible cross-resistance to everninomycin, which was investigated for use in humans (EU, 1998; Aarestrup, 2000; Butaye, 2003).

Bacitracin

Bacitracin is a polypeptide product of *Bacillus licheniformis* and *Bacillus subtilis*, and is commonly used in the topical treatment of human and animal skin infection. It is also commonly used as growth promoter in poultry, swine, and feedlot beef production (Aarestrup, 1998; Prescott, 2000b; FDA 2003c). The European Union banned bacitracin as animal growth promoter in 1998 because analogs of bacitracin can sometimes be used for the treatment of vancomycin-resistant enterococci (EU, 1998; Chia, 1995; O'Donovan, 1994). Relatively low levels of *S. aureus* resistance to bacitracin in cattle were reported in Europe before the ban (Devriese, 1980; Aarestrup, 1998), and reduced susceptibility to bacitracin (83% with MIC≥32μg/*ml* by e-test) was also reported in *S. aureus* from human skin and soft-tissue infections in Norway (Afset, 2003).

Ceftiofur

Ceftiofur, a third-generation cephalosporin, has been developed strictly for veterinary use (Hornish, 2003). It is approved for treatment of pneumonia in dairy cattle with zero withdrawal time, as well as for acute bovine interdigital necrobacillosis (Online Green book, 2003). Ceftiofur has worldwide approvals for respiratory disease in swine, ruminants (cattle, sheep and goats) and horses and has also been approved for foot rot and metritis infections in cattle. Since the extralabel use of ceftiofur for severe mastitis is not very efficacious (Erskine, 2002), ceftiofur is not widely used for mastitis. However, the zero withdrawal time of milk and meat still make the drug attractive.

Chloramphenicol

Chloramphenicol has been prohibited for food producing animal in the US since January 1986 (Veterinary Newsletter, 1996) and has been rigorously enforced by the FDA. The resistance to chloramphenicol is rendered by the inactivation with chloramphenicol acetyl transferase (CAT) (Prescott, 2000a) and by enhanced efflux. White et al. (2000) investigated 48 *E. coli* recovered from calves with diarrhea and found the majority of resistant *E. coli* had enhancing efflux genes (*flo* and *cmlA*), which may be disseminated via plasmids or a mobile trasposon(s). They suggested the extra-label use of florfenicol in calves was the major selection pressure of those resistant traits. Florfenicol is a structurally similar antimicrobial to chloramphenicol, and was approved for bovine respiratory treatment in 1996 (Online Green book, 2003).

Fluoroquinolone

Fluoroquinolone is not commonly used in dairy industry. Sarafloxacin was approved in the United States for the poultry in 1995, but the approval was withdrawn in 2001 (Federal Register, 2001). The Center for Veterinary Medicine of FDA proposed to withdraw approval of enrofloxacin for poultry use because of the possible transfer of fluoroquinolone-resistant *Campylobacter* spp. from poultry to human (Federal Register, 2000b). Enrofloxacin belongs to the fluoroquinolones group of antimicrobials and has been approved for treatment of bovine respiratory disease since 1998 in the United States (FDA, 2003a). The extralabel use of any fluoroquinolones in dairy cattle has been prohibited by the FDA in the United States (FDA, 2003b). However, extralabel drug use is practiced if the product being used is approved in that species for a different disease or if the product is used at a different dose or with an altered withdrawal time (Bateman, 2000).

E. coli

Osterblad et al. (2000) isolated the *Enterobacteriaceae* from human fecal samples and compared the antimicrobial resistance in *E. coli* to other enterobacteria, such as *Citrobacter*, *Klebsiella* and *Proteus*. They found little antimicrobial resistance in other enterobacteria and speculated that *E. coli* were the main carrier of antimicrobial resistance in fecal flora. Oppegaard et al. (2001) also suggested that *E. coli* is a major carrier of resistance traits in the coliform flora of cattle. *E. coli* have an ability to horizontally transfer their resistant determinants to other genera (Kruse, 1994). *E. coli* have been used in this and other studies as indicator organisms for antimicrobial

resistance monitoring activities among Gram-negative bacteria (Bager, 2001; NARMS, 2000). *Escherichia coli* are conditional pathogens causing urinary tract infections, wound infections, septicemia, and also are important agents of environmental clinical mastitis in dairy cows. They are present in the normal intestinal tract flora of most animals (Sorum, 2001). Indicator isolates of *E. coli* from Danish cattle (n=85) had resistance to tetracycline 4.7%, streptomycin 1.2% (modified based on our breakpoints), sulfamethoxazole 3.5%, and ampicillin 0% in DANMAP 2001.

Extended-spectrum β-lactamase (ESBL) producing strains of *Salmonella* have been noted in many countries since 1990s (Jing-Jou, 2003). ESBL is produced by transmissible plasmids, which could be acquired from other multidrug –resistant enterobacteriaceae, such as *Klebsiella pneumoniae* or *Escherichia coli*. Fey et al. (2000) reported that the ceftriaxone-resistant *Salmonella* isolate from the child was indistinguishable from one of the isolates from cattle, which was also resistant to ceftriaxone. Winokur et al. (2001) reported in their broad study of human isolates that high levels of co-resistance to aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, and ciprofloxacin were observed in ESBL producing strains of *E. coli*.

Campylobacter

Campylobacter spp. has been recognized as a cause of septic abortion, infectious infertility and diarrhea in cattle and sheep (Radostits, 2000). Abortions in cattle can be caused by *Campylobacter fetus* subsp. *veneralis* or *C. fetus* subsp. *fetus*, however *C. jejuni* and *C. coli* also recognized as causal agents of abortions (Larson, 1992; Welsh, 1984). *Campylobacter hyointestinalis* was reported as a cause of ileitis in pigs (Gebhart,

1983), bovine diarrhea (Diker, 1990) and human gastroenteritis (Gorkiewicz, 2002). Some *Campylobacter* species, such as *C. fetus*, were known cause of mastitis (Akhtar, 1993; Logan, 1982).

Campylobacter was not recognized as a cause of human enteritis until the mid-1970s when selective isolation media were developed for human stool culture. At present, campylobacteriosis is the most commonly reported human bacterial gastroenteritis in the United States, and the majority of infections are with *C. jejuni* (Nachamkin, 1999). The incidence of laboratory-diagnosed campylobacteriosis was 15.7 per 100,000 person-years in FoodNet surveillance sites (CDC, 2001) and an estimated 2 to 2.4 million infections occur in the United States each year (Friedman, 2000). The majority of sporadic cases of *Campylobacter* infections are foodborne and undercooked poultry is the most likely source of infections (Friedman, 2000; Pearson, 2000). Contaminated water and unpasteurized milk are common sources of outbreaks; nine percent of bulk tank milk was found culture positive for *Campylobacter jejuni* in a study of 131 dairy herds in South Dakota and Minnesota (Jayarao, 2001).

The *Campylobacter* spp. isolations were decreased approximately 16% by storing feces at 4°C for 24 hours (Ladron de Guevara, 1989). Cary-Blair transport medium with icepacks enabled *Campylobacter* spp. to maintain sufficient viability to be isolated at the laboratory (Luechtefeld, 1981; Wang, 1983, Wasfy, 1995). Nielsen (2002) found 9 out of 77 positive samples were only positive after growth in enrichment broth. The use of enrichment broth may increase the rate of *Campylobacter* spp. isolation (Bolton, 1982; Martin, 1983). *Campylobacter jejuni* subsp. *doylei*, *C. fetus* subsp. *fetus*, *C. upsaliensis*, and *C. hyointestinalis* are known to be inhibited by cephalothin (Nachamkin, 1999).

Other studies of *Campylobacter* spp. used an incubation temperature of 42°C to optimize the growth of thermophilic *Campylobacter* species, such as *C. jejuni, C. coli* or *C. lari*, with the decreased ability to isolate non-thermophilic species (*C. fetus*, and *C. jejuni* subsp. *doylei*). Atabay et al. (1998) used three kinds of media, enrichment technique, membrane filtration technique, and at three different incubation temperatures. They found 62% overall prevalence in 136 cattle in three farms in the U. K. Giacoboni et al (1993) found *C. fetus* subsp. *fetus* in 17% of cattle and *C. hyointestinalis* in 19% of cattle, whereas dominant species was *C. jejuni* found in 29% of 94 cattle in Japan.

Nielsen (2002) found that 23% of 332 animals, and 83% of 24 farms were positive for *Campylobacter jejuni* in Denmark. The prevalence was significantly higher in calves (42%) than young cattle (20%) or cows (9%). Wesley, et al. (2000) found *Campylobacter jejuni* in fecal specimens from 37.7% of 2,085 individual dairy cattle fecal samples, and 80.6% of 31 farms in the USA. Samples collected before May 1 contains more *C. jejuni* in cull cows than those taken later in the year. They also reported that *C. jejuni* were more frequently recovered from large herds (more than100 cattle) than from smaller dairy herds. Hoar, et al. (2001) also observed a higher prevalence in larger beef herds in California than from smaller herds, although the prevalence of *Campylobacter* spp. in these beef herds was much lower (5%).

Heuer et al. (2001) reported a 100% broiler flock prevalence of thermophilic *Campylobacter* spp. in Danish organic farms compared with a 36.7% prevalence in conventional broiler flocks. They suggested that the high prevalence of *Campylobacter* spp. in organic flocks may have been due to the aggregate effects of age, breed, housing, feed, and rearing system, and not related to the use of antimicrobials.

Antimicrobial resistance in Campylobacter

The agar dilution test was recently set by NCCLS as a reference standard susceptibility testing method for veterinary isolates of Campylobacter spp. (NCCLS, 2002). Ge, et al. (2002) recently reported that MIC values measured with the Etest were generally lower than the results obtained with the agar dilution method. Huang, et al. (1992) also compared the Etest method with the agar dilution method and reported slightly lower MIC values with the Etest than with the agar dilution.

Resistance to ciprofloxacin in human isolates of *Campylobacter jejuni* is reportedly increasing (Allos, 2001; Gaudreau, 1998; Smith, 1999). Ten percent of 604 *C. jejuni* isolated in NARMS EB 2000 program were resistant on Etest to ciprofloxacin, 52% were resistant to tetracycline, 0.5% were resistant to erythromycin and none were resistant to gentamicin (USDA, 2000).

Staphylococcus

Numerous reports have been published on antimicrobial susceptibility in *Staphylococcus* from dairy cattle, which were usually collected from individual quarter milk samples from cows with clinical mastitis (Devriese, 1980; De Oliveira, 2000). Bulk tank cultures for *S. aureus* could also be used as inexpensive and convenient monitoring methods for udder health (Jayarao, 2003). Antimicrobial resistance in *S. aureus* has primarily been studied on isolates from clinical mastitis that had been submitted to various veterinary diagnostic laboratories (Makovec, 2003; Erskin 2002b; De Oliveira, 2000; Bager, 2002). Multiple samples from a single farm may be common, and clonal isolates might have been included in the data. Most surveys did not account for multiple

observations from the same cow or herd, so clonal isolates of resistant bacteria from the same cow or herd might have greatly influenced the results. To avoid these complicated issues, De Oliveira et al. (2000) used only one isolates from a single herd. Samples submitted for diagnosis of clinical mastitis cases tend to exhibit more AR, probably because cases that are refractory to treatment are more likely to be cultured in an attempt to identify the causative agent and its AR traits (Aarestrup, 1998). Also, such studies have mainly attributed AR to antibiotics commonly used for mastitis therapy (Rossitto, 2001; Watts, 1995).

Effect of management factors on resistance

Langlois et al. (1988) reported that pigs on pasture have lower AR *E. coli* prevalence than pigs in finishing or farrowing house. They speculated the exposure to antimicrobials is not the only factor that influences the prevalence of AR bacteria. Hinton et al. (1985) reported a high prevalence of resistant *E. coli* in calves which did not receive oral antimicrobials. Bennedsgaard et al. (Bennedsgaard, T. W., S. M. Thamsborg, F. M. Aarestrup, C. E. Enevoldsen, and M. Vaarst, submitted for publication) recently reported a higher prevalence of AR *E. coli* in calves as compared to cows. Higher percentages of resistant bacteria in young animals, as compared with adults, were also observed in pigs (Hinton, 1987; Langlois, 1988; Larsen, 1972).

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CHAPTER THREE

MILK PRODUCTION AND MASTITIS ON MIDWESTERN ORGANIC DAIRY FARMS

ABSTRACT

Organic dairy farms in and around Wisconsin (n=101) were contacted in July 1999 with a one page questionnaire. A total of 69 responses were sufficiently complete for analysis. Herd size averaged 61.3 cows. Mean milk production was estimated as 17.2 kg per cow per day, which is less than the Wisconsin state average of 20.73 kg per cow per day. The average bulk tank somatic cell count was 329,000 cells per ml, which was almost identical to the Wisconsin average of 335,000 cells per ml. The overall rate of mastitis was 42 cases per 100 cow years-at-risk, which is within the range of rates reported in the literature. The cull rate based on July 1999 was 25% per year, which is low-normal for the Midwestern dairy industry. Only 16% of responding organic farms usually used non-conventional remedies, and 50% of farms never used non-conventional remedies. This preliminary survey suggested that the responding organic herds had similar herd size, mastitis rate and SCC as did conventional herds in the same geographic area. In contrast, their milk production and culling rates were lower than those reported for most conventional herds.

INTRODUCTION

Organic dairy production is drawing increasing attention because of public concerns about food safety, animal welfare and the environmental impacts of intensive livestock systems (Weller and Cooper, 1996). Though the U. S. organic food market is approximately $6 billion, which is less than 1% of total food consumption in the USA, the organic market has been growing at 20-30 percent per year (Greene, 2000). In contrast, the organic milk market in Denmark is approximately 14% of the total milk consumption (Mann, 1999). More than 25% of total sales of dairy products in Switzerland is labeled as organic (Busato et al., 2000). In the UK, a 30 to 40% per annum increase of organic products was observed (Weller and Bowling, 2000). Organic agriculture is being recognized by governmental bodies as a tool to improve rural income diversity and stability (Anonymous, 2000a).

The definition of "organic" has varied from country to country and among U. S. certifying organizations, causing consumer confusion, farms were certified as organic dairies by independent certifying organizations based on their own standards. The USDA has attempted to establish federal standards since 1990, and a revised National Organic Standard was recently published in the Federal Register (Anonymous, 2000b). Organic dairies must use organic feedstuffs which are grown without any chemically synthesized fertilizer, herbicides or insecticides. The use of antibiotics for therapy or prophylaxis and hormones for growth promotion or production enhancement are prohibited. Since many conventional veterinary prophylactic and therapeutic medicines are not permitted, organic dairy farmers have adopted a variety of management practices to prevent clinical disease.

It has been reported that organic dairies had higher culling rates, primarily due to

the development of udder infections and reproductive problems (Anonymous, 1998). However, Wellers et. al (2000) reported that the rate of clinical mastitis in 10 organic dairies in the UK was not significantly different from the rate of clinical mastitis in conventional dairies. Busato et al. (2000) reported the prevalence of subclinical mastitis in organic dairies in Switzerland to be lower than the national average.

The purpose of this survey was to determine if herd size, milk production, mastitis incidence, SCC and culling rate of organic dairy farms differ from values reported for conventional dairy farms in the same region.

MATERIALS AND METHODS

In August 1999, a one-page questionnaire regarding milk production, animal movement and mastitis was mailed to 101 dairies (80 in Wisconsin, 13 in Minnesota, seven in Iowa and one in Illinois). Non-responders received a second questionnaire six weeks later. Wisconsin state averages were used as comparison data. Statistical analysis was performed using Excel[(a)]. A Z test was used to test the hypothesis that the organic farms did not differ from the Wisconsin state average for milk production and herd size.

RESULT

A total of 73 responses were received, four of which were excluded due to missing data. There were 3,581 cows contributing bulk tank milk on responding farms in July 1999. Based on an assumed 13 months calving interval and two months dry period (Anonymous, 1998), the total number of milking cows (cows which have had at least one calf) was estimated at 4,232 or 61.3 head per farm. Only three farms had more

than 100 milking cows, and two farms had less than 20 milking cows (Figure 3-1). In 1999, the average number of milking cows per farm was 62.3 and 82.1 for Wisconsin and the U. S. dairy herds, respectively (Anonymous, 2000c). Therefore, the organic farms we studied had about the same herd size as the average dairy farm in Wisconsin (p=0.87), however they were much smaller than US average (p=0.0011). Production parameters were summarized in table 3-1.

The amount of bulk tank milk produced per day per cow in the 69 organic dairy farms was 17.16 Kg (75.6 metric ton / 4,232 cows) which was lower than the Wisconsin state average of 20.73 kg (p=0.001) (*8*). There was considerable variation reported for milk yield; herd average milk production per cow ranged from 8.5 Kg to 25.6 Kg (Figure 3-2). Herd bulk tank SCC averaged 329,000 cells/*ml* of milk. The lowest SCC was 104,000 cells/*ml* and the highest SCC was 935,000 cells/*ml*. Two farms reported SCC >900,000 cells/*ml* during single month, and five farms had approximately 500,000 cells/*ml* (Figure 3-3). The mean bulk tank SCC in Wisconsin for 1995 to 1998 was 335,000 cells/*ml* for grade A herd and 480,000 cells/*ml* for grade B herd (Ruegg and Tabone, 2000); SCC values for responding organic herds approximated the mean reported values for all Wisconsin dairy herds.

The producers were asked to recall the number of mastitis cases in the "last month" (July 1999) and also the number of mastitis cases in the "last year" (June 1998 - July 1999). Clinical mastitis was defined in this questionnaire as "a period of disease when a cow has noticeable clots or strings in its milk". When based on "last month", the mastitis rate was estimated at 42.4 cases per 100 cow-years at risk, but when based on "last year", the mastitis rate was only 17.7 cases per 100 cow-years at risk.

When the organic producers found mastitic quarters, 72% usually treated the quarter by keeping it milked out. Sixty two percent of responding farms usually massaged the mastitic quarter, but 22 producers (33%) occasionally massaged the quarter and three farms (5%) never massaged the quarter. Sixty three percent of producers usually or sometimes used vitamins, but 36% of producers never used vitamins for mastitis treatment. Whey colostrum products for mastitis treatment were usually used by 28% of producers, 29% sometimes used whey products and 43% of producers never used these products. Sixty-four percent of producers had never used any udder infusions for mastitis treatment, but 36% of producers occasionally used various kind of non-antibiotic udder infusions, such as aloe products. Only 16% usually used non-conventional remedies, 34% sometimes used non-conventional remedies and half (50%) of the organic farms never used non-conventional remedies for mastitis.

Sixty nine responding organic producers reported the number of cows sold for slaughter in the month of July 1999 and between June 1998 and July 1999. The total number of milking-age cows sold for slaughter were 88 in July 1999 and 795 in the previous year. The cull rate was therefore 25.0 % (88/4232*12) and 18.8 % (795/4232) for July 1999 and for the previous year, respectively.

Four organic producers reported selling a total of 13 milking-age cows to other organic dairy farms during 12 months in 1998. Eight organic producers sold a total of 32 cows to conventional dairy farms in 1998. Five organic dairy producers treated a total of nine organic cows with antibiotics and sold them to conventional farms.

Organic producers reported that 15 cows died in the month of July 1999, and 92 died in previous year. Fifty four producers reported no dead cattle in July 1999 and 19

reported no death in the previous year. The mortality rate was estimated at 4.3% (15*12/4232) based on "last month" and 2.2% (92/4232) based on the "previous year".

DISCUSSION

The data for this study was from the membership of one particular organic dairy cooperative in Wisconsin and surrounding states. Therefore, the results of this analysis may not be applicable to other organic dairy organizations. The response to our questionnaire was moderately high (72%). However, non-respondents may have differed from respondents in their management and production parameters (Bartlett, 1992). Although defined on the survey form, the definition of clinical mastitis is subjective and open to interpretation, therefore reporting bias may have occurred.

Our study found that organic dairy farms were about the same size as other farms in the region. Ogini et al. (1999) studied six organic dairies in Ontario and found that organic dairy producers had comparable tillable land base and herd size (48 cows per herd) as did conventional dairies. The study in Switzerland indicated the organic herd size was equal to the national average (Busato et al., 2000). Herd size may be determined by the regional conditions rather than the type of dairy husbandry. Though the organic dairies in our study were much smaller than the U. S. average, this does not imply that larger organic dairy operations are impractical; large organic dairy operations can be found in other parts of the U. S. (Anonymous, 1999a).

The mean milk production reported by the organic dairy farms was lower than the Wisconsin state average. Lower milk production per cow when compared to the conventional dairies in the same region was supported by other studies (Busato et al.,

2000, Krutzinna et al., 1996, Reksen et al., 1999). Lower milk yield per cow is likely attributable to more pasture and less grain being used for organic dairy production (Krutzinna et al., 1996). Grazing farms usually produce less milk per cow than do nongrazing farms (Hanson et al., 1998).

The rate of clinical mastitis based on data from July 1999 was slightly higher than rates reported from other regions (Bartlett et al., 1992, Weller and Bowling, 2000); however, the results based on data from the previous year were lower than what has been reported for other regions. The mastitis rates from other populations are difficult to compare because of the regional differences, poor standardization of case definition and high variance in diagnostic acumen among the studies (Bartlett et al. 2001). The mastitis rate, based on July data, may be higher than the rate based on the "previous year" because the more recently occurring cases were more easily recalled (recall bias). Also, mastitis incidence is usually highest in July and August (Erskine et al., 1988). In and around Wisconsin, July 1999 was a record breaking hot months. Therefore, the mastitis rate may be overestimated when the July rate is extrapolated to a whole year.

The bulk tank SCC average of the 69 organic dairies, based on July, was within the range of the Wisconsin state average. The SCC data for the organic herds may be overestimated because it was based on the month of a year (July) which has been reported to have the highest SCC (Anonymous, 1999b; Erskine et al., 1988). Although Weller and Davies (1998) reported a higher SCC in one organic herd in a six-year longitudinal study as compared to conventional herds, other studies reported lower SCC in organic herds than what was found in conventional herds (Busato et al., 2000). The

SCC data reported here suggested that responding organic dairies may have lower average bulk tank SCC than do most conventional dairies.

