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USE OF ANTIMICROBIAL AGENTS IN DAIRY CALVES AND THE PUBLIC HEALTH CONCERN

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# USE OF ANTIMICROBIAL AGENTS IN DAIRY CALVES AND THE PUBLIC HEALTH CONCERN

By

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## ABSTRACT

USE OF ANTIMICROBIAL AGENTS IN DAIRY CALVES AND THE PUBLIC HEALTH CONCERN

By

James J. Averill, DVM

Since their inception, antimicrobial agents have been used in multiple ways to enhance the health and well-being of humans and animals. Today, in developed countries, we presume that antimicrobial agents will cure the vast majority of bacterial diseases. However, bacterial resistance to antimicrobial agents is an emerging problem that challenges the biomedical professions and public health. Additionally, the use of antimicrobial agents in animal agriculture has been implicated as contributing to bacterial resistance in human medicine. Veterinary medicine can play a key role in helping mitigate the potential increase of antimicrobial resistance.

This study was developed with the overall goal to enhance knowledge on the appropriate use of antimicrobial agents in veterinary medicine. This overall goal was addressed through the four following objectives: 1) Evaluate growth, morbidity, and mortality of Holstein heifer calves fed milk replacer with or without oxytetracycline and neomycin add, 2) Determine the steady state pharmacokinetics of oxytetracycline administered in milk replacer, 3) Compare minimum inhibitory concentrations of *Escherichia coli* and *Pasteurella multocida* isolated from calves fed milk replacer with or without antimicrobial agents, and 4) Develop a computer-aided learning program on the appropriate use of antimicrobial agents in veterinary medicine to be used in veterinary school curricula.

Findings are briefly summarized below. Calves fed medicated milk replacer (MMR) were heavier at 42 and 150 days of age than calves fed non-medicated milk replacer. Risk factors that influenced weight gain were treatment group, birth weight, season of birth, and having an episode of respiratory disease. Though there was no difference in morbidity between treatment groups. There was a numeric difference with mortality, as the medicated group had lower mortality rates.

Plasma concentrations of oxytetracycline in dairy calves fed medicated milk replacer were below minimum inhibitory concentration (MIC) values for *Escherichia coli* and *Pasteurella multocida*. For *Pasteurella multocida,* the medicated group did have a higher MIC90 compared to the non-supplemented calves, while for *Escherichia coli* there was no difference.

A website was developed which contains a 'Principle' module that goes over the basics of antimicrobial resistance and the role of antimicrobial use in animal and human health. There are also multiple 'Case Study' modules that apply these principles to specific clinical situations involving dairy and beef cattle, small animals, pocket pets and swine. Each module is about how a veterinary student or veterinarian investigates an issue concerning the appropriate use of antimicrobial agents. Within each module there are videos, animations, and questions to engage the learner.

In conclusion, MMR increases growth in dairy calves and plasma levels of oxytetracycline do not reach MIC values for either pathogen tested. Feeding MMR during the first few weeks of life may be beneficial to reduce mortality but given the concern regarding potential development of resistance, MMR not be feed for the entire suckling period.

To my parents David and Sandra.
Without your unconditional love and guidance
this would never have been possible.

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# INTRODUCTION

## RATIONALE

The discovery of antimicrobial agents advanced medicine from the inability to treat complications from a simple scratch to the ability to cure life-threatening diseases such as syphilis, cholera, tuberculosis and pneumonia. Since their inception, antimicrobial agents have been used in multiple ways to enhance the health and well-being of humans and animals. Today, in developed countries, we presume that antimicrobial agents will cure the vast majority of bacterial diseases. However, bacterial resistance to antimicrobial agents is an emerging problem that challenges the biomedical professions and public health. Additionally, the use of antimicrobial agents in animal agriculture has been implicated as contributing to bacterial resistance in human medicine.

Antimicrobial agents were added to animal feed as early as 1948. Soon after, antimicrobial agents were added to milk replacer to improve the growth of dairy calves and for disease prevention. By the mid 1950s research demonstrated that medicated milk replacer increased growth 10 to 30 percent in the first eight weeks of life. As animal husbandry and preventive management improved over the last half century, the literature has offered paradoxical results as to the benefit of feeding medicated milk replacer. National Animal Health Monitoring System (NAHMS) surveys of the dairy industry in 2002 and 2007 reported that supplementing milk replacer with antimicrobial agents is a still a common practice as over fifty percent of all milk replacers include antimicrobial agents. The most common drugs added are oxytetracycline and neomycin.

As antimicrobial resistance has increased, questions have arisen regarding the need to add antimicrobial agents to milk replacer and other animal feeds. Scrutiny over the use of antimicrobial agents began in 1969 when the British government released the Swan report, calling for antimicrobial agents in animal agriculture to be used by prescription only and for drugs used as feed additives be prohibited if used in human medicine. The World Health Organization released several reports in the late 1990s and into the 21st century stating concern regarding the use of antimicrobial agents in animal feed and potential transfer of resistance to humans. In 2006, the European Union banned the use of all antimicrobial agents in animal agriculture for growth promotion, including ionophores.

## PROBLEM STATEMENT

With the emergence of antimicrobial resistance as a public health concern, veterinary medicine plays a critical role in mitigating further development of resistance. Although there have been advancements in animal husbandry and preventive medicine practices (vaccines, nutrition and biosecurity) over the past fifty years, medicated milk replacer is still commonly used in the dairy industry. There is a need to investigate the necessity of adding antimicrobial agents to milk replacer for aid in growth and/or prevent disease given the concern regarding the development of antimicrobial resistance.

## RESEARCH QUESTIONS TO BE ADDRESSED

The overall aim of this dissertation is to enhance scientific knowledge on the appropriate use of antimicrobial agents in dairy cattle. Specifically, to address the use of

antimicrobial agents in dairy calves. Key research questions that should be answered by the studies conducted;

1) Do calves fed milk replacer supplemented with antimicrobial agents grow faster and have a lower disease burden?
2) Do plasma-steady state pharmacokinetic values for oxytetracycline as fed in medicated milk replacer achieve minimum inhibitory concentrations for *Escherichia coli* and *Pasteurella multocida*?
3) Will a computer-aided learning module be useful to teach veterinary students concepts about appropriate use of antimicrobial agents?

**HYPOTHESES TO BE TESTED**

To address the above overall aim and research questions the following hypotheses were developed. They are:

1) Holstein heifer calves fed a milk replacer supplemented with oxytetracycline and neomycin will gain more weight and have a lower morbidity and mortality rate than calves fed the same milk replacer without antimicrobial agents.
2) Plasma concentration of oxytetracycline in calves fed a milk replacer supplemented with oxytetracycline will reach minimum inhibitory concentrations for *Escherichia coli* from fece and *Pasteurella multocida* from nasal swab.

## OVERVIEW OF RESEARCH

A literature review regarding discovery and development of antimicrobial resistance and the use of antimicrobial agents in milk replacer are provided in **chapter one**. **Chapter two** addresses hypothesis 1 via a field trial where calves' growth and health records were monitored weekly until weaning and then again at five months of age. Hypothesis 2 is addressed in **chapter three** which describes an investigation of the steady-state pharmacokinetics of oxytetracycline in six calves and compares minimum inhibitory concentrations of oxytetracycline to two common bacterial pathogens to plasma drug levels in calves fed milk replacer with or without oxytetracycline and neomycin. **Chapter four** focuses on hypothesis 3, which describes a website was on the appropriate use of antimicrobial agents in veterinary medicine to be used as an adjunct to a veterinary students education.

# CHAPTER 1

# USE OF ANTIMICROBIALS IN DAIRY CALVES AND PUBLIC HEALTH CONCERNS: A REVIEW

## INTRODUCTION:

The discovery of antimicrobial agents advanced medicine from the inability to treat complications from a simple scratch to the ability to cure life-threatening diseases such as syphilis, cholera, tuberculosis and pneumonia. Since their inception, antimicrobial agents have been used in multiple ways to enhance the health and well-being of humans and animals. Today, in developed countries, we presume that antimicrobial agents will cure the vast majority of bacterial diseases. However, bacterial resistance to antimicrobial agents is an emerging problem that challenges the medical professions and endangers public health. The use of antimicrobial agents in animal agriculture has been implicated as contributing to bacterial resistance in human medicine.

Antimicrobial agents were added to animal feed as early as 1948 (Jukes and Williams, 1953). Soon after, antimicrobial agents were added to milk replacer to improve the growth of dairy calves and for disease prevention. As animal husbandry and preventive management improved over the last half century, questions arose regarding the need to add antimicrobial agents to milk replacer, and other animal feeds.

The purpose of this literature review is to impart a historical and current understanding of the use of antimicrobial agents in animal feed. In particular, topics will include: 1) discovery of antimicrobial agents, 2) the mechanisms of bacterial resistance, 3) public health concerns regarding the use of antimicrobial agents in animal agriculture, 4) the practice of using medicated milk replacer, 5) pharmacokinetics of oxytetracycline in milk replacer, and 6) factors associated with dairy calf growth.

## DISCOVERY OF ANTIMICROBIAL AGENTS:

Antimicrobial agents are substances that inhibit microorganism viability, and derive their name from the Greek words *anti*, meaning "against," *micros*, meaning "little," and *bios*, meaning "life." Substances used to treat bacterial infections in animals and humans are typically referred to as antibiotics. The terms antimicrobial agents and antibiotics are not interchangeable; antibiotics refer to substances of microbial origin that act upon other microorganisms, while the broader term, antimicrobial agents, also includes synthetic compounds.

In recalling the discovery of penicillin, Sir Ernest Chain stated that it "is practically impossible for anyone growing bacteria not to come across chance contaminants with antagonistic properties" (Chain, 1980). During the 1870s, scientific emphasis on the germ theory derived the term "abiogenesis." Roberts documented this in his "*Studies of Abiogenesis*" in 1874, where he noted antagonism between fungi and bacteria (Roberts, 1874). The use of such an antagonist for therapy was not suggested until 1885 by Cantani's paper in *Medical News* (Cantani, 1885). In 1889, a French biologist, P. Vuillemin, described the destruction of one organism by another with the adjective "antibiotic" (Chain, 1980). Later in that century, Paul Ehrlich, who was later proclaimed by some as the father of antibacterial therapy, determined that the chemical dye salvarsen had potential antimicrobial effects in patients with syphilis. However, salvarsen was later found to have unpleasant side effects as the compound contained an arsenic derivative (Amyes, 2001). In his early work, Ehrlich suggested that dyes might be the "magic bullet" for treating bacterial infections. Ehrlich's work was rediscovered in 1929 by Gerhard Domagk who took a more systematic approach to discovery of

chemicals to treat bacterial infections by using a mouse model (Amyes, 2001). Domagk's vigilance paid off in 1932 when a new drug, the red dye Prontosil, was successfull in treating *Streptococcus* in mice (Amyes, 2001). Prontosil was tested against a variety of bacteria and was shortly discovered not to be effective against gram-negative bacteria. Domagk's discovery was not well accepted by the medical community until 1936, when the son of Franklin D. Rooosevelt was cured from tonsillitis by Prontosil (Amyes, 2001). The press hailed the miracle drug. Later research by a French laboratory discovered that the active compound in Prontosil was a sulphanilamide, the first of this class of antimicrobial agents (Amyes, 2001).

In his St. Mary's laboratory during the 1920's, Alexander Fleming was investigating the antibacterial properties of body secretions (Chain, 1980). In these experiments he demonstrated that a teardrop on a culture caused rapid lysis of certain bacterial organisms. This lytic enzyme was named lysozyme, and was Fleming's first major scientific finding (Amyes, 2001). Further studies found that lysozyme did not readily kill pathogenic bacteria; this did not deter Fleming from continuing to look for new antiseptics. Fleming was not known for being a tidy, well-organized bacteriologist, as any visitor to his laboratory would see clutter and colonized petri dishes on the bench tops. One day in early September 1928, Fleming was going through some old petri dishes inoculated with *Staphylococcus* when he noticed mold contaminating one area of a particular plate (Wainwright, 1990). Around the area of contamination was a wide circle of inhibited growth of Staphylococci. Fleming named this inhibitory substance penicillin, after the mold *Penicillium notatum* that was growing on the plate (Wainwright, 1990). Fleming was able to reproduce this phenomenon for other pathogenic gram-

positive bacteria, but not for gram-negative bacteria (Wainwright, 1990). Further studies not only demonstrated that penicillin was effective against staphylococci but also non-toxic to mice and rabbits (Chain, 1980). One question that Fleming did not answer with his studies was the potential chemotherapeutic effect of penicillin.

Ironically, his discovery went unnoticed by the medical community for almost 10 years until 1936 when Florey and Chain attempted to determine the structure and chemotherapeutic benefit of Fleming's discovery (Chain, 1980). In 1940, they succeeded with the help of Heatley to purify penicillin and later tested it in mice infected with streptococci. After receiving penicillin doses every three hours, all but one survived, as compared to all control mice dying within 16 hours (Chain, 1980). Fleming was pleased with these findings and was quoted as saying, "they have turned out to be the successful chemist I should have liked to have with me in 1929." (Amyes, 2001). The next step was to test the compound on humans and mass-produce penicillin via fermentation. Florey struggled to get pharmaceutical companies to explore large vat fermentation even after several successful treatments in humans. Finally, John L. Smith, president of Chas Pfizer Company was intrigued by the success of the compound and Chas Pfizer became the first company to begin large-scale production of the drug in 1942 (Amyes, 2001).

Penicillin's breakthrough in the United States took place when a devastating fire at the Coconut Grove Nightclub in Boston occurred on November 29, 1942 (Levy, 2002). Merck and Company sent a small supply of penicillin to Massachusetts General Hospital for patients receiving skin grafts. This became one of the most important clinical trials demonstrating the safety and efficacy of penicillin to the United States government. The media touted penicillin as the "miracle drug" due to its ability to control infectious

bacteria that previously were not treatable (Levy, 2002). Thus, the discovery of clinical applications for sulfonamides and penicillin revived the quest for what Paul Ehrlich called the "magic bullet", a drug that could kill bacteria without harming humans (Levy, 2002). For the discovery of penicillin, mass production, and success in treating soldiers' wounds during World War II, Fleming, Florey, and Chain received the Nobel Prize for Medicine in 1945 (Amyes, 2001).

In 1941, S.A. Waksman introduced the term antibiotics, a noun, as "chemical substances that are produced by microorganisms and that have the capacity, in dilute solution, to selectively inhibit the growth of and even destroy other microorganisms" (Aarestrup, 2006). This term was commonly used from this time forward when referring to a compound used to treat bacterial infection(s). Through the mid 1940s into the early 1960s, many antimicrobial agents were discovered and brought into clinical practice (Table 1).

**DEVELOPMENT OF ANTIMICROBIAL RESISTANCE:**

Alexander Fleming, in a 1945 interview with The New York Times, warned that misuse of penicillin could lead to bacterial resistance (Levy, 2002). Fleming had observed this phenomenon in his laboratory; strains of previously susceptible organisms, in the presence of low concentrations, were no longer inhibited by penicillin (Levy, 2002). To avoid such development, Fleming spoke out saying that complete courses of therapy were necessary to avoid initiating resistance. Little was done to address Fleming's concern due to the overwhelming benefit antimicrobial agents had on

**Table 1.1** Historical Timeline of Antimicrobial Development, Introduction and Important Events

| Dates | Antimicrobial Drug Development | Antimicrobial Drug Introduction and Important Events |
|---|---|---|
| 1925 – 1930 | Penicillin discovered by Alexander Fleming | |
| 1931 – 1935 | Sulfonamides discovered | |
| 1936 – 1940 | | Sulfonamides introduced into food animal use |
| 1941 – 1945 | Streptomycin discovered | Penicillin used in World War II to treat wounds |
| 1946 – 1950 | Bacitracin, chloramphenicol, neomycin, polymyxin, streptogramins, and tetracycline were discovered, discovery of antibiotics for growth promotion | Release of penicillin and streptomycin for use in human and veterinary medicine |
| 1951 – 1955 | Erythromycin discovered | Neomycin and nitrofurans introduced to clinical practice, tetracyclines and chloramphenicol used therapeutically in animals, intrammamry use of antibiotics for mastitis, extensive use of antibiotics for growth promotion |
| 1956 – 1960 | Vancomycin and tylosin discovered | Virginiamycin used as a growth promoter |
| 1961 – 1965 | Methicillin, other penicillinase-resistant penicillins, and gentamicin discovered | Discovery of transmissible, plasmid muti-drug resistance in *Enterobacteriaceae* |
| 1966 – 1970 | Cephalothin, ampicillin, amikacin | Flavomycin introduced as growth promoter, new drug analogs to address resistance problem |
| 1971 – 1975 | Carbenicillin discovered | Narrow-sprectrum cephalosporins and trimethoprim-sulfonamide introduced |
| 1976 – 1980 | Cefoxitin and moxalactam discovered | Avoparcin introduced as growth promoter in Europe, chloramphenicol use banned in food animals |

**Table 1.1, con't.** Historical Timeline of Antimicrobial Development, Introduction and Important Events, cont.

| | | |
|---|---|---|
| 1981 – 1985 | Cefotaxime, other broad-sprectrum cephalosporins, beta-lactamase inhibitors combined with aminopenicillins, imipenem-cilastatin discovered | Ban on nirtofuran and nitroimidazole drugs in food animals in United States |
| 1986 – 1990 | Quinolones, fluoroquinolones, ceftiofur discovered | Development of Food Animal Residue Avoidance Database, moratorium on sulfamethazine use in dairy cows and aminoglycoside in food animals in United States |
| 1991 – 1995 | | Tilmicosin, tiamulin and florfenicol introduced to selective food animals, enrofloxacin introduced to poultry |
| 1996 – 2000 | | Fluroquinolones introduced for use in cattle for respiratory disease, vancomycin resistant enterococci linked to avoparcin use in food animals in Europe, ban of avoparicin and four other growth promoters in Europe |
| 2001 – 2005 | Danofloxacin discovered for respiratory disease in food animals | Withdrawal of fluoruquinolones for use in poultry in United States |
| 2006 – | Will new drugs be discovered? | |

Modification of chapter 2 'History of Antimicrobial Usage in Agriculture: an Overview' in Antimicrobial Resistance In Bacteria of Animal Origin, Edited by Frank M. Aarestrup, 2006 ASM Press, Washington DC

medicine. However, by 1946, resistance to penicillin was noted in a London hospital where 14% of the isolated strains were resistant to the drug, and by the end of the decade, the frequency of resistant strains had increased to 59 percent in the same hospital (Levy, 2002).

The proportion of penicillin-resistant organisms continued to increase into the 1950's, as the drug was sold over-the-counter and incorporated into numerous products to treat various ailments. By 1955, most countries had documented an increase in penicillin resistance, which prompted many countries to require a prescription for human use (Levy, 2002). During this period, emerging resistance among pathogens in animal agriculture was also documented, and most believed this evolution was strictly chromosomally mediated (Elam et al., 1951a, b; Jones and Ricket, 2003). Barnes confirmed these earlier studies (mentioned above), and determined that antimicrobial agents in animal feed altered the microflora and increased the proportion of organisms resistant to antimicrobial agents (Barnes, 1958). The concern over potential transfer of antimicrobial resistance to zoonotic pathogens came to a head during an outbreak of chloramphenicol-resistant *Salmonella* Typhimurium in dairy calves, which was implicated to cause infections in humans (Anderson, 1968).

**DEVELOPMENT OF PUBLIC HEALTH CONCERNS:**

As antimicrobial agents were being rapidly discovered, they were being employed therapeutically or non-therapeutically, for animals and humans, and via a variety of routes of administration and therapeutic regimens. In 1969, the "*Swann Report*," was released by the British government regarding animal husbandry and the use of

antimicrobial agents by veterinary medicine in animal agriculture (Swann, 1969). This report was prompted by: 1) an outbreak of resistant *Salmonella* in calves that infected humans (Anderson, 1968), 2) an outbreak of chloramphenicol-resistant *Salmonella typhi* in Central America (Randall, 1969), 3) evidence that antimicrobial agents used in animal feed for growth promotion were causing the development of resistant bacteria isolated from pigs and chickens (Barnes, 1958; Smith, 1975, 1977) and 4) demonstration of transferable resistance (Anderson, 1968). This committee concluded that therapeutic use of antimicrobial agents in farm animals should be used by prescription only and that feed additive use of antimicrobial agents should only be permitted if the drug is not used in humans (Swann, 1969). Tetracycline and penicillin did not meet the feed additive use guidelines and were banned in the United Kingdom from such use (Aarestrup, 2006).