Radke and Lloyd (2000) reported that culling rates are difficult to compare because of different definitions of culling. The culling rate in our study was defined as number of cows culled per year divided by the average milking herd size. The culling rate in the reports of the Dairy Herd Improvement Associations (DHIA) include the average herd size plus the number of animals culled in the denominator (Radke and Lloyd, 2000), so the rates become lower than they would be using our definition. The statewide DHIA culling rate of Holstein cattle in Wisconsin was reported as 37.6% (AgSource CRI, personal communication). Therefore, the estimated culling rate of the organic dairies was considerably lower than the Wisconsin average. The estimated culling rate in the organic dairies based on "last month" was almost identical to the national average of 25.0% (Anonymous, 1996), but the 18.8% estimate based on "last year" was lower than the national average. Recall bias in failing to remember animals culled 2 to 12 months ago may have caused some of the difference between our two rates estimates. Also important is the previously discussed issues of extrapolating data from a particularly hot month of July to an annual rate. Reksen et al. (1999) reported a significant difference between organic and conventional dairy farms in annual replacement. Norwegian organic dairies had a 23% cull rate, whereas conventional dairies had a 35% cull rate, which resulted in more multiparous cows in organic dairies as compared with conventional dairy farms. A culling rate of 20-30% optimizes producer profit (Radke and Lloyd, 2000), therefore the culling rate in the organic dairies (18.8-25.0%) may be economically optimized.

This preliminary survey suggested that organic dairy farm in Midwestern have lower or at least similar bulk tank SCC and mastitis rates as compared conventional dairy farms in the same region. Lower milk yield per cow may reduce stress on the udder, causing a lower mastitis rate in organic dairies. Culling rates appeared to be lower, or at most similar, in the organic herds as compared with state and national averages. Non-Non-conventional remedies were not widely used by the organic farms, and there was little movement of sick cows from organic to conventional farms.

(a) Microsoft Excel 2000, Microsoft Corp., Redmond, Wash

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Figure 3-1. Frequency distribution on number of lactating cows in July 1999

Number of farms (y axis) for each herd size (x axis).

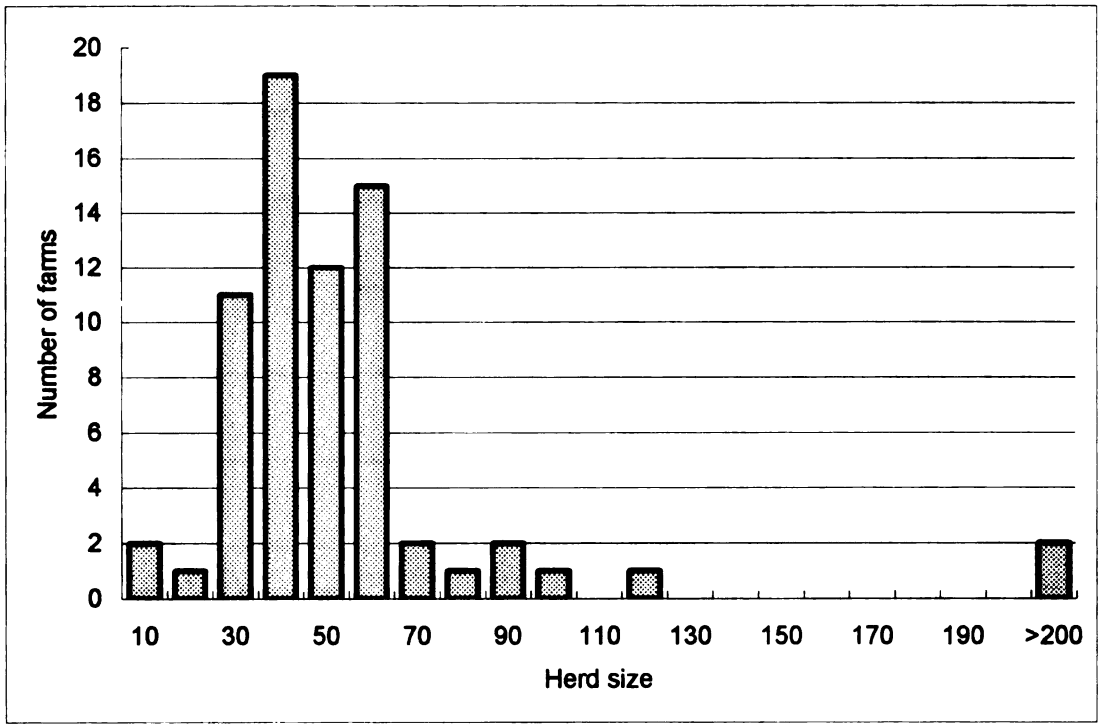

Figure 3-2. Frequency distribution of average milk production per cow per day

Number of farms (y axis) for each production class (x axis).

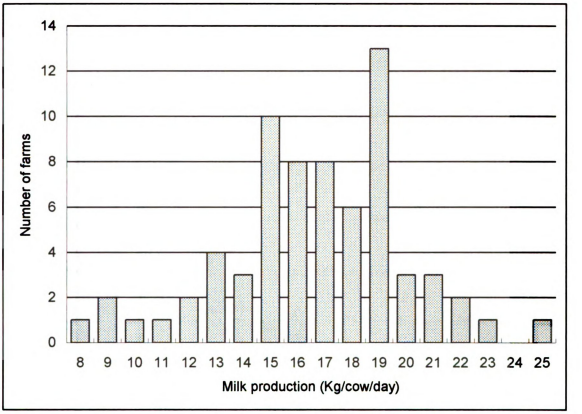

Figure 3-3. Frequency distribution on bulk tank SCC in July 1999

Number of farms (y axis) for each SCC class (x axis).

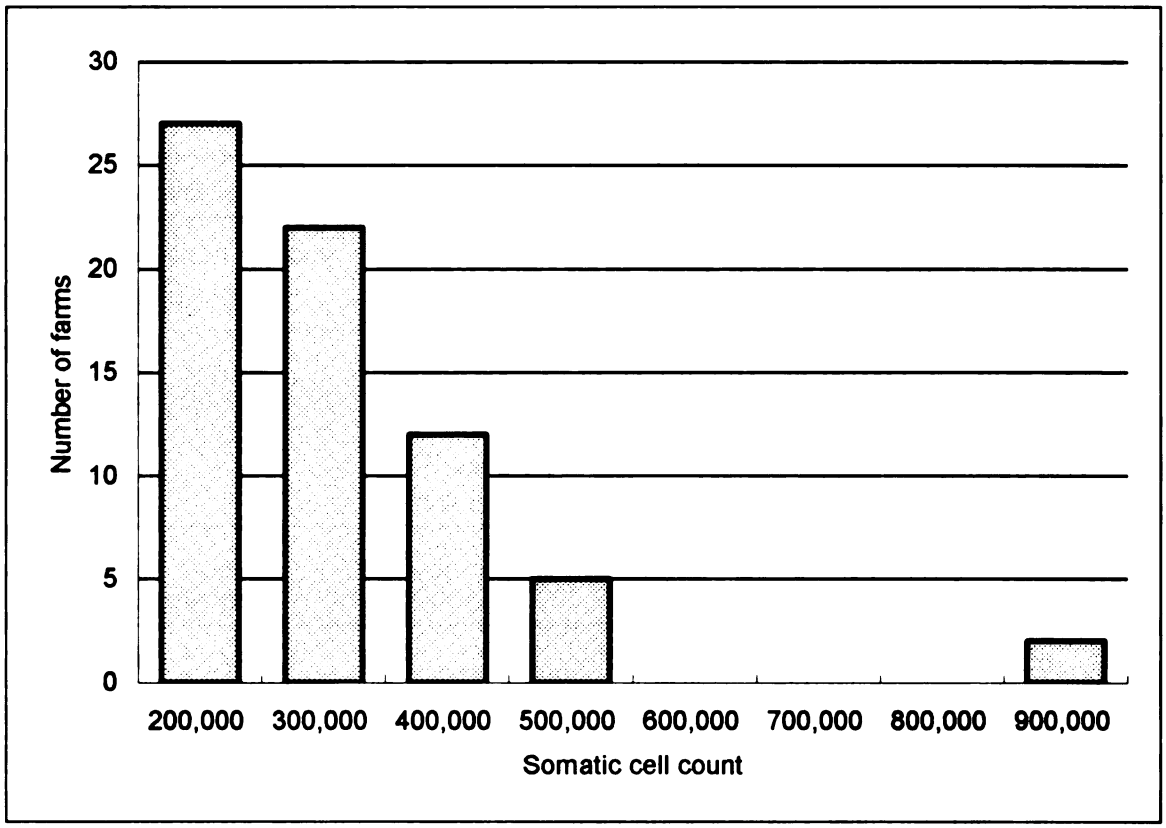

Table 3-1. Estimated production parameters based on a presumable calving interval of 13 months and the dry period of two months with available Wisconsin state averages. The Wisconsin state average was extracted from USDA_NASS Agricultural Statistics 2000 (Anonymous, 2000c) and SCC from Ruegg and Tabone (2000).

	Organic farms (n=69)		Wisconsin state average
Number of cattle per herd	61.3		62.3
Milk production per cow (Kg per day)	17.8		20.3
SCC (cells/*ml*)	329,000		328,000-335,000
	(Based on last month)	(based on last year)	
Mastitis (case/100 cow-year)	42.4	17.7	Not Available
Culling rate (%)	25.0	18.8	Not Available
Mortality rate (%)	4.3	2.2	Not Available

Appendix 1. Questionnaire

Initial Survey - August 1999

How many cows are now contributing milk to your bulk tank? _____

How many years has your dairy farm been organic? _____years

If a "case" of mastitis is defined as a period of disease when a cow has noticeable clots or strings in its milk, about how many cases of mastitis have you had in:

the past week? _____ the past month? _____ the past year? _____

About how many milking-age cows:

	last month?	last year?
died	_____	_____
were sold for slaughter	_____	_____
were sold to an organic farm	_____	_____
received antibiotics and were sold to a non-organic farm	_____	_____
were NOT treated, but were sold to a non-organic dairy farm	_____	_____
Other _____	_____	_____

Please indicate how you usually treat your routine cases of clinical mastitis in which the cow's milk has clots or strings, but the cow is not sick.

		Circle one	
Keep the quarter milked out.	usually	sometimes	never
Massage the quarter.	usually	sometimes	never
Use vitamins (B, C, etc.)	usually	sometimes	never
Use Impro (or similar whey colostrum product)	usually	sometimes	never
Use other udder infusions (describe type)	usually	sometimes	never
Other	usually	sometimes	never

CHAPTER FOUR

COMPARISON OF PRODUCTION AND MANAGEMENT BETWEEN ORGANIC AND CONVENTIONAL DAIRY HERDS IN WISCONSIN

ABSTRACT

An observational study was conducted in Wisconsin to compare production and management on organic and conventional dairy farms. Thirty organic dairy herds, where antimicrobials are rarely used for calves and never used for cows, were compared with 30 neighboring conventional dairy farms on which antimicrobials were routinely used for animals of all ages. A seven-page questionnaire regarding milk production, milking practices, housing, incidence of the major dairy diseases and medical treatments was used to assess management and production during 2000-2001. Body condition scores of lactating cows and environmental and animal sanitation scores were also collected on each of two farm visits. The organic herds had significantly fewer cattle than did the conventional herds ($p=0.017$). The average daily milk production per cow in organic dairy herds (20.2 kg/day) was lower than that of conventional herds (23.7 kg/day). The incidence of clinical mastitis on organic farms (28 cases per 100-cow-years at risk) was not statistically different from that of on conventional farms (32 cases per 100-cow-year at risk). No significant difference in bulk tank somatic cell count was observed between organic (262,000 cells per ml) and conventional farms (285,000 cells per ml) farms. The average annual cull rate was 18.0 cases per 100-cow-years for the conventional farms and 17.2 for the organic farms ($p = 0.426$). There was little evidence of other fundamental differences between two farm types in other major management and production parameters.

INTRODUCTION

Organic dairy production is drawing increasing attention because of public concerns about food safety, animal welfare and the environmental impacts of intensive livestock systems (Weller, 1996; Sundrum, 2001). The organic food market is approximately $6 billion, which is less than 1% of total food consumption in the USA. However, the organic market has been growing at 20-30 percent per year (Green, 2000; 2001). In contrast, the organic (økologisk) milk market in Denmark is approximately 14% of the total milk consumption (Mann, 1999) and more than 25% of total sales of dairy products in Switzerland is labeled as organic (Busato, 2000). In the UK, a 30 to 40% per annum increase of organic products was observed (Weller, 2000). Organic agriculture is increasingly being recognized by governmental bodies as a tool to improve rural income diversity and stability (FAO, 2000).

The standards for using antibiotics in organic dairies in the EU are less strict than those in the USA. The USDA Organic Standard prohibits the use of any animal drug in the absence of demonstrated clinical illness (USDA, 2001). The standard also stipulates that all appropriate medications and treatments must be applied to restore an animal to health when methods acceptable to organic production standards fail, however, this means that the animal will lose its organic status.

Mastitis is a major cause of economic loss in the dairy industry and the primary reason for which antibiotics are used in dairy operations (Kaneene, 1992). Field studies in Pennsylvania, Ohio, and California indicated that the average annual herd incidence of CM was 45 to 50 cases per 100 cows (Hady, 1993). Milking hygiene and environmental sanitation are traditional ways to prevent the disease (Bartlett, 1992a). Antimicrobial use

for treating CM is a common practice on most US dairy farms. Routine intramammary treatment for all cows with long-acting antimicrobials after the end of lactation (dry-cow-treatment) is widely adopted in the US as a preventive method. (Jayarao 1999; Hardeng 2001). Though mild cases of CM may not always receive antibiotic treatment during the lactational period, dairy producers and veterinarians often treat severe cases with supportive therapies and intramammary antibiotic infusion or injection.

Organic dairy herds may have higher culling rates, primarily due to the development of intramammary infections and reproductive problems. However, Weller and Bowling (2000) reported that the incidence of CM in 10 organic dairies in the UK was not significantly different from the rate of CM in conventional dairies. Busato et al. (2000) reported that the prevalence of subclinical mastitis in organic dairies in Switzerland was lower than the national average. Few studies have compared the incidence of CM on organic and conventional dairy farms in the US. Barlow (2001) reported bacteriological analysis of 109 CM quarters on 6 organic farms in Vermont, however, no incidence of CM was given or compared with conventional dairy farms.

In Wisconsin, the organic dairy farmers sell their milk for almost twice as much as what conventional farmers receive. As such, organic milk production has created a niche market that has allowed many small dairy farms to stay in business during a time when profit margins are small, the dairy industry is consolidating and many small and moderately sized dairy herds are going out of business.

In general, the organic restrictions on the use of insecticides and herbicides for producing animal feed have induced most Midwestern organic dairy farms to employ grazing to a much greater extent than do conventional farms. This generally creates less

intensive feeding and housing management systems compared with what is seen in the mainline dairy industry. It is generally assumed by the dairy industry that organic farms have lower milk production and higher culling and disease rates because farmers are not allowed to treat sick cows with antibiotics. However, valid comparisons are not available and are confounded by comparisons between small extensive organic dairies and large total-confinement dairy operations which employ a very different intensive nutritional and management strategy.

The overall purpose of this study was to compare the major health, management and milk production parameters between organic and neighboring conventional Wisconsin farms.

MATERIALS AND METHODS

A geographical cluster of 30 organic dairy farms in Southwestern Wisconsin were selected from 110 members of an organic dairy association (Sato, 2002). All farms were certified as complying with the US national organic standards and had been selling organic milk through their organic association for at least 3 years before the start of our study. For each organic farmer selected, a neighborhood "conventional" dairy farmer was asked to volunteer their farm to serve as a control. Thirty of 37 organic farmers who were contacted, agreed to participate in the study. The conventional dairy farms were chosen from the nearest neighboring conventional farms, with only five cases of a farmer declining to participate.

All herds were visited twice; once in March and once in September. Fecal specimens were collected for comparison of antimicrobial resistance, as described

elsewhere (Sato, K., submitted for publication). Numbers of cows sold or culled were recorded. Herd average milk production, Bulk Tank Somatic Cell Count (BTSCC) and bacteria counts of the previous month were obtained from the milk production receipts or other production records whenever available. Average daily milk production per cow was computed from the amount of bulk tank milk per day divided by number of lactating cows. A seven-page questionnaire regarding milk production, milking practices, housing, incidence of the major dairy diseases, medical treatments and other management factors was conducted at the first visit; 10 pairs of herds in the Spring of 2000, 10 pairs in the Fall of 2000 and 10 pairs in the Spring of 2001. On the second farm visit, previously collected management and production data were reviewed and verified.

Clinical mastitis (CM) was defined in this study as a cow having a swollen or hard udder or noticeable clots or strings in its milk . The dairy producers were asked to retrieve from their records (or recall from memory) the number of CM cases in the three months before the interview. Recurrent episodes of disease were counted as one case if the episodes occurred within two weeks after the initial case. Cows with multiple quarters affected with CM were counted as one case. The CM rate for each herd was calculated as the number of CM cases per 100-cows-years at risk.

Bulk tank milk samples from each of the two visits were collected and sent to the laboratory at Atlantic Veterinary College, University of Prince Edward Island for examination of antibodies against *Ostertagia ostertagi* by an indirect enzyme-linked immunosorbent assay (ELISA) (Guitian, 2000). The optical density (OD) values of ELISA test were adjusted with positive and negative controls, and the adjusted OD data were used for the rest of analysis.

At each visit, the body condition score (BCS) was measured on ten systematically selected lactating cows. Cows were scored from 1 (thin) to 5 (fat), with increments of 0.25, (Wildman, 1982). A Body Condition Score Guide with photographs (Church & Dwight Co. Inc., Princeton, NJ) was used for standardization. The mean of ten BCSs was used as a representative value for each farm for each season.

Environmental and animal sanitation was measured with subjective scores of cow cleanliness and the amount of moisture and manure in the bedding and exercise areas, as previously described (Bartlett, 1992a). Subjective sanitation score were graded as 1 (presumed upper 1/3 of all dairy farms in Wisconsin), 2 (middle 1/3) and 3 (lower 1/3), given current weather conditions. The scores were considered to be discrete variable rather than ordered categorical data, and mean value of all environmental and animal sanitation score (EASS) were computed for each farm in each season.

Statistical Analysis

The questionnaire was proofread twice for correct data entry and were evaluated for reasonableness. No data were excluded as being unreasonable or impossible responses. The milk production per cow, CM rate, BTSCC, BCS, EASS, and adjusted OD data from ELISA test were tested for normality with the Shapiro-Wilk test and no transformations were deemed necessary ($W > 0.95$ in each instance). Bacteria count and total cow per herd (herd size) were transformed with a log function and the transformations yielded W-values of 0.96 and 0.98, respectively. A mixed linear model with random effects was used to estimate the difference in continuous production variables between organic and conventional dairy farms. Farm type, season, and

interaction of farm type and season were included in the model as fixed effects. Pairs (each organic farm and its conventional neighbor farm) were included as a random effect to reflect the pairwise matching. For discrete variables, such as grazing intensity, milking methods and mastitis treatments, McNemar's test was used to examine differences between the two farm types. For culling and mortality analysis, Poisson regression was used to estimate the difference between farm types. The adjusted OD values of ELISA test for each season were tested by paired t-test, and then analyzed with mixed liner model which included farm type, season, grazing intensities and milk production per cow as fixed effects and pairs as a random effect. All statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC).

RESULTS

The mean herd size was 72 cows (range 35 - 223) for conventional herds and 51 cows (range 37 - 132) for the organic herds. The organic herds were significantly smaller than the conventional herds ($p=0.017$). Eighteen organic herds and 22 conventional dairy herds consisted of 100 % Holstein breed, whereas the other 12 organic and 8 conventional herds consisted of Jersey, Brown Swiss, Guernsey or mixed breed. McNemar's test showed there was no statistical difference in breed choice between the two types of farms. However, estimations of herd breed composition (percentage of Holstein breed) showed preference on organic dairy farms for non-Holstein and mixed breeds ($p=0.10$).

Organic farms had lower milk production per cow (20.2 kg/day) compared to conventional farms (23.7 kg/day)($p=0.013$). The CM rates were 27.7 (SD=20.7) and

32.1 (SD=21.8) cases per 100-cows-year at risk for organic and conventional herds (p=0.65), respectively. The frequency distribution of herd CM rates is shown at figure 4-1. The overall mean BTSCC was 274,000 cells /ml (SD=89,700) and was approximately normally distributed (Shapiro-Wilk = 0.968). The arithmetic mean BTSCC for organic herds was 263,000 cells /ml (SD= 101,000) and 285,000 cells /ml (SD= 137,000) for conventional herds (p=0.333), as shown in figure 4-2. Half of the organic herds (15 farms) applied intensive grazing during summer, whereas only two conventional herds used intensive grazing (p=0.008). Free stalls were used on 9 conventional herds, in contrast to being used on only four organic herds (p=0.059).

The total number of milking-age cows culled for any reason were 367 per year from the conventional herds and 247 per year from the organic herds. The average annual cull rate were thereby calculated as 18.0 cases per 100-cows-years (SD=9.00) for the conventional farms and 17.2 cases per 100-cows-years (SD=9.76) for the organic farms ($p > 0.426$). The incidence of cow deaths on farms were 90 (4.2 cases per 100-cow-yeasr at risk) on conventional and 47 (3.1 cases per 100-cow years) on organic dairy herds (p=0.082). Only four cows in two organic farms were sold to conventional farms as milking cows in a year.

The most common type of milking systems were stanchion or tie stall systems (51/60). Only one organic farm used a milking parlor compared to 8 conventional farms (p=0.008). There was no significant difference in 9 milking procedures which were assessed and compared between paired organic and conventional dairy farms (Table 4-2). Fourteen organic farms and 17 conventional farms used pre-milking teat disinfections

(pre-dipping) and most farms (27 organic and all conventional farms) use post-milk teat disinfections.

Though 26 conventional dairy farms use udder infusions for dry cow treatment, only 18 producers reported regular use of antimicrobial udder infusion for CM treatment. Udder infusion of cephapirin was frequently used by nine conventional producers and penicillin was frequently used by four conventional producers, whereas the remaining others conventional producers did not report which specific antibiotic products were used. One conventional producer did not know what treatments were given by his veterinarian and two producers use only injectable antimicrobials for treatment of CM. Seven other conventional dairy producers reported that they usually do not use antimicrobials for CM. Thirteen conventional producers used oxytocin and 25 conventional producers reported frequent stripping out (frequent emptying of the udder) as CM treatment. None of the organic producers used antimicrobials to treat CM. They reported using anti-inflammatory drugs and stripping out the quarters at frequent intervals as the most common CM treatments. On 19 organic farms, natural remedies (whey products, herb, mineral oil, vinegar), vitamin E (parenteral), C and selenium were occasionally used. Two organic producers allocated mastitis cows for sucking by calves as the mastitis therapy and one organic producer provided no special care for CM for several years. Table 4-2 shows the major differences among the two farming types in the treatment of CM.

In the Spring, organic dairy farms had significantly lower (thinner) BCS than did the conventional herds ($p=0.001$), however no significant difference was observed in September ($p=0.66$). We did not find any difference in environmental and animal

sanitation score among the two farm types. Neither CM nor BTSCC had shown any association with BCS and EASS in the mixed liner analysis. A paired t-test of adjusted optical density data from ELISA test had shown significant difference between organic and conventional farms in both seasons ($p=0.0059$ for March and $p=0.0067$ for September). However, a mixed liner analysis indicated that the type of farm (organic or conventional) was not significant ($p=0.8730$) when controlling season, grazing intensity and milk production. The season ($p=0.0081$), grazing intensity ($p=0.0217$) and average milk production per cow ($p=0.0053$) were highly associated with adjusted OD value of the milk ELISA test.

DISCUSSION

Though using neighboring conventional herds as a comparison group helped control for regional differences, the selection of the control farms was not random and was certainly not representative of Wisconsin conventional herds or the national dairy industry. Due to their proximity, it is likely that organic and conventional farms shared many management characteristics. For example, milking procedures were very similar between matched pairs, so the effect of milking procedures on CM rate or BTSCC could not be estimated. According to the NAHMS study (USDA, 1996), 88 % of dairy operations use udder infusion for all four quarters on almost all cows, which agrees relatively closely with our observation that 26 conventional dairy farms (87%) used udder infusions for dry cow treatment. However, the percent of operations which use injectable antimicrobials for milking cows was lower in our study (60%) compared to the 1996 NAHMS study (93.5%). It was difficult to identify the amount and type of antimicrobials

used on conventional farms. Two conventional dairy producers in our study claimed they had not used any antimicrobials for any purpose for several years.

Animal numbers per herd (71.7 cows) and milk production per cow (23.7 kg/day) in our conventional herds were similar to the Wisconsin average (67.0 cows and 25.5kg/day), but were smaller than the national average (93.4 cows and 27.0 kg/day; USDA, 2002). We did see some evidence that organic farms tended to have smaller cows. They had a greater proportion of smaller non-Holstein breeds. They were less likely to use artificial insemination, which tends to result in a gene pool of smaller cows compared with what is commonly seen in today's herds that have used AI to produce large cows with high milk production per cow. Therefore, because smaller cows eat less feed, comparisons in milk production between organic and conventional herds must consider this lower cost of production. Also, other differences in nutrition and management most likely act to reduce the per-cow cost of feeding and maintaining smaller grazing cows, as compared with larger genetically selected cows being fed a high energy diet in total confinement.

Dairy cow mortality (4.2 deaths per 100 cow-years) on the conventional dairy herds was compatible with the U.S. average (4.4 for less than 100 herd size), however the culling rate (18.0) was somewhat lower than the U. S. average (24.9 for less than 100 herd size; USDA, 2002). Another unique feature of this region of Wisconsin regarded the preponderance of the more traditional style of tie stall or stanchion housing. Twenty two percent (13/60) of operations used freestall housing, which was lower than the national average of 31 %, and 75 % (45/60) of the operations used tie stall or stanchion

housing, which was higher than the national average of 52% (USDA, 2002). This probably is reflective of the small herd size and harsh winter climate in Wisconsin.

We found significantly lower BCS in organic dairy cows in early spring. Because organic dairy producers are required to feed "organic feed", which must be grown without herbicides and pesticides. Purchase of feed is therefore difficult, and it may sometimes be difficult for organic producer to prepare sufficient concentrated rations for a long winter. Cows in year-round confinement may have a less variable feed supply as compared with herds which employ substantially more grazing.

Internal parasites are one of the main causes for lower heifer growth and reduced milk production in older cows. Anthelmintic treatment is prohibited on organic dairy farm, so higher prevalence of gastrointestinal nematodes in organic cows could expectedly be higher than what is commonly found in conventional dairy herd. The frequency and type of deworming was not measured in our conventional farms. According to a recent NAHM study, over 60% of dairy operations in the U. S. normally use dewormers for at least some lactating cattle (USDA, 2002).

Fecal egg counts (FEG) are commonly used for detecting gastrointestinal nematodes, however the test is time consuming and expensive, and the result is highly variable. The ELISA test uses a crude antigen of *Ostertagia ostertagi* that has been evaluated in number of previous studies (Kloosterman, 1996; Borgsteede, 2000). The optical density value of ELISA has been found to be a reasonable overall measure of parasite burden, and bulk tank milk is useful for testing whole-worm antigen (Guitian, 2000; Sanchez, 2002). Our paired t-test results indicated significantly higher parasite burden on organic dairy farms, however, no significant difference between the two farm

types when controlling for season (March and September), grazing intensity (no grazing, little grazing, grazing with access to housing, and grazing only) and herd average milk production per cow. The result may indicate that the organic farms may have a greater worm burden because of the increased use of grazing. Our data shows significant association with seasons, highest in late summer and lowest in spring. The observation agrees with the study result on serum antibody (Borgsteede, 2000). Herd average milk production per cow was also a significant predictor of the adjusted OD value in our study (i.e. higher producing herds were associated with lower worm burdens).

Though mean CM incidence on organic farms was lower than that of conventional farms (28 and 32), the difference was not statistically significant. The measure of herd CM in this study may have been affected by reporting bias. Conventional farmers must keep meticulous records of antimicrobial treatments, especially mastitis treatments, in order to avoid the substantial penalty resulting from contaminating an entire milk truck with antimicrobial residues. Organic farms have no such impelling need to record or remember their non-antimicrobial treatments and, therefore, incomplete reporting may have existed for organic farms. Also, farmers generally record or remember episodes of clinical treatment rather than episodes of disease. Clinical signs not sufficiently advanced to warrant medical treatment may not considered to be case of that particular disease. As such, disease reports on both organic and conventional farms likely reflect episodes for which the farmer decided that clinical signs were sufficiently severe to warrant treatment. Diagnostic acumen may therefore differ between organic and conventional farms because antimicrobial treatment generally entails withholding milk from sale for several days, and is usually not given to mild cases. Although we have no

evidence of this speculation, Berry (2002) reported that farmers converting to organic status in the UK were less likely to report cases of CM. It is therefore certainly possible that underreporting of clinical mastitis may have been greater on the organic farms as compared to conventional farms.

The arithmetic mean of BTSCC on 30 organic farm (263,000 cells per ml) was lower than that of conventional herds (285,000), however the difference (7.9% lower) was not statistically significant. Reporting of BTSCC from the milk receipts was uninfluenced by farmer reporting. Busato (2000) studied 152 certified organic farms in Switzerland and found the geometric mean of 85,600 cells/ml, which was 15% lower than the Swiss average of 100,000 cells/ml. The difference we found in BTSCC between organic and conventional farms (7.9%) was not statistically significant given the relatively small number of herds that we studied.

Despite of the possible reporting bias speculated for CM, the results of both CM rate and BTSCC suggested that organic dairy farms managed to successfully control mastitis without the use of antibiotics. For some mastitis pathogens such as *S. aureus* and non-severe coliform, antibiotics are often ineffective. (Kirk, 1994). Dry cow therapy infusions are effective against contagious mastitis pathogen, but are ineffective against environmental coliforms (Berry, 1997). A retrospective study of 9,007 cases of subclinical mastitis cases in New York and northern Pennsylvania showed the overall spontaneous bacteriological cure rate was 65% and the cure rate with antimicrobial treatment was not much better at 75% (Wilson, 1999). The majority of CM may be cured by udder immune mechanisms without much benefit of antimicrobial treatment, as witnessed by the fact that only 18 conventional producers reported regular use of

antimicrobial udder infusion for CM treatment. Some organic producers claimed that they keep their cows at a less stressful milk production level and thus maintain high immune function. Effective mastitis control should rely on prevention rather than treatment (Erskine, 1993) and early identification, culling, and segregation is probably the best management approach for controlling mastitis (Kirk, 1994).

Higher producing cows and herds tend to have greater problems with mastitis. Grohn et al. (1995) studied 8,070 cows in 25 herds and found high producing cows are more susceptible to mastitis. Schukken (1990) studied 125 herds and found a higher incidence of CM in herds with high milk production. The average daily milk production per cow in organic dairy herds was about 15% lower than that of conventional dairy herd in our study. This lower milk production in organic herds could account for at least some of the non-significant trend to reduction in mastitis and BTSCC which we observed for organic farms. Milk production without increasing CM may be attained by those principles in the organic herds.

CONCLUSION

The study showed that organic dairy farms were producing milk without significantly increasing reported CM rate, BTSCC or culling rate as compared with matched conventional farms. Notable differences between organic dairy farms and conventional farms were lower milk production per cow and smaller herd size. Organic herds were more likely to use intensive grazing, which may have accounted for their higher rate of gastrointestinal nematodes as compared with conventional herds.

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Table 4-1. Comparison between organic and conventional dairy farms. Means of continuous variables regarding management and production variables. The data was analyzed by PROC MIXED procedure with a random factor of "pairs".

Continuous variables	Organic (n=30)	Conventional (n=30)	Type III test p-value
Number of lactating and dry cows	51.1	71.7	0.017
Average milk production per cow (kg/day)	20.2	23.7	0.025
Mastitis rate per 100 cow-years	27.7	32.0	0.654
BTSCC (cells/ml)	263,000	285,000	0.328
Bacteria count of bulk tank milk (cells/ml)	4,200	4,800	0.433
Body condition score in March	2.58	2.81	0.001
Environmental Sanitation score in March	1.88	1.79	0.548
Body condition score in September	2.81	2.84	0.662
Environmental Sanitation score in September	1.83	2.02	0.157
Average labor (person-minutes) per cow per milking	2.66	2.68	0.925

Table 4-2. Comparison of discrete variables between organic and conventional dairy farms.

	Organic (n=30)	Conventional(n=30)	McNemar's test p-value
100% Holstein breed	18	22	0.248
Purchase of any cows in previous 12 months	4	9	0.132
Dry cow treatment with antibiotic infusion	0	26	NA
Grazing			
No grazing	1	7	0.096
Outside exercise area with little grazing	3	8	0.593
Grazing pasture with access to housing	11	13	0.001
Intensive grazing (grazing only)	15	2	0.008
Housing			
Free stalls	4	9	0.059
Loose housing	2	0	NA
Tie stalls	24	21	0.257
Bedding			
No bedding	3	1	0.317
Straw or corn shredder	20	13	0.090
Wood shavings or sawdust	3	10	0.008
Sand	3	5	0.480
Rubber mat	1	1	1.000
Milking			
Cows were milked in stanchion/tie stalls	29	22	0.008
Dry massage or wipe with no water used	6	6	1.000
Wash bucket usually used	10	6	0.206
Individual cow paper towel or cloths used for washing	14	11	NA
Shared towel or cloths used for washing	3	1	0.317
Pre-milking teat dipping usually used	14	17	0.405
Post-milking teat dipping always used	27	30	NA
Gloves used for washing udder	7	12	0.197
Individual towels (cloth or paper) used for drying	15	21	0.058
Teats are usually not dried before milking	4	1	0.180

Table 4-2 (cont'd)

Mastitis prevention and treatment			
Dry cow treatment with antibiotic infusion	0	26	NA
Strip out the quarter at frequent intervals	20	25	0.132
Anti-inflammatory or antipyretics drugs	10	6	0.317
Administer oxytocin to assist milkout	1	13	0.002
Udder infusions of antibiotics	0	18	NA
Systemic antibiotic injection	0	8	NA

NA: McNemar's test cannot be performed because some cells contain zero.

Figure 4-1. Frequency distribution of mastitis rate (100-cow-year) of organic farms (n=30) and conventional farms (n=30). Mean mastitis rate were not statistically different among two farm types (p=0.432)

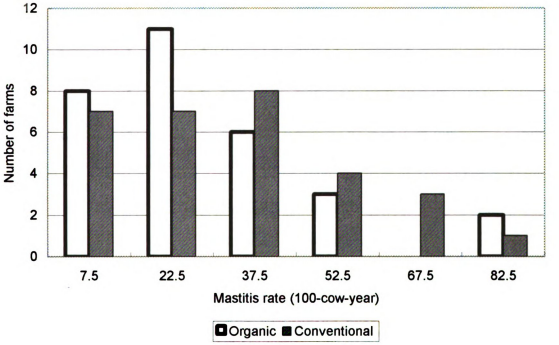
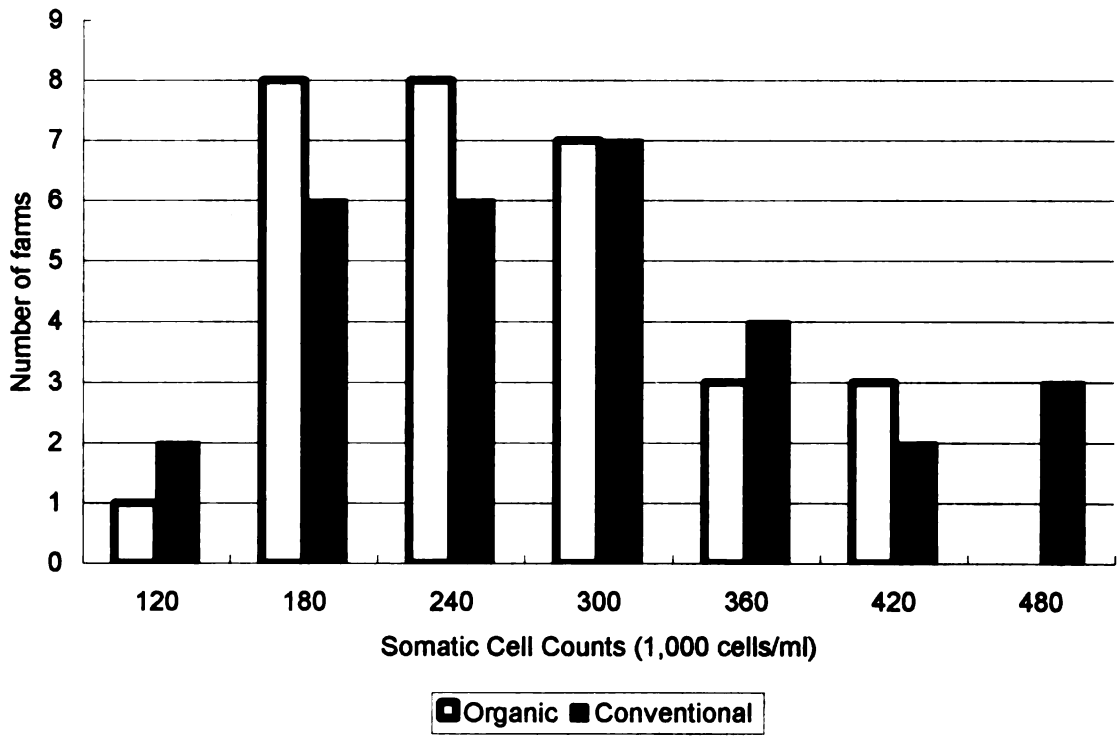


Figure 4-2. Frequency distribution of milk somatic cell count (SCC) of organic farms (n=30) and conventional farms (n=30). Arithmetic mean were not statistically different between two farm type (p=0.333).



CHAPTER FIVE

COMPARISON OF ANTIMICROBIAL SUSCEPTIBILITY OF *E. Coli* ISOLATED FROM ORGANIC AND MATCHED CONVENTIONAL DAIRY CATTLE IN WISCONSIN

ABSTRACT

A cross-sectional study was conducted in Wisconsin to compare antimicrobial resistance patterns in fecal *Escherichia coli* on 30 organic dairy farms, where antimicrobials are rarely used for calves and never used for cows, and 30 neighboring conventional dairy farms, where antimicrobials are routinely used for animals of all ages. Fecal specimens from ten cows and ten calves on each farm yielded 1,120 *E. coli* isolates, which were tested for resistance to 17 antimicrobials using a micro-broth dilution test. The highest overall rates of resistance were to tetracycline (26%), streptomycin (15%), kanamycin (14%), sulfamethoxazole (14%), and ampicillin (13%). Prevalence of antimicrobial resistance in *E. coli* was lower in organic dairy herds for ampicillin, amoxicillin-clavulanic acid, streptomycin, kanamycin, chloramphenicol, tetracycline, sulphamethoxazole, and trimethoprim-sulphamethoxazole. Although the organic farms had converted to organic farming methods at least 3 years before our study (mean = 8.0 years), antimicrobial resistance clearly presented long after antimicrobial selective pressure had been withdrawn.

INTRODUCTION

Some resistant bacteria isolated from human infections have reportedly been traced back to farm animals (Fey, 2000; Larsen, 1972; Molbak, 1999, O'Brien, 1982; Spika, 1987). Farm-level studies have shown an association between antimicrobial usage in food animals and rates of antimicrobial resistant in animals (Mathew, 2001; Van Den Bogaard, 2001). Generally, farms with higher antimicrobial usage have a higher proportion of resistant bacteria, as well as the presence of multi-drug resistance strains. However, the magnitude of the contribution of livestock production practices to the growing antimicrobial resistant problem is unclear. (Sorum, 2001; Torrence, 2001).

Antimicrobial agents are used less frequently in dairy cattle than they are in other food-producing animals. Because milk from antibiotic-treated cows must be withheld from sale, most antimicrobials are used relatively conservatively in the dairy industry. Most antibiotic use in adult cows is for treatment and prevention of clinical mastitis (Hady, 1993). In dairy calves, most antimicrobial usage is for treatment and prophylaxis of diarrhea/pneumonia, and as a medicated milk replacer (McEwen, 2002; NAHMS, 2002).

Escherichia coli most commonly are conditional pathogens causing urinary tract infections, wound infections, septicemia, and hemorrhagic colitis in humans and also are important agents of environmental clinical mastitis in dairy cows. They are present in the normal intestinal tract flora of most animals (Sorum, 2001). As commensal bacteria, *E. coli* are of concern to human health because they are likely to be transferred to humans through food or water. Osterblad et al. (2000) isolated *Enterobacteriaceae* from human fecal samples and compared the antimicrobial resistance in *E. coli* to other enterobacteria

such as *Citrobacter*, *Klebsiella* and *Proteus*. They found lack of antimicrobial resistance in other enterobacteria and speculated that *E. coli* were a principal carrier of antimicrobial resistance in fecal flora. Oppegaard et al. (2001) also suggested that *E. coli* is a major reservoir of resistance traits in the coliform flora of cattle. *Escherichia coli* have an ability to horizontally transfer their resistant determinants to other genera (Kruse, 1994). *Escherichia coli* have been used in this and other studies as indicator organisms for antimicrobial resistance monitoring activities among Gram-negative bacteria (Bager, 2001; NARMS, 2000).

Our primary research interest was to determine if antimicrobial resistant in *E. coli* isolates on dairy farms is associated with the type and the quantity of antimicrobials being used. A second objective was to provide background information on antimicrobial resistance of *E. coli* on dairy farms and to estimate the degree of AR persistence after farms converted from conventional to organic farms.

MATERIALS AND METHODS

Cattle fecal samples were collected from 30 organic dairy farms and 30 conventional farms in southwestern Wisconsin. The organic farms were members of an organic dairy association of 325 dairy farms. All organic farms were certified by a certification agency as not having used antimicrobials on cows for at least 3 years before the start of our study. For each organic farm selected, the geographically closest "conventional" dairy farm, for which the farm owner would agree to participate, was included in this study as a control. All herds were visited twice; once in March and once in September, during 2000-2001.

Management information was collected at the first visit using an orally administered questionnaire. Questions were administered and observations were made regarding milk production, milking practices, housing, incidence of the major dairy diseases, medical treatments and other management factors. At each visit, fecal samples were collected from five lactating cows and five calves under approximately 6 months of age. Animals were excluded if they had diarrhea or were under treatment for another illness. Adult cows were sampled by walking among the cows and waiting for one to defecate. The fecal sample was taken from the freshly voided fecal pile, taking care to not contact the ground beneath. If this could not be done with certainty, the next cow to defecate was sampled. Fecal samples were obtained directly from calves when they defecated following anal stimulation. A clean sterile latex glove was used for each specimen to avoid cross-contamination. Approximately five grams of fecal sample were placed into a Cary-Blair Transport Media tube (Medical Chemical Corp., Torrance, CA) and another five grams were placed into a sterile plastic tube. The specimens were kept on ice and sent to the Michigan Department of Community Health laboratory by overnight courier service.

The fecal samples were processed within 72 hours of sample collection by streaking directly on McConkey agar plates (REMEL, Lenexa, KS) and incubating at 35 °C for 24 hours. If no suspect *E. coli* colonies were evident, the raw fecal sample was inoculated in a McConkey broth tube and incubated at 35 °C for 24 hours and then streaked on McConkey agar. One typical *E. coli* colony was selected and identified following standard methods (Gray, 1995).

Escherichia coli isolates were tested for AR against 17 antimicrobial agents (Table 5-1) by semi-automatic micro broth dilution methods (Sensititre; Trek diagnostic Systems Inc., OH). The MICs were tested in accordance with the manufacture's instruction. In summary, *E. coli* were inoculated on TSA with 5% sheep blood agar (REMEL, Lenexa, KS) and incubated at 35 °C for 24 hours. The sub-cultured colonies were examined for purity and emulsified in 4 *ml* de-mineralized distilled water, adjusting the turbidity to that of a 0.5 McFarland standard. A 10 micro-liter suspension was transferred into a Mueller Hinton broth tube (11 *ml*) and 50 μl of the broth suspension was transferred to Sensititre panels, which were incubated at 35 °C for 18 hours prior to determining the minimum inhibitory concentration (MIC). Quality control was performed by testing *E. coli* of American Type Culture Collection (ATCC) 29212, *Staphylococcus aureus* (ATCC29213) and *Pasturella aeruginosa* (ATCC27853).