The Swann report received mixed reviews, as few believed such recommendations were warranted (Freeman, 1970; Prescott et al., 2000), but it did initiate debate as to how antimicrobial agents should be used in animal agriculture. However, as time passed, scrutiny of non-therapeutic use of antimicrobial agents in animal agriculture from public health and human medical communities increased. A report from the U.S. Food and Drug Administration (FDA) in 1972 concluded that use of antimicrobial agents in food animals could promote resistance in *Salmonella* and required manufacturers to demonstrate that their product did not increase the prevalence of resistant *Salmonella* in animals (FDA, 1972). This report was the first in the United States to suggest that there was enough scientific information to justify a ban on non-therapeutic use of penicillin and chlortetracycline, except under veterinary prescription. However, this recommendation was not accepted (Force, 1972).

In 1980, the National Research Council of the U.S. released "The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animals" (IOM, 1980). This report stated that there was not adequate information to prove or disprove a relationship between the use of antimicrobial agents in animal feed and increased bacterial resistance in human medicine. This report further commented that the United Kingdom had seen little benefit from changes recommended in the Swann Report of 1969 (IOM, 1980), though the authors did recognize that non-therapeutic use of antimicrobial agents increased isolation of resistant *E. coli* and *Salmonella*. Similar results were found in the Institute of Medicine report in 1989, which determined a lack of substantial evidence linking the use of penicillin and tetracycline at non-therapeutic levels in animal feed to any impact on human health (IOM, 1989). Both of these studies did state that there was a need for more information, as was reiterated in 1995 by the Office of Technology Assessment (OTA, 1995).

A report in 1981 stated that banning the use of antimicrobial agents in animal feed at non-therapeutic levels would cost the United States economy 3.5 billion dollars (Technology, 1981). The authors went on to say that in their opinion, banning non-therapeutic antimicrobials would only be beneficial if therapeutic use was banned, too. This might result in unethical and inhumane treatment of livestock.

After considerable debate, Sweden in 1985, prohibited non-therapeutic use of antimicrobials in animal feed (SOU, 1997). This had economic ramifications, as the mortality rate increased for piglets at weaning and necrotic enteritis emerged in broiler chickens (Wierup, 2001). A self-study in 1997 concluded that the benefits out-weighed

the risks of banning the use of antimicrobial agents for growth promotion, and non-therapeutic use (SOU, 1997).

The World Health Organization recommended the discontinuation of antimicrobial agents in animal feed for growth promotion in 1997 (WHO, 1997). Specifically, antimicrobials used in human medicine that were also used in animal feed for growth promotion and evolved towards cross-resistance were to be prohibited from animal use. The following year the Ministry of Agriculture, Fisheries and Food (U.K.) released a report stating that resistance in animal pathogens is due to antimicrobial use. Specifically *Campylobacter* and *Salmonella* with regards to certain antimicrobial agents, can reach humans through the food supply and cause disease or colonize humans allowing resistance to be transferred (Aarestrup, 2006).

Public health concerns continued to develop over the use of antimicrobials in animal feed at non-therapeutic levels, and numerous reports were released stating that such practice was leading to increased resistance in human medicine and decreasing therapeutic success (Barza and Gorbach, 2002; JETACAR, 1999; WHO, 2003b). These findings were strengthened by the Danish experience on banning antimicrobials in animal feed for growth promotion and disease prevention; that ban resulted in a greater than 50 percent reduction in the use of antimicrobial agents in animal agriculture and decreased prevalence of resistant organisms (WHO, 2003a). However, there was an increased morbidity in weaned pigs and decreased feed efficiency that lead to a 1% increase in production cost (WHO, 2003b). Over a five-year period, the FDA and pharmaceutical industries debated the use of a fluoroquinolone drug in poultry because of concerns that

feeding this drug resulted in antimicrobial resistance in bacteria that cause infections in humans, and the license for this use was withdrawn in September 2005 (FDA, 2005).

Given the overwhelming public health concern and regulatory response, the scientific community continues to seek an objective viewpoint on the ban of antimicrobials in animal feed for growth promotion and disease prevention. The National Research Council and Institute of Medicine concluded that there was not enough information to demonstrate an immediate public health concern (IOM, 1989; NRC and IOM, 1999). The NRC/IOM report did acknowledge that there were many gaps and further information may change their conclusions. It was also estimated that banning non-therapeutic use of antimicrobial agents in animal feed would add $5-$10 a year to the cost of food for each United States citizen (NRC and IOM, 1999). Bywater et al reported in 2000 a qualitative assessment of antimicrobial resistance and animal agricultures role. Results of a questionnaire sent to experts in the United Kingdom found that use of antimicrobial agents in animal agriculture contributed only 3.88 percent of the resistance in major bacterial pathogens affecting humans, while the rest was due to use of such drugs in human medicine (Bywater and Casewell, 2000).

## TYPES AND MECHANISMS OF ANTIMICROBIAL RESISTANCE:

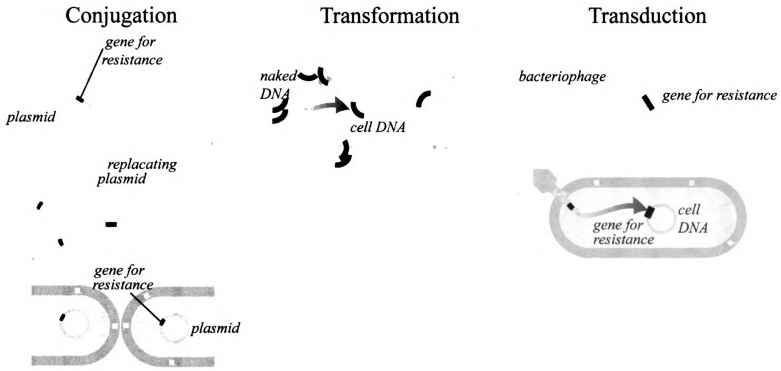
Bacteria can be intrinsically resistant to antimicrobial agents from a lack of targets for drug activity, the inability of the drug to enter the bacteria, genetically coded pumps to export the drug, or the presence of an enzyme that inactivates the drug (Sefton, 2002). Intrinsic resistance occurs in many bacteria to various antimicrobial agents, for example, glycopeptides are not able to enter the outer membrane of gram-negative bacteria

(Aarestrup, 2006). Perhaps of greater concern is acquired resistance, as this causes the emergence and spread of resistance in what was once considered a susceptible organism.

Acquired resistance arises from a genetic mutation of an organism or via acquisition of genetic material through horizontal transmission (Sefton, 2002; Tenover, 2006). Mutations that confer resistance spontaneously (endogenous resistance) occur at varying rates among species and among strains within a species. As an example, *Staphylococcus aureus* is approximately 100-fold more likely to develop resistance to rifampin than *Escherichia coli*, making the treatment of staphylococci with rifampin inappropriate (Aarestrup, 2006).

Exogenous resistance, or transferable resistance, can occur by one of three possible mechanisms (Figure 1): transformation, transduction, and conjugation (cell to cell transfer of genetic material) (Aarestrup, 2006; Sefton, 2002; Tenover, 2006). Transformation and transduction do not require contact between organisms, however acquisition of antimicrobial resistance by these mechanisms is limited to closely related species or genus, as homology is required between donor and recipient (Aarestrup, 2006). Transduction occurs via plasmid DNA incorporated into a bacteriophage that attaches to bacteria and transfers DNA into the bacteria, which potentially carries genes coding for antimicrobial resistance, although this is believed to be a rare event (Tenover, 2006). Transformation is the transfer of naked DNA from one cell to another (Prescott et al., 2000). This form of transferable resistance is becoming recognized as an important source of emerging resistance, e.g. *Strepotococcus* transferring genes for penicillin-binding-protein 2 (Aarestrup, 2006; Prescott et al., 2000).

**Figure 1.1** Methods of transferable resistance for antimicrobial agents; conjugation, transformation, transduction



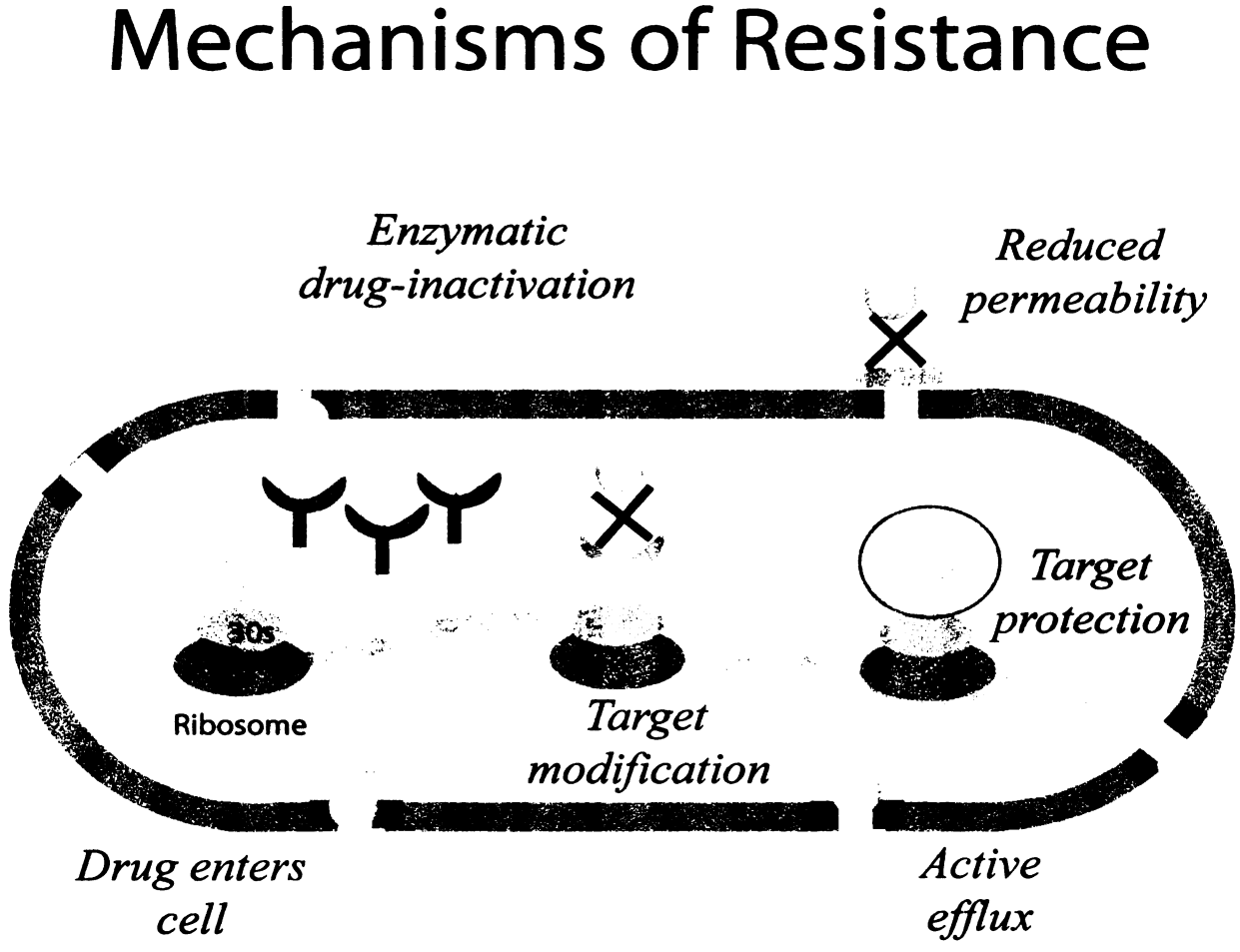
Conjugation is likely to play the biggest role in acquired resistance as genes for antimicrobial resistance are commonly found on conjugative genetic elements; plasmids, transposons, and integrons (Prescott et al., 2000; Sefton, 2002). Plasmids are extrachromosomal circular DNA in bacteria, which replicate independently of chromosomal DNA, but at the same time (Prescott et al., 2000). Plasmids can confer resistance from 1 to 10 different antimicrobial agents. Transposons are short sequences of DNA, which readily transfer between plasmids, or between plasmids and chromosomes (Prescott et al., 2000). In order for transposons to integrate into foreign DNA, insertion genes are vital; these gene sequences flank both ends of the transposon (Salyers and Whitt, 2002). Frequency of transposition is highly dependent on the transposon and bacterial strain. Integrons are mobile genetic elements that are often found on plasmids that are associated with antimicrobial resistance and other bacterial changes (Prescott et al., 2000). In order for integrons to function and transfer resistance they must contain an integrase enzyme for site-specific recombination, a gene-capture site, and a captured gene (mobile element that contains the gene for antimicrobial resistance) (Salyers and Whitt, 2002). Plasmids develop multi-drug resistance by incorporation of multiple integrons conferring antimicrobial resistance (Prescott et al., 2000; Salyers and Whitt, 2002).

Cross-resistance is the final type of antimicrobial resistance, which occurs when a bacterium becomes resistance to an antimicrobial agent, and in doing so becomes resistant to another (Aarestrup, 2006). For example, macrolides, lincosamides, and streptogramins act on ribosomes, and bacterial adaptation of the 50s ribosomal RNA

confers resistance not just to one of the antimicrobial agents, but to all three (Aarestrup, 2006).

Mechanisms of antimicrobial resistance can be classified on a biochemical basis into the following classifications (figure 2); modifying enzyme, impermeability, active expulsion of drug, and modification of target. Modifying enzymes that inactivate the drug can occur naturally and/or be plasmid mediated (Aarestrup, 2006; Sefton, 2002). This mechanism is commonly seen with resistance to β-lactams and aminoglycosides; an example is β-lactamase that binds to penicillin to deactivate the drug. Resistance to β-lactams and fluoroquinolones can also arise from reduced permeability of the drug into the bacteria because membrane porins are too small or the drug is unable to diffuse through the cytoplasmic membrane (Aarestrup, 2006; Sefton, 2002). An example of this is the lack expression of OmpF porin in *E. coli,* which reduces susceptibility to β-lactams, tetracyclines, and quinolones (Jensen et al., 1999). An efflux pump is a transmembrane protein that works by active expulsion of the drug and is typically plasmid mediated (Aarestrup, 2006; Sefton, 2002). This mechanism is often seen with tetracycline where the drug enters the cytoplasm of resistant bacteria and is pumped back out via the efflux pump (Sefton, 2002). The final major mechanism is modification of the drug target from structural changes, replacement or protection (Prescott et al., 2000). This mechanism is often seen in penicillin, macrolide, lincomycin, streptomycin, and quinolone families. As an example, methicillin-resistant *S. aureus* expressing the *mecA* gene synthesize a penicillin-binding-protein that has a lower affinity for methicillin (Walsh, 2003).

**Figure 1.2** Mechanisms of resistance for antimicrobial agents



## USE OF ANTIMICROBIALS IN ANIMAL FEED:

The idea of using antimicrobial agents in animal feed was presented in a report by Moore et al in 1946 (Moore et al., 1946) in a study where chicks fed streptomycin and sulfasuxidine in purified diets had accelerated growth. This idea was further supportedby another study in 1948 that found a similar result (Stokstad et al., 1949). During this time, research was focused on an "animal protein factor" (APF) as a growth promtant, the activity of which was derived from vitamin $B_{12}$. Stokstad's study found that an APF fermented from *Steptomyces aureofaciens* contained an unidentified growth factor, which caused greater growth than in chicks fed diets only supplemented with vitamin $B_{12}$ (Stokstad et al., 1949). Similar results were observed in pigs and turkeys when fed the same fermented material used by Stokstad et al (Cunha et al., 1949; McGinnis et al., 1949). The fermented product was later found to contain aureomycin, otherwise known as chlortetracycline (McGinnis et al., 1949; Stokstad and Jukes, 1950). Finding that antimicrobials increased the growth rate in animals was somewhat surprising to scientists, as work with sulfonamides in rats lead to vitamin deficiencies and decreased growth rates (Jukes, 1971). It was eventually determined that diets containing sulfonamides fed to rats suppress normal intestinal bacterial flora that synthesize vitamins. Although numerous studies with chickens and pigs determined the beneficial effect of antimicrobial agents in animal feed on growth, it remains unclear what mechanisms are involved.

Although early studies in dairy calves found no benefit of antimicrobial agents in calf feed (Rusoff and Haq, 1950; Williams and Knodt, 1950), Loosli and Wallace determined APF, or aureomycin, in milk fed to dairy calves increased weight gain and decreased the frequency of diarrhea when compared to the controls (Loosli and Wallace,

1950). Subsequent trials determined dairy calf feed supplemented with either APF or aureomycin increased weight gain (Bartley et al., 1950a; Bartley et al., 1950b; Hogue et al., 1957b; Lassiter, 1955; Loosli and Wallace, 1950; Rushoff, 1950; Rusoff et al., 1951).

These findings led to multiple studies of antimicrobial agents in feed of young dairy calves. In 1955, Lassiter conducted a review of the published literature regarding antimicrobial agents in dairy calves and as a growth promotant (Lassiter, 1955). In this review, there was an attempt to discuss all antimicrobials being studied in dairy calves (mainly aureomycin and terramycin), the effective antibiotic level, the effect of route of administration on growth, and the effect of combinations of antimicrobial agents. Lassiter concluded that when antimicrobial agents were added to feed, especially in milk, that growth benefits were likely. The growth benefits ranged from a 10 to 30 percent increase over the controls in the first 16 weeks of life. Most of the benefit was observed in the first 8 weeks. Addition of antimicrobial agents to feed also led to decreased frequency of diarrhea and mortality. These benefits were reported when the antimicrobial agents where fed at 0.33 to 0.44 mg/kg or 15-20 mg per 45 kilograms of body weight daily; and higher levels had no additional benefit (Lassiter, 1955).

Studies investigating antimicrobial agents in milk replacer continued to demonstrate benefits in growth (Brown et al., 1960; Everett et al., 1958; Felsman et al., 1973; Hogue et al., 1957a; Hvidsten, 1959; Jorgensen et al., 1964; Morrill et al., 1977; Radisson et al., 1956; Rusoff et al., 1959; Swanson, 1963; Thomas et al., 1959). However, over this period of time there were also reports that demonstrated no statistically significant benefit of antimicrobial agents in milk fed to dairy calves (Bush et al., 1959; Edwards, 1962; Gabrilidis, 1972; Lassiter et al., 1958; Preston et al., 1959). As

is seen in a report with mixed review on the benefit of antimicrobial agents based on studies conducted in 1956 and 1960, in which a statistically significant higher rate of growth was observed in a single group for both years when compared to controls, while all other groups and treatments showed no increased growth (Edwards, 1962). Preston et al and Bush et al found a benefit in growth once the calves were consuming adequate grain that contained antimicrobial agents (Bush et al., 1959; Preston et al., 1959). Preston et al proposed that the addition of chlortetracycline to milk did not benefit growth because of reduced effectiveness of the drug when added to milk versus grain.

More recent literature has also offered paradoxical results as to the effects of antimicrobial agents in milk replacer. Tomkins reported in 1991 that antimicrobial agents improved performance and decreased the incidence of diarrhea in dairy calves (Tomkins and Jaster, 1991). Quigley et al found a trend towards increased growth when feeding medicated milk replacer at 57 mg/day of oxytetracycline (OTC), but there was no statistically significant difference over the controls (Quigley et al., 1997). Additionally, two other studies found no differences in growth between antibiotic or control groups (Donovan et al., 2002; Heinrichs et al., 2003) when fed at 64mg and 403 mg per day. The authors offered no hypothesis for why there was no difference between antibiotic and control groups. However, there are multiple factors that affect calf growth; such as sex, stress, environment, nutrition, and it is unknown if a higher proportion of gut flora in the calves in the later studies may have been resistant to the antimicrobial agent(s) selected for the study.