The MIC results were dichotomized based on the clinical breakpoints set by National Committee for Clinical Laboratory Standards (NCCLS). Isolates with intermediate susceptibility were categorized as being susceptible. Logistic regression was used to estimate the odds ratio for each antimicrobial, comparing the organic and conventional farms. Animal age (cow/calf) and season (September/March) were also included in these models as possible confounders. Management factors, such as the number of cattle, amount of milk produced, intensive grazing, animals purchased during the past year (yes/no), mastitis rate, calf mortality rate and the number of years that each farm had been organic were tested as predictors of AR prevalence in logistic regression analysis.

The dichotomized data were used to construct tables of the multiple drug resistance. The number of *E. coli* isolates which were resistant to 0, 1, 2, and ≥3 antimicrobials were compared among conventional and organic dairy farms. Each table was analyzed separately by extended Mantel-Haenszel mean score statistic (Stokes, 2000).

All statistical analysis was performed using SAS statistical software (version 8.02; SAS Institute, Cary, NC).

RESULTS

Nine fecal samples were not available from five farms due to an insufficient number of calves on farms. A total of 1,120 *E. coli* were isolated from 1,191 fecal samples (94.1%) collected at the 120 farm visits. *Escherichia coli* were not isolated from 45 of 596 organic samples (7.6%) and 25 of 595 conventional samples (4.2%).

Each organic dairy had converted to organic farming methods at least 3 years before our study (mean=8.0 years). Organic farmers indicated that no antimicrobials were used on their dairy cows, but four organic farmers reported antimicrobials were used for calves with serious diarrhea or pneumonia. In 26 of the 30 conventional dairy herds, cows routinely received antimicrobial infusions into the udder at the cessation of each lactation cycle (dry-cow treatment). Cephapirin or penicillin were the most frequently used products. Eighteen conventional dairy producers reported using udder infusion of antimicrobials for the treatment of clinical mastitis. For the severe clinical mastitis cases, eight conventional dairy producers used systemic antimicrobials.

The frequency distributions of *E. coli* MIC and the NCCLS breakpoints for 17 antimicrobial agents were shown in Table 5-1. There were no clinically resistant isolates to ceftriaxone, amikacin, nalidixic acid, or ciprofloxacin. Though not resistant at NCCLS break points, five isolates from calves on three conventional dairy farms had MIC of more than 4 *μg/ml* of ceftriaxone. Those five isolates also had MIC of more than 4 *μg/ml* of ceftiofur and were resistant to eight other antimicrobials; ampicillin, amoxicillin-Clavulanic acid, cephalothin, cephoxitin, streptomycin, chloramphenicol, tetracycline, and sulphamethoxazole. Two of the decreased susceptibility isolates to ceftriaxone were from one farm, but were collected six month apart from each other. Another notable finding was that 27 resistant isolates to chloramphenicol were isolated from calves on 18 farms. All but two of these isolates were from conventional dairy farms and all 27 isolates had multiple AR patterns (3-11 drugs, median=5). Among those 27 chloramphenicol resistant isolates, 26 isolates, 25 isolates, 22 isolates and 22 isolates were resistant to sulphamethoxazole, tetracycline, streptomycin and kanamycin, respectively.

Table 5-2 summarizes the logistic regression analysis of the NCCLS dichotomized data. The type of farm (conventional/organic) and age (cow/calf) were included in all logistic regression models. The season and the interaction between farm type and age (cow/calf) were evaluated in the model, but were not included in the final model because they were not statistically significant. The farm size (number of cattle), amount of milk produced, intensive grazing, animals purchased during the past year (yes/no), mastitis rate, calf mortality and the number of years that each farm had been organic, were not significant predictors for E. coli antimicrobial resistance to each

antimicrobial. The results of the analyses show that conventional dairy farms as compared to organic farms had a significantly higher rates of resistant isolates of *E. coli* to each of seven antimicrobials: ampicillin, streptomycin, kanamycin, gentamicin, chloramphenicol, tetracycline, sulphamethoxazole, and trimethoprim-sulfamethoxazole. The analysis of multiple antimicrobial resistance (Table 5-3), using the extended Mantel-Haenszel mean score statistic, indicated that prevalence of multiple AR was not significantly different between *E. coli* isolates from conventional and organic dairy herds (Q_{SMH}=0.6125, p=0.4338). However, multiple AR in *E. coli* isolates from calves was significantly higher in conventional dairy herds than it was in organic dairy herds (Q_{SMH}=24.1193, p<0.0001).

DISCUSSION

The fecal samples were collected from presumably healthy cows and calves after having excluded animals with obvious diarrhea or that, to our knowledge, were under treatment for some other disease. This selection policy may have resulted in lower measures of antimicrobial resistance in our study compared to studies based on diagnostic submissions from ill animals, many of which may have recently undergone antimicrobial therapy (Schroeder, 2002). The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP, 1996 - 2001) reported a higher prevalence of resistant bacteria in diagnostic submission samples as compared with specimens from animals at slaughter. For example, a high proportion (80-86%) of *E. coli* isolates from Denmark were resistant to ampicillin in diagnostic submission, however only 0-8 % of *E. coli* from presumably healthy slaughter cattle were resistant to ampicillin (Bager, 1997).

The higher prevalence of antimicrobial resistance in diagnostic bacterial isolates may be due to the selection pressure from antimicrobial treatments.

Like DANMAP and NARMS, we avoided the difficult issue of clustering of isolates in cows within herds by taking only one representative *E. coli* isolate from each animal. A non-differential selection bias may have existed if the *E. coli* isolates that we were able to culture were, in some unknown way, not representative of all the *E. coli* that were present. Such a non-differential bias should not have effected our measures of effect.

Our organic dairy farm isolates were from a geographic cluster of about 110 organic dairy farms belonging to a particular organic dairy association at farm selection. The conventional dairy farms were not selected randomly, but were the nearest, in sequence of proximity, to the selected organic dairy farms that agreed to participate. A study conducted by Singer et al. (1998) suggested AR might be clustered in time and space, though their data was derived from a clinical diagnostic database. The logistic regression analysis indicated that the season of sampling was not significant predictor of antimicrobial resistance prevalence on dairy farms, although the six-month interval may be too short to detect the change of antimicrobial resistance *E. coli* prevalence in time.

Assessing the amount of antimicrobials used on the farms was difficult. Generally, in spite of our requests, farmers may not have recorded the details of antimicrobials used. Some farmers may not have known what treatments were rendered by veterinarians. Also, our assumption that organic dairy herds used no antimicrobials was dependent upon the farmers' compliance to the rules of their organic association. The certified organic dairy farms maintained the compliance records. If there were

serious compliance issues, the dairy farm would have lose their organic status and would not be able to sell the milk at the premium organic price. Moreover, the majority of organic producers started organic dairy production based on an individual philosophy of environmental impacts and biological soundness. The farms' compliance to the rules of their organic association is assumed to be well regarded. Regardless of issues of absolute compliance, the evidence is overwhelming that antimicrobial use was much greater on the conventional farms than it was on the organic farms.

Adult dairy cattle almost never receive antimicrobials in the feed over long periods of time, but medicated milk replacer is often used for calves. Antimicrobial use as feed additive is probably a principal reason why the total amount of antimicrobials used in swine and poultry production is much greater than what is used in the dairy industry. Consequently, a higher proportion of resistant bacteria are found in swine and poultry isolates as compared with isolates from dairy cattle (Salmon, 1995; Bager, 1997; Mathew, 1998; Schroeder, 2002).

Our five *E. coli* isolates with reduced susceptibility to ceftriaxone were resistant to all beta-lactams and cephalosporins, and were co-resistant to aminoglycosides, chloramphenicol, tetracycline and sulfonamide. Winokur et al. (2001) reported in their study of human isolates that high levels of co-resistance to aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, and ciprofloxacin were observed in extended-spectrum β-lactamase (ESBL) producing strains of *Enterobacteriaceae*. Our isolates shared the resistant pattern with the human ESBL strains. Farm could be a source of resistant plasmids in human ESBL isolates. It must be noted that all five isolates came from conventional dairy calves, to which substantial amount of antimicrobials are administered

as feed additives and injections. Ceftiofur was developed for veterinary use, and is approved for treatment of pneumonia in dairy cattle with zero withdrawal time, as well as for acute bovine interdigital necrobacillosis (FDA, Green book). Since the extralabel use of ceftiofur for severe mastitis is not very efficacious (Erskine, 2002), ceftiofur is not widely used for mastitis. However, the zero withdrawal time of milk and meat make the drug attractive. Though we could not assess the amount of ceftiofur used on the farms, we speculate the use of third-generation cephalosporin is the selective pressure for ESBL isolates on these farms.

Chloramphenicol has been prohibited for food producing animal in the US since January 1986 (FDA Veterinarian Newsletter, 1996) and has been rigorously enforced by the USDA. The resistance to chloramphenicol is rendered by the inactivation with the chloramphenicol transacetylase (Prescott, 2000) and by enhanced efflux. White et al. (2000) investigated 48 *E. coli* recovered from calves with diarrhea and found the majority of resistant *E. coli* had enhancing efflux genes (*flo* and *cmlA*), which may be disseminated via plasmids and/or a mobile trasposon(s). They suggested the extra-label use of florfenicol in calves was the major selection pressure of those resistant traits. Florfenicol is a structurally similar antimicrobial to chloramphenicol, and was approved for bovine respiratory treatment in 1996 (Online Green book, 2003). Other possible selection pressure on farm is the co-selection by other antimicrobials. Our observation of high multiple AR supports the co-selection by non-chloramphenicol antimicrobials. Further research is required to identify the selective pressure on farm that has caused chloramphenicol resistant trait to be reserved for so long on both organic and conventional dairy farms.

The overall prevalence of resistant bacteria in calf fecal samples was significantly higher than that of cows in both conventional and organic dairy herds. Calf milk replacers, which may contain antimicrobials such as chlortetracycline, oxytetracycline and neomycin, are widely used by dairy producers in the United States (USDA/APHIS, 2002; Heinrichs et al., 1995). The possible use of medicated milk replacer in conventional dairies may, in part, explain the high prevalence of resistant *E. coli* in calves. Though organic dairy producers may use antimicrobials in animals under one year of age for life threatening situations, medicated milk replacer is prohibited. The organic dairy producers usually treated their sick calves with oral electrolyte solutions, whey products and probiotic products. Only four of the 30 organic producers responded that they had used antimicrobials for sick calves in the past one year.

Hinton et al. (1985) reported a high prevalence of resistant *E. coli* in calves which did not receive oral antimicrobials. They found a continual turn-over of *E. coli* strains in the fecal flora, the majority of which were isolated only a few times. The prevalence of antimicrobial resistant isolates became highest on approximately the 10th day of age, and gradually reduced in following 140 days to the level observed in the cows. Bennedsgaard et al. (Bennedsgaard, T. W., S. M. Thamsborg, F. M. Aarestrup, C. E. Enevoldsen, and M. Vaarst, submitted for publication) recently reported a higher prevalence of AR *E. coli* in calves as compared to cows. This was observed in both organic and conventional dairy herds in Denmark, where milk replacer with antimicrobials have been prohibited since the early 1970's (Larsen, 1972). Higher percentages of resistant bacteria in young animals, as compared with adults, were observed in pigs (Hinton, 1987; Langlois, 1988; Larsen, 1972). Larsen et al. (1972) speculated that bacteria in young animals have the

increased potential for resistance transfer, and Langlois et al. (1988) suggested the AR *E. coli* can more easily colonize in intestinal tract in young animals than adult. In any case, animal age could be an important factor when investigating AR in enteric *E. coli* in populations.

Our analysis did not find any significant effect of most management factors on AR *E. coli* prevalence when controlling for farm type (organic or conventional) and animal age. Langlois et al. (1988) reported that pigs on pasture have lower AR *E. coli* prevalence than pigs in finishing or farrowing house. They speculated the exposure to antimicrobials is not the only factor that influences the prevalence of AR bacteria. When we do not control for farm type and animal age in logistic regression analysis, intensive grazing becomes a significant predictor for AR prevalence. However, this is probably because intensive grazing is highly correlated with farm type, i.e. intensive grazing was a confounding factor.

Less than 2% of indicator *E. coli* from cattle were multi-resistant in Denmark where a similar set of antimicrobials was tested. In our study, 23.9% (268/1,120) had multiple antimicrobial resistant isolates in cows and calves combined, and 7.6% (37/486) in cows. Differences in multiple-AR between the Danish study and our study may be due to a more restrictive policy on use of antimicrobial agents in Denmark. Indicator isolates from Danish cattle (n=85) had resistance to tetracycline 4.7%, streptomycin 1.2% (modified based on our breakpoints), sulfamethoxazole 3.5%, and ampicillin 0% in DANMAP 2001. Our AR results for *E. coli* in cow was tetracycline 7.3%, streptomycin 2.5%, sulfamethoxazole 3.6% and ampicillin 2.0%. AR proportions in our Wisconsin isolates were slightly higher than in Danish isolates (α=0.05).

CONCLUSION

Our study shows significantly lower prevalence rates of AR for seven antimicrobials in organic dairy herds as compared to conventional herds. However, the odds ratios for having resistant *E. coli* were relatively small, suggesting that AR persists for many years in organic herds after exposure to antimicrobials is withdrawn. Differences in AR prevalence between cows and calves was large in the both organic and conventional herds; therefore animal age (cow/calf) should be taken into account when AR *E. coli* prevalence in animals is studied.

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Table 5-1. Frequency distributions of antimicrobial resistance *E. coli*.

		Conventional		Organic	
Antimicrobials	MIC	Cow	Calf	Cow	Calf
Beta-lactam					
Ampicillin	<=2	109	74	105	102
	4	158	112	147	121
	8	10	11	12	7
	16	2	1	2	3
	32	0	2	0	1
	>32	7	84	4	47
Amoxicillin Clavulanic Acid	<=0.5	0	0	1	0
	1	11	2	9	11
	2	49	27	44	37
	4	174	144	176	155
	8	45	96	34	70
	16	6	6	5	4
	32	1	2	1	3
	>32	0	7	0	1
Cephalosporins (1st generation)					
Cephalothin	2	9	2	7	11
	4	60	53	43	51
	8	154	153	147	161
	16	54	62	64	44
	32	4	5	4	8
	<32	5	9	5	6
(2nd generation)					
Cefoxitin	<=4	247	236	216	252
	8	36	36	49	23
	16	2	5	5	4
	32	1	3	0	2
	>32	0	4	0	0
(3rd generation)					
Ceftiofur	0.5	283	276	266	277
	1	0	1	0	3
	2	1	1	0	1
	4	1	1	3	0
	8	0	4	1	0
	16	0	1	0	0
	>16	1	0	0	0
Ceftriaxone	<=0.25	284	278	270	280
	0.50	1	1	0	1
	2.00	1	0	0	0
	4.00	0	2	0	0
(resistance ≥ 32μg/ml)	8.00	0	3	0	0
Aminoglycosides					
Streptomycin	<=32	279	186	263	220
	64	5	46	4	35
	128	1	27	1	18
	256	1	22	2	7
	>256	0	3	0	1

Table 5-1 (cont'd).

		Conventional		Organic	
Antimicrobials	MIC	Cow	Calf	Cow	Calf
Kanamycin	<=16	281	179	269	230
	32.00	0	1	0	0
	64.00	4	104	1	51
Gentamicin	<=0.25	25	16	21	21
	0.50	198	185	192	192
	1.00	59	57	54	59
	2.00	2	3	2	2
	4.00	0	1	1	0
	8.00	0	2	0	0
	16.00	2	5	0	0
	>16	0	15	0	7
Apramycin	<=2	12	19	15	10
	4	183	184	171	179
	8	81	72	76	87
	16	7	5	5	5
	32	2	1	2	0
	>32	1	3	1	0
Amikacin	<=4	282	282	269	277
(resistance ≥ 64 *μg/ml*)	8	4	2	1	4
Chloramphenicol					
Chloramph	<=4	261	252	249	267
	8	25	10	21	9
	32	0	6	0	0
	>32	0	16	0	5
Tetracycline					
Tetracycline	<=4	252	113	249	169
	8	9	16	5	12
	16	0	2	1	4
	32	0	24	3	21
	>32	25	129	12	75
Sulfonamide/Trimethoprim					
Sulphamethoxazole	<=128	277	201	259	230
	256	0	2	0	0
	512	0	15	4	7
	>512	9	66	7	44
Trimethoprim-Sulfamethoxazole	<=0.12	264	196	250	227
	0.25	19	40	12	26
	0.5	2	22	6	21
	1	0	8	0	1
	2	0	1	0	0
	>4	1	17	2	6
Fluoroquinolones					
Nalidixic acid	<=4	264	266	253	269
(resistance ≥ 32 *μg/ml*)	8.00	22	18	17	12
Ciprofloxacin	<=0.015	283	278	269	280
	0.030	2	6	1	1
(resistance ≥ 4 *μg/ml*)	0.060	1	0	0	0

Table 5-2. Logistic Regression Analysis of resistance to antimicrobials among *E coli* isolates from organic and conventional dairy farms.

Antimicrobials	Conventional (Resistant/ Susceptible)	Organic (Resistant/ Susceptible)	Odds Ratio	Chi-square Probability
Beta-lactams				
Ampicillin	93/477	52/499	2.055	0.0002
Amoxicillin - Clavulanic acid	10/560	5/546	1.98	0.2138
Cephalosporins (1st generation)				
Cephalothin	23/547	23/528	0.969	0.9178
(2nd generation)				
Cefoxitin	8/562	2/549	3.997	0.0811
(3rd generation)				
Ceftiofur	6/564	1/550	5.910	0.1006
Ceftriaxone	0/570	0/551	NA	NA
Aminoglycosides				
Streptomycin	105/465	68/483	1.755	0.0018
Kanamycin	107/462	52/499	2.588	<0.0001
Gentamicin	22/548	7/544	3.241	0.0077
Apramycin	7/563	3/548	2.262	0.2386
Amikacin	0/570	0/551	NA	NA
Chloramphenicol				
Chloramphenicol	22/548	5/546	4.384	0.0031
Tetracycline				
Tetracycline	180/390	116/434	2.009	<0.0001
Sulfonamide/Trimethoprim				
Sulphamethoxazole	90/480	63/488	1.531	0.0209
Trimethoprim - Sulfamethoxazole	18/552	37498	2.272	0.0573
Fluoroquinolones				
Nalidixic Acid	0/570	0/551	NA	NA
Ciprofloxacin	0/570	0/551	NA	NA

NA: Not available because there was no resistant bacteria.

Table 5-3. Frequency distribution of multiple antimicrobial resistance among 1,120 *E. coli* isolates

	Cow		Calf	
Number of antimicrobials to which isolates were resistant	Conventional (n=284)	Organic (n=270)	Conventional (n=285)	Organic (n=281)
0	245	241	121	167
1	19	12	20	27
2	11	10	24	20
3	7	6	36	24
4	2	0	43	19
5	0	1	11	17
6	0	0	14	3
7	0	0	7	2
8	0	0	4	1
9	0	0	1	0
10	0	0	1	1
11	0	0	2	0
12	0	0	1	0

The frequency of multiple antimicrobial resistant in cow and calf isolates were analyzed separately with the extended Mantel-Haenszel mean score statistic. The frequency distribution of cow isolates was not different among conventional and organic (Q_{SMH}=0.6125, p=0.4338), however the distribution of calf isolates was significantly different (Q_{SMH}=24.1193, p<0.0001).

CHAPTER SIX

COMPARISON OF PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *CAMPYLOBACTER SPP.* ISOLATED FROM ORGANIC AND CONVENTIONAL DAIRY HERDS IN WISCONSIN, USA

ABSTRACT

The prevalence and antimicrobial susceptibility of *Campylobacter spp.* isolated from bovine feces was compared between organic and conventional dairy herds. Thirty organic dairy herds, where antimicrobials are rarely used for calves and never used for cows, were compared with 30 neighboring conventional dairy farms, where antimicrobials were routinely used for animals for all ages. Fecal specimens from ten cows and ten calves on 120 farm visits yielded 332 *Campylobacter* isolates. The prevalence of *Campylobacter spp.* in organic and conventional farms were 26.7% and 29.1%, and the prevalence was not statistically different between the two types of farms. *Campylobacter* prevalence was significantly higher in March than in September, higher in calves than in cows, and higher in smaller farms than in large farms. The rates of retained placenta, pneumonia, mastitis and abortion rate were associated with the proportion of *Campylobacter* isolation from fecal samples. Gradient disk diffusion minimal inhibitor concentration method (Etest) was used for testing susceptibility to four antimicrobial agents: ciprofloxacin, gentamicin, erythromycin and tetracycline. Two isolates were resistant to ciprofloxacin and none of isolates were resistant to gentamicin or erythromycin. Resistance to tetracycline was 45% (148/332 isolates). Tetracycline resistance was found more frequently in calves than in cows ($p=0.042$), but no difference

was observed between organic and conventional farms. We saw no evidence that restriction of antimicrobial use on dairy farms was associated with prevalence of antimicrobial resistance in *Campylobacter spp.* to ciprofloxacin, gentamicin, erythromycin and tetracycline.

INTRODUCTION

Campylobacter spp. has been recognized as a cause of septic abortion, infectious infertility and diarrhea in cattle and sheep (Radostits, 2000). Abortions in cattle can be caused by *Campylobacter fetus* subsp. *veneralis* or *C. fetus* subsp. *fetus*, however *C. jejuni* and *C. coli* also recognized as causal agents of abortions (Larson, 1992; Welsh, 1984). *Campylobacter hyointestinalis* was reported as a cause of ileitis in pigs (Gebhart, 1983), bovine diarrhea (Diker, 1990) and human gastroenteritis (Gorkiewicz, 2002). *Campylobacter jejuni*, and *C. coli* can be found in the rumens and small intestines of normal calves and adult cattle (Stanley, 1998), so that the bacteria are considered commensal in cattle.