**PHARMACOKINETICS OF OXYTETRACYCLINE IN MILK REPLACER:**

The National Animal Health Monitoring System (NAHMS) Dairy Study of 2002 and 2007 reported that oxytetracycline and neomycin are the most frequently used antimicrobial agents in milk replacer for dairy calves (USDA, 2005, 2008a). Oxytetracycline is active against susceptible Gram-positive and Gram-negative bacteria and is readily absorbed after oral administration to fasting animals (Plumb, 2002). However, the presence of food or dairy products can significantly reduce the ability of oxytetracycline to be absorbed by an animal from the gastrointestinal tract (Plumb, 2002). Because of their lipophilic nature, tetracyclines have wide distribution throughout the body except for cerebrospinal fluid, and are eliminated unchanged primarily via glomerular filtration (Plumb, 2002). This drug is bacteriostatic and inhibits protein synthesis by binding to the 30S ribosomal subunit of susceptible organisms.

Pharmacokinetic studies of oxytetracycline in dairy calves are limited, especially those including administration in milk replacer (Palmer et al., 1983; Schifferli et al., 1982). Red Holstein- Simmental crossed calves, administered oral oxytetracycline in milk at a dose of 50 mg/kg of body weight, attained peak serum concentrations of 3.10 to 6.39 μg/ml between 6 and 12 hours after administration (Schifferli et al., 1982). Bioavailability of oxytetracycline via oral administration was 46% and the elimination half-life ranged from 7.95 to 15.20 hours (Schifferli et al., 1982).

A study of calves fasted overnight and fed 9 mg/kg of oxytetracycline in milk replacer had statistically significantly lower serum concentrations than calves administered the drug in water or an electrolyte solution (Palmer et al., 1983). Palmer et al. also determined the area under curve (AUC) was statistically different for milk

replacer, water and electrolyte solution: 570, 754, and 1306 μg/ml*min respectively. Sixty-three percent of oxytetracycline was bound to the milk replacer, and therefore not available for absorption (Palmer et al., 1983). Luthman et al reported similar results to Palmer et al. with a higher dose of oxytetracycline; 50 mg/kg in cows milk, milk replacer or water (Luthman and Jacobsson, 1983). In a later study, Luthman et al administered tetracycline chloride at 25 mg/kg in milk replacer twice a day into dairy calves resulting in serum concentrations above 1μg/ml for most of the day, these values are similar to Palmer et al (Luthman et al., 1989). This study also evaluated bolus administration of tetracycline chloride at 50 mg/kg in milk replacer or 4 hours post feeding in water and found that serum concentrations peaked at four hours in both groups, although the milk replacer group had statistically higher serum concentrations, which contradicts the reports cited above. Luthman did hypothesize that the lower serum concentrations attained with the water bolus may be in part due to grain and hay consumption during the 4-hour time frame, which may have reduced absorption of tetracycline chloride.

Young pigs also have been used as a model for oral administration of oxytetracycline pharmacokinetics and are similar to pre-ruminant neonatal calves both are essentially physiological monogastrics. Fasted pigs, tube fed oxytetracycline at a dose of 45 mg/kg, had increased plasma concentrations and areas under the curves than pigs fed before administration. However, bioavailability was low for both fasted and fed pigs; 18 and 5 percent respectively (Nielsen and Gyrd-Hansen, 1996). These pharmacokinetic values are lower than those reported by Schifferli et al who fed the calves at a similar level, 50 mg/kg (Schifferli et al., 1982). Similar results were seen in earlier studies by Mevis et al and Hall et al, in which drug levels after oral administration

**Table 1.2** Pharmacokinetic Studies of the Tetracycline Family in Dairy Calves and Pigs

| | **Schifferli *et al* 1983** | | **Luthman *et al* 1983** | | **Palmer *et al* 1983** | | **Luthman *et al* 1989#** | |
|---|---|---|---|---|---|---|---|---|
| **Species** | Bovine | Bovine | Bovine | Bovine | Bovine | Bovine | Bovine | Bovine |
| **Dose** | 5 mg/kg | 50 mg/kg | 50 mg/kg | 50 mg/kg | 9 mg/kg | 9 mg/kg | 25 mg/kg | 50 mg/kg |
| **Study Design** | In milk replacer for 5 days | In milk replacer | In water | In milk replacer | In milk replacer | In water | In milk for 5 days | In milk for 1 feeding |
| **$T_{max}$** | ~6 hours | 9.16 hours | 2 hours | 4 hours | 6 hours | 2 hours | 4 hours | 4 hours |
| **$C_{max}$** | ~1.2 ug/ml | 4.99 ug/ml | ~3.5 ug/ml | 1.2 ug/ml | 0.5 ug/ml | 1.0 ug/ml | 2.27 ug/ml | 3.25 ug/ml |
| | | | | | | | | |
| | **Hall *et al* 1989** | | **Mevius *et al* 1986** | | **Nielsen & Gyrd-Hansen 1996** | | **Pijpers *et al* 1991** | |
| **Species** | Porcine | | Porcine | Porcine | Porcine | Porcine | Porcine | Porcine |
| **Dose** | 27 mg/kg | | 20 mg/kg | 29 mg/kg | 45 mg/kg | 45 mg/kg | 13 mg/kg | 55 mg/kg |
| **Study Design** | In feed for 4 days | | Drench | In feed for 3 days | Drench in fasted pigs | Drench in pigs on feed | Steady state in feed | Steady state in feed |
| **$T_{max}$** | 48 hours | | 3 hours | 30 hours | 3.8 hours | 4.3 hours | | |
| **$C_{max}$** | 0.4 ug/ml | | 1.27 ug/ml | 0.2 ug/ml | 0.7 ug/ml | 0.4 ug/ml | 0.13-0.22 ug/ml* | 0.39-1.14 ug/ml* |

*plasma concentration range
# study conducted using tetracycline chloride

of oxytetracycline in feed peaked at 0.2 and 0.4 μg/ml in serum respectively (Hall et al., 1989; Mevius et al., 1986). The three studies mentioned above did have varied time to peak concentration, ranging from 4 to 48 hours. A steady state kinetic study from the Netherlands determined that pigs administered a dose of 54.5 mg/kg of body weight in the feed attained plasma concentrations from 0.39-1.14 μg/ml, which is similar to studies in pigs and calves fed at comparable levels (Pijpers et al., 1991a).

In diseased animals, the administration of antimicrobial agents through feed or water is typically not recommended as consumption of the drug may be reduced. Pijpers et al determined that administration of oxytetracycline in feed to pigs at 50mg/kg that were challenged with respiratory pathogens had shorter times to peak plasma concentration and higher volumes of distribution and AUC when compared to the non-challenged pigs (Pijpers et al., 1991b). A similar finding was reported following intravenous administration of oxytetracycline in feedlot cattle that were challenged with a respiratory pathogen when compared to healthy cattle (Ames et al., 1983).

A few studies have proposed that serum or plasma concentrations of oxytetracycline following feeding of calves or pigs may be high enough to treat some bacterial pathogens, based on typical minimum inhibitory concentrations (Hall et al., 1989; Mevius et al., 1986; Schifferli et al., 1982). Schifferli et al looked at serum drug concentrations to determine if they were high enough to therapeutically treat an *Escherichia coli* infection in gastrointestinal tract of calves fed at 50 mg/kg. Oxytetracycline via oral administration did attain and maintain serum drug concentrations, peak concentration between 6 to 12 hours was 3.10 to 6.39 μg/ml and concentration remained above 2.0 μg/ml at 24 hours; these levels were above the

minimum inhibitory concentration (Morrill et al., 1977) of *E. coli* (0.5 μg/ml) for 24 hours (Schifferli et al., 1982). However, this study was conducted in the early 1980s. In 2004 the National Antimicrobial Resistance Monitoring System in the United States reported that 65 percent of bovine *E. coli* isolates from feces were resistant with MIC ≥16 μg/ml for tetracycline (NARMS, 2004). Mevis et al reported that feeding oxytetracycline to pigs at 400 ppm resulted in plasma concentrations that may be effective for some bacterial pathogens whose MIC ranged from 0.1 to 0.5 μg/ml, such as *Streptococci* spp, and *Fusibacterium necrophorum*, but not for *Pasteurella* and *Bordetella* spp whose MIC's ranged from 0.2 to 3.0 μg/ml (Mevius et al., 1986). Hall et al made a similar conclusion as plasma oxytetracycline concentration did not exceed 0.4 μg/ml when fed at 0.55 mg/kg (Hall et al., 1989).

**FACTORS AFFECTING CALF GROWTH:**

Holstein heifers should attain a weight of 84 kg and height of 87 cm at the withers by 60 days of age (Heinrichs and Hargrove, 1987). Optimal growth is positively associated with reproduction and lactation performance once a heifer enters the lactating herd (Hoffman and Funk, 1992). Additionally, the cost of raising replacement heifers ranges from 15 to 20 percent of the cost of producing milk in United States dairy herds (Heinrichs, 1993). Several factors impact optimal growth of dairy replacement heifers, including nutrition, passive transfer of immunoglobulins, disease, housing, environment, dam, parity, and season.

Dairy replacement heifers in the United States are generally fed 8 to 10 percent of their body weight in milk on a daily basis until they are able to consume calf-starter grain

at approximately 0.75 kg per day (Jurgens, 1993; NRC, 2001). This diet allows calves to be weaned as early as 4 weeks of age. A milk replacer should contain a minimum of 20 percent crude protein, 10 percent crude fat, and a maximum of 1 percent crude fiber, with adequate vitamins and minerals (Table 2) (Adams et al., 1995; Heinrichs et al., 2003; NRC, 2001). However, calf growth is enhanced when they are fed milk replacers that contain 26-30 and 20 percent protein and fat, respectively (Nonnecke et al., 2003). Calf-starter grain should contain 16 to 20 percent crude protein and three percent crude fat with balanced vitamins and minerals (Table 3) (Heinrichs and Jones, 2003).
Milk-based products; dried skim milk, buttermilk, whey, and casein are the preferred protein ingredients of a milk replacer (Adams et al., 1995; Heinrichs and Jones, 2003). Plant proteins and other sources of protein are inferior ingredients because of the immature digestive system of a newborn calf that has limited ability to digest non-milk proteins until after 3 weeks of age (NRC, 2001). The primary source of energy in milk replacer is tallow, as either white grease or lard (NRC, 2001).

Dry matter intake of milk, milk replacer and/or grain affects calf growth (Bar-Peled et al., 1997; Brown et al., 2005; Jasper and Weary, 2002; Jenny et al., 1982; Place et al., 1998; Quigley et al., 2006; Thomas and Tinnimit, 1976). An Israeli study allowed calves to suckle on a cow three times a day for 42 days while the control calves were fed a limited amount of milk replacer daily. Suckled calves had a 0.85 kg average daily gain for the first 6 weeks, which was almost 0.2 kg higher then the control calves (Bar-Peled et al., 1997). Milk replacer with different crude protein and energy levels fed at either 1.1 percent or 2.0 percent of body weight, resulted in calves with an average daily gain of 0.668 kg for high intake diet and 0.379 kg for the low intake diet at 8 weeks of age

**Table 1.3** Recommended milk-replacer ingredients for replacement calves

| Nutrient | Amount |
|---|---|
| Crude protein, min (%) | 20 – 28 |
| Fat, min (%) | 10 – 22 |
| Crude fiber, max (%) | 1 |
| Vitamin A, (IU/lb) | 4091 |
| Vitamin D, (IU/lb) | 273 |
| Vitamin E, (IU/lb) | 22.7 |
| Iron (ppm) | 100 |
| Selenium (ppm) | 0.3 |
| Calcium (%) | 1.0 |
| Phosphorus (%) | 0.7 |
| Magnesium (%) | 0.07 |

Source: Adapted from Heinrichs, AJ and Jones CM. "Feeding the Newborn Calf". Extension Circular. www.pubs.cas.psu.edu

**Table 1.4** Recommended calf-starter composition

| Nutrient | Amount |
|---|---|
| Crude protein (%) | 18 – 20 |
| Fat (%) | 3 |
| ADF (%) | 11.6 |
| NDF (%) | 12.8 |
| ME (Mcal/lb) | 1.49 |
| Vitamin A, (IU/lb) | 1818 |
| Vitamin D, (IU/lb) | 273 |
| Vitamin E, (IU/lb) | 11.4 |
| Manganese (ppm) | 40.0 |
| Iron (ppm) | 50.0 |
| Copper (ppm) | 10.0 |
| Zinc (ppm) | 40.0 |
| Cobalt (ppm) | 0.10 |
| Iodine (ppm) | 0.25 |
| Selenium (ppm) | 0.30 |
| Calcium (%) | 0.70 |
| Phosphorus (%) | 0.45 |
| Magnesium (%) | 0.10 |
| Sulfur (%) | 0.20 |
| Potasium (%) | 0.65 |

Source: Adapted from Heinrichs, AJ and Jones CM. "Feeding the Newborn Calf". Extension Circular. www.pubs.cas.psu.edu

(Brown et al., 2005). A Pennsylvania study investigating factors that affect dairy heifer growth included dry matter intake as part of their final model (Place et al., 1998). Jasper and Weary demonstrated that calves fed milk replacer *ad libitum* were 10.5 kg heavier at 35 days of age than calves fed conventionally, and that this difference in weight continued until the study ended on day 63 (Jasper and Weary, 2002). However, other studies question the benefits of higher dry matter intake on calf performance. Higher dry matter intake was associated with increased frequency of diarrhea and treatment compared to feed-limited controls (Quigley et al., 2006). This agreed with an earlier report that found increased dry matter intake prior to weaning resulted in higher body weight initially, but was followed by decreased average daily gains after weaning (at 4 weeks of age), and ultimately, no difference in body weight gained by 6 weeks of age (Jenny et al., 1982).

Calves rely on passive transfer of immunoglobulins in colostrum for maternal antibodies due to a lack of *in-utero* transfer of these antibodies in the bovine. Calves should receive immunoglobulins via colostrum within the first 24 hours of life to allow absorption of macromolecules, such as immunoglobulins, before the gut wall becomes impermeable. After 24 hours of age, the passive transfer of antibodies across the gut wall ceases or is limited (Franklin et al., 1998; Stott et al., 1979). Serum immunoglobulin G (IgG) concentration in calves is positively correlated to calf health and growth (Berge et al., 2005; Davidson et al., 1981; DeNise et al., 1989; Donovan et al., 1998a; Nocek et al., 1984; Pare' et al., 1993; Robison et al., 1988; Van Donkersgoed et al., 1993; Virtala et al., 1996a; Wittum and Perino, 1995). Nocek et al determined calves fed high quality colostrum, ($\geq$ 60 mg/ml of immunoglobulin in milk) had higher growth rates in the first

four days of life compared to those fed low quality colostrum (Nocek et al., 1984). A study of 1000 Holstein calves found that those high serum concentration of immunoglobulins (>8 mg/ml) had higher average daily gains than calves with low serum immunoglobulin concentration (Robison et al., 1988). High concentrations of immunoglobulins in serum were also associated with a decreased number of days that calves were affected with diarrhea (Pare' et al., 1993). More recently, a California study determined decreased morbidity and mortality in calves with serum IgG $\geq$ 1000 mg/dL (Berge et al., 2005). However, while positively impacting calf health, a Florida study suggested that colostral immunoglobulins did not impact calf growth (Donovan et al., 1998b). A possible reason for this discrepancy was that earlier studies did not control for disease in their analysis, and if included, immunoglobulin concentrations were not associated with calf growth. Additionally, growth was only monitored for a short period of time in some of the studies (Davidson et al., 1981; Nocek et al., 1984), and if measured for longer durations (greater age), the benefit of colostral immunoglobulins on calf growth may diminish.

Infectious diseases also impairs calf growth; diarrhea typically is the primary concern during the first two weeks of life, and pneumonia is a concern for the remainder of their growing phase until first calving (Roy, 1980). In review, Simensen and Norheim emphasized the impact of enteric and respiratory disorders on calf health and growth (Simensen and Norheim, 1983a). Multiple studies have demonstrated the impact of disease on calf health and growth (Berge et al., 2005; Curtis et al., 1989; Donovan et al., 1998a; Ganaba et al., 1995; Heinrichs et al., 2005; Lundborg et al., 2005; Lundborg et al., 2003; Place et al., 1998; Van Donkersgoed et al., 1993; Virtala et al., 1996a; Waltner-

Toews et al., 1986). A study of Holstein calves in Florida documented the negative effect of diarrhea on weight gain through six months of age (Donovan et al., 1998b). Calves with diarrhea in the first 90 days of life are 2.5 times more likely to leave the herd, and if remaining, are three times more likely to calve after 900 days of age (Waltner-Toews et al., 1986). Contrary to the above study, Curtis et al (Curtis et al., 1989) found that diarrhea had no effect on calves leaving the herd, which may have resulted from differences in recording morbidity between the studies. Similar findings were observed in a Quebec study; calves that were ill from diarrhea during the first two weeks of life compensated for lost weight gain by weaning age (Ganaba et al., 1995). Lundborg et al found diarrhea and pneumonia were both risk factors for decreased calf growth (Lundborg et al., 2003). Van Donkersgoed et al also determined pneumonia affected chest girth, but diarrhea did not (Van Donkersgoed et al., 1993). A New York study reported that pneumonia verified by a veterinarian was associated with decreased weight gain of 3.8 kg for the first 90 days, while producer identified pneumonia was not (Virtala et al., 1996a). The above studies suggest that both the diagnosis and severity of diarrhea and pneumonia is subjective, and the impact of these diseases on calf growth is difficult to assess without standard clinical benchmarks.

Calves in the U.S. are traditionally raised in individual hutches, and most studies support the concept that this type of housing increases weight gain, while decreasing disease and cross-sucking (Maatje et al., 1993; Simensen, 1982; Tomkins, 1991; Van Putten, 1982). In review, Simensen and Norehim summarized that individual housing in hutches improved growth compared to calves housed within a stanchion barn with cows (Simensen, 1982), although some studies suggested group housing was not detrimental to

calf health. A Canadian study reported that housing calves in pairs did not affect calf health, performance and behavior (Chua et al., 2002; Kung et al., 1997). Additionally, Kung et al, determined that suckling calves raised in groups performed as well as calves in individual hutches (Kung et al., 1997). Several of the above mentioned authors suggested that more research is needed to determine the importance of behavioral patterns and social interactions when comparing group and individual housing impact on growth (Chua et al., 2002; Simensen, 1982; Wilson et al., 1999).

Evidence for environmental factors such as temperature, relative humidity, ammonia content in air, and air movement on calf growth is mainly anecdotal. The optimum environmental temperature for raising calves is between 15 to 25°C, with a relative humidity of 0.6 to 0.8, though these ranges vary with age of animal (Davis and Drackley, 1998; Roy, 1990). Environmental temperatures outside the thermoneutral zone (5 to 20°C) and especially below the lower critical temperature require more energy for maintenance (Davis and Drackley, 1998; Roy, 1990). A Wisconsin study demonstrated that calves housed at 21°C versus 3°C on the same diet would gain 586g and 20g daily, respectively (Gebremedhin et al., 1981). Similar findings where also observed in a study from Pennsylvania (Scibilia et al., 1987). Calves maintained at a relative humidity of 0.95 on wooden slats had an increased incidence of diarrhea compared to calves maintained at a relative humidity of 0.75 (Roy, 1990). Others did not find an impact on calf growth with varying relative humidity and temperatures (Place et al., 1998; Roy, 1990). What may be more detrimental to calf health are large variations in environmental temperature and relative humidity in short periods of time (Roy, 1990). Proper ventilation, of four complete air exchanges per hour, is believed to decrease the exposure

of the calf's respiratory tract to ammonia, pathogens, and dust (Davis and Drackley, 1998). Natural ventilation, as with hutches, promotes healthier calves (Davis and Drackley, 1998), although drafty conditions should be avoided (Roy, 1990).

A common practice in the United States is to use wheat straw for dairy calves as bedding. An Arkansas study found no difference in calf growth when other bedding material was used (Panivivat et al., 2004), although calves bedded with sand and granite fines were treated more during the first two weeks of life for diarrhea. Simensen and Norheim cited that calves housed above liquid manure pits had increased morbidity and decreased growth (Simensen and Norheim, 1983a). A study of 51 herds in Norway demonstrated that calves between 31 to 90 days of age raised on solid floor with limited bedding, or with deep bedding (sawdust/wood shavings/straw), grew slower compared to calves raised on slotted floors with or without litter (Simensen and Norheim, 1983a).

Limited literature is available regarding the growth of dairy calves that are born as twins. There is general agreement that twin calves are smaller at birth and will grow slower (Ganaba et al., 1995; Lundborg et al., 2003). A study in beef cattle demonstrated that single calves had a 15 percent greater chance of surviving to 200 days of age then twin calves (Gregory et al., 1996). The single calves were 8.8 kg heavier at birth and 28 kg heavier at 200 days of age.

Other risk factors impacting dairy calf growth have been investigated, although reports are limited. A Florida study determined that there was no association between season of birth (summer, winter) and weight gain (Donovan et al., 1998b). A Norwegian study of 51 dairy herds found chest girth size increased for the first 90 days of life for calves born during late summer and early fall which contradicts the findings of 795

Holstein calves in Pennsylvania that had improved growth when born in winter and spring (Place et al., 1998; Simensen and Norheim, 1983b). These same two studies also reported an association between parity of the dam (first lactation) and decreased growth of the calf (Place et al., 1998; Simensen and Norheim, 1983b). Reports have suggested the benefit of family members as compared to hired staff in caring for calves (Hartman et al., 1974; Jenney et al., 1981; Martin et al., 1975; Simensen, 1982; Speicher and Hepp, 1973) while others have reported no benefit (Hagstad et al., 1984; James et al., 1984; Lundborg et al., 2005; Oxender et al., 1973).

## CONCLUSION:

Research over the past 50 plus years has generally shown that when antimicrobial agents are added to milk replacer, dairy calf growth is increased. However, there are limited studies in the past 20 years showing this benefit, especially since preventive medicine practices have been implemented (quality colostrum, proper nutrition, housing and use of vaccines) to improve the health and well being of dairy calves. Secondly, drug concentrations of oxytetracycline in milk replacer have varied over time and there are no pharmacokinetic studies with the current dose used by the dairy cattle industry in milk replacer to benefit growth and disease prevention. Finally, the public health community scrutinizes the use of antimicrobial agents in animal feed, as antimicrobial resistance is a public health concern. Using antimicrobial agents at non-therapeutic doses, as is practiced in animal feed, there is a potential that resistance can develop due to survival of the fittest, as not all bacteria will die when exposed to drugs at low levels.

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# CHAPTER 2

# EFFECTS OF FEEDING MEDICATED OR NON-MEDICATED MILK REPLACER TO HOLSTEIN CALVES ON GROWTH, MORBIDITY AND MORTALITY

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## ABSTRACT

Three hundred and one Holstein heifer calves were randomly assigned to be fed a milk replacer with 440 mg/kg of neomycin and 220 mg/kg of oxytetracycline, or a milk replacer with no antimicrobial agents added. Calves were fed approximately 220g of milk replacer twice a day starting at three days of age and were provided *ad libitum* access to water and grain until weaning at 7 weeks of age. Occurrence of health events and weekly gains in height and weight were recorded until weaning and were again obtained at 150 days of age. Height at birth, 42- and 150-days of age did not differ between treatment groups ($P>0.4$). However, the medicated treatment group had higher weights at 42- and 150-days of age ($P<0.05$), and weeks 5 through 8 ($P<0.01$). Calves with a pre-weaning episode of respiratory disease gained less weight than calves without respiratory disease ($P<0.01$). Calves born in the fall and winter months had higher weights than calves born in the summer and spring ($P\leq 0.05$). There was no difference in morbidity rates for enteritis, respiratory, and other diseases but there was a trend towards overall lower mortality rates in the medicated group ($P= 0.099$). Our results suggest that calves fed medicated milk replacer grow faster than calves fed non-medicated milk replacer, although there was no difference in morbidity for any particular disease.

## INTRODUCTION

In 1955, a review of the published literature concluded that antimicrobial agents added to feed, especially in milk, would likely increase growth rates in dairy calves (Lassiter, 1955). Growth rates increased from 10 to 30 percent in the first 8 weeks of life. Over the next 20 years, additional studies reported improved growth from the feeding of antimicrobial agents in milk replacer (Felsman et al., 1973; Hvidsten, 1959; Morrill et al., 1977; Radisson et al., 1956; Swanson, 1963). However, other studies demonstrated no statistically significant benefit of antimicrobial agents in milk fed to dairy calves (Bush et al., 1959; Edwards, 1962; Gabrilidis, 1972; Lassiter et al., 1958; Preston et al., 1959).

More recent literature has also offered paradoxical results as to the effects of antimicrobial agents in milk replacer. Tomkins and Jaster reported that antimicrobial agents improved performance and decreased the incidence of diarrhea in dairy calves (Tomkins and Jaster, 1991). Quigley et al found a trend towards increased growth in feeding medicated milk replacer, but there was no statistically significant difference compared to the controls (Quigley et al., 1997). Two additional reports have found no differences in growth between antibiotic or control groups (Donovan et al., 2002; Heinrichs et al., 2003).

Animal agriculture has received scrutiny from the feeding of antimicrobial agents at subtherapeutic levels because of the potential for development of antimicrobial resistance (WHO, 2003b). The European Union has banned all antimicrobial agents for growth promotion (EUROPA, 2002). Thus, the use of antimicrobials in milk replacer fed to dairy calves, especially if benefits are equivocal, may be cause for concern. Over the

years the concentration of antimicrobial agents in milk replacer have varied. Industry standards today use 440 mg/kg of neomycin and 220 mg/kg of oxytetracycline in milk replacer fed to dairy calves, as medicated milk replacers are used in over 50% of dairy farms in the United States. Studies demonstrating a benefit in growth promotion for feeding medicated milk replacer have generally only followed the calves to weaning, so there is no literature demonstrating if the difference in growth is maintained further into the growing heifers life.

The objectives for this study was to determine the effect of feeding milk replacer supplemented with or without antimicrobial agents on the height, weight, morbidity, and mortality of dairy calves from birth to five months of age between the two treatment groups. Secondly, apply a repeated measures model to determine risk factors affecting growth in these calves and determine age at which two treatment groups differ in growth.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Michigan State University approved the experimental protocol of this study.

Herd: A commercial 600-cow dairy farm was selected based on willingness to participate and their ability to keep records. At birth, all calves had navels dipped with iodine, weight recorded, were placed in an individual hutch bedded with straw, and then received one gallon of quality colostrum, immunoglobulin level >50 mg/ml.
Three hundred and one heifer calves were enrolled over a 13-month period. Each animal was enrolled from three days until five months of age. Inclusion criteria for the study were: Holstein heifer, non-twin, no signs of birth defects, and over 250 days *in-utero*.

The farm was blinded as to which milk replacer was medicated; all feeding equipment, milk replacer bags and hutches were color coded to maintain consistent feeding of the correct treatment assignment.

Milk Replacer: At birth, calves were randomly assigned to one of the two study groups: milk replacer without antimicrobial agents (n = 151) or milk replacer with antimicrobial agents (n = 149). The medicated milk replacer had 440 mg/kg of neomycin and 220 mg/kg of oxytetracycline. Milk replacer was fed at approximately 220g, dissolved in 2 liters of water, twice a day until one week prior to weaning when they were reduced to once a day feeding. Calves received a total dose of about 45 mg of oxytetracycline (1mg/kg twice a day) and 90 mg of neomycin (2 mg/kg twice a day) at each feeding. The composition of the milk replacer included 20 percent crude protein, 20 percent crude fat and a crude fiber less then 0.15 percent. The protein was entirely derived from milk derivatives.

Calf Management: Within the first day, the calves received injections of Vitamin A, D and E, selenium, and an intranasal modified-live Infectious Bovine Rhinotracheitis and Parainfluenza-3 vaccine. All calves in the study received vaccines per the farm's vaccine management schedule for replacement heifers. Calves received two liters of colostrum twice a day until being placed on milk replacer at three days of age. Calves were offered fresh water and a calf-starter, *ad libitum*, beginning on day 3. Once weaned, calves were moved into group housing with other heifers of the same age and size. Calves remained in this transition group until approximately 5 months of age. At weaning, they were initially fed calf starter and hay and slowly transitioned to a corn-silage-based total mixed ration (TMR). .

Data Collection: Height and weight data were collected weekly until weaning and once at five months of age before leaving the transition barn. Health records of individual animals were also monitored during weekly visits to the farm. Body weight was obtained with a digital scale that was calibrated each week. Wither height was obtained with a sliding ruler; measurements were taken three times and averaged.

Assessment of Passive Transfer: In order to assess serum total protein, a blood sample was collected from each calf via jugular venipuncture with a 20G needle into a 10 ml vacutainer during the first week of birth. The sample was placed on ice, and allowed to clot. The vacutainers were centrifuged at 1,000 x g for 10 minutes. Serum was harvested and a total solid was determined with a refractometer (Reichert TS-Meter, Model 1310400A, Depew NY). The remaining serum was frozen at -80°C until radial immunodiffusion was performed by a commercial kit (VMRD, Inc., Pullman WA). Briefly, 3 µl of serum was placed into each well and held at room temperature for 18 to 24 hours. Controls and specimen diameters were measured and plotted on a semi-log graph to determine immunoglobulin G concentration.

Case definitions: We used the following case definitions of clinical disease in calves for this study.

An ENTERIC CASE at least exhibited loose stool and rectal temperature >39.5°C, and may also show signs of depression, anorexia, off feed and/or dehydrated.

A RESPIRATORY CASE presented with coughing or abnormal thoracic sounds, mucous discharge, and rectal temperature >39.5°C, and may also shown signs of depression.

OTHER INFECTIOUS CASES included: calves that did not match the enteric or respiratory definition but had an elevated temperature or keratoconjunctivitis.

OTHER NON-INFECTIOUS CASES included calves that were anorexic, lame, or bloated.

Statistical analysis: Weekly data (weight, height, and health records), passive transfer of antibodies and management practices were entered into a Microsoft Access database (2000, Microsoft Corporation, Redmond, WA). Separate investigators reviewed the data to detect data entry errors. Microsoft Excel (2000, Microsoft Corporation, Redmond, WA) was used to calculate morbidity and mortality rates. Average daily gain, and adjusted 42-day and 150-day weight and height were calculated with SAS v.9.1 (Statistical Analysis System Institute, Cary, NC). PROC ANOVA procedure was performed with SAS v.9.1 and used to make comparisons in growth variables between the two treatment groups at birth, weaning and 5 months of age. Weekly weight was analyzed as a repeated measure by PROC MIXED procedure with first-order autoregressive covariance structure. Effect variables treatment and week were forced into the model while all others were included if they initially had a P-value of 0.20 or less. The final model included effect variables with a P-value less than or equal to 0.05. All values from each calf were used in the modeling, even if the calf died. Categorical data, such as morbidity and mortality, was compared using EPI INFO v.3.4.3 (Centers for Disease Control and Prevention, Atlanta GA). Results were expressed as relative risk with confidence intervals. Statistically significant differences were determined when the confidence interval did not include 1.0.

## RESULTS

There was no difference in starting wither height of 76.41 and 76.52 cm (P=0.72) and birth weight of 42.45 and 42.35 kg (P= 0.85) for the medicated and non-medicated groups, respectively (Table 2.1). Birth weight of thirty-seven calves were not recorded, 19 for the medicated group and 18 for non-medicated group. A mean weight of 42.4 kg was used for these calves to calculate their growth from birth. The first recorded height, either at birth or within week born, was used as the starting height for calculating change in height for each calf.

Sixty-six calves did not receive any treatments during the study, thirty-three from each treatment group (Table 2.3). There was no statistical difference between the two groups for the number of respiratory, enteric, other infectious, or other non-infectious cases, as the relative risk confidence intervals included 1.0 (Table 2.2). A majority (135/139) of the enteric cases occurred prior to weaning, and a total of four cases occurred post-weaning. Conversely, respiratory cases (110/147) tended to occur more frequently after 42 days. Calves had fewer other cases of disease during the post-weaning period, compared to the pre-weaning period. Non-infectious cases were predominantly bloat and lameness while cases in the other infectious category included elevated temperature of unknown origin, infectious bovine keratoconjunctivitis and/or otitis. The medicated milk replacer treatment had 50 calves with two or more disease cases during the study, while the non-medicated treatment had 37, relative risk of 1.14 (CI=0.98 to 1.32) (Table 2.3).

A numeric difference in mortality rate occurred by eight weeks of age, 7.4% and 13.2 % for the medicated and non-medicated groups, respectively, Mantel-Haenszel Chi-

Square (MH) of 2.72 (P=0.099). At five months of age, mortality rate for the medicated group was 8.7% and 15.1% for non-medicated group, respectively MH of 2.92 (P=0.087). Sixty-nine percent of deaths occurred during the first two weeks of life (Figure 2.1). Twenty-three deaths (Table 2.4) occurred in the non-medicated group and thirteen in the medicated group. For the medicated group, the case fatality rates for respiratory, enteric, and other cases are; 1.32, 12.9 and 10.7 % respectively, while the non-medicated group had similar results; 1.41, 23.2, and 16.2 % for respiratory, enteric, and other cases, respectively.

Initial wither height measurements were not different among treatment groups (Table 2.1). There was no statistical difference in wither height between treatment groups at 42- and 150-days of age (Table 2.1). Initial body weight did not differ between treatment groups (Table 2.1). However, calves fed the medicated milk replacer had higher mean body weights at 42 days (P= 0.011), and at 150 days (P= 0.0015) (Table 2.1). Calves in both treatment groups experienced a positive weight gain throughout the first 8 weeks of age except for the second week. Modeling weight of calves at weekly intervals from birth to 8 weeks of age by repeated measures determined that the medicated group had higher weights from week five through eight than the non-medicated group (P<0.01). The final model had a Bayesian Information Criteria (BIC) of 11707.5 and included the following variables; treatment, week, weight at birth (wtb), season born (season), respiratory case (resp), and interaction terms: treatment*week and week*resp (Table 2.5).

Since the occurrence of a respiratory case was included in the model, weights were analyzed for those with respiratory cases (n=59) or no respiratory cases (n=242)

pre-weaning. Weights of calves with a pre-weaning respiratory case were lower compared to calves without a pre-weaning respiratory case (P=0.008) at 8-weeks of age when treatment groups were combined (Figure 2.2). There also was a difference in weight between treatment groups at 8 weeks of age for calves with a pre-weaning respiratory case as the medicated group was 5 kg (P=0.036) heavier, and for the non-respiratory cases as the medicated group gained an additional 1.4 kg (P=0.01).

Weight gained at 42 days of age was stratified by season of birth. There was no difference in weight gained between treatments groups within season born (P>0.05). However, there were statistical differences in weight gained between seasons (Figure 2.3), as spring born calves were lightest, followed by summer and fall born calves and winter born calves gained the most weight (P<0.05). Mean total protein for the medicated group was 5.59 g/dl (range: 4.3 to 7.2) and did not differ (P=0.39) from the non-medicated group (5.65g/dl; range: 4.2 to 7.4). Thirty-three and 40 calves were below 5.2 g/dl for the medicated and non-medicated groups respectively. There is an association between a total protein less than 5.2 g/dl to death (RR=1.99 (1.07-3.68)).

## DISCUSSION

The judicious use of antimicrobial agents has been a topic of increasing concern for animal agriculture. The American Veterinary Medical Association (AVMA) and the Food and Drug Administration (FDA) have developed guidelines on the judicious use of antimicrobial agents in veterinary medicine to help mitigate antimicrobial resistance (FDA, 2001). In particular, the use of antimicrobial agents in a non-therapeutic manner in farm animals has been criticized (WHO, 1998, 2003b). The American College of

Veterinary Internal Medicine has developed a position statement that voluntary actions should be taken by the veterinary profession to conservatively use antimicrobial agents to minimize adverse effects on animal or human health (Morley et al., 2005)

Our study is the largest conducted to date investigating the relationship between dairy calf growth and health, and milk replacer with or without antimicrobial agents added. This study was conducted on a well-managed 600-cow commercial dairy farm in Michigan and there was no difference in crude morbidity between the two treatment groups. This is contrary to previous studies that showed a difference in morbidity in calves fed milk replacer with antimicrobial agents compared to the controls (Berge et al., 2005; Braidwood and Henry, 1990; Heinrichs et al., 2003). Lundborg et al 2005 found morbidity on Swedish farms ranged from 0 to 57.6% with a median of 21.6% (Lundborg et al., 2005). Our overall morbidity rate (78%) was also higher than the NAHMS 2007 Dairy study (38.5% in unweaned calves and 9.5% in weaned calves) (USDA, 2008c). The mortality rate in the present study of 11.9% is higher than previously reported in other studies as they ranged from 5.6 to 9.4% (Losinger and Heinrichs, 1997; Tyler et al., 1998; Virtala et al., 1996b; Waltner-Toews et al., 1986; Wells et al., 1997). The higher overall morbidity and mortality rates in our trial can be attributed to calves that had a serum total protein below 5.2 g/dl and two disease outbreaks; a week-long respiratory outbreak during the fall in weaned calves, and an enteritis outbreak in suckling calves that occurred late fall into early winter. Increased mortality rate in this study is suggestive of a more severe infection, though diagnostics was not conclusive on causative agent for enteric cases. Morbidity rates may also be higher in this study as they

were producer diagnosed and are subjective based on the caretaker's interpretation of clinical signs.

Calf weight in the first eight weeks was below standard weight curves, compared to a Pennsylvania (Heinrichs and Hargrove, 1987) and a national study (Heinrichs and Losinger, 1998). However, calves in both treatment groups exceeded the standard growth curves by 150 days of age. Standard height was attained for calves in this study at 8 weeks and 150 days of age (Heinrichs and Hargrove, 1987; Heinrichs and Losinger, 1998). Suckling calves were being fed approximately 220g of milk replacer twice a day prior to weaning, which is below the standard recommendation of 250g per feeding (Tomkins and Jaster, 1991) for a 50-kg calf, which in part may explain the sub-standard weight gain.

Medicated calves had a higher weight at 42 days and 150 days of age. This confirmed previous studies that determined antimicrobial agents improve growth of suckling calves (Berge et al., 2005; Morrill et al., 1977; Quigley et al., 1997). Other studies found no statistical increase in weight gain following feeding of medicated milk replacer, although numerical differences were observed (Donovan et al., 2002; Heinrichs et al., 2003). Today, the dairy cattle industry adds oxytetracycline and neomycin at 220 mg/kg and 440 mg/kg to milk replacer and when feed according to the label calves receive 1 mg/kg and 2 mg/kg twice a day for oxytetracycline and neomycin respectively. Caution should be applied in comparing earlier studies to our results as all studies used higher concentrations of oxytetracycline in the milk replacer. With studies using different drug concentrations may explain the previous paradoxical results regarding the benefit of antimicrobial agents in milk replacer. To the best of our knowledge, there are

no previous studies that compared weight between calves fed medicated versus non-medicated milk replacers post-weaning.

In our repeated measures model all data was included until the calf left the study (including data from calves prior to their death). There was no difference between treatment groups in weight until after four weeks of age in the repeated measures model. This is similar to findings reported by Morrill et al, which reported no difference until 3 weeks of age (Morrill et al., 1977). Other significant variables that affected weight at weaning included birth weight, respiratory cases, and season of birth, which have been shown to impact growth in previous studies (Place et al., 1998; Virtala et al., 1996a). Virtala et al demonstrated that for each week a calf had a respiratory case, weight was decreased by 0.8 kg (Virtala et al., 1996a). Dairy calves treated by producers for pneumonia had an average weekly growth (chest girth) of 2.00 cm versus 2.47 cm in non-treated calves (Van Donkersgoed et al., 1993).

Virtala et al reported that calves born during the winter had higher weight gain than calves born in the summer (Virtala et al., 1996a), which was similar to our findings. Place et al hypothesized that differences in growth relative to season of birth may be due to the availability of the caretaker to spend time with the calves (Place et al., 1998). Seasonal differences were also reported by Donovan et al, but saw the inverse of the reports stated above, with higher gains in summer rather than winter-born calves (Donovan et al., 2002). A likely factor in season being included in our final model may have been due to the enteritis outbreak that started in October and extended into February.