Campylobacter was not recognized as a cause of human enteritis until the mid-1970s when selective isolation media were developed for human stool culture. At present, campylobacteriosis is the most commonly reported human bacterial gastroenteritis in the United States, and the majority of infections are with *C. jejuni* (Nachmkin, 1999). The incidence of laboratory-diagnosed campylobacteriosis was 15.7 per 100,000 person-years in FoodNet surveillance sites (CDC, 2001; Friedman, et. al, 2000) and an estimated 2 to 2.4 million infections occur in the United States each year (Friedman, 2000). Though antimicrobials are not essential for the treatment of most routine human cases of

campylobacteriosis, severe or prolonged cases are usually treated with fluoroquinolone or erythromycin. Resistance to ciprofloxacin in human isolates of *Campylobacter jejuni* is reportedly increasing (Allos, 2001; Gaudreau, 1998; Smith, 1999).

The majority of sporadic cases of *Campylobacter* infections are foodborne and undercooked poultry is the most likely source of infections (Friedman, 2000; Pearson, 2000). Contaminated water and unpasteurized milk are common sources of outbreaks; nine percent of bulk tank milk was found culture positive for *Campylobacter jejuni* in a study of 131 dairy herds in South Dakota and Minnesota (Jayarao, 2001).

Critical control points are largely unknown for reducing pre-harvest *Campylobacter* prevalence. Most animal-specific factors (age, gender, breed, etc.) are not amenable to intervention. Herd-level management factors (bedding, sanitation, feeding, stocking rate, etc.) can often be changed, albeit sometimes only with considerable investment in labor and physical facilities. The influence on *Campylobacter* prevalence of the management factors that constitute "organic dairy production" has not heretofore been investigated.

Organic dairy milk production has been previously described (Sato, 2002). Organic farms in Wisconsin usually graze their cattle during the warm season, do not use hormones, herbicides, insecticides, or anthelmintics, and no antibiotics are permitted for one year before milk is marketed. This antibiotic restriction means that dairy calves may receive antibiotics, but antibiotic usage for calves is reportedly very low due to the overall management philosophy of these farmers. It is not known to what extent the management practices embodied in the organic approach may lead to a lower rate of antimicrobial resistance among *Campylobacter* isolates from cattle on these farms.

The objective of the study was to describe the prevalence and antimicrobial resistance patterns of *Campylobacter spp.* in healthy calves and cows in organic and conventional dairy farms in Wisconsin.

MATERIALS AND METHODS

Data and fecal sample collection

Cattle fecal samples and management & production data were collected from 30 organic dairy farms and 30 conventional farms in southwestern Wisconsin. The organic farms were from an association of about 325 organic dairy farms. All organic farms were certified by an approved certification agency as not using antimicrobials for cows for at least 3 years (mean = 8.0 years) before the start of our study. For each organic farm selected, the nearest "conventional" dairy farmer (in sequence of geographical proximity) was asked to serve as a control farm. All herds were visited twice; once in March and once in September.

Management and production information was collected at the first visit using an orally administered questionnaire. Questions and investigator observations regarded milk production, milking practices, housing, grazing, incidence of the major diseases, medical treatments and other management factors. Also at each visit, environmental and animal sanitation was assessed with a subjective score of cow cleanliness and the amount of moisture and manure in the bedding and exercise areas, as previously described (*4*).

At each of the two visits, fecal specimens were collected from five lactating cows and five calves (under approximately 6 months of age). Animals were excluded if they had obvious diarrhea or were under treatment for another illness. Adult cows were

sampled by walking among the cows and waiting for one to defecate. The fresh fecal sample was taken from the freshly voided fecal pile, taking care to not contact the ground beneath. Fecal samples were obtained from calves when they defecated following anal stimulation. A sterile latex glove was used for each specimen to avoid cross-contamination. Approximately five grams of fecal sample were collected into a Cary-Blair Transport Media tube (Medical Chemical Corp., Torrance, CA). The specimens were kept on ice and mailed to the Michigan Department of Community Health (MDCH), by overnight courier service for processing within 32 hours from the time of sampling.

Bacteria isolation

The fecal samples from the Cary-Blair tube were streaked directly on Campy Blood Agar (REMEL, Lenexa, KS). The inoculated plates were incubated under microaerophilic atmosphere (Campy-Pak, BBL Microbiology Systems, Cockeysville, MD) at 37 °C for 48 hours. One typical colony was selected and identified to genus level by testing by Gram stain, microscopic cell morphology, catalase production, oxidase production, and hippurate hydrolysis in accordance with the standard methods at MDCH (*24*).

Antimicrobial susceptibility testing

Bacterial isolates were tested for resistance using gradient disc diffusion minimal inhibitory concentration to ciprofloxacin (CI: 0.002-32.0 *μg/ml*), erythromycin (EM: 0.016-256 *μg/ml*), gentamicin (GM: 0.016-256 *μg/ml*) and tetracycline (TC: 0.016-256 *μg/ml*) by Etest (AB Biodisk, Piscataway, NJ). Sample bacteria were streaked from the frozen stock onto SBA (SBA) plates (REMEL, Lenexa, KS) and incubated for 48 hours at 37 °C under microaerophilic atmosphere. The colonies were restreaked to a new SBA

and incubated for another 24 hours to allow recovery after being frozen. The sub-cultured colonies were examined for purity and emulsified in 4 ml Mueller-Hinton broth, adjusting the turbidity to that of a 1.0 McFarland standard. The suspension was then inoculated evenly on 150 mm Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood (REMEL, Lenexa, KS) by swabbing evenly in accordance with the Etest manufacture's instructions. Etest strips containing CI, GM, EM and TC were placed on the surface of agar plate in a radial pattern with the lowest concentration toward the center. The plates were incubated for 72 hours at 37 °C under the microaerophilic conditions and the MICs were read directly from the test strip point where the growth inhibition zone intersected with the test strip, in accordance with manufacture's instruction. Quality control was performed daily using *Campylobacter coli* of American Type Culture Collection (ATCC) 33559.

Since no break points for *Campylobacter* MIC were defined by National Committee for Clinical Laboratory Standards (NCCLS), our test results were dichotomized based on the breakpoints used by the National Antimicrobial Resistance Monitoring System (NARMS): CI ≥4 *μg/ml*, GM ≥16*μg/ml*, EM ≥8*μg/ml*, and TC ≥16*μg/ml*.

Statistical Analysis

The prevalence of *Campylobacter spp.* in herds was analyzed using a generalized linear model with logit link function, based on the binomial distribution. The outcome variable was *Campylobacter* negative (0) or positive (1). Explanatory (independent) variables were farm type (organic/conventional), cow or calf, season, herd size (number of milking cows), purchase of animals during the past year (yes/no), grazing intensity

during summer (no grazing/ little grazing / intensive grazing), abortion rate (per 100-cows/year), metritis rate, retained placenta rate (retained over 12 hours after calving), calf population, calf mortality rate, and calf diarrhea rate. "Farm" was included as a random effect variable with an independent correlation matrix.

A regression model (generalized linear model with logit link function) was used to estimate the effect of farm type, animal age and season on the prevalence of antimicrobial resistant bacteria. The data were also analyzed using a proportional odds model with a generalized estimating equation (GEE). The proportional odds model with GEE provides a method to analyze an ordinal-level repeated dependent variable and several categorical and continuous-level explanatory variables with fixed or random effects (Stokes, 2000). Farm type, season and animal age were included as fixed effects and the farm was included as a random effect. All statistical analysis was performed using SAS statistical software (version 8.02. SAS Institute, Cary, NC).

RESULTS

The organic dairies had converted to organic farming methods at least 3 years before the initiation of our study (mean=8.0 years). Organic farmers indicated that no antimicrobials were used for cows on their dairy farms, but four organic farmers reported using antimicrobials for calves if they had serious diarrhea or pneumonia. In 26 of the 30 conventional dairy herds, cows routinely received antimicrobial infusions into the udder at the cessation of each lactation cycle ("dry-cow treatment"). Cephapirin or penicillin was used most for this purpose. Eighteen conventional dairy producers reported using

udder infusion of antimicrobials for the treatment of clinical mastitis. For the severe clinical mastitis cases, eight conventional dairy producers used systemic antimicrobials.

A total of 332 *Campylobacter spp.* isolates were obtained from 1,191 fecal specimens (27.9%). A total of 234 (70.5%) was identified as *Campylobacter jejuni*, however the remainder of isolates, which were hippurate-negative, were not identified to species level. *C. jejuni* and others distributed evenly in both organic and conventional dairy herd (Mantel-Haenszel p=0.25). No *Campylobacter* isolates were obtained from one conventional farm or from three organic farms, thus 6.7% of farms were culture negative. On the 56 *Campylobacter*-positive farms, 5% to 70% of the collected specimens were culture positive. The prevalence was significantly higher in calves (32.7%) than in cows (23.2%), and significantly higher in March (36.8%) than in September (18.9%; Table 6-1).

There was no significant difference in *Campylobacter* prevalence between organic and conventional farms in the multivariate analysis (p=0.5253; Table 6-3). Rates of retained placenta, pneumonia incidence rate, and abortion were positively associated with *Campylobacter* prevalence, whereas herd size (number of lactating cows and dry cows) and mastitis rate were negatively associated with *Campylobacter* prevalence ($p < 0.05$). The calf mortality was nearly significantly associated with the prevalence (p=0.0511).

Only two isolates of *Campylobacter spp.* from geographically distant conventional dairy herds were resistant to ciprofloxacin (>32 and 24 *μg/ml*). The other 330 isolates had MIC values between 0.012-0.25 *μg/ml*. None of the 332 isolates was resistant to gentamicin or erythromycin. The ranges of MIC were 0.047 - 2 *μg/ml* for

gentamicin and 0.047 - 4 *μg/ml* for erythromycin (Table 6-4). A total of 148 resistant isolates (44.6%) to tetracycline were obtained (Table 6-4).

The analysis of the dichotomized tetracycline resistant data indicated a higher prevalence of resistant *Campylobacter spp.* in calf isolates as compared with cow isolates ($p=0.0419$), with the estimated odds ratio of 1.81 ($1.0221 < OR < 3.2059$). Farm type (organic or conventional) and season of specimen collection were not significant predictors of tetracycline resistance ($p=0.4971$ and 0.1729, respectively). The proportional odds model analysis using all antimicrobial dilution levels did not find significant difference of MIC distributions to tetracycline between two types of farm (Fig 6-1). For ciprofloxacin, gentamicin and erythromycin resistance, the proportional odds model found no significant effect on MIC distribution by farm type (organic or conventional), animal age or season of specimen collection.

DISCUSSION

The estimation of *Campylobacter spp.* prevalence may be affected by factors, such as location, season, use of transport medium, time before processing, use of enrichment media, and the use of various isolation methods (media, temperature, atmosphere, and time). The selection of farms in the current study was not random, but rather constituted a cluster of organic herds and neighboring conventional herds in a particular region of Wisconsin. The fecal samples were collected from presumably healthy cows and calves, after having excluded animals with obvious diarrhea or that were under treatment for some other disease. This selection strategy may have resulted in lower measures of *Campylobacter spp.* prevalence in our study as compared with other

studies, if cows with diarrhea are more likely to have been infected with *Campylobacter spp.* It has been reported that *Campylobacter spp.* isolation rate was decreased approximately 16% by storing feces at 4°C for 24 hours (Ladron de Guevara, 1989) and our samples took 24 to 36 hours to be transported to the laboratory. However, the Cary-Blair transport medium with icepacks should have enabled *Campylobacter spp.* to maintain sufficient viability (Luechtefeld, 1981; Wang, 1983; Wasfy, 1995).

The enrichment techniques are beneficial for the detection of *Campylobacter spp.* when present in low concentration. Perhaps our measured prevalence estimate would have been higher had we used enrichment technique (Bolton, 1982; Martin, 1983). Nielsen (2002) found 9 out of 77 positive samples were only positive after growth in enrichment broth. We used Campy Blood Agar plate, which contains cephalothin, polymyxin B, vancomycin, trimethoprim and amphotericin B. The culture media is optimized for *C. jejuni* and *C. coli*, but not for other *Campylobacter spp.* in cattle. *Campylobacter jejuni* subsp. *doylei*, *C. fetus* subsp. *fetus*, *C. upsaliensis*, and *C. hyointestinalis* are known to be inhibited by cephalothin (Nachamkin, 1999). Though we used an incubation temperature of 37 °C, other studies of *Campylobacter spp.* used an incubation temperature of 42°C to optimize the growth of thermophilic *Campylobacter* species, such as *C. jejuni*, *C. coli* or *C. lari*, with the decreased ability to isolate non-thermophilic species (*C. fetus*, and *C. jejuni* subsp. *doylei*). The incubation temperature of 37 °C may have resulted in lower prevalence of *Campylobacter* in our study. Atabay et al. (1998) used three kinds of media, enrichment technique, membrane filtration technique, and three different incubation temperatures. They found 62% overall prevalence in 136 cattle in three farms in the U. K. The major species in their study were

C. hyointestinalis (32%), *C. sputorum* biovar *paraureolyticus* (21%), *C. fetus* subsp. *fetus* (11%) and *C. jejuni* subsp. *jejuni* (7%). Giacoboni et al. (1993) also found *C. fetus* subsp. *fetus* in 17% of cattle and *C. hyointestinalis* in 19% of cattle, whereas dominant species was C. jejuni found in 29% of 94 cattle in Japan. Our study design emphasized the isolation of *C. jejuni* and *C. coli* which are species of public health importance.

Higher *Campylobacter spp.* prevalence was found on dairy farms in March compared to September, and higher in calves compared to cows. These observations generally agreed with previous population-based studies (Nielsen, 2002; Wesley, 2000). The housing and grazing styles in our study were very different between organic and conventional dairy herds. Free stalls were used on 9 conventional herds, in contrast to being used on only four organic herds. Half of the organic herds (15 farms) applied intensive grazing during summer, whereas only two conventional herds used intensive grazing. We saw no evidence that antimicrobial use on dairy farms had any effect on Campylobacter spp. prevalence, since farm type was not significantly associated with prevalence after controlling for housing and grazing in the regression analysis.

It may be reasonable to find retained placenta rate and abortion rate were positively associated with *Campylobacter spp.* prevalence, since *Campylobacter spp.* was originally found as a cause of abortion, and even *C. jejuni* and *C. coli* were recognized as causal agents of abortions (Larson, 1992; Wesley, 2000). Positive association between pneumonia and *Campylobacter spp.* prevalence may be explained by a shared common cause such as poor environmental sanitation. Some *Campylobacter* species, such as *C. fetus*, were known cause of mastitis (Akhtar, 1993; Logan, 1982). However, the negative

association between mastitis rates and Campylobacter prevalence was observed with borderline significance (p=0.0486) in our study.

Antimicrobial Resistance in *Campylobacter spp.*

Agar disk diffusion, broth dilution, agar dilution and the gradient disk diffusion (Etest) have commonly been used to determine *Campylobacter* susceptibilities *in vitro*. The agar dilution test was recently set by NCCLS as a reference standard susceptibility testing method for veterinary isolates of *Campylobacter spp.* (Nccls, 2002), however the agar dilution test requires more materials and considerable labor, and therefore is not ideal for most surveillance purposes. Ge, et al. (2002) recently reported that MIC values measured with the Etest were generally lower than the results obtained with the agar dilution method. The agreement (±1 dilution range) between MICs between two test methods depended on antimicrobials used: Ciprofloxacin (85%), Gentamicin (92.6%), Erythromycin (65.6%), and tetracycline (57.7%). The Etest MIC results of the quality control strain (*C. jejuni* ATCC33560) were consistently one to several dilutions lower than the corresponding agar dilution results. Huang, et al. (1992) also compared the Etest method with the agar dilution method and reported slightly lower MIC values with the Etest than with the agar dilution. The percent agreement (±1 dilution range) were 90.4% for ciprofloxacin, 83.0% for gentamicin, 94.1% for erythromycin, and 77.5% for tetracycline. In our study, any bias due to the testing procedure should not have affected our comparison between organic and conventional farms. Any such systematic error or bias would have been a non-differential misclassification bias that would have equally affected the organic and conventional farms (Rothman, 1998). However, direct

comparisons of MIC values obtained from different methods should be interpreted with caution.

We isolated two ciprofloxacin resistant *Campylobacter spp.* from conventional dairy farms. Fluoroquinolone is not commonly used in dairy industry. Sarafloxacin was approved in the United States for the poultry in 1995, but the approval was withdrawn in 2001 (Federal Register, 2001). The Center for Veterinary Medicine of FDA proposed to withdraw approval of enrofloxacin for poultry use because of the possible transfer of fluoroquinolone-resistant *Campylobacter spp.* from poultry to human (Federal Register, 2000). Though the use of enrofloxacin in beef cattle is approved for treatment of bovine respiratory disease, the extralabel use of any fluoroquinolones in dairy cattle has been clearly prohibited by the FDA. The resistance to fluoroquinolone is rendered by (a) decreased permeability of bacterial cell wall; (b) increased efflux pump activity; and (c) mutation of the DNA gyrase. Thus, the decreased permeability and/or the increased efflux pump can also confer resistance to other antimicrobial agents, such as tetracycline (Walker, 2000). Since our ciprofloxacin resistant *Campylobacter spp.* were not resistant to other three antimicrobials, it is speculated that the resistant isolates were arose by point mutation.

CONCLUSION

The prevalence of *Campylobacter spp.* was not significantly different between organic and conventional dairy farms in Wisconsin. Campylobacter prevalence was significantly higher in March than in September, higher in calves than in cows, and higher on smaller farms than on larger farms. Rates of retained placenta, pneumonia, and

abortion were positively associated with the *Campylobacter spp.* prevalence. The proportion of tetracycline-resistant *Campylobacter spp.* was higher in isolates derived from calves. The prevalence of resistance to ciprofloxacin, gentamicin and erythromycin was very low. We saw no evidence that restricted antimicrobial use on dairy farm had any association with antimicrobial resistance to ciprofloxacin, gentamicin, erythromycin and tetracycline in *Campylobacter spp.*

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Table 6-1. Number of *Campylobacter* isolates (percent of culture positive samples) in each group of samples.

	Conventional dairy farms (n=30)		Organic dairy farms (n=30)	
	Calf	Cow	Calf	Cow
March	56 (37.8%)	54 (36.0%)	65 (43.3%)	45 (30.0%)
September	42 (28.6%)	21 (14.0%)	30 (20.5%)	19 (12.7%)

Table 6-2. Odds ratios for *Campylobacter sp.* isolation (Generalized Linear Model analysis)

Risk factors	*Campylobacter* positive / negative	Odds	Odds ratio	95% confidence interval	Type 3 GEE Chi-Sq (p-value)
Animal age					
Calf	193 / 398 (48.5%)	0.485	1.635	1.180 > OR > 2.656	0.0031
Cow	139 / 461 (30.2%)	0.302	1		
Season					
March	220 / 378 (58.2%)	0.582	2.524	1.748 > OR > 3.646	<0.0001
September	112 / 481 (23.3%)	0.233	1		
Farm type					
Conventional	173 / 422 (41.0%)	0.410	1.138	0.742 > OR > 1.743	0.5541
Organic	159 / 437 (36.4%)	0.368	1		

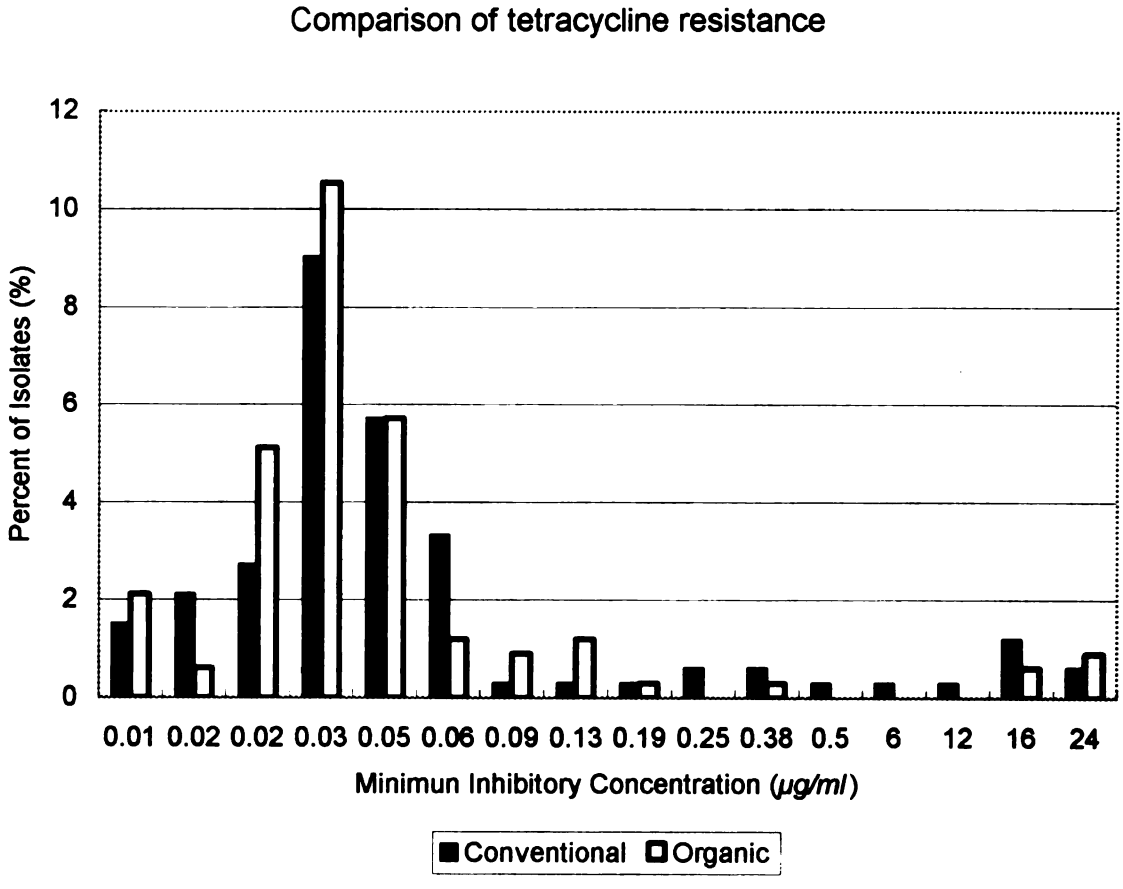
Table 6-3. Generalized Linear Model analysis of management factors on *Campylobacter sp.* Prevalence.