Total serum protein was not included in our final model as a variable affecting weight gain. Studies have adequately shown that the failure of passive transfer increases the risk of calves becoming ill and therefore decreasing growth (Jarmuz et al., 2001; Vann and Baker, 2001; Virtala et al., 1996a). We did see that low serum total protein of less than 5.2 g/dl is associated with death in agreement with previous studies. The non-significant effect of an enteritis case is in agreement with previous studies (Sivula et al., 1996; Van Putten, 1982). However, other studies have demonstrated that morbidity due to enteritis does impact calf growth (Donovan et al., 1998b; Waltner-Toews et al., 1986). Additionally, Waltner-Toews reported that calves with enteritis were 2.5 times more likely leave the herd in first 90 days (Waltner-Toews et al., 1986). Enteritis may have not impacted growth in our study due to almost identical number of cases between two treatment groups in the first 8 weeks of life or the fact that calves were followed for more than four weeks and were able to compensate for weight lost in first few weeks of life.

## CONCLUSION

Calves fed milk replacer with antimicrobial agents gained more weight by 42 and 150 days of age than calves fed non-medicated milk replacer, with numerical differences being seen starting at 4 weeks of age. Risk factors that influenced weight gain were treatment group, birth weight, season of birth, and having a respiratory case. Though there was no difference in morbidity between treatment groups, there was a non-significant association with mortality. The benefit of feeding medicated milk replacer may have resulted, in part, from the occurrence of an enteric outbreak during the trial as more calves survived and/or consumed more feed. Calves receiving the non-medicated

milk replacer had higher mortality and decreased weight gain while having equal morbidity rate compared to the medicated milk replacer group. This suggests that the medicated milk replacer calves recover better from enteric disease, especially since a majority of mortalities were of enteric disease.

Given the benefit in growth for calves receiving medicated milk replacer, is a 6 kg difference at 150-days of age biologically significant. This study is not able to answer that question, as the calves were not followed through puberty and into their first lactation. However, one could argue that the difference is not large enough for the added cost of feeding medicated milk replacer. More importantly, this study shows that calves fed medicated milk replacer have a lower mortality rate, which is very important to a producer. With the increased scrutiny over the use of antimicrobial agents in animal feed this study would show the benefit in growth and prevention of death. If one was to conclude that the growth differences are not biologically significant than using medicated milk replacer throughout the pre-weaning period may not be necessary. This would decrease the use of antimicrobial agents and may decrease the chance for antimicrobial resistance to develop, as the drugs would be used less frequently.

**Table 2.1**. Mean growth for calves

| | Medicated | Non-Medicated | P-value |
|---|---|---|---|
| **# Calves Enrolled** | 149 | 152 | |
| **# Calves Weaned** | 139 | 131 | |
| **# Calves Finished Trial** | 136 | 129 | |
| **Birth Weight** (kg) | 42.35 | 42.45 | 0.85 |
| **Adj. 42-Day Weight** (kg) | 64.18 | 61.99 | 0.011 |
| **42-Day ADG**[1] (kg) | 0.513 | 0.464 | 0.012 |
| **Adj. 150-Day Weight** (kg) | 181.4 | 175.7 | 0.0015 |
| **150-Day ADG**[1] (kg) | 0.925 | 0.888 | 0.0018 |
| **Starting Height** (cm) | 76.41 | 76.52 | 0.72 |
| **Adj. 42-Day Height** (cm) | 82.99 | 82.74 | 0.43 |
| **42-Day ADHG**[2] (cm) | 0.129 | 0.125 | 0.60 |
| **Adj. 150-Day Height** (cm) | 104.6 | 104.6 | 0.85 |
| **150-Day ADHG**[2] (cm) | 0.188 | 0.187 | 0.93 |

[1]Average Daily Gain
[2]Average Daily Height Gain

**Table 2.2** Calf Morbidity Data

| | Medicated | Non-Medicated | Relative Risk (CI) |
|---|---|---|---|
| **# Total Illnesses** | 174 | 177 | 1.00 (0.92-1.08) |
| **Respiratory** | 76 | 71 | 1.10 (0.87-1.40) |
| **Enteric** | 70 | 69 | 1.05 (0.82-1.34) |
| **Other_NI** | 15 | 20 | 0.86 (0.45-1.66) |
| **Other_I** | 13 | 17 | 0.66 (0.34-1.27) |
| **# Cases ≤42 days** | 116 | 125 | 0.98 (0.85-1.13) |
| **Respiratory** | 27 | 32 | 0.86 (0.54-1.36) |
| **Enteric** | 67 | 66 | 1.03 (0.80-1.70) |
| **Other_NI** | 11 | 14 | 0.80 (0.37-1.70) |
| **Other_I** | 11 | 11 | 1.02 (0.46-2.28) |
| **# Cases > 42 days** | 58 | 52 | 1.13 (0.84-1.52) |
| **Respiratory** | 49 | 39 | 1.19 (0.84-1.68) |
| **Enteric** | 3 | 1 | 2.85 (0.30-27.01) |
| **Other_NI** | 4 | 6 | 1.81 (0.34-9.72) |
| **Other_I** | 2 | 6 | 0.94 (0.31-2.85) |

Other_NI is Other_Non-Infectious case classification
Other_I is Other_Infectious case classification

**Table 2.3** Number of cases per animal

| # Cases/Animal | Medicated | Non-Medicated |
|---|---|---|
| **0** | 33 | 33 |
| **1** | 66 | 82 |
| **2** | 44 | 22 |
| **3** | 4 | 10 |
| **4** | 2 | 4 |
| **5** | 0 | 1 |

**Table 2.4** Type and number of mortalities per treatment group

| | Medicated | Non-medicated | Relative Risk (CI) |
|---|---|---|---|
| **# Total Mortalities** | 13 | 23 | 0.58 (0.30-1.10) |
| **Enteric** | 9 | 16 | 0.57 (0.26-2.00) |
| **Respiratory** | 1 | 1 | 1.02 (0.06-16.16) |
| **Other_Infectious** | 0 | 0 | -- |
| **Other_Non-infectious** | 3 | 6 | 0.51 (0.13-2.00) |
| **# Mortalities <42 Days** | 10 | 20 | 0.51 (0.25-1.05) |
| **Enteric** | 9 | 16 | 0.51 (0.26-1.26) |
| **Respiratory** | 0 | 0 | -- |
| **Other_Infectious** | 0 | 0 | -- |
| **Other_Non-infectious** | 1 | 4 | 0.26 (0.03-2.26) |
| **# Mortalities >42 Days** | 3 | 3 | 0.97 (0.20-4.72) |
| **Enteric** | 0 | 0 | -- |
| **Respiratory** | 1 | 1 | 0.98 (0.06-15.48) |
| **Other_Infectious** | 0 | 0 | -- |
| **Other_Non-infectious** | 2 | 2 | 0.95 (0.14-6.63) |

**Table 2.5.** Fixed Effects values for Repeated Measure Model with Weight as Outcome

*Type 3 Tests of Fixed Effects*

| Effect | Num DF | Den DF | F Value | Pr>F |
|---|---|---|---|---|
| Treatment | 1 | 999 | 2.75 | 0.0973 |
| Week | 8 | 1799 | 372.31 | <.0001 |
| Month | 12 | 443 | 6.74 | <.0001 |
| WTB | 1 | 779 | 46.68 | <.0001 |
| Resp | 1 | 2226 | 0.50 | 0.4748 |
| Treatment*Week | 8 | 1797 | 1.79 | 0.0755 |
| Week*Resp | 8 | 1797 | 2.25 | 0.0217 |

**Figure 2.1** Calf mortalities by treatment group

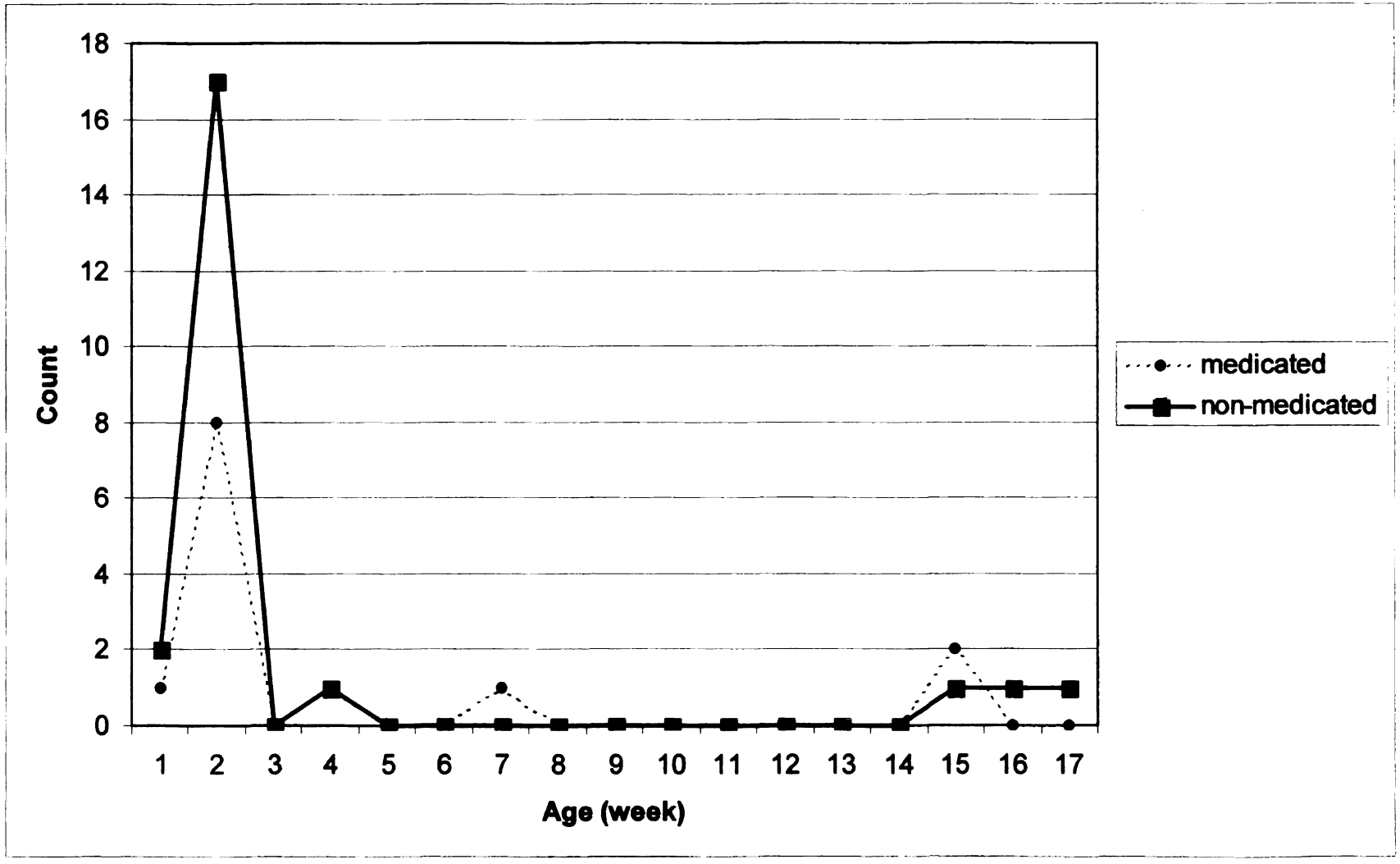


**Figure 2.2** Weight Gained in the First 8-Weeks for Calves With or Without a Respiratory Case

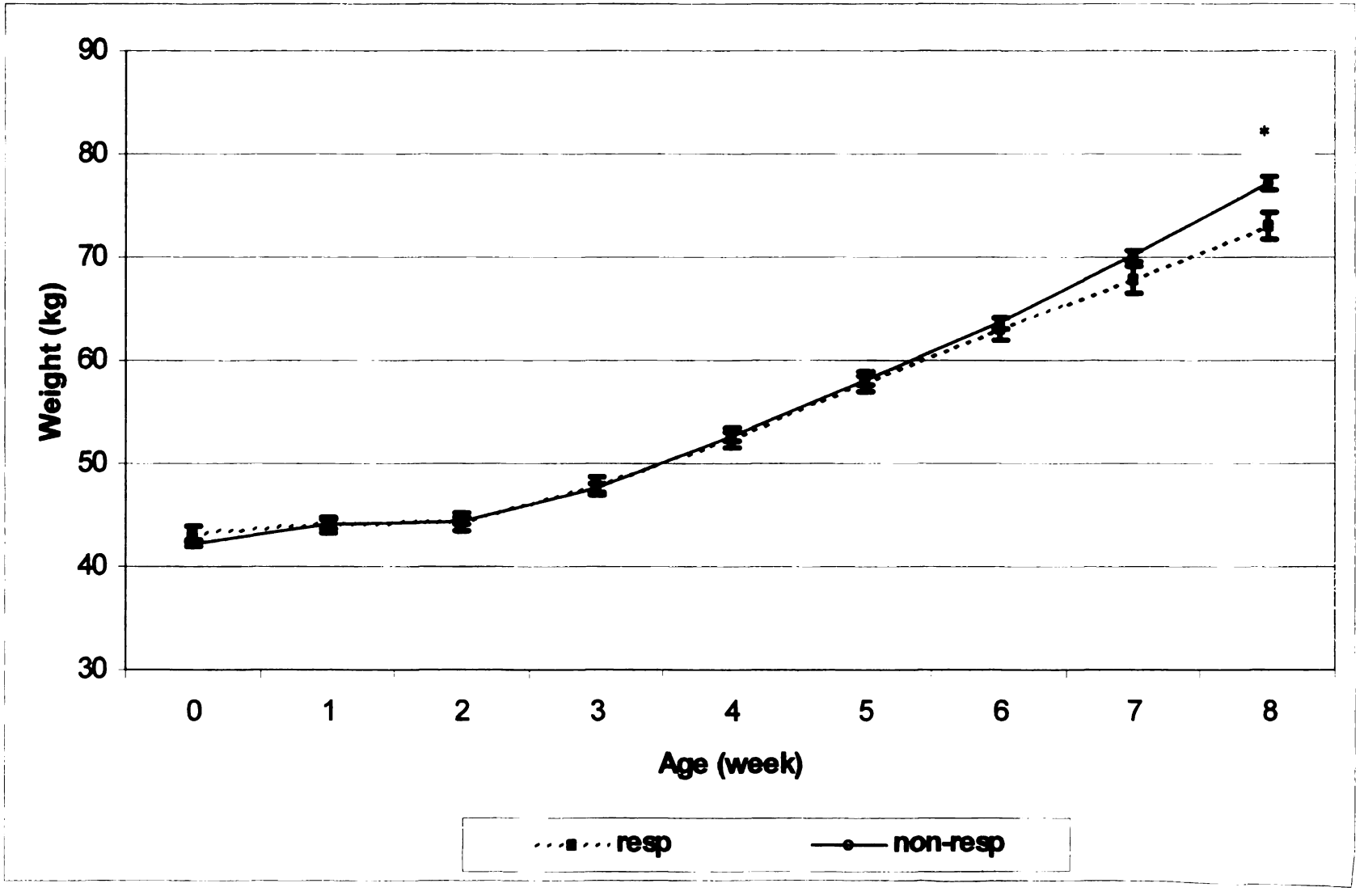
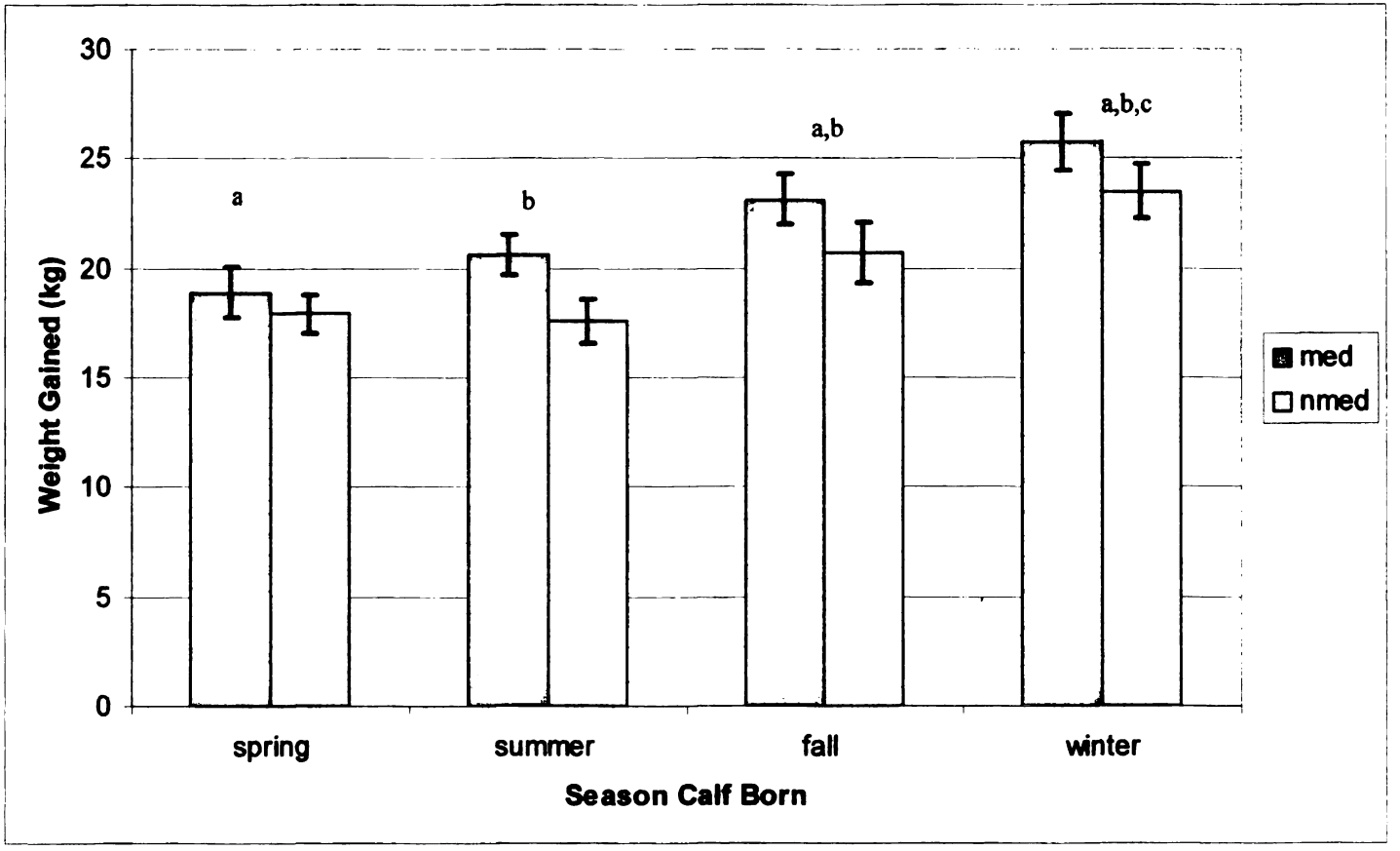


**Figure 2.2** Weight Gained at 42 Days of Age




[a] Statistically significant difference between spring & fall, and spring & winter calves ($p<0.05$)

[b] Statistically significant difference between summer & fall, and summer & winter calves ($p<0.050$

[c] Statistically significant difference between fall & winter calves ($p<0.05$)

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# CHAPTER 3

# PHARMACOKINETICS OF OXYTETRACYCLINE IN MILK REPLACER FED TO HOLSTEIN CALVES

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## ABSTRACT

Six healthy Holstein heifer calves were fed approximately 45 mg of oxytetracycline in milk replacer twice a day beginning at 3 days of age in order to determine steady state pharmacokinetics for the drug. At 10 days of age, plasma samples were collected over a 9-hour period (0, 60, 90, 120, 150, 180, 210, 300, 390, 480, 540 minutes) after the morning feeding. Liquid chromatography was used to determine oxytetracycline concentration in plasma. Mean $C_{max}$ of oxytetracycline was 0.178 μg/ml and mean $T_{max}$ was 290 minutes post feeding. Additionally we compared minimum inhibitory concentrations of oxytetracycline for *Pasteurella multocida* and *Escherichia coli* isolated from calves that were fed medicated or non-medicated milk replacer. *E. coli* and *P. multocida* were isolated from fecal samples and nasal swabs of calves at 2 and 8 weeks of age, respectively. Samples were ollected from 15 calves fed medicated milk replacer (220 mg/kg oxytetracycline and 440 mg/kg neomycin) and 15 calves fed non-medicated milk replacer at each age group, with susceptibility to oxytetracycline evaluated using microbroth dilution. The $MIC_{50}$ and $MIC_{90}$ were identical for *E. coli* in both groups, >8 μg/ml, while for *P. multocida* the $MIC_{50}$ of 2 μg/ml was identical in both groups. The *P. multocida* $MIC_{90}$ for the non-medicated group was 2 μg/ml, while the medicated group had a one-fold higher dilution of 4 μg/ml ($P < 0.05$). The steady-

state pharmacokinetic values in this study are lower than previously reported for oxytetracycline in milk replacer. Peak plasma concentrations of oxytetracycline were below the $MIC_{50}$ for *E. coli* and *P. multocida*. Higher concentration of the drug in milk replacer may be necessary for therapeutic success.

## INTRODUCTION

The National Animal Health Monitoring System (NAHMS) Dairy Study of 2002 and 2007 reported that oxytetracycline and neomycin are the most frequently used antimicrobial agents in milk replacer for dairy calves (USDA, 2005, 2008b). Tetracyclines are also the most commonly used drug to treat enteritis in unweaned dairy calves, while florfenicol is for respiratory disease (USDA, 2008c). Oxytetracycline is effective against susceptible Gram-positive and Gram-negative bacteria and is readily absorbed after oral administration to fasting animals (Plumb, 2002). However, the presence of food or dairy products can reduce the ability of oxytetracycline to be absorbed by an animal in the gastrointestinal tract (Plumb, 2002). Because of their lipophilic nature, tetracyclines have wide distribution throughout the body, and they are eliminated unchanged primarily via glomerular filtration (Plumb, 2002).

Pharmacokinetic studies of oxytetracycline in dairy calves are limited, especially those studying administration via milk replacer (Palmer et al., 1983; Schifferli et al., 1982). Studies that have been conducted were administered at doses not in use by the dairy industry today, as manufacturers of milk replacer commonly supplement their product with 220 mg/kg of oxytetracycline and 440 mg/kg of neomycin. Since neonatal calves are physiologically monogastric, young pigs are another animal model that can be

used to assess pharmacokinetic values of oxytetracycline after oral administration (Hall et al., 1989; Mevius et al., 1986; Nielsen and Gyrd-Hansen, 1996; Pijpers et al., 1991a).

Animal agriculture has received scrutiny from the feeding of antimicrobial agents at subtherapeutic levels because of the potential for development of antimicrobial resistance (WHO, 2003b). Studies in calves and pigs have hypothesized that serum or plasma concentrations of oxytetracycline administered *per os* in feed may be high enough to treat some common bacterial pathogens, based on typical minimum inhibitory concentrations (MIC) (Hall et al., 1989; Mevius et al., 1986; Schifferli et al., 1982). With antimicrobial resistance emerging, this may no longer be true.

The objectives for the current investigation are to evaluate the steady state pharmacokinetics of oxytetracycline administered to dairy calves in milk replacer and determine if the plasma concentration of the drug is greater than the in vitro minimum inhibitory concentration (MIC). The second objective will be based on oxytetracycline MIC for *Escherichia coli* from fecal samples and *Pasteurella multocida* from nasal swabs as both pathogens are commensal organisms within their respective body organs.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee (IACUC) at Michigan State University approved all procedures involving live animals, as well as the trial protocol.

**Pharmacokinetics:**

Calves: Six healthy Holstein heifer calves aged 9 to 14 days and of similar weight (42.5 to 47.3 kg) were used in a steady state pharmacokinetic study.

Milk Replacer: Calves received quality colostrum (immunoglobulin level >50 mg/ml) at birth and until 3 days of age. At three days of age, calves were switched to a commercial medicated milk replacer with 220 mg/kg of oxytetracycline and 440mg/kg of neomycin (Nutrena™ Snowflakes 20-20 All-Milk Medicated Milk Replacer). The milk replacer was labeled to contain 20 percent crude protein (milk based) and crude fat, and 0.15 percent crude fiber. Milk replacer was fed as approximately 220 g dissolved in 2 liters of water twice a day. Thus, at each feeding medicated calves received approximately 45 mg of oxytetracycline (1 mg/kg twice daily) and 90 mg of neomycin (2 mg/kg twice daily).

Specimen Collection and Handling: Whole blood samples were collected via jugular venipuncture from each calf prior to the morning feeding and at 60, 90, 120, 150, 180, 210, 300, 390, 480, and 540 minutes post feeding. Specimens were transported to the laboratory at Michigan State University on ice and were spun at 1000 x g for 10 minutes. Plasma was separated and frozen in 5mL polypropylenes tubes at -80°C.

Liquid Chromatography: Plasma oxytetracycline levels were determined by liquid chromatography (LC). A modification of the Association of Analytical Committees (AOAC® Official Methods℠) method 995.09 for chlortetracycline, oxytetracycline and tetracycline in edible animal tissues was used (AOAC International, 2000). One ml of plasma was mixed with 10ml of McIlvaine buffer and passed through a C18 solid-phase extraction column for cleanup. The oxytetracycline was eluted from the solid phase extraction column with ethyl acetate and 5:95, methanol:ethyl acetate. The elution fractions were concentrated to dryness under nitrogen and resolvated in 100 ul of methanol and sent to LC. A set of standards (50, 100, 200, 500, 1000, and 2000 ppb

oxytetracycline) in a blank plasma matrix were also solid phased extracted and used as a standard curve. Standards and samples sent to LC were chromatographed using 3x3 Perkin Elmer C18 guard column and an Axiom ODS 5 micron 150mm x 3mmm analytical column. Detection of oxytetracycline was at 350 nm by UV light. Results were quantified against the plasma matrix standard curve. The retention time of the oxytetracycline was 6.5 minutes. For this method the oxytetracycline limit of detection (LOD) was 10 ppb and the limit of quantification (LOQ) was 25 ppb.

**Antimicrobial Susceptibility Testing**

Calves: Thirty healthy calves with no previous illnesses were selected from two different age groups, suckling (about 2 weeks of age) and just-weaned heifer calves (about 8 weeks of age). These calves were from the same farm mentioned above. Within each age group 15 calves fed medicated milk replacer and 15 fed an identical milk replacer, but without antimicrobial agents were randomly selected for specimen collection. Calves in the suckling group were used to collect fecal specimens for culture and isolation of *Escherichia coli* while those calves in just-weaned group were used for nasal swab specimens to culture and isolate *Pasturella multocida.*

Specimen Collection: Approximately 10 grams of fecal matter were collected from each calf via digital rectal palpation; using a separate glove for each animal. Approximately 1 gram of fecal matter was placed in Cary-Blair transport media for transport to the laboratory on ice. Nasal specimens were collected using a sterile guarded swab. Swab and guard was inserted into the right nostril approximately 10 cm, then the swab was inserted another 5 cm, rotated 360°, retracted back into the guard and removed. After sampling, each swab was inserted into transport media (BBL Culture Swab media,

Becton, Dickinson and Company, Sparks, MD) and placed on ice for transport to the laboratory at Michigan State University.

Bacterial Culturing: We cultured all specimens within 6 hours of collection. Fecal specimens were inoculated on a MacConkey agar gel plate, triple sugar iron agar slant, and urea slant and incubated at 37°C with 5% $CO_2$ overnight. Suspect *E. coli* colonies underwent further biochemical tests for confirmation; indole, methyl red, vogues-proskauer, citrate, sorbitol, and motility. Quality control of biochemical tests were performed using *Salmonella* Java (CDC control), *Enterococcus aerogenes* ATCC® 13048, and *Escherichia coli* ATCC® 25922. Nasal swab specimens were inoculated on a blood agar plate and incubated at 37°C with 5% $CO_2$ overnight. Suspect colonies of *Pasteurella multocida* were then gram stained to confirm a small Gram-negative rod. Confirmatory biochemical testing was performed using API20NE strips following the manufacture's instructions (bioMerieux, Inc, Durham, NC). Quality control of API20NE strips was performed using *Sphingobacterium multivorum* ATCC® 35656, *Aeromonas hydrophilia* ATTCC® 35654, *Pseudomonas aeruginosa* ATCC® 27853 and *Alcaligenes faecalis* ATCC® 35655. Isolates were frozen at -80°C in skim milk until susceptibility testing was performed.

Susceptibility Testing: MIC values were determined using a microbroth dilution system. A sterile inoculating loop was used to obtain a sample of *E. coli* or *P. multocida* from hand thawed skim milk and streaked onto MacConkey agar for *E. coli* or blood agar for *P. multocida*. Plates were incubated at 37°C for 24 hours. A single colony from each plate was isolated then streaked onto a Mueller Hinton agar and incubated at 37°C for 24 hours. Minimum inhibitory concentration was determined for the bacteria using the

Sensititre semi-automated antimicrobial susceptibility testing system following the manufacturer's instructions (Trek Diagnostic Systems, Westlake OH). Sensititre antimicrobial susceptibility plates used for *E. coli* and *P. multocida* were BOPO1F and CMV1ABPF respectively. The minimum dilution of antimicrobial agent that inhibited growth was recorded as the MIC. Quality control was conducted using *Escherichia coli* ATCC® 25922. Results were entered into Microsoft Access database (2000, Microsoft Corporation, Redmond, WA) and data entry reviewed for errors by separate individuals.

**Statistical Analysis**

Microsoft Excel (2000, Microsoft Corporation, Redmond, WA) was used to calculate pharmacokinetic values, and the mean MIC values to inhibit growth for 50 percent of the isolates ($MIC_{50}$) or for 90 percent of the isolates ($MIC_{90}$) of *P. multocida* and *E. coli* to oxytetracycline. When exact MIC50's and 90's could not be calculated, a conservative approach was followed, by rounding up to the next isolate in the order. Mantel-Haenszel Chi-square analysis for trend (SAS 9.1, Cary, NC) was used to compare MIC values between the medicated and non-medicated milk replacer groups. Statistical significance was designated a priori as a *P*-value of 0.05 or less.

## RESULTS

Mean weight was 44 kg (range 42 to 47) and mean age 11 days (range 9 to 14), for the six calves used for the pharmacokinetic portion of this study. All calves were healthy and had not received any medications prior to enrollment. Individual and mean (±SEM) plasma concentrations at each time point following feeding of milk replacer with oxytetracycline is presented in Table 3.1. Plasma concentrations generally increased

until 300 minutes post feeding and then declined to 0.135 μg/ml at 540 minutes (Fig. 3.1). Mean peak serum concentration occurred at 290 minutes (range 90 to 540). The mean plasma concentration for oxytetracycline over the nine-hour period was 0.178 μg/ml and ranged from 0.098 to 0.275 μg/ml (Table 3.2).

Antimicrobial susceptibility results were obtained on bacterial isolates from 60 calves; 15 fed medicated milk replacer and 15 fed non-medicated milk replacer within the suckling and just-weaned groups. For the 13 *P. multocida* isolates from the medicated group their MIC ranged from 1.0 – 4.0 μg/ml while 11 isolates from the non-medicated calves ranged from 0.5 – 2.0 μg/ml (Fig. 3.2). The difference in MIC of isolates collected from the medicated group compare to non-medicated was significant (Mantel-Haenszel Chi-Square of 4.83; $p < 0.028$). The $MIC_{50}$ was 2 μg/ml and the $MIC_{90}$ was 4 μg/ml for isolates from the medicated calves, while isolates from the non-medicated calves were 2 μg/ml and 2 μg/ml, respectively (Table 3.3). All *E. coli* isolates had an MIC >8 μg/ml except for one isolate in the non-medicated group (Fig. 3.3).

MIC values for oxytetracycline against *P. multocida* and *E. coli* exceeded the plasma concentration that this drug attained in calves receiving the medicated milk replacer at all collection times for all calves. $MIC_{50}$ was 2.0 μg/ml for *P. multocida* while the highest individual calf plasma concentration only reached 0.275 μg/ml.

## DISCUSSION

Oxytetracycline is approved by the U.S. Food and Drug Administration as a supplement to feed for growth promotion, to improve feed efficiency, and to aid/control

in treatment of bacterial pathogens when administered at 0.11-0.22 mg/kg/day. For the treatment of bacterial enteritis and pneumonia due to an infection with pathogens susceptible to oxytetracycline, the dose to be administered is 22 mg/kg/day (Bayley, 2006). For this study, the calves received approximately 45 mg at each feeding in the milk replacer, thus receiving approximately 1 mg/kg per feeding. On the manufacturer's label, it states that this product is to *"aid in the treatment of bacterial enteritis (scours)"* (Nutrena™ Snowflakes 20-20 All-Milk Medicated Milk Replacer).

Previous pharmacokinetic studies of oxytetracycline in calves were conducted with doses ranging from 5 to 50 mg/kg in milk replacer (Luthman and Jacobsson, 1983; Palmer et al., 1983; Schifferli et al., 1982). The steady state plasma concentrations of oxytetracycline obtained in our study (Table 3.1) were low compared to previous work. Schifferli et al reported serum levels of oxytetracycline ranged from 0.75 to 1.2 μg/ml in calves administered 5 mg/kg in milk replacer twice a day for five days (Schifferli et al., 1982). Curve simulation in this study approximated a $T_{max}$ post-feeding of approximately 6 hours, which is comparable to our results (Schifferli et al., 1982). In our study, the average concentration for oxytetracycline was 0.125 μg/ml. A steady-state kinetic study in feeder pigs fed oxytetracycline in feed at 13 mg/kg of body weight (Pijpers et al., 1991a), resulted in a $C_{max}$ of 0.13 to 0.22 μg/ml, which is similar to our findings. Pijpers et al did not calculate $T_{max}$ as plasma samples were only collected prior to feeding and 3 hours post feeding (Pijpers et al., 1991a).

A study in calves fasted overnight and administered a single dose of oxytetracycline at 9 mg/kg in milk replacer reported results similar to ours (Palmer et al., 1983), $T_{max}$ of 6 hours and a $C_{max}$ of 0.5 μg/ml. As one would expect, studies

administering 50 mg/kg of oxytetracycline for one dose in milk replacer fed to calves achieved higher $C_{max}$ and longer $T_{max}$. Schifferli et al and Luthman et al published results with a $C_{max}$ of 4.99 and 1.2 μg/ml and a $T_{max}$ of 9.16 and 4 hours in the serum, respectively (Luthman and Jacobsson, 1983; Schifferli et al., 1982).

The high level of resistance in our *E. coli* specimens to oxytetracycline correlates with previous studies (Catry et al., 2007; Khachatryan et al., 2004; NARMS, 2004; Sato et al., 2005). However, previous reports used tetracycline for their susceptibility testing, while we used oxytetracycline. We reported, at a breakpoint of 16 μg/ml, 100 percent resistance as did Catry et al (Catry et al., 2007). Data from the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH) from 1998 to 2002 reported a high level of resistance in *E. coli* to tetracycline, as 23 percent of isolates were susceptible (Averill, 2005). These results are higher than those reported by Sato et al and Khachatryan et al, 55 and 79 percent, respectively (Khachatryan et al., 2004; Sato et al., 2005). The age in which calves were sampled may have been a factor between these studies. Khachatryan et al demonstrated that as calves aged, the resistance to tetracycline of *E. coli* isolates collected from them decreased, so that by 6 months only 17% of isolates were resistant to tetracycline (Khachatryan et al., 2004).

The mean MIC for oxytetracycline to *P. multocida* in calves previously reported by Catry et al in two separate studies were 0.25 and 0.5 μg/ml respectively, values lower than our $MIC_{50s}$ (Catry et al., 2006; Catry et al., 2005). Though our $MIC_{90}$ values were lower than what Catry reported in 2005 and 2006, which were 64 and 32 μg/ml respectively (Catry et al., 2006; Catry et al., 2005). DCPAH data from 1998 to 2002 had a high level of susceptibility, 80 percent, to tetracycline for *P. multocida*, which is similar

to our results as all isolates were $\leq 4$ μg/ml. A study reporting the MIC values of *P. multocida* to oxytetracycline in ill calves also had higher values than observed in our study, as the $MIC_{50}$ and $MIC_{90}$ were both $\geq 16$ μg/ml (Mevius et al., 1990). The higher MIC values reported in the above studies may be due to multiple reasons; including unknown drug use on farm and treatment of calves before bacterial sampling.

In our study, oxytetracycline MIC values for *P. multocida* were higher for isolates collected from medicated calves. This difference may be due to the fact that the medicated calves were receiving milk replacer containing oxytetracycline since three days of age. However, the sample size was limited, as isolates were only obtained from 11 and 13 calves from the control and medicated groups, respectively. At the time of study design, the NCCLS recommended that 10 isolates for an antibiogram were sufficient to make valid comparisons between two groups. Since then, the NCCLS, now the CLSI (Clinical and Laboratory Standards Institute), has changed their recommendation to state that 30 isolates are necessary to report susceptibility results of an organism to an antimicrobial agent in an antibiogram (CLSI, 2005). There is a possibility of type 1 error where the MICs are truly similar between the two groups.

Previous studies have looked at serum or plasma concentrations of oxytetracycline administered in the feed to determine if therapeutic levels are achieved to treat various bacterial pathogens (Hall et al., 1989; Mevius et al., 1986; Schifferli et al., 1982). Schifferli et al reported that feeding 50 mg/kg of oxytetracycline in milk replacer to calves could achieve and maintain a serum concentration above 1.0 μg/ml for 24 hours, and exceeded the MIC of 0.5-1.0 μg/ml for respiratory pathogens (Schifferli et al., 1982). Within the same study, steady-state kinetic values from feeding calves

oxytetracycline at 5 mg/kg achieved serum peak concentrations around 1 μg/ml, which is above the reported MIC of 0.5 μg /ml. However, it was demonstrated in our study, that feeding oxytetracycline at the levels typically added to milk replacer are inadequate to maintain effective concentrations in plasma for therapeutic efficacy of pneumonia.

In treating animals with antimicrobial agents *per os* there is no literature, to the investigators knowledge, that states the concentration that is obtained and maintained in the lumen of the gastrointestinal tract. Nor is there information stating how plasma drug concentration relates to gastrointestinal drug concentration. With the lack of literature to demonstrate what drug concentration is needed to treat bacterial enteritis, it is speculative to predict whether or not medicated milk replacers offer an effective treatment regimen for enteritis.

## CONCLUSION:

Plasma concentration of oxytetracycline in milk replacer fed to calves does not reach MIC values for *Escherichia coli* from feces and *Pasteurella multocida* from nasal swabs. Therefore, supplementing milk replacer with oxytetracycline at low levels will likely not be effective in treating bacterial enteritis or pneumonia caused by the pathogens tested in this study. Higher concentrations of the drug in milk replacer, or by separate administration or feeding, may be necessary for therapeutic success.