Parameters	Estimates	*p*-values
Season (March/September)	1.0225	<0.0001
Retained placenta incidence rate	0.0460	<0.0001
Herd size	-0.0234	0.0031
Cow or calf (Calf/Cow)	0.5304	0.0032
Pneumonia incidence rate	0.0266	0.0187
Mastitis rate	-0.0131	0.0486
Abortion rate	0.0531	0.0437
Calf mortality	0.5909	0.0511
Metritis rate	0.0133	0.1532
Open herd (cow purchased)	0.1691	0.4359
Milk production per cow	0.0001	0.5165
Organic or conventional	0.1166	0.5253
Grazing with housing	-0.1697	0.5204
No grazing (tie stall, free stall)	0.1178	0.7051
SCC	0.0003	0.7693
Cow mortality	-0.0062	0.8391

Table 6-4. Proportion (%) of isolates which were inhibited by antimicrobials at each concentration. The dotted lines indicate the NARMS breakpoints (CI ≥4 *μg/ml*, GM ≥16*μg/ml*, EM ≥8*μg/ml*, and TC ≥16*μg/ml*).

Antimicrobial concentration (*μg/ml*)	Ciprofloxacin (n=332)	Gentamicin (n=332)	Erythromycin (n=332)	Tetracycline (n=332)
0.012	0.3			3.6
0.016	1.8			2.7
0.023	6.9			7.8
0.032	28.6			19.6
0.047	29.2	0.6	0.3	11.4
0.064	22.9	0.9	0.9	4.5
0.094	6.0	7.5	1.8	1.2
0.125	2.4	17.2	7.2	1.5
0.19	0.9	18.1	24.7	0.6
0.25	0.3	28.0	23.2	0.6
0.38		13.3	14.5	0.9
0.5		6.9	12.7	0.3
0.75		3.9	6.6	
1		0.9	3.3	
1.5		1.5	1.8	
2		1.2	1.8	
3	----------		0.9	
4			0.3	
6			----------	0.3
8				
12		----------		0.3 ----------
16				1.8
24	0.3			1.5
32				3.9
48				2.1
64	0.3			3.3
96				3.3
128				2.4
192				0.6
256				0.6
>256				25.0
	100%	100%	100%	100%

Figure 6-1. Distribution of MICs to tetracycline of *Campylobacter spp.* from conventional and organic dairy farms. The proportional odds model analysis using all antimicrobial dilution levels did not find significant difference of MIC distributions to tetracycline between two types of farm.



CHAPTER SEVEN

COMPARISON OF ANTIMICROBIAL SUSCEPTIBILITY OF *Staphylococcus aureus* ISOLATED FROM BULK TANK MILK IN ORGANIC AND CONVENTIONAL DAIRY HERDS IN MIDWESTERN USA AND DENMARK

ABSTRACT

An observational study was conducted to compare the antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from bulk tank milk in organic and conventional dairy farms in Wisconsin, U. S. A. and in Southern Jutland, Denmark. Bulk tank milk samples and data regarding management and production were collected from 30 organic dairy farms and 30 conventional farms in Wisconsin and 20 organic and 20 conventional dairy herd in Denmark. *S. aureus* isolates were tested for resistance against 15 antimicrobial agents by semi-automatic micro broth dilution methods in each country. Of the 118 bulk tank milk samples in Wisconsin, 71 samples (60%) yielded at least one *S. aureus* isolate, and a total of 331 isolates were collected. Of the 40 bulk tank milk samples from Denmark, 27 samples (55%) yielded at least one *S. aureus* isolate and a total of 152 isolates were collected. Significant difference between organic and conventional dairy was detected to only ciprofloxacin in Wisconsin and only avilamycin in Denmark. Significant difference between two countries was detected to nine antimicrobials. Denmark had higher probability of having reduced susceptibility to ciprofloxacin and streptomycin ($p=0.015$ and 0.003). Wisconsin isolates had higher probability of having reduced susceptibility to other seven antimicrobial agents

(bacitracin, gentamicin, kanamycin, penicillin, sulphamethoxazole, tetracycline, and trimethoprim). We found small differences between organic and conventional farms types in each country and larger differences between the two national agricultural systems.

INTRODUCTION

Staphylococcus aureus is a common cause of contagious mastitis in dairy cattle. Such infections often persist for weeks, months or years, with infected animals becoming the main source and reservoir of infection for herd mates. Antibiotics are commonly used to control *S. aureus* infection, by treating cases of clinical mastitis and by udder infusion at the cessation of each lactation cycle (dry-cow treatment). This type of mastitis treatment and prevention is the predominant reason for antibiotic use in dairy industry (Kaneene, 1992).

Food animal agriculture has been suspected of fostering antimicrobial resistant (AR) bacteria, which can then be transmitted to people through direct contact, food of animal origin, and environment contamination (McEwen, 2002). Generally, farms with higher antimicrobial usage have been found to have a higher proportion of resistant bacteria, and farm-level studies have usually shown an association between antimicrobial usage in food animals and rates of antimicrobial resistance in several bacterial species (Mathew, 2001; Van Den Bogaard, 2001).

The use of antibiotics has been prohibited for organic dairy cows, and now this prohibition is specified by the National Organic Standard (USDA, 2001). Though sick cows have to be treated with all necessary medications including antimicrobials, these

treated cows have to be excluded from organic herds in the United States. In Denmark, national certification of organic "økologisk" farms began in 1988 and organic farms now comprise 8.4% of all Danish dairy farms and produced about 22% of the fluid (drinking) milk in 2000 (Bennedsgaard, 2003). In contrast to the organic farms in the USA, antimicrobial drugs can be used for Danish økologisk cows, although the økologisk standards require a three fold longer milk withdrawal time than cows on conventional farms. In both Denmark and the US, it is not known to what extent the restriction or ban on antimicrobial use in the organic or økologisk approach may lead to a lower rate of antimicrobial resistance among bulk tank *Staphylococcus* isolates from these farms in two very different agricultural systems.

Antimicrobial resistance in *S. aureus* has primarily been studied on isolates from clinical mastitis that had been submitted to various veterinary diagnostic laboratories (Bager, 2002; De Oliveira, 2000; Erskine, 2002; Makovec, 2003). Such studies have mainly attributed AR to antibiotics commonly used for mastitis therapy (Rossitto, 2001; Watts, 1995). In most studies, antimicrobial susceptibility was determined by agar disk diffusion method. Results have usually been reported as being either positive or negative, so susceptibility changes below the clinical breakpoints have usually gone unreported.

The objective of the current study was to determine and compare the prevalence and antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from bulk tank milk in organic and conventional dairy farms in Wisconsin and Denmark.

MATERIALS AND METHODS

Bulk tank milk samples and data regarding management and production were collected from 30 organic dairy farms and 30 conventional farms in Wisconsin, U.S.A. The organic farms were from an organic association with about 120 member dairy farms at the start of the study. All 30 organic farms had been organic for at least 3 years before the start of our study. For each organic farmer selected, a neighborhood "conventional" dairy farmer was asked to allow their farm to serve as a control. All herds were visited twice; once in March and once in September. Management and production data were collected at the first visit using in-person administered questionnaires. Questions and investigator observations included milk production, milking practices, housing, grazing, incidence of the major diseases, medical treatments, and other management factors (Sato, Bartlett, Erskine, and Kaneene, submitted for publication). Also at each visit, environmental and animal sanitation was assessed with a subjective score of cow cleanliness and the amount of moisture and manure in the bedding and exercise areas, as previously described (Bartlett, 1992).

In Southern Jutland, Denmark, 20 organic herds which had converted to organic dairy management before 1995 were selected for the study. Twenty conventional dairy herds were randomly selected from a group of 120 eligible herds which had participated in a previous research project. Information on parturition, culling, veterinary diagnostics, and disease treatments were readily available from the Danish Cattle Database (Bartlett, 2001). These dairy herds were visited by investigators from April to May 2000 and bulk tank milk samples were collected and processed (Danish Veterinary Institute) in accordance with the same procedure used in the U.S.A.

The bulk tank milk was agitated for about five minutes before collecting samples. At each farm visits, approximately 10 *ml* of bulk tank milk (BTM) were collected from the top of the bulk tank with a stainless steel dipper into a sterile tube. The sample tubes were kept in ice and sent to a laboratory by overnight courier service. The BTM samples were frozen within 24 hours and stored for variable time at –72 °C until processing.

Sample tubes were brought to room temperature, placed into water bath at 35-37 °C for 10 minutes, and mixed thoroughly with a mechanical platform shaker. One milliliter of milk was diluted to ten (10^{-1}) and hundred (10^{-2}) dilutions with physiological saline. An inoculum of 0.3 *ml* from each dilution was spread with a bent-glass streaking rod on the surface of 140 *mm* Baird-Parker Agar plate (Bacto/Difco, Detroit, Michigan, USA) supplemented with egg yolk tellurite (Ollis, 1995). The plates were incubated at 32 °C for 48 hours. Typical black, shiny, convex colonies with white edge surrounded by a clear zone were counted as presumptive staphylococci and the number of colonies on each dilution plate were recorded. Presumptive colonies were collected, starting from the more diluted plates (10^{-2}) and moving to the undiluted plate, until up to ten isolates had been obtained. Then each colony was transferred to trypticase soy agar (TSA) with 5% sheep blood (REMEL, Lenexa, KS) and incubated at 32 °C for 24 hours. The colonies were examined for purity and identified as *Staphylococcus aureus* by Gram stain, catalase production, and tube coagulase test (coagulase rabbit plasma with EDTA, Bacto/Difco, Detroit, Michigan, USA) in accordance with the standard methods (Flowers, 1993). The material and microbiological procedures were standardized among American and Danish portions of the study as much as possible by Danish investigators visiting the project site in the USA at the initiation of the study.

Staphylococcus aureus isolates were tested for AR against 15 antimicrobial agents by semi-automatic micro broth dilution methods (Sensititre; Trek diagnostic Systems Inc., Cleveland, OH). The minimum inhibitory concentrations (MIC) of *S. aureus* were tested for the same antimicrobials and the same range of concentrations in both countries in accordance with the manufacture's instruction, except that avilamycin was only tested in Denmark and cephapirin was only tested in the U. S. In summary, *S. aureus* were inoculated on TSA with 5% sheep blood agar and incubated at 35 °C for 24 hours. The sub-cultured colonies were examined for purity and emulsified in 4 ml de-mineralized distilled water, adjusting the turbidity to that of a 0.5 McFarland standard. A 10 micro-liter suspension was transferred into a Mueller Hinton broth tube (11 *ml*) and 50 *µl* of the broth suspension was transferred to Sensititre panels, which were incubated at 35 °C for 18 hours prior to determining the MIC. Quality control was performed by testing *Staphylococcus aureus* (ATCC29213) and *Pasteurella aeruginosa* (ATCC27853).

A initial descriptive statistical analysis ignored the clustering of clonal isolates within the same farm and summarized the MIC results by country and type of farm. The interpretive standards set by National Committee for Clinical Laboratory Standards (NCCLS, 2002) and the breakpoints used by DANMAP 2001 (Bager, 2002) were utilized. Because the majority of isolates had MIC values far below threshold values for clinical resistance, the minimum inhibitory concentration for 90% of all isolates tested (MIC_{90}) to each antibiotic was used as a break point upon which to dichotomize the susceptibility for purposes of comparison between the conventional and organic/økologisk farms. Isolates with MIC values lower than the MIC_{90} value were categorized as a "high susceptibility", otherwise categorized as a "reduced susceptibility" (Table 7-4 and 7-5). Logistic

regression analysis was used to estimate the effect of farm type (organic/økologisk or conventional) on rates of reduced *S. aureus* susceptibility to the different antimicrobials. The data were considered unbalanced with repeated values for each farm. All statistical analysis was performed using SAS statistical software (version 8e. SAS Institute, Cary, NC).

RESULTS

Of the 118 bulk tank milk samples in Wisconsin, 71 samples (60%) yielded at least one *S. aureus* isolate, and a total of 331 *S. aureus* were collected. Twenty six organic farms (87%) yielded at least one isolate in comparison to 22 conventional farms (73%) which yielded at least one isolate. Of the 40 bulk tank milk samples from Denmark, 27 samples (55%) yielded at least one *S. aureus* isolate: 10 (50%) for økologisk farms and 17 (85%) for conventional farms, and a total of 152 *S. aureus* were collected (Table 7-1).

The MIC data for the *S. aureus* isolates is shown in Table 7-2. Resistant isolates were only found to bacitracin, penicillin, streptomycin, sulphamethoxazole, and trimethoprim based on NCCLS or DANMAP interpretive standards in our samples, and resistance to streptomycin and trimethoprim were only found in Wisconsin samples. A notable finding was that a very large proportion of isolates (162/331, 49%) exhibited high resistance to sulphamethoxazole (512 μg/ml) in Wisconsin, whereas only one Danish isolates (0.7%) had the same resistance level.

Only one significant difference between organic and conventional/økologisk farms in each country was found by a logistic regression with the correlated data model.

Conventional dairy herds in Wisconsin had 3.3 times higher probability (95% confidence interval [1.21 – 9.14]) of having *S. aureus* isolates with reduced susceptibility to ciprofloxiacin compared with organic dairy herds in Wisconsin (Table 7-4). In Denmark, økologisk dairy farms had 6.78 time higher probability (95% confidence interval [1.30 – 35.31]) of having isolates with reduced susceptibility to avilamycin compared with Danish conventional dairy farms (Table 7-5).

The logistic regression model with farm type, country and interaction of farm type and country was used for comparing the sub-therapeutic susceptibility differences between Wisconsin and Denmark. No difference was found among the two countries for chloramphenicol, erythromycin, oxacillin, quinupristin/dalfopristin and vancomycin. Denmark had higher probability of having reduced susceptibility to ciprofloxacin and streptomycin ($p=0.015$ and 0.003). Wisconsin isolates had higher probability of having reduced susceptibility to other seven antimicrobial agents (bacitracin, gentamicin, kanamycin, penicillin, sulphamethoxazole, tetracycline, and trimethoprim at $p<0.05$; Table 7-6).

DISCUSSION

Organic dairy farmers in Wisconsin were chosen by geographical clusters and were asked to participate. Eight of 38 organic farmers who were contacted refused to participate in the study. The conventional dairy farms were chosen from neighboring farms, with only five cases of farmer declining to participate. Antimicrobial usage on conventional farms was difficult to quantify. Thus, the dose, route of administration, and frequency of dosing was unknown. Although somewhat larger than the organic farms, the conventional farms were small by U.S. standards and their philosophy regarding

antibiotic usage may have been influenced by the many organic dairy farms in the region. The farms in Denmark were selected randomly from their prescribed location, and are therefore presumably representative of Danish økologisk and conventional farms.

Bulk tank cultures for *S. aureus*, and somatic cell count (SCC) are widely used as inexpensive and convenient monitoring methods for udder health (Jayarao, 2003). Isolates of *S. aureus* from bulk tank milk are generally presumed to have come from the cow's teats or udders. However, it is also possible that they may have originated from contamination or even from the humans working in the milking parlor, although given the sanitation of the milking equipment after each milking, it is probable that the overwhelming number of *S. aureus* isolates originated in cows' teats or udders. Numerous reports have been published on antimicrobial susceptibility in *Staphylococcus* from dairy cattle, which were usually collected from individual quarter milk samples from cows with clinical mastitis (De Oliveira, 2000; Devriese, 1980). These studies focused on antimicrobial susceptibility of *S. aureus* as mastitis pathogens and the focus of the studies has been on the therapeutic implications for mastitis therapy. Study outcomes have usually been reported as a percentage of resistant isolates. Most surveys did not account for multiple observations from the same cow or herd, so clonal isolates of resistant bacteria from the same cow or herd might have greatly influenced the results. To avoid these complicated issues, De Oliveira et al. (2000) used only one isolates from a single herd. Our study included up to 10 isolates from a bulk tank on each of two separate sampling, separated by approximately a half year. Given the mixing of milk in the bulk tank, some of the isolates for one particular herd may have come from the same cow. Our logistic regression analysis for correlated data (repeated data) should have

helped to control for the possible effect of clonal isolates having an undue effect on the results .

The proportion of resistant *S. aureus* in our study were generally much lower than what was found in recent studies of isolates from clinical submission from Wisconsin (Makovec, 2003) and Michigan (Erskine, 2002). Samples submitted for diagnosis of clinical mastitis cases tend to exhibit more AR, probably because cases that are refractory to treatment are more likely to be cultured in an attempt to identify the causative agent and its AR traits (Aarestrup, 1998). Multiple samples from a single farm may be common, and clonal isolates might have been included in the data.

Organic/økologisk farms and conventional farms differed statistically with respect to: (1) reduced susceptibility to ciprofloxacin in conventional farms in Wisconsin and (2) reduced susceptibility to avilamycin in økologisk farms in Denmark. The reduced susceptibility to ciprofloxacin in Wisconsin could be related to the use of enrofloxacin in beef cattle. Enrofloxacin belongs to the fluoroquinolones group of antimicrobials and is approved for treatment of bovine respiratory disease since 1998 in the United States (FDA, 2003a). The extralabel use of any fluoroquinolones in dairy cattle has been prohibited by the FDA in the United States (FDA, 2003b). However, extralabel drug use is practiced if the product being used is approved in that species but for a different disease, or if the product is used at a different dose or with an altered withdrawal time (Bateman, 2000). The reduced susceptibility to ciprofloxacin on conventional dairies relative to organic dairies could be explained by the use of enrofloxacin on conventional farms for dairy or beef cattle, by exposure to purchased feeds on conventional farms, or exposure to other sources of resistant bacteria.

Even though all isolates were susceptible to avilamycin with the interpretive standard of DANMAP, the difference of MIC distributions between organic and conventional farms in Denmark is hardly explained. Avilamycin is a mixture of oligosaccharides and had been used for growth promoter primarily of broilers and some for pigs, but not for dairy cattle in Denmark (Aarestrup, 1998; Aarestrup, 2001). Avilamycin use as growth promoter for broilers and pigs was voluntarily stopped by the end of 1999 in Denmark, to avoid the possible cross-resistance to everninomycin, which was investigated for use in humans (Aarestrup, 2000; Butaye, 2003; EU, 1998). Organic dairy regulation in Denmark permits sick cows to be treated with antimicrobials. These cows are permitted to stay in their herds while they are receiving treatment, although the milk must be withheld from sale for three times longer than it is for conventional cows. Recent work showed that økologisk herds had less frequently asked veterinarians to treat their mastitis cows, however treatment frequency and antibiotic selection is not substantially different among two type of herds (Bennedsgaard, 2003). Avilamycin and its structurally related substances were generally not used on either økologisk or conventional dairy farms. Thus, the statistical significance we observed in avilamycin could have been by chance alone.

Though bacitracin is not commonly used for dairy cattle in either country, we found that *S. aureus* from dairy herds in Wisconsin were more likely to have reduced susceptibility to bacitracin than were herds in Denmark (Figure 7-1). Bacitracin is a polypeptide product of *Bacillus licheniformis* and *Bacillus subtilis*, and is commonly used in the topical treatment of human and animal skin infection. It is also commonly used as growth promoter in poultry, swine, and feedlot beef production (Aarestrup, 1998; FDA,

2003c; Prescott, 2000). The European Union banned bacitracin as animal growth promoter in 1998 because analogs of bacitracin can sometimes be used for the treatment of vancomycin resistant enterococci (Chia, 1995; EU, 1998; O'Donovan, 1994). Relatively low levels of *S. aureus* resistance to bacitracin in cattle were reported in Europe before the ban (Aarestrup, 1998; Devriese, 1980), however, reduced susceptibility to bacitracin (83% with MIC≥32μg/*ml* by e-test) was also reported in *S. aureus* from human skin and soft-tissue infections in Norway (Afset, 2003). In the United States, five antibiotics (i.e., bacitracin methylene disalicylate, chlortetracycline, oxytetracycline, tylosin, and virginiamycin) are approved and commonly used for beef cattle or calves for prevention of liver abscesses in feedlot cattle (Nagaraja, 1998). It is speculated that, although bacitracin is not used for dairy animals in the US, dairy cattle may become colonized with resistant strains by environmental and foodborne exposure to their surrounding agricultural environment. Besides bacitracin, our study found significantly higher probability of having reduced susceptibility to other seven antimicrobials in Wisconsin, in contrast to Danish isolates which showed reduced susceptibility to only two antimicrobials. Differences in reduced susceptibility patterns between Wisconsin and Denmark may be due to a more restrictive policy on use of antimicrobial agents in Denmark.

In general, we found small differences between organic/økologisk and conventional farms types in each country and larger differences between the two national agricultural systems.

CONCLUSION

The difference between Wisconsin and Denmark was large and isolates from Wisconsin had higher probability of reduced susceptibility to 7 out of 14 comparable antimicrobials, whereas Danish isolates had higher probability of reduced susceptibility to only two drugs. Differences in antimicrobial susceptibility between organic/økologisk and conventional farms were small relative to the differences observed between the two countries. Significant difference was detected to only one drug in each country.

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Table 7-1. Number of *Staphylococcus aureus* isolates examined in this study.

	Wisconsin, USA					
	March			September		
	Number of farms	Positive milk samples	*S.aureus* isolates	Number of farms	Positive milk samples	*S.aureus* isolates
Conventional farms	29	15	64	30	18	88
Organic farms	29	18	73	30	20	106

	Denmark		
	April-May		
	Number of farms	Positive milk samples	*S.aureus* isolates
Conventional	20	17	77
	20	10	75

Table 7-2. Antimicroibal susceptibility to 15 drugs in *Staphylococcus aureus* which were isolated from bulk tank milk samples collected from Wisconsin organic and conventional dairy farms.