**Table 3.1.** Plasma concentration of oxytetracycline in calves following oral administration as part of a medicated milk replacer

| | **Plasma Concentration of Oxytetracycline** (μg /ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Collection Time** (minutes) | | | | | | | | | | |
| | 0 | 60 | 90 | 120 | 150 | 180 | 210 | 300 | 390 | 480 | 540 |
| **Calf ID** | | | | | | | | | | | |
| 4761 | 0.070 | 0.107 | 0.123 | 0.141 | 0.114 | 0.127 | 0.124 | -- | 0.111 | 0.107 | 0.099 |
| 4763 | 0.095 | 0.111 | 0.142 | 0.101 | 0.107 | 0.103 | 0.099 | 0.128 | 0.113 | 0.086 | 0.084 |
| 4767 | 0.119 | 0.089 | 0.148 | 0.189 | 0.180 | 0.139 | 0.187 | 0.232 | 0.182 | 0.060 | 0.176 |
| 4777 | 0.083 | 0.096 | 0.116 | 0.117 | 0.110 | 0.132 | 0.128 | 0.140 | 0.140 | 0.136 | 0.181 |
| 4779 | 0.173 | 0.119 | 0.089 | 0.065 | 0.181 | 0.154 | 0.188 | 0.201 | 0.275 | 0.170 | 0.192 |
| 4781 | 0.071 | 0.072 | 0.072 | 0.095 | 0.086 | 0.094 | 0.095 | 0.098 | 0.092 | 0.091 | 0.080 |
| **Min.** | 0.070 | 0.072 | 0.072 | 0.065 | 0.086 | 0.094 | 0.095 | 0.098 | 0.092 | 0.060 | 0.080 |
| **Max.** | 0.173 | 0.119 | 0.148 | 0.189 | 0.181 | 0.154 | 0.188 | 0.232 | 0.275 | 0.170 | 0.192 |
| **Mean** | 0.102 | 0.099 | 0.115 | 0.118 | 0.130 | 0.125 | 0.137 | 0.160 | 0.152 | 0.108 | 0.135 |
| **SE** | 0.0160 | 0.0069 | 0.0121 | 0.0175 | 0.0166 | 0.0092 | 0.0169 | 0.0246 | 0.0277 | 0.0160 | 0.0216 |



**Table 3.2.** Pharmacokinetic values for oxytetracycline following oral administration as part of a medicated milk replacer

| *Steady-State Kinetic Values for Oxytetracycline in Milk Replacer* | | | |
|---|---|---|---|
| | **Mean** | **Maximum** | **Minimum** |
| $T_{max}$ (minutes) | 290 | 540 | 90 |
| $C_{max}$ (μg/ml) | 0.178 | 0.275 | 0.098 |
| **Average Concentration, Over 9 hours** (μg/ml) | 0.125 | 0.164 | 0.086 |

**Figure 3.1.** Steady State Kinetics, Mean Plasma Concentration (± SEM) for Oxytetracycline

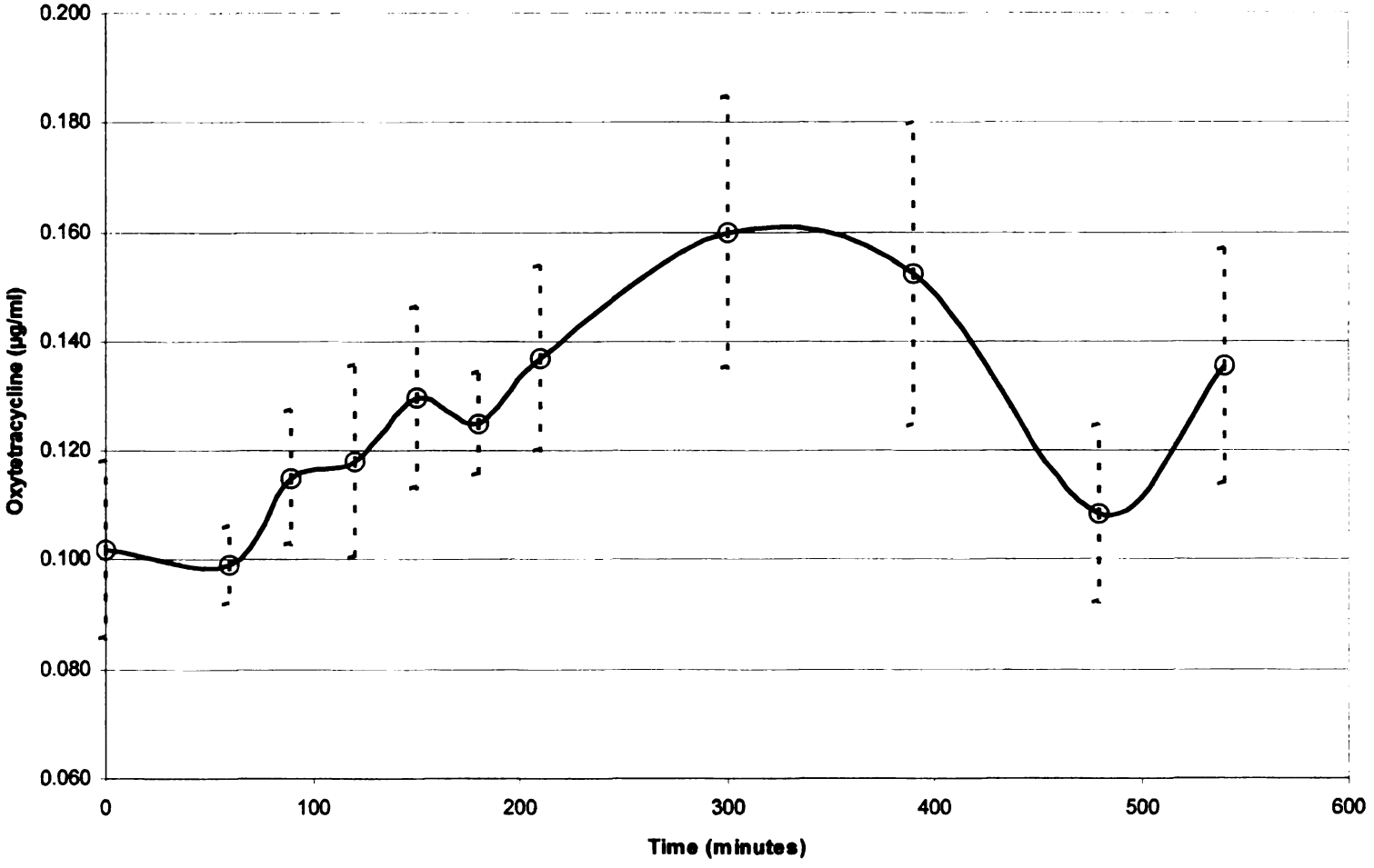
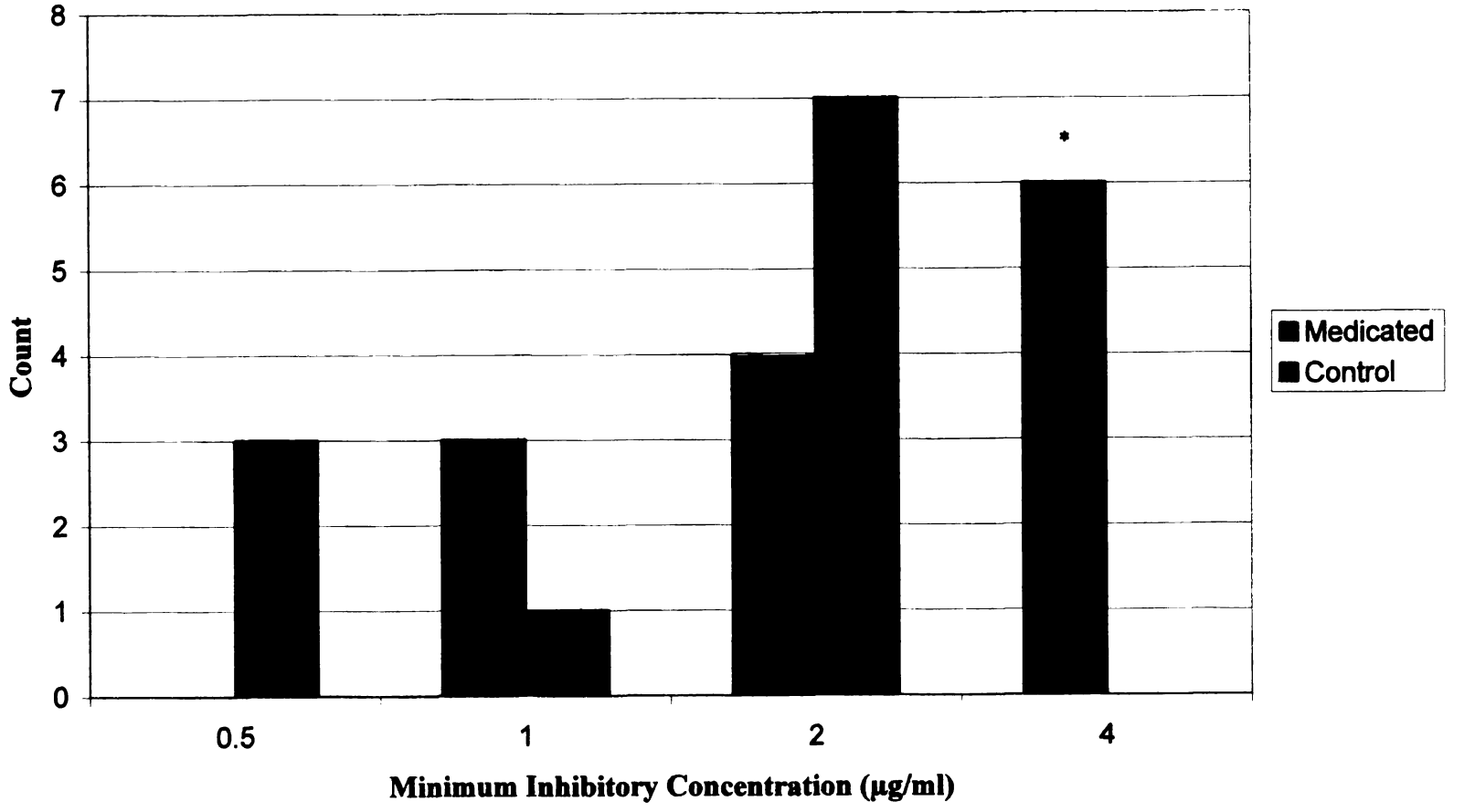


**Figure 3.2.** MIC of oxytetracycline for *Pasteurella multocida*




* Statistically significant trend towards higher MIC value in the medicated group, p=0.028

**Figure 3.3** MIC values of Oxytetracycline for *Escherichia coli*

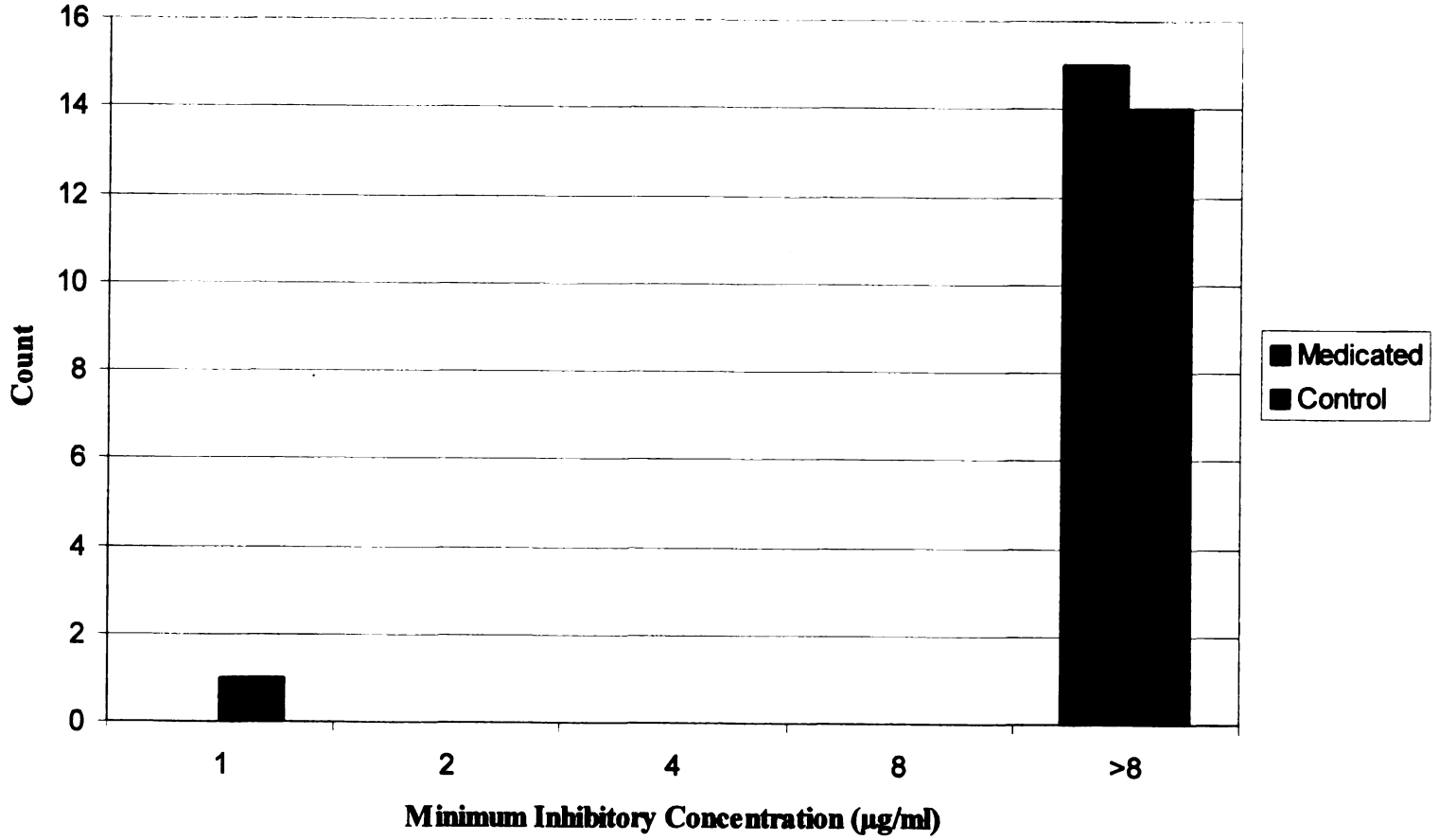


**Table 3.3** $MIC_{50}$ and $MIC_{90}$ values of Oxytetracycline for *P. multocida* and *E. coli*

| Organism | Treatment | Count | MIC50 | MIC90 |
|---|---|---|---|---|
| *Escherichia coli* | Medicated | 15 | >8 | >8 |
| *Escherichia coli* | Control | 15 | >8 | >8 |
| *Pasteurella multocida* | Medicated | 13 | 2 | 4* |
| *Pasteurella multocida* | Control | 11 | 2 | 2 |

* MIC 90 different between medicated and control p=0.028

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# Chapter 4

# TEACHING ANTIMICROBIAL RESISTANCE VIA COMPUTER AIDED LEARNING

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## ABSTRACT

Veterinary medicine plays a key role in helping mitigate antimicrobial resistance. A computer aided learning tool was developed at the following website: http://old.cvm.msu.edu/cdc, as an aid for teaching veterinary students about antimicrobial resistance. This CAL allows for active learning that is student driven and interactive. Usability testing was conducted to ensure learners could easily navigate the CAL and follow the logical flow of the teaching objectives and information being presented. The CAL contains a module regarding the basic 'Principles' of antimicrobial resistance, which summarizes the microbiology, pharmacology and epidemiology concepts in regards to antimicrobial resistance, and the role of antimicrobial use in animal and human health. There are also multiple 'Case Study' modules that apply these principles to specific clinical situations involving dairy and beef cattle, small animals, and swine. These modules relate to a specific scenario regarding antimicrobial usage in veterinary medicine/animal agriculture. Within each module, there are videos, animations, and questions to engage the learner. The materials within this CAL can be used as an adjunct to traditional styles of learning in pre-clinical or clinical settings.

## INTRODUCTION

Veterinary school curricula are over-extended as the breadth and depth of veterinary knowledge continues to expand. Little opportunity exists to add new courses

regarding emerging issues, such as antimicrobial resistance (AMR) (Hird et al., 2002). With the reemergence and interest in the 'One Health, One Medicine' concept, AMR is also an excellent example of how such a concept can be used to address a medical issue that impacts animals, humans and the environment. If AMR is to be taught in veterinary curricula, information must be integrated into existing veterinary courses and course moderators may not know who is teaching specific issue(s). Computer-Aided Learning (CAL) is one potential tool that can help educate students on AMR.

The American Veterinary Medical Association (AVMA) and American Medical Association (AMA) are working together towards a common goal 'One Health, One Medicine' (AVMA, 2008). The 'One Health, One Medicine' concept brings animal health, human health and environmental health together as one. AMR is an excellent example of how this concept can be used to mitigate drug resistance, as physicians, veterinarians, microbiologist, ecologist and others are working to understand the development, spread and control of AMR. Antimicrobial agents, since their inception, have been used in multiple ways (therapeutically or sub-therapeutically via injections, *per os*, lotions, and other formats) to enhance the health and well-being of animals and humans. However, bacterial resistance to antimicrobial agents is an emerging problem that challenges the biomedical professions and public health (WHO, 1999). Additionally, the use of antimicrobial agents in animal agriculture has been implicated as contributing to bacterial resistance in human medicine (JETACAR, 1999; WHO, 2003a). The time is now for the veterinary profession to educate all veterinarians about the prudent use of antimicrobial agents and antimicrobial resistance (Morley et al., 2005). Similar steps are

also being in the United Kingdom by the human medical community (Davey and Garner, 2007).

Advancement of information and technology is likely to continue to impact veterinary education into the future not only in how information is delivered but also the location of the learner (Short, 2002). These developments are opportunities to enhance learning and will effect how we teach and learn in the future. Computer-Aided Learning (CAL) also known as computer-assisted learning, computer-assisted instruction, or computer-based learning, is a form of self-instruction with no direct interaction with the instructor. It relies on computer-based lessons that include text, images, video, three-dimensional photos and simulated (virtual) reality. A simple lesson in CAL may consist of text or visual information, such as radiographs, while more complex CAL lessons have a greater level of interactive learning. For example, students are given a clinical case where they choose their own pathway to solve the scenario and later explain why they proceeded in such a manner.

The effectiveness of CAL in teaching is still being debated, but there is general agreement that it can serve as an adjunct to traditional didactic learning (Nerlich, 1995; Rosenberg et al., 2005; Valcke and De Wever, 2006). The downside of CAL is the up-front cost of development, programming and training of faculty on how to use such tools effectively for teaching (Childs et al., 2005; Short, 2002). Advantages of CAL include the ability of the student to learn at their own pace, the learning can take place anywhere around the world, many students can be taught simultaneously, and collaboration among experts can easily be facilitated to design lessons (Childs et al., 2005; Short, 2002). At least five principles must be met in order for CAL or the use of information technology to

be effective in teaching, 1) just-in time, personalized learning, 2) student centered learning, 3) self-paced learning, 4) learning anytime, anywhere and; 5) experimental, discovery learning (Smith, 2003).

The purpose of this project was to enhance veterinary education on the appropriate use of antimicrobial agents in veterinary medicine and thereby mitigate the further development and spread of antimicrobial resistance via a CAL tool based on the five principles mentioned above. The following objectives were developed for the learner: 1) Understand the need to appropriately use antimicrobial agents, 2) Learn to formulate strategic choices regarding the use of antimicrobial agents, 3) Explore the relationship between animal and human health with respect to use of antimicrobial agents.

## MATERIALS AND METHODS

The CAL titled "Appropriate Use of Antimicrobial Agents" was a collaborative effort between Michigan State University College of Veterinary Medicine (MSU CVM), the Centers for Disease Control and Prevention (CDC), and Michigan Department of Community Health through an Epidemiology and Laboratory Capacity grant from CDC. This CAL also included participants from several other colleges of veterinary medicine in United States as authors or editors of modules.

This CAL is intended to be used as an adjunct to didactic learning, such as in courses of public health, pharmacology and microbiology, and also in clinical settings such as production medicine clerkships. Individual modules or the entire CAL can be used by instructors as an adjunct to learning specific topics for students through videos,

animations and questions about microbiology, pharmacology, public health and management of livestock.

In the process of developing this CAL initial discussions centered on what delivery format should be used, ie. a website or a course management/learning management system (CMS/LMS) such as Blackboard, Web CT, or Angel. We chose to use a website, instead of a CMS/LMS, since the tool could be used by anyone and accessed around the world. The downside to using a website is that an individual whom knew HTML programming had to enter the content; this was overcome by using trained personnel at MSU CVM Information Technology Center. A standard template was developed for the CAL with three panes within the monitor screen; navigation and a 'Tools' tab which contain a glossary, links, references, and credits are in the left pane, the right pane was for additional information/links, and the middle pane contains the text, video, animation and questions about the lesson. The CAL was constructed using Adobe® Dreamweaver®.