Antimicrobial agents	Farm type	Percent of isolates at each indicated MIC (μg/*ml*)															
		0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		
Bacitracin	Con								8.4	21.7	23.8	39.9	6.3				
	Org								17.2	15.9	11.9	39.7	15.2				
Cephapirin	Con					92.1	7.9										
	Org					93.9	5.6	0.6									
Chloram-phenicol	Con						0.7	17.1	76.3	5.9							
	Org							26.8	70.4	2.8							
Ciprofloxacin	Con			83.6	15.1	1.3											
	Org			94.4	5.6												
Erythromycin	Con			40.8	52.0	6.6	0.7										
	Org			35.2	60.3	4.5											
Gentamicin	Con					93.4	6.6										
	Org					95.0	5.0										
Kanamycin	Con							86.2	13.8								
	Org							87.7	11.2	1.1							
Oxacillin	Con				99.3	0.7											
	Org				100												
Penicillin	Con	67.1	10.5	1.3	2.0	7.2	5.9	3.3	1.3	1.3							
	Org	72.1	12.3	5.0	1.1	0.6	0.6	6.7	1.7								
Streptomycin	Con							52.0	44.1	3.3	0.7						
	Org							55.9	36.9	5.6	1.7						
Sulpha-methoxazole	Con									5.9	1.3	2.0	1.3	5.3	84.2		
	Org									10.1	4.5	4.5	1.7	4.5	74.9		
Quinupristin / Dalfopristin	Con				100												
	Org				100												
Tetracycline	Con				86.2	13.2		0.7									
	Org				91.1	8.9											
Trimethoprim	Con					3.3	19.7	42.1	17.1	5.3	12.5						
	Org					2.2	33.0	32.4	14.0	5.0	13.4						
Vancomycin	Con					98.7	1.3										
	Org					96.1	3.9										

Con: Conventional farms, Org: Organic farms
*Interpretive standards were based on NCCLS M31-A2, otherwise based on DANMAP 2001.
Dilution range were indicated as un-shaded cells and break points were shown as a vertical border.

Table 7-3. Distribution of antimicrobial susceptibility to 15 drugs in *Staphylococcus aureus* which were isolated from bulk tank milk samples collected from Danish organic and conventional dairy farms.

Antimicrobial agents	Farm type	Percent of isolates at each indicated MIC (µg/ml)														
		0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	
Avilamycin	Con					1.3	5.3	86.8	6.6							
	Org					1.4	21.6	44.6	32.4							
Bacitracin	Con								77.9	9.1	5.2	6.5	1.3			
	Org								57.3	10.7	17.3	14.7				
Chloramphenicol	Con							7.8	92.2							
	Org							24.0	76.0							
Ciprofloxacin	Con			79.2	13.0	7.8										
	Org			58.7	24.0	17.3										
Erythromycin	Con			28.6	71.4											
	Org			45.3	53.3	1.3										
Gentamicin	Con					98.7	1.3									
	Org					100										
Kanamycin	Con							90.9	9.1							
	Org							98.7	1.3							
Oxacillin	Con				100											
	Org				100											
Penicillin	Con	94.8	1.3	1.3	1.3		1.3									
	Org	82.7		8.0	4.0			1.3	4.0							
Streptomycin	Con							31.6	46.1	22.4						
	Org							40.0	44.0	16.0						
Sulpha-methoxazole	Con								22.1	27.3	49.4					1.3
	Org								32.4	29.7	32.4	5.4				
Quinupristin / dalfopristin	Con				100											
	Org				100											
Tetracycline	Con				97.4	2.6										
	Org				97.3	2.7										
Trimethoprim	Con					14.3	66.2	19.5								
	Org					33.3	50.7	16.0								
Vancomycin	Con					98.7	1.3									
	Org					94.7	2.7	2.7								

Con: Conventional farms, Org: Organic farms

*Interpretive standards were based on NCCLS M31-A2, otherwise based on DANMAP 2001. Dilution range were indicated as un-shaded cells and break points were shown as a vertical border.

Table 7-4. Logistic regression analysis with correlated data on dichotomized susceptibility result in *Staphylococcus aureus* from Wisconsin. Odds ratio (OR) of having reduced susceptibility in conventional dairy herds

		Logistic regression with repeated observation	
	Break point (μg/*ml*)	OR	95% Wald confidence interval
Bacitracin	64	0.89	[0.32 – 2.47]
Cephapirin	2*	1.31	[0.55 – 3.14]
Chloramphenicol	8	1.70	[0.77 – 3.75]
Ciprofloxacin	0.5	3.33	[1.21 – 9.14] [1]
Erythromycin	0.5	0.79	[0.40 – 1.56]
Gentamicin	2*	1.33	[0.49 – 3.64]
Kanamycin	8	1.14	[0.63 – 2.08]
Penicillin	1	2.25	[0.41 – 12.37]
Streptomycin	8	1.17	[0.66 – 2.09]
Sulphamethoxazole	512	1.79	[0.76 – 4.23]
Tetracycline	1*	1.63	[0.73 – 3.67]
Trimethoprim	16	0.96	[0.42 – 2.16]
Vancomycin	2*	3.05	[0.74 – 12.51]

1 : Isolates from **conventional dairy herd** had significantly higher probability to have reduced susceptibility

* : MIC_{90} were used to dichotomize the MIC value, except for those antimicrobials with only two or three MIC categories.

Table 7-5. Logistic regression analysis with correlated data on dichotomized susceptibility result in *Staphylococcus aureus* from Denmark. Odds ratio (OR) of having reduced susceptibility in conventional dairy herds.

	Break point (μg/*ml*)	Logistic regression with repeated observation	
		OR	95% Wald confidence interval
Avilamycin	8	0.15	[0.028 – 0.769] [1]
Bacitracin	64	0.49	[0.09 – 2.56]
Chloramphenicol	8	3.74	[0.99 – 14.17]
Ciprofloxacin	0.5	0.37	[0.09 – 1.49]
Erythromycin	0.5	2.07	[0.55 – 7.87]
Gentamicin	2*	NE	NE
Kanamycin	8	7.40	[0.93 – 58.92]
Penicillin	1	0.23	[0.013 – 4.26]
Streptomycin	8	1.39	[0.42 – 4.57]
Sulphamethoxazole	64†	1.72	[0.37 – 7.94]
Tetracycline	1*	1.03	[0.07 – 15.72]
Trimethoprim	4†	1.27	[0.18 – 9.10]
Vancomycin	2*	0.23	[0.02 – 2.40]

NE : Not Estimable, because all økologisk isolates had the same response.

1 : Isolates from **organic dairy** herd had significantly higher probability to have reduced susceptibility.

* : MIC_{90} were used to dichotomize the MIC value, except for those antimicrobials with only two or three MIC categories.

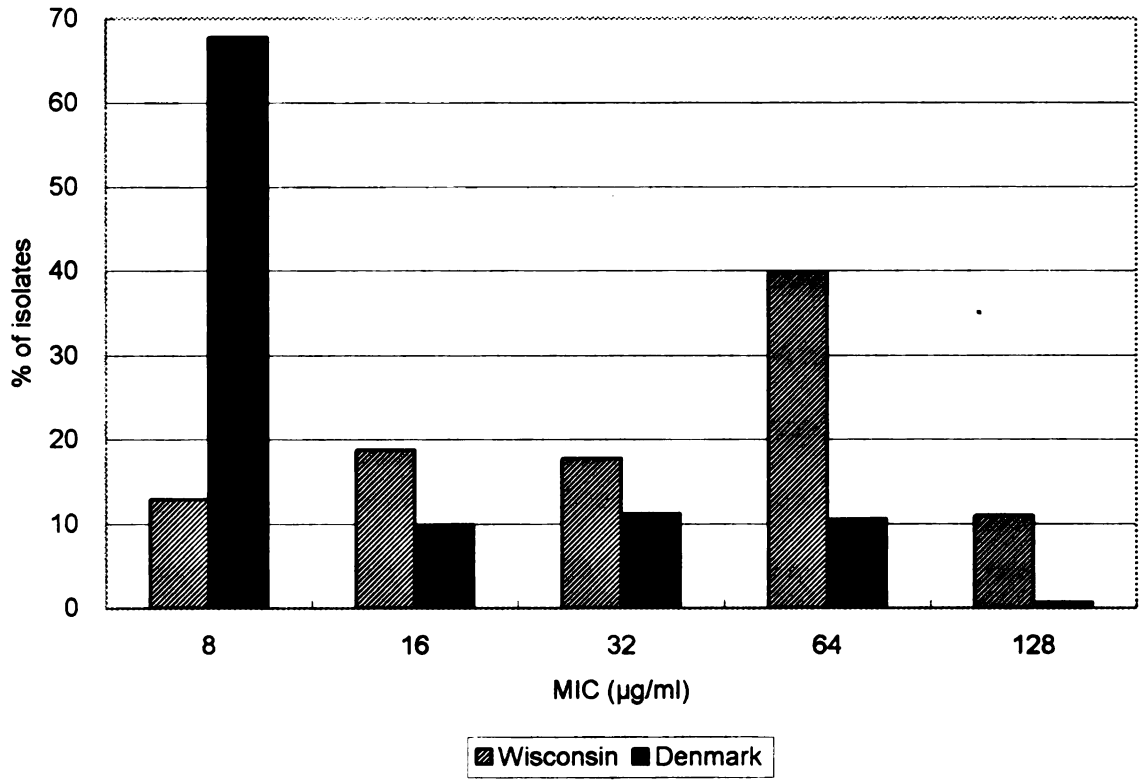
† : MIC_{90} of Danish isolates only were used to dichotomize the data.

Table 7-6. Logistic regression analysis with correlated data on dichotomized susceptibility result in *Staphylococcus aureus*. Odds ratio (OR) of having reduced susceptibility in Wisconsin, compare to Denmark.

	Break point (μg/*ml*)	Logistic regression with repeated observation	
		OR	95% Wald confidence interval
Bacitracin	64	6.76	[2.56 – 17.82] [1]
Chloramphenicol	8	0.58	[0.27 – 1.26] [3]
Ciprofloxacin	0.5	0.25	[0.11 – 0.59] [2]
Erythromycin	0.5	0.94	[0.44 – 1.99] [3]
Gentamicin	2*	9.20	[1.16 – 72.28] [1]
Kanamycin	8	4.08	[1.39 – 12.00] [1]
Oxacillin	-	NE	NE [3]
Penicillin	1	5.78	[1.07 – 31.11] [1]
Streptomycin	8	0.48	[0.25 – 0.94] [2]
Sulphamethoxazole	-	NE	NE [1]
Quinupristin/Dalfopristin	-	NE	NE [3]
Tetracycline	1*	4.64	[1.12 – 19.25] [1]
Trimethoprim	-	NE	NE[1]
Vancomycin	2*	0.85	[0.21 – 3.34] [3]

NE: Not Estimable. However, the difference of susceptibility to sulphamethoxazole and trimethoprim among two countries were determined from the Table 7-1 and 7-2.
1: Isolates from the U. S. A. had significantly higher probability to have reduced susceptibility.
2: Isolates from Denmark had significantly higher probability to have reduced susceptibility.
3: No significant difference were observed.
*: MIC_{90} were used to dichotomize the MIC value, except for those antimicrobials with only two or three MIC categories.

Figure 7-1. Comparison of minimum inhibitory concentration to bacitracin in *Staphylococcus aureus* isolated from bulk tank milk in Wisconsin and Denmark



CONCLUSIONS

Organically produced milk sells for almost twice the price of conventionally produced milk, creating an interesting economic comparison between the two different management styles of milk production. Our findings suggest the need to more completely assess the cost effectiveness of some interesting differences between the organic and conventional farms. Our study showed that organic dairy farms were producing milk without significantly increasing reported CM rate, BTSCC or culling rate as compared with matched conventional farms. For organic and conventional farms, respectively, mastitis rate (28 and 32 cases per 100-cow-years at risk), bulk tank milk somatic cell count (262,000 and 285,000 cells per ml) and culling rate (17.2 and 18.0 cases per 100-cow years) were lower in organic than in the conventional farms, although the differences were not statistically significant. The milking procedures were very similar between matched pairs, so the effect of milking procedures on CM rate or BTSCC could not be estimated. The organic herds were significantly smaller than the conventional herds (51.1 and 71.1 cows) and lower milk production per cow (20.2 and 23.7 kg/day). Organic herds were more likely to use intensive grazing, which may have accounted for their higher rate of gastrointestinal nematodes as compared with conventional herds. We subjectively observed that organic farms tended to have smaller cows and had a greater proportion of smaller non-Holstein breeds. Organic farms used less artificial insemination, which may have created a gene pool of smaller cows compared with what is commonly seen in today's mainstream dairy industry where AI has been used to produce large cows with high milk production per cow. Because smaller cows eat less feed, comparisons in milk production between organic and conventional herds must consider this lower cost of

production. Also, other differences in nutrition and management most likely act to reduce the per-cow cost of feeding and maintaining the smaller grazing cows, as compared with larger cows being fed a high energy diet in total confinement.

E. coli

We used *E. coli* as an indicator organism to represent the selective pressure on a commensal Gram negative bacteria. Other antimicrobial resistance studies, e.g. DANMAP and NARMS, have used *E. coli* as their Gram negative indicator bacterial species, thereby allowing us to compare our results. Our study shows significantly lower prevalence rates of AR for seven antimicrobials (ampicillin, streptomycin, kanamycin, gentamicin, chloramphenicol, tetracycline, and sulphamethoxazole) in organic dairy herds, as compared to conventional herds. However, the odds ratios for having resistant E. coli were relatively small (OR=1.5 – 4.3). Although the organic farms had converted to organic farming methods at least 3 years before our study (mean = 8.0 years), antimicrobial resistance clearly persisted long after antimicrobial selective pressure had been withdrawn. Antimicrobial resistance tended to be larger in calves than in cows in the both organic and conventional herds; therefore animal age (cow/calf) should be taken into account when AR in animals is studied.

Salmonella spp.

We had only 7 (seven) isolates (4 from organic & 3 from conventional) from 1,191 samples. Three isolates were from cows, and four isolates from calves. Only two isolates were from the same organic farm, and the rest of isolates were from different farms. The lower rate of Salmonella isolation may be the real prevalence on farm, or due to the low detecting power of our sampling method. One isolate from a

conventional farm showed high resistance to Amoxicillin+Clavulanic acid (>32), Ampicillin (>32 *μg/ml*), Apramycin (>32 *μg/ml*), Cefoxitin (32 *μg/ml*), Ceftiofur (16 *μg/ml*), Ceftriaxon (8 *μg/ml*), Cephalotin (>32 *μg/ml*), Kanamycin (>64 *μg/ml*), Streptomycin (256 *μg/ml*), Sulphamethoxazole (>512 *μg/ml*), tetracycline (>32 *μg/ml*), but was susceptible to ciprofloxacin, and nalidixic acid.

Campylobacter spp.

The prevalence of *Campylobacter spp.* was not significantly different between organic and conventional dairy farms in Wisconsin. *Campylobacter* prevalence was significantly higher in March than in September, higher in calves than in cows, and higher on smaller farms than on larger farms. Rates of retained placenta, pneumonia, and abortion were positively associated with the Campylobacter spp. prevalence.

The proportion of tetracycline-resistant Campylobacter spp. was higher in isolates derived from calves. Two isolates from conventional dairy farms were resistant to ciprofloxacin and none of the isolates were resistant to gentamicin or erythromycin. Resistance to tetracycline was 41.5% (66/159) for organic and 47.4% (82/173) for conventional herds, which was not statistically significant. We saw no evidence that restricted antimicrobial use on dairy farm had any association with antimicrobial resistance to ciprofloxacin, gentamicin, erythromycin and tetracycline in *Campylobacter* spp.

Staphylococcus aureus

A significant difference in antimicrobial resistance in *Staphylococcus aureus* was detected to only one antimicrobial in our Wisconsin study and in the parallel study in Denmark. Our study showed that Wisconsin organic dairies had more

susceptible isolates to ciprofloxacin as compare to conventional farms. Danish conventional dairies had more susceptible isolates to avilamycin as compared to Danish organic (økologisk) dairy farms. The differences in AR were large between Wisconsin and Denmark. Isolates from Wisconsin had higher probability of reduced susceptibility to 7 out of 14 comparable antimicrobials (bacitracin, gentamicin, kanamycin, penicillin, sulphamethoxazole, tetracycline, and trimethoprim), whereas Danish isolates had higher probability of reduced susceptibility to only two drugs (ciprofloxacin and streptomycin). Differences in antimicrobial susceptibility between organic/økologisk and conventional farms were small relative to the differences observed between the two countries.

Enterococcus spp.

Isolation, identification and MIC measurement was done at William Beaumont Hospital. However, the result shows the rates of resistance were very low for Quinupristin / dalfopristin (9 for organic and 16 for conventional) and Gentamicin (2 for organic and 7 for conventional), and no isolates were resistant to Vancomycin, which make a further analysis difficult. Though it was not included in the original plan, we tested a stratified random sample (type of farms, cow/calf, faecalis/faecium/others, and season, in total 600) on a custom Sensititre panel (CMV5ACDC) which is similar to the Danish gram positive panel. This will facilitate comparisons with the Danish data and give us more information regarding *Enterococci* as an indicator species of gram positive bacteria. The results will be published later.

This study confirmed that milk can be produced without antibiotics, and that disease rates and measures of production are roughly comparable between herds that don't use antibiotics and small conventional dairy farms that do use antibiotics. Generally, AR prevalence in dairy animals in Wisconsin was very low compared to previous reports from poultry and swine production. We found lower rate of AR in organic herds for some "bug-drug" combinations, but not for others. The impact on public health caused by use of antimicrobials for dairy industry was assessed as relatively very low. In general, differences in AR between organic and conventional farms were mild to moderate, if they existed at all. Our results indicated that the persistence of AR on organic farms was such that AR remained for many years after antibiotics were no longer being used.

APPENDICES

QUESTIONNAIRE

Producer's name: _____ Date of interview:

Farm name:

Farm address:

Tel: _____ Fax:

E-mail:

Type of farm: ___ Organic ___ Not organic

---

1) Usual number of animals making milk

2) Usual number of dry cows _____

3) Breed of cattle:

Holstein _____ Jersey _____ Guernsey _____

4) Number of times per day cows are milked: ___ 2 ___ 3

Based on monthly bulk tank report of CO-OP

5) Pounds of bulk tank milk

6) Usual SCC in cells/*ml*

7) Bacterial count (SPC or Raw)

On the average, how many times per year does each cow have:

8) The hair clipped around her teats and udder? _____ 9) Feet trimmed? _____

Mastitis:

On the average day, how many lactating cows would not be put in the bulk tank because of mastitis or treatment for other diseases?

10) In Summer_____ 11) In Winter

Dry cow treatment:

12) Udder infusions are used for drying cow? Yes / No

13) What is your treatment?

Milking:

14) Usual milking procedure (check all that apply)

Winter	Summer	
____	_____	Dry massage or wipe with no water used
____	_____	Wash bucket usually used
____	_____	Running water (hose) usually used
____	_____	Pre-milking teat dipping usually used
____	_____	Individual-cow paper towels or cloths used for washing (each towel used for only one cow)
____	_____	Shared towels or cloths used for washing
____	_____	Gloves used for washing?
____	_____	Individual towels (cloth or paper) used for drying
____	_____	Teats are usually not dried before milking
____	_____	Post-milking teat dipping almost always used

15) Are cows milked in a stanchion or in a parlor?

____ Stanchion/Tie stalls ____ Milking Parlor

16) For each milking, it usually takes _____ people about _____ hours to milk the cows

using _____ milking units (claws) at a time.

17) Approximately what percent of the labor for milking and cow-care is hired (non-family) labor?

_____ %

18) How many years old is your milking machine? _____ years old

Cattle numbers and movement:

19) About how many animals were purchased last year?

How many cows:	**last month?**	**last year?**
20) died	_____	
21) culled	_____	
22) were sold to an organic farm	_____	
23) were sold to a non-organic farm or auction	_____	

24) Not counting heifers, what percent of your cows were sired by:

Herd bull _____ %

Artificial Insemination _____ %

25) Average days in milk? _____ days

26) Usual herd calving interval? _____ months

Housing:

27) WINTER Lactating cow housing (Check all that apply)

__ Strictly pasture __ Pasture with access to housing

__ Tie stalls ___ Loose housing (manure pack/straw pack)

__ Free stalls ___ other _____

28) SUMMER Lactating cow housing (check all that apply)

__ Outside exercise area with little grazing

__ Grazing pasture with access to housing

__ Strictly grazing pasture

__ Tie stalls ___ Loose housing (manure pack/straw pack)

__ Free stalls ___ other _____

29) If pastured, how many months do cows have access to grazing pasture? _____

30) What type of bedding is usually used for the **milking herd**?

no bedding __ % sand __ % straw __ %

wood shavings or sawdust __ % other _____ __ %

Feed

31) Do you feed a ration balanced for protein,energy, fiber and minerals?
Yes ____ No

32) Do you calculate dry matter intake for each cow/milking group?
Yes ____ No

33) Do you feed a TMR? Yes ____ No

34) Are any feeds fed separately? Yes ____ No

35) Do you get forage analyis done? Yes ____ No

36) Do you feed a separate balanced ration for dry cows? Yes ____ No

37) For pre-calving (pre-fresh, close-up, steam-up)? Yes ____ No

Diseases and Treatments:

38) How many cases of the following diseases were seen in your herd **in the past 3 months?**

_____ Mastitis (flakes or strings in the milk or a swollen hard udder)

_____ Metritis (smelly vaginal discharge usually following calving)

_____ Displaced abomasum

_____ Retained placenta (retained over 12 hours after calving)\

_____ Abortion (observed loss of the fetus)

39) What is your standard treatment for routine cases of mastitis in which the cow **IS NOT** systemically sick with a fever, depression or other signs outside of the udder?

___ Strip out the quarter at frequent intervals

___ Anti-inflamatory or antipyretics drugs (steriods, aspirin, banamine, etc.)

___ IV fluids

___ Administer oxytocin to assist milkout

___ Udder infusions of antibiotics? _____ other?

___ Systemic Antibiotics

___ other _____

___ other _____

40) What is your standard treatment for routine cases of mastitis in which the cow **IS** systemically sick with a fever, depression or other signs outside of the udder?

___ Strip out the quarter at frequent intervals

___ Anti-inflamatory or antipyretics drugs (steriods, aspirin, banamine, etc.)

___ IV fluids

___ Administer oxytocin to assist milkout

___ Systemic Antibiotics

___ Udder infusions of antibiotics? _____ other?

___ other _____

___ other _____

41) What is your standard treatment for metritis (smelly vaginal discharge after calving)?

___ Infuse iodine

___ Manually remove as much fluid as possible and remove any placenta which may be retained.

___ Antibiotics

___ Prostagrandin F2 (Lutalyse®)

___ Estradiol (ECP®)

___ Other _____

42) What do you do when cows with severe disease do not respond to treatment?

number of cows do not respond to treatment last year

number of cows I sold the cow for slaughter

number of cows I sold the cow to a non-organic dairy herd

Calf Section:

43) About what percent of live-born calves die before they are weaned? _____%

44) What percent of the calves get colostrum by the following methods?

__ Only by nursing the dam

__ Always bottle or tube fed even if seen nursing the dam

__ Bottle or tube fed only if not seen nursing

__ Other _____

45) How many cases of calf diarrhea do your farm have per year?

number of calves _____ number of cases

46) How do you treat these diarrhea calves?

I use

47) How many cases of calf pneumonia do your farm have per year?

number of calves _____ number of cases

48) How do you treat these pneumonia calves?

I use

Pre-weaning calf housing:

49) What kind of calf housing do you use?

	Calf housing (winter)	Calf housing (summer)
individual hutches	_____	_____
group hutches or pens	_____	_____
indoor individual pens	_____	_____
indoor group housing	_____	_____
on pasture	_____	_____
Other _____	_____	_____

Direct Observations of Facilities by the Investigator:
Random Selection of 10 cows

Cow	Body Cond. Score	Lameness Score
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____
5.	_____	_____
6.	_____	_____
7.	_____	_____
8.	_____	_____
9.	_____	_____
10.	_____	_____

Lactating Cow Environmental Sanitation Scores:

		Top third	Middle third	Bottom third	
Lactating cow bedding area: Amount of moisture and manure	Clean	___	___	___	dirty
Lactating cow exercise area: Amount of moisture and manure	Clean	___	___	___	dirty
Lactating cow cleanliness: Amount of manure above the knees	Clean	___	___	___	dirty

Pre-weaning calf scores:

Pre-weaning: housing Amount of moisture and manure	Clean	___	___	___	dirty
Pre-weaning: manure on calves	Clean	___	___	___	dirty

Cow numbers or names of all cows present on the farm. This includes all dry and lactating animals which have had at least one calf in their lifetimes.

SAS Control Commands

SAS Control Command for Table 4-1
Pair wise analysis for continuous variables

```
options nocenter;

DATA mastitis;
        INFILE 'c:\SAS\mastitis.csv' DLM = ',' DSD MISSOVER FIRSTOBS=2 OBS=61;
        INPUT pair type season cow drycow Holstein milk SCC  bacteriaNB_S NB_W
        drycowInfuse Drywipe bucket predip ind_towel sha_towel glove dry_towel nodry  postdip
        stanchion people hours  laborpercow unit hired purchase OPENHIRD ai dim
        house grazing bedding straw sand wood corn mastitis Stripout antiinf Oxytocin  infusions
        AB BC_Mar Env_Mar BC_Sep Env_Sep;

        BTM=milk*0.4536;  /* milk in Kg */
        totalcow=cow+drycow;
        masrate=mastitis*400/totalcow;
        milkpercow=BTM/cow;  /*milk per cow in Kg */
        if holstein=1then breed=1; else breed=0;

/* type=1 is organic and type=0 is conventional*/
proc mixed;
 class pair type season;
 model  totalcow = type /solution;
 random pair;
proc mixed;
 class pair type season;
 model  ai = type /solution;
 random pair;
proc mixed;
 class pair type season;
 model   milkpercow = type /solution;
 random pair;
proc mixed;
 class pair type season;
 model  laborpercow = type /solution;
 random pair;
proc mixed;
 class pair type season;
 model  masrate = type /solution;
 random pair;
proc mixed;
 class pair type season;
 model  SCC = type /solution;
 random pair;
proc mixed;
 class pair type season;
 model  bacteria = type /solution;
 random pair;

proc chart data=mastitis;
hbar SCC /group=type freq;
```

```
proc chart;
hbar masrate /group=type freq;


proc univariate;
var totalcow;
by type;
run;
quit;
```

SAS Control Command for Table 4-2
Comparison of discrete variable between organic and conventional dairy farms
Pair wise analysis (McNemar's test)

```
options nocenter;

DATA paired;
        INFILE 'c:\SAS\paired.csv' DLM = ',' DSD MISSOVER FIRSTOBS=2 OBS=31;
        INPUT pair      type    season cow drycow Holstein milk SCC  bacteriaNB_S NB_W
                drycowInfuse Drywipe bucket predip ind_towel sha_towel glove dry_towel nodry
                postdip stanchion people hours laborpercow unit hired purchase OPENHIRD ai
        dim     house grazing bedding straw sand wood corn mastitis Stripout antiinf Oxytocin
        Infusions AB BC_Mar Env_Mar BC_Sep Env_Sep o_cow o_drycow o_Holstein o_milk
        o_SCC o_bacteria o_NB_S o_NB_W o_drycowInfuse o_Drywipe o_bucket o_predip
        o_ind_towel o_sha_towel o_glove o_dry_towel o_nodry o_postdip o_stanchion o_people
        o_hours o_laborpercow o_unit o_hired o_purchase o_OPENHIRD o_ai o_dim o_house
        o_grazing o_bedding o_straw o_sand o_wood o_corn o_mastitis o_Stripout o_antiinf
        o_Oxytocin o_Infusions o_AB o_BC_Mar o_Env_Mar o_BC_Sep o_Env_Sep;

/*assign 100% holstein as 1, else hostein=0*/
if holstein=1 then breed=1; else breed=0;
if o_Holstein=1 then o_breed=1; else o_breed=0;

/*assign housing to each category */
if house='0' then freestall='1'; else freestall='0';
if house='1' then loose='1'; else loose='0';
if house='2' then tiestall='1'; else tiestall='0';
if o_house='0' then o_freestall='1'; else o_freestall='0';
if o_house='1' then o_loose='1'; else o_loose='0';
if o_house='2' then o_tiestall='1'; else o_tiestall='0';

/*assign bedding to each category*/
if bedding='0' then nobedding='1'; else nobedding='0';
if bedding='1' then strawg='1'; else strawg='0';
if bedding='2' then woodg='1'; else woodg='0';
if bedding='3' then sandg='1'; else sandg='0';
if bedding='4' then rubberg='1'; else rubberg='0';

/*assign bedding to each categoly*/
if o_bedding='0' then o_nobedding='1'; else o_nobedding='0';
if o_bedding='1' then o_strawg='1'; else o_strawg='0';
if o_bedding='2' then o_woodg='1'; else o_woodg='0';
if o_bedding='3' then o_sandg='1'; else o_sandg='0';
if o_bedding='4' then o_rubberg='1'; else o_rubberg='0';
```

```
proc freq data=paired;
        tables breed*o_breed /agree;

        tables drycowinfuse*o_drycowinfuse /agree;
        tables openhird*o_openhird /agree;
        tables freestall*o_freestall /agree;
        tables loose*o_loose /agree;
        tables tiestall*o_tiestall /agree;

        tables stanchion*o_stanchion /agree;
        tables drywipe*o_drywipe /agree;
        tables bucket*o_bucket /agree;
        tables predip*o_predip /agree;
        tables postdip*o_postdip /agree;
        tables ind_towel*ind_towel /agree;
        tables sha_towel*o_sha_towel /agree;
        tables glove*o_glove /agree;
        tables dry_towel*o_dry_towel /agree;
        tables nodry*o_nodry /agree;
        tables stripout*o_stripout /agree;
        tables antiinf*o_antiinf /agree;
        tables oxytocin*o_oxytocin /agree;
        tables infusions*o_infusions /agree;
        tables AB*o_AB /agree;

        tables nobedding*o_nobedding /agree;
        tables strawg*o_strawg /agree;
        tables woodg*o_woodg /agree;
        tables sandg*o_sandg /agree;
        tables rubberg*o_rubberg /agree;
run;
quit;
```

SAS Control Command for Table 5-2

Logistic Regression Analysis of resistance to antimicrobials among E. coli isolates from organic and conventional dairy farms

---

```
options nocenter;

Title 'The Analysis of MIC data with proportional odds model & GEE (for Table 4)';

%MACRO ecoli (variable1=);
proc genmod data=ecoli descending;
        class farm organic CowCalf collection;
        model &variable1 = organic CowCalf / link=clogit dist=mult type3;
        repeated subject=farm / type=ind;
        estimate 'OR:O-C' organic 1 -1 /exp;
        estimate 'OR:O-C' CowCalf 1 -1 /exp;

%MEND ecoli;

DATA ecoli;
        INFILE 'c:\SAS2\ecoli03.csv' DLM = ',' DSD MISSOVER FIRSTOBS=2;
        INPUT SampleID Farm Organic CowCalf season collection Amikacin AmoxClav Ampicillin
```

```
        Apramycin Cefoxitin Ceftiofur Ceftriaxone Cephalothin Chloramph Ciproflox Gentamicin
        Kanamycin Nalidixic Streptomycin Sulphamethox Tetracycline TrimetSulf;

%ecoli (variable1= Amikacin )
%ecoli (variable1= AmoxClav )
%ecoli (variable1=Ampicillin)
%ecoli (variable1=Apramycin)
%ecoli (variable1=Cefoxitin)
%ecoli (variable1=Ceftiofur)
%ecoli (variable1=Ceftriaxone)
%ecoli (variable1=Cephalothin)
%ecoli (variable1=Chloramph)
%ecoli (variable1=Ciproflox)
%ecoli (variable1=Gentamicin)
%ecoli (variable1=Kanamycin)
%ecoli (variable1=Nalidixic)
%ecoli (variable1=Streptomycin)
%ecoli (variable1=Sulphamethox)
%ecoli (variable1=Tetracycline)
%ecoli (variable1=TrimetSulf)
run;
quit;
```

SAS Control Command for Table 5-3

```
title 'Multiple antimicrobial resistance Analysis with the extended Mantel-Haenszel mean score
statistic';
/* Table 5 was analyzed according to SAS manual Categorical Data Analysis p73- */

data multidrugcalf;
        input cowcalf $ organic $ response count @@;
datalines;
calf conv 6 30 calf conv 5 11 calf conv 4 43 calf conv 3 36 calf conv 2 24 calf conv 1 20 calf conv 0
121 calf org 6 7 calf org 5 17 calf org 4 19 calf org 3 24 calf org 2 20 calf org 1 27 calf org 0 167;

proc freq data=multidrugcalf order=data;
        weight count;
        tables cowcalf*organic*response /cmh nocol nopct;
run;
quit;

data multidrugcow;
        input cowcalf $ organic $ response count @@;
datalines;
cow conv 3 9 cow conv 2 11 cow conv 1 19 cow conv 0 245
cow org 3 7 cow org 2 10 cow org 1 12 cow org 0 241;

proc freq data=multidrugcow order=data;
        weight count;
        tables cowcalf*organic*response /cmh nocol nopct;
run;
quit;
```

SAS Control Command for Table 6-2

```
options nocenter;

Title 'The Analysis of Campylobacter MIC data';

DATA campy;
        INFILE 'c:\SAS3\isolates02.csv' DLM = ',' DSD MISSOVER FIRSTOBS=2;
        INPUT SampleID Organic CowCalf farm  campy Season;

proc freq;
        table organic*campy/ chisq relrisk;
        table cowcalf*campy/ chisq relrisk;
        table season*campy/ chisq relrisk;

proc genmod data=campy descending;
        class farm organic CowCalf season campy;
        model campy = organic cowcalf season /LINK=LOGIT DIST=BINOMIAL type3;
        repeated  subject=farm / type=ind;
        estimate 'OR:Org-Convl' organic 1 -1 /exp;
        estimate 'OR:cow-calf' CowCalf 1 -1 /exp;
        estimate 'OR:mar-sep' season 1 -1 /exp;
run;
quit;
```

SAS Control Command for Table 6-3

```
options nocenter;

Title 'The Analysis of Campylobacter MIC data';

DATA campy;
        INFILE 'c:\SAS3\manage01.csv' DLM = ',' DSD MISSOVER FIRSTOBS=2;
        INPUT SampleID Organic CowCalf farm campy  Season cow
        milk SCC openherd die_year grazing mastitis metritis placenta
        abortion calf_die Calf diarrha pneumo;

Proc sort;
        by organic cowcalf;

/*Overall estimates;  Backward selection was performed*/
Proc genmod data=campy descending;
 class farm organic cowcalf campy season openherd grazing;
 model campy = Organic CowCalf Season cow milk SCC openherd die_year grazing mastitis
metritis placenta abortion calf_die Calf diarrha /LINK=LOGIT DIST=BINOMIAL type3;
 repeated  subject=farm / type=ind;

/*Only Calf data;  Backward selection was performed*/
Proc genmod data=campy descending;
 where cowcalf=0;
 class farm organic cowcalf campy season openherd grazing;
 model campy = season calf_die calf diarrha /LINK=LOGIT DIST=BINOMIAL type3;
 repeated  subject=farm / type=ind;
```

```
/*Only Cow data; Backward selection was performed*/
Proc genmod data=campy descending;
 where cowcalf=1;
 class farm organic cowcalf campy season openherd grazing;
 model campy = season cow openherd grazing metritis placenta abortion/LINK=LOGIT
DIST=BINOMIAL type3;
 repeated  subject=farm / type=ar;
 estimate 'OR:grazing' grazing 1 0 -1 /exp;
run;
quit;
```

SAS Control Command for Table 7-4

```
options nocenter;

Title 'The Analysis of Staphylococcus MIC data';

DATA staph;
        INFILE 'c:\SAS4\SAUSDEN.csv' DLM = ',' FIRSTOBS=2;
        INPUT Number ID country Farm Organic Season Avilamycin Bacitr Cepha Chlora Ciprof
Eryth Gentam Kanamy Oxacil Penici Strept Sulfam Synerc Tetra Trim Vancom;

If Bacitr=4 then Bacitr=8;
If country=1 and organic=1 then group=1; /*Organic in Wisconsing*/
if country=1 and organic=0 then group=2; /*Conventional in Wisoconsin*/
if country=0 and organic=1 then group=3; /*Organic in Denmark*/
if country=0 and organic=0 then group=4; /*Conventional in Denmark*/

/*dichotomized by MIC90, high sensitibity=<MIC90, low sensitibity>MIC90 */
If Avilamycin >=8 then AVI = 1; If Avilamycin < 8 then AVI = 0;
If Bacitr >=64 then BAC = 1; If Bacitr < 64 then BAC = 0;
If Cepha >=2 then CEF = 1; If Cepha < 2 then CEF = 0;
If Chlora >= 8 then CHL = 1; If Chlora < 8 then CHL = 0;
If Ciprof >=0.5 then CIP = 1; If Ciprof < 0.5 then CIP = 0;
If Eryth >=0.5 then ERY = 1; If Eryth < 0.5 then ERY = 0;
If Gentam >=2 then GEN = 1; If Gentam < 2 then GEN = 0;
If Kanamy >=8 then KAN = 1; If Kanamy < 8 then KAN = 0;
If Oxacil >=1 then OXA = 1; If Oxacil < 1 then OXA = 0;
If Penici >=1 then PEN = 1; If Penici < 1 then PEN = 0;
If Strept >=8 then STR = 1; If Strept < 8 then STR = 0;
If Sulfam >=512 then SUL = 1; If Sulfam < 512 then SUL = 0;
If Tetra >=1 then TET = 1; If Tetra < 1 then TET = 0;
If Trim >=16 then TRI = 1; If Trim < 16 then TRI = 0;
If Vancom >=2 then VAN = 1; If Vancom < 2 then VAN = 0;

proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model BAC = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
```

```
        where country=1;
        class farm organic;
        model CEF = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model CHL = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph  descending;
        where country=1;
        class farm organic;
        model CIP = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;

proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model ERY = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model GEN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model KAN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model PEN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model STR = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
```

```
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model SUL = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model TET = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model TRI = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=1;
        class farm organic;
        model VAN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
quit;
```

SAS Control Command for Table 7-5

```
options nocenter;

Title 'The Analysis of Staphylococcus MIC data';

DATA staph;
        INFILE 'c:\SAS4\SAUSDEN.csv' DLM = ',' FIRSTOBS=2;
        INPUT Number ID      country Farm   OrganicSeason Avilamycin
Bacitr  Cepha Chlora Ciprof  Eryth  GentamKanamy        Oxacil
Penici  Strept Sulfam Synerc Tetra  Trim   Vancom;

If Bacitr=4 then Bacitr=8;
If country=1 and organic=1 then group=1; /*Organic in Wisconsing*/
if country=1 and organic=0 then group=2; /*Conventional in Wisoconsin*/
if country=0 and organic=1 then group=3; /*Organic in Denmark*/
if country=0 and organic=0 then group=4; /*Conventional in Denmark*/

/*dichotomized by MIC90, high sensitibity=<MIC90, low sensitibity>MIC90 */
If Avilamycin >=8 then AVI = 1; If Avilamycin < 8 then AVI = 0;
If Bacitr >=64 then BAC = 1; If Bacitr < 64 then BAC = 0;
If Cepha >=2 then CEF = 1; If Cepha < 2 then CEF = 0;
If Chlora >= 8 then CHL = 1; If Chlora < 8 then CHL = 0;
If Ciprof >=0.5 then CIP = 1; If Ciprof < 0.5 then CIP = 0;
If Eryth >=0.5 then ERY = 1; If Eryth < 0.5 then ERY = 0;
```

```
If Gentam >=2 then GEN = 1; If Gentam < 2 then GEN = 0;
If Kanamy >=8 then KAN = 1; If Kanamy < 8 then KAN = 0;
If Oxacil >=1 then OXA = 1; If Oxacil < 1 then OXA = 0;
If Penici >=1 then PEN = 1; If Penici < 1 then PEN = 0;
If Strept >=8 then STR = 1; If Strept < 8 then STR = 0;
If Sulfam >=64 then SUL = 1; If Sulfam < 64 then SUL = 0;
If Tetra >=1 then TET = 1; If Tetra < 1 then TET = 0;
If Trim >=4 then TRI = 1; If Trim < 4 then TRI = 0;
If Vancom >=2 then VAN = 1; If Vancom < 2 then VAN = 0;


proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model AVI = organic / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;

run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model BAC = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;


proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model CEF = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model CHL = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model CIP = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;

proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model ERY = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
```

```
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model GEN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model KAN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model PEN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model STR = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model SUL = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model TET = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model TRI = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
run;
proc genmod data=staph descending;
        where country=0;
        class farm organic;
        model VAN = organic/ link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'organic versus conventional' organic 1 -1/exp;
```

```
run;
```

SAS Control Command for Table 7-6

```
options nocenter;

Title 'The Analysis of Staphylococcus MIC data';

DATA staph;
        INFILE 'c:\SAS4\SAUSDEN.csv' DLM = ',' FIRSTOBS=2;
        INPUT Number ID       country Farm   OrganicSeason Avilamycin
Bacitr  Cepha  Chlora Ciprof  Eryth   GentamKanamy        Oxacil
Penici  Strept  Sulfam Synerc Tetra   Trim    Vancom;

If Bacitr=4 then Bacitr=8;
If country=1 and organic=1 then group=1; /*Organic in Wisconsing*/
if country=1 and organic=0 then group=2; /*Conventional in Wisoconsin*/
if country=0 and organic=1 then group=3; /*Organic in Denmark*/
if country=0 and organic=0 then group=4; /*Conventional in Denmark*/

/*dichotomized by MIC90, high sensitibity=<MIC90, low sensitibity>MIC90 */
If Avilamycin >=8 then AVI = 1; If Avilamycin < 8 then AVI = 0;
If Bacitr >=64 then BAC = 1; If Bacitr < 64 then BAC = 0;
If Cepha >=2 then CEF = 1; If Cepha < 2 then CEF = 0;
If Chlora >= 8 then CHL = 1; If Chlora < 8 then CHL = 0;
If Ciprof >=0.5 then CIP = 1; If Ciprof < 0.5 then CIP = 0;
If Eryth >=0.5 then ERY = 1; If Eryth < 0.5 then ERY = 0;
If Gentam >=2 then GEN = 1; If Gentam < 2 then GEN = 0;
If Kanamy >=8 then KAN = 1; If Kanamy < 8 then KAN = 0;
If Oxacil >=1 then OXA = 1; If Oxacil < 1 then OXA = 0;
If Penici >=1 then PEN = 1; If Penici < 1 then PEN = 0;
If Strept >=8 then STR = 1; If Strept < 8 then STR = 0;
If Sulfam >=512 then SUL = 1; If Sulfam < 512 then SUL = 0;
If Tetra >=1 then TET = 1; If Tetra < 1 then TET = 0;
If Trim >=16 then TRI = 1; If Trim < 16 then TRI = 0;
If Vancom >=2 then VAN = 1; If Vancom < 2 then VAN = 0;

/*
proc freq data=staph;
where country=1;
table organic*Avilamycin /chisq nocol nopct;
table organic*Bacitr /chisq nocol nopct;
table organic*Cepha /chisq nocol nopct;
table organic*Chlora /chisq nocol nopct;
table organic*Ciprof /chisq nocol nopct;
table organic*Eryth /chisq nocol nopct;
table organic*Gentam /chisq nocol nopct;
table organic*Kanamy /chisq nocol nopct;
table organic*Oxacil /chisq nocol nopct;
table organic*Penici /chisq nocol nopct;
table organic*Strept /chisq nocol nopct;
table organic*Sulfam /chisq nocol nopct;
table organic*Tetra /chisq nocol nopct;
```

```
table organic*Trim /chisq nocol nopct;
table organic*Vancom /chisq nocol nopct;
run;
quit;
*/
proc genmod data=staph;
        class farm organic country;
        model BAC = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;

proc genmod data=staph;
        class farm organic country;
        model CHL = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;

proc genmod data=staph;
        class farm organic country;
        model CIP = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
proc genmod data=staph;
        class farm organic country;
        model ERY = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
proc genmod data=staph;
        class farm organic country;
        model GEN = country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
proc genmod data=staph;
        class farm organic country;
        model KAN = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
proc genmod data=staph;
        class farm organic country;
        model PEN = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
proc genmod data=staph;
        class farm organic country;
        model STR = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
proc genmod data=staph;
```

```
        class farm organic country;
        model SUL = country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
proc genmod data=staph;
        class farm organic country;
        model TET = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;
/*
proc genmod data=staph;
        class farm organic country;
        model TRI = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;*/
proc genmod data=staph;
        class farm organic country;
        model VAN = organic country organic*country / link=logit dist=binomial type3;
        repeated subject=farm / type=ind;
        estimate 'US versus Denmark' country 1 -1/exp;
run;

quit;
```