In constructing this tool, a team (veterinarians, instructional designer and web designers) approach was used to develop key themes to be covered within the 'Principle' and 'Case Study' modules to help with flow and reduce replication. Intended flow of this tool is for the learner to proceed through the 'Principles' module first to obtain the key concepts regarding antimicrobial resistance then move into species specific topics in the 'Case Study' modules (Fig 4.1). Each module or lesson was scripted and storyboarded by the main author and designers before recording video, creating animations and finalizing the lesson. This allowed for the authors and designers to work together on the lesson and decide on the appropriate technology for incorporation into the module. Also

included within the storyline were questions for students to answer and evaluate their understanding of the issue in various formats ranging from multiple-choice to drag-and-drop using Adobe® Flash®. Once a working draft was agreed upon a shot list/storyboard was developed. After footage was captured, the author(s) reviewed it, and all shots were cataloged to expedite editing. Video was edited using Adobe® Premiere®, and compressed for the CAL using either Windows Media® Player format or Adobe® Flash® Player format. Adobe® Flash® was also used to develop animations to explain or reinforce a specific topic. Still photos were also used in the CAL and were edited with Adobe® Photoshop®. Once a module was completed the estimated time to complete the module was placed at the beginning.

Six veterinary students participated in Usability Testing of the CAL using a method similar to Hinchliffe et al (Hinchliffe and Mummerv, 2008). The Institutional Review Board approved the use and participation of human subjects to test the CAL. Student selection was based on their willingness to participate in the process and their comfort with using the computer/internet. There were two students for each of the following categories; novice (can turn computer on and run basic programs), intermediate (novice skills plus ability to browse internet and download programs), experienced (intermediate skills plus ability to design websites/programs). Combinations of quantitative (time taken) and qualitative (administrator's observations and subjective user preferences) techniques were used to collect data from students while completing eleven pre-defined tasks (Table 4.1). These tasks focused on the ease of navigating through the CAL and flow of the material. The administrator explained the process to each student at the beginning; once the student began the tasks the administrator could not answer any

questions to eliminate any potential bias. To record their responses, students were videotaped with a digital camcorder and by a screen capture program Camtasia Studio™. Students were instructed to express verbally their thoughts and explain what they were doing as they completed the eleven tasks. Each student's video was evaluated by the development team to identify any difficulties they had in navigating the CAL. Areas of difficulty were recorded, along with comments from the participants, and used to improve navigation of the CAL.

## DESCRIPTION OF COMPUTER AIDED LEARNING TOOL

This CAL is accessible at http://old.cvm.msu.edu/cdc. A broadband internet connection is recommended, due to the number of embedded video and audio clips. Most modules are based on a storyline with a veterinary student or veterinarian addressing an antimicrobial resistance issue. Within the lesson are videos, cartoons, and animations to further explain a topic. As learners proceed through the CAL they have opportunities to answer questions to determine their comprehension of the topic with immediate feedback as to what is the correct answer. The main module is called 'Principles', which includes an overview of key concepts in microbiology, pharmacology and epidemiology/public health regarding AMR. Other lessons specific to one species are contained within 'Case Study' section. The case studies offer more detailed information about antimicrobial resistance topics pertinent to dairy cattle, beef cattle, swine and small animal. Each module also underwent peer review by veterinarians who are experts in the given topic to ensure accuracy of the material.

Principles: This lesson follows Jeff, a veterinary student and his personal experience with a *Salmonella* infection and how he collaborated with the State Public Health Veterinarian to determine the source of his infection. Jeff's journey leads him to reflect on and investigate his past food consumption along with small and large animal exposures. Along the way, he learns about the microbiology, pharmacology and epidemiology/public health of antimicrobial resistance. To complete the investigation, Jeff visits the State Diagnostic Laboratory to review culture and susceptibility testing and also visits with his pharmacology professor to discuss antimicrobial agents, and how to use MICs (minimum inhibitory concentrations). Finally, Jeff explores the use of antimicrobial agents within animal agriculture and their role in developing resistance in human pathogens.

Case Studies: This section contains case studies in dairy cattle, beef cattle, swine, pocket pets and companion animals. Each module covers a specific topic within that specie in greater detail as compared to the 'Principles'. Below is a brief description of each of the dairy cattle modules.

**Medicated Milk Replacer:** This module regards a veterinary student, Gretchen, who is riding with a veterinarian, Dr. Jessup, and visits a farm with an enteritis problem in suckling calves. Objectives of this module are to understand what medicated milk replacer is, the different antimicrobial agents added to milk replacer, the advantages and disadvantages of medicated milk replacer and the importance of a colostrum management program. During Gretchen's history taking, she discovers a bag of milk replacer with a label. She notices that oxytetracycline and neomycin are included in the milk replacer. Gretchen is surprised by this and asks the farmer how long and why they have been using

the product. Gretchen then questions Dr. Jessup about the practice and purpose of supplementing antimicrobial agents in milk replacer. Dr. Jessup attempts to answer all of Gretchen's questions regarding the use of antimicrobial agents in feed.

**Neonatal Scours:** Pertains to Dr. Karl's response to a farm call where the farmer, Chuck Erby, has tried numerous drugs to treat enteritis in his dairy calves while experiencing increased mortality. This module focuses on when antimicrobial agents should or should not be used to treat enteritis and on management practices to reduce drug use on a farm. Learners are able to view the farm operation via video, interpret physical exam findings of healthy and ill calves, determine which specimens to collect for submission to the laboratory, review herd health records and discuss treatment options with the farmer. Discussions with Mr. Erby are centered on his desire to give the ill calves medication while Dr. Karl explains the need to know the pathogen(s) before determining if antimicrobial agents are necessary, especially in calves not demonstrating signs of systemic disease. Dr. Karl explains to Mr. Erby that antimicrobial agents are not always appropriate for treating enteritis in calves. In fact, management practices can play a major role in preventing neonatal calf diseases. A suggestion that Dr. Karl makes to Mr. Erby is to improve husbandry practices to minimize antimicrobial use.

**Contagious Mastitis:** This module profiles a veterinarian, Dr. Susan Keller, working with a client, Mr. Oliver McCormick, who is concerned about a high somatic cell count (SCC). Main topics of discussion are preventive measures to mitigate mastitis, importance of culturing mastitis cases, the proper use of antimicrobials in treating mastitis and how treatment duration is important for effective therapy. During the herd visit, the last milk test and SCC reports are reviewed and analyzed. Dr. Keller and Mr.

McCormick discuss options and agree to a return visit to monitor the milking procedure and collect milk samples for culture from cows with high SCC. Once culture results are reported, they discuss treatment options with antimicrobial agents ranging from intramammary infusions to systemic therapy. Dr. Keller also emphasizes the importance of management practices to mitigate disease transmission of contagious mammary pathogens from cow to cow.

**Farm Based Mastitis Program:** This is a 22-minute video written and filmed in the style of a local news story regarding how one large dairy farm uses an evidence-based approach to preventing and controlling mastitis. Learner objectives for this module include the role of a veterinarian in developing standard operating procedures, how to use a farm antibiogram to determine treatment regimens, the five key topics to consider when selecting a drug and the importance of preventive measures to mitigate mastitis. The news reporter, Ms. Becky Dewitt, interviews the farm veterinarian, about their mastitis program. An evidence-based approach is taken for each case: identify causal pathogen, determine appropriate drug to use based from the farm antibiogram, treat the cow and monitor. This is the standard operating procedure for treating mastitis at this operation. Antimicrobial agents are only a part of the mastitis program on this farm. This evidence-based plan that is presented, decreased antimicrobial agent use by 74 percent and the number of cows treated and duration in the mastitis pen.

## USABILITY TEST RESULTS

Six veterinary students completed the 11 tasks in a reasonable amount of time. Overall feedback was that the CAL could be navigated and material flowed smoothly.

However there were a few suggestions or areas of difficulty that were identified by the administrators. Three key issues were identified: students looked for content only within the middle pane, a need for page advancement buttons and change the format of interactive questions. Participants frequently used the left pane to navigate throughout the CAL and looked for content within the middle pane. Only two participants, in their first attempt, completed a question that required the participant to go to the right-side pane and click on a link for more information, while two never answered the question. To correct this, the right pane of the CAL is reserved only for remedial, advanced or additional information that is not critical for the learner. The usability testing also demonstrated a need for easier navigation with 'page forward' and 'page backward' buttons on each screen, and to limit the amount of material within each screen to reduce scrolling. The final change was in regard to question formatting, as there was a need to give more clear directions to 'drag and drop' or just 'click and point'. Additionally, questions with two parts were removed as all six of the participants were confused with them. Conducting usability testing early in the development process allowed for a standard template to by created which saved time in creating further modules, as author(s) knew the appropriate layout and placement of content.

## DISCUSSION

This CAL was designed using the five principles of information technology in teaching; 1) just-in time (detailed information about a current topic that is not incorporated in the classroom or clinical teaching), personalized learning, 2) student centered learning, 3) self-paced learning, 4) learning anytime, anywhere and; 5)

experimental, discovery learning (Smith, 2003). Antimicrobial resistance is a current issue within the medical fields, and is an excellent example of how a 'One Medicine' concept can be used to address and resolve this issue. Veterinary medicine plays an important role in this topic, as there is a potential for the use of antimicrobial agents in animals to impact the ecosystem and human health (WHO, 1998, 2003b).

This CAL allows learners to expand upon what they have learned in the classroom. Learners are likely to have some prior knowledge regarding antimicrobial agents and/or AMR, and will have an idea of what information he/she needs to learn. Also, the learners are able to work at their own pace, choose which modules they wish to view, and the location where they prefer to learn. These parameters are what make the CAL student-centered and allow it to function as an active learning environment (Arseneau and Rodenburg, 2004; Smith, 2003). This is in great contrast to a teacher-centered, passive learning atmosphere, such as lectures in a large classroom.

Active learning is a major component of this CAL. Incorporating multiple forms of media, variety in content delivery, and use of different question formats allows the learners to be more engaged, compared to traditional formats of learning (Arseneau and Rodenburg, 2004). In addition, learners receive immediate feedback from the questions they answer, allowing for self-evaluation and reinforcement of learning objectives. There is a chance that learners may only view this CAL at a superficial level; if true, the learner is still dictating what is viewed and the direction taken through the CAL, and is thus engaged in an active learning process.

Experimental learning is another key factor for this site. Learners are able to discover unknown facts and enhance their knowledge throughout each module's

storyline, by the questions asked, or investigating the various links and references for more information. Experimental learning is the primary way in which adult learners further their knowledge (Kolb, 1984).

## CONCLUSION

This CAL is meant to be an adjunct to traditional formats of learning in the classroom or clinical settings. The exact role of CAL in veterinary education is still being developed, as there are several complicating factors: a sharp learning curve, unwillingness of faculty to develop material, and the start-up costs for implementing the technology (Childs et al., 2005; Dale et al., 2005; Short, 2002). At a minimum, CAL is an excellent addition to traditional learning (Nerlich, 1995; Rosenberg et al., 2005). At the onset of this project, the intention was for this CAL to be used in veterinary school curriculums, but there is potential for this to be used in continuing education for graduate veterinarians too. Further evaluation of this tool is needed to determine its effectiveness as a teaching tool.

This CAL tool continues to be enhanced and evolve and the most recent version is now located at the following website: http://arls.cvm.msu.edu. Additional modules have been added regarding basic concepts of microbiology, pharmacology and public health beyond what is mentioned in the 'Principles' section. Another change that has been made is that the left-side pane has been moved to the top of the CAL, allowing the storyline to contain more of the screen. These changes were made by the design team, which includes Michigan State University and CDC personnel, to enable authors of the modules to more easily edit content in the future.

**Figure 4.1** Intended Flow of the CAL and Themes Covered in Each Module

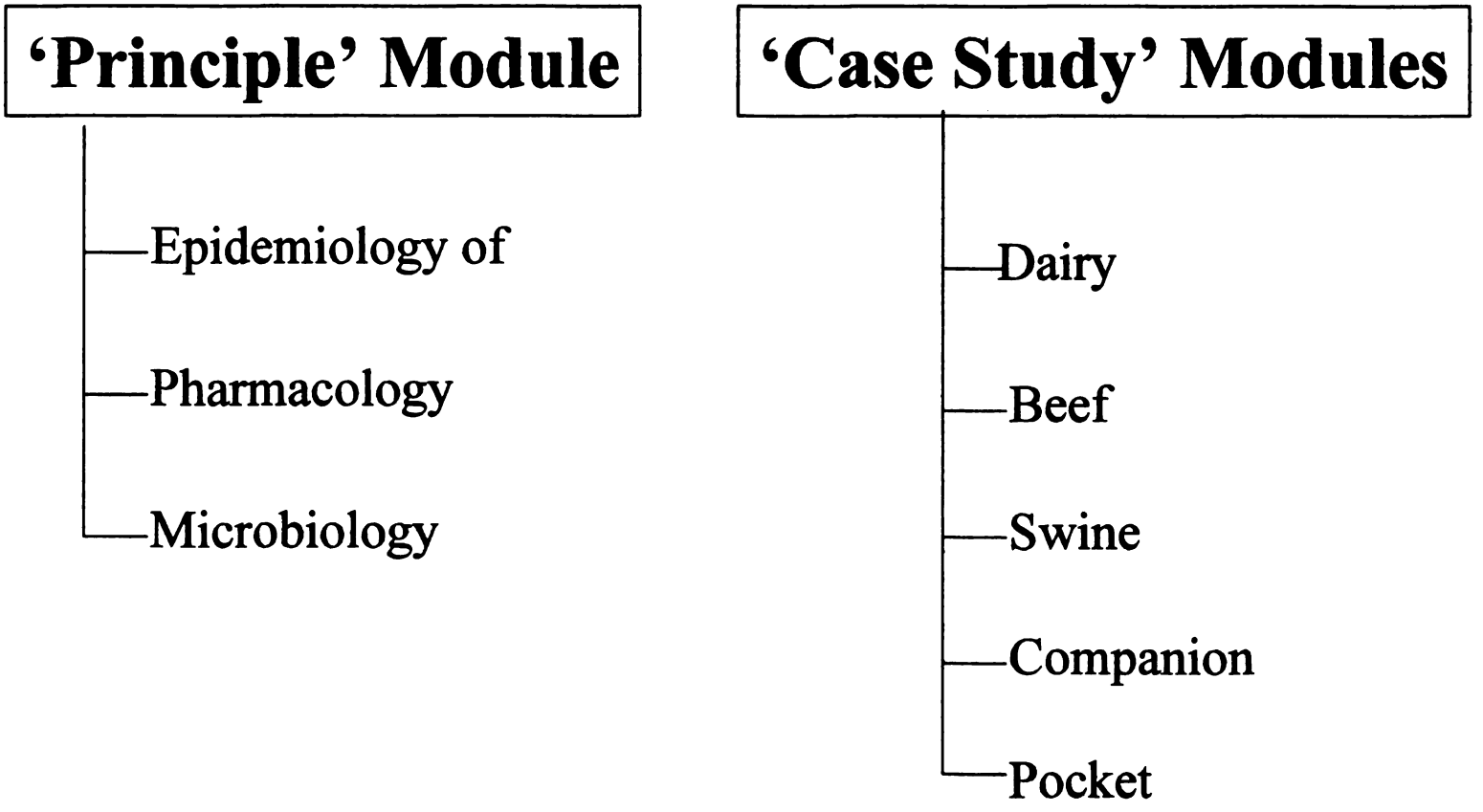

**Table 4.1** Usability Testing Task List for the CAL

Go to the following website http://www.cvm.msu.edu/cdc the username and password is "cdcmsu".

1. Find objectives for this program
2. Start the Investigation
3. Use the side bar to list some superbugs
4. In the next page locate and define 3 methods for resistant genes to be transferred
5. Go to "Use in Agriculture" and answer the first question
6. On the next page work through the first question, determine if Tetracycline is an over the counter drug
7. Answer the first two questions in the "Small Animal Clinic" section
8. In the "Microbiology" section review the video on "Bacterial Culturing"
9. In the "Pharmacology" section locate the dose formula for an antibiotic
10. Use the glossary to define pharmacokinetics
11. Go to the "Milk Replacer" case study

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# CONCLUSIONS

# CONCLUSIONS

The overall aim of this dissertation was to enhance scientific knowledge regarding the appropriate use of antimicrobial agents in dairy cattle. To meet this aim there were a few questions; 1) Do calves fed milk replacer with antimicrobial agents grow faster and have a lower disease burden, 2) Do plasma-steady state pharmacokinetic values for oxytetracycline as fed in medicated milk replacer (MMR) achieve minimum inhibitory concentrations for *Escherichia coli* and *Pasteurella multocida*, and 3) Will a computer-aided learning module be useful to teach veterinary students concepts about appropriate use of antimicrobial agents? All of these questions have been answered in the previous chapters.

In thinking about the results of my research, the first two studies (chapters 2 and 3) compliment each other well. The milk replacer study evaluates the on farm benefit of antimicrobials in feed, while the pharmacokinetic study investigates the benefit of oxytetracycline in milk replacer. What is interesting to me is that there are paradoxical results and differences that are either biologically but not statistically significant or vice versa. For example, oxytetracycline and neomycin benefits growth yet there is no difference in morbidity between treatment groups. While there is a numeric difference in mortality rates between treatments but was not statistically significant.

From a production standpoint there is a benefit to feeding MMR but is it biologically significant with a 6 kg difference at 150 days of age? To me this is not a large enough difference for the economic investment of an additional 5 dollars per calf from feeding MMR. This statistical difference is in part likely due to being the largest study to date as I calculated a sample size to determine a 10-gram difference in average

daily gain, instead it was 37 g difference. The difference in weight could have been higher if there was no enteric disease outbreak, but in looking at the data prior to and after the outbreak the difference in weight between treatments was almost the same.

In my opinion the most important finding in these studies is that there is no difference in morbidity but there were 28 deaths in the first two weeks of life with almost all of them during the enteric outbreak. This is almost 80% of the deaths for the whole year, of which 19 were in the non-medicated milk replacer group. Though there is no statistically significant difference between treatments, there is a definite biological significance. For me to walk onto a farm and tell them not to feed MMR, when there is a 2 fold increase in the number of deaths in calves fed non-medicated milk replacer, would be hard to do.

When you look at the pharmacokinetic values of oxytetracycline there clearly is no benefit for the treatment of systemic infections, such as pneumonia. Nor did it reduce the morbidity rate in MMR calves. Yet there may be some benefit to feeding MMR as it may mitigate the severity of illness, shown by the decrease in mortality between treatment groups. In this study, we only know the plasma concentrations that were attained for oxytetracycline. However, we were not able to evaluate what was going on in the gastrointestinal tract with oxytetracycline. Secondly, we only looked at one drug, as neomycin may also be playing a major role in reducing mortality. We know that aminoglycosides, like neomycin, are not well absorbed from the gut lumen. Therefore drug concentrations may be high enough in the gastrointestinal tract to minimize the severity of enteric disease. Neomycin may also be playing more of a role as there was increased growth in pre-weaning calves with no respiratory disease compared to calves

with respiratory disease pre-weaning, while oxytetracycline plasma concentrations in calves fed the MMR did not reach MIC values for *P. multocida*.

If I was to walk onto a farm and was asked if feeding MMR is of any benefit, I would have to say YES but with some careful considerations. Results from my research are only from one farm and extrapolating these results to other farms is hard to do, but in combination with previous studies you can make some decisions. From an animal welfare point it would be hard to argue against the use of MMR during an enteric disease outbreak. Now do calves need to be on MMR for the entire suckling period? This is where veterinary medicine has to look at the pros and cons of such a practice. There is likely to be few or no new classes of antimicrobial agents coming to market and therefore the profession needs to preserve and prolong efficacy of the current drugs for treating ill animals. Given that I would recommend only using MMR during the first few weeks and switch over to a product that contains no antimicrobial agents.

Now switching gears, developing the CAL tool (chapter 4) was a great learning experience in respect to working in a team environment, but also exciting to be part of educating students in a non-traditional way. This tool can be very beneficial to a veterinary curriculum as it can be used in different ways: to supplement class material for various courses (microbiology, pharmacology and public health), to clinical settings as a review or learn about specific issues. There is also a possibility that veterinarians and/or producers could use the tool for continuing education.

A concern of mine is that I left this project over two years ago and the tool has yet to be released to the general public. This is mainly due to the bureaucratic process at the Centers for Disease Control and Prevention (CDC) to review and approved material.

Given this, this leads to my biggest concern as to who is going to ensure information stays current. For instance in the 'Principles' module there is a question regarding the likely source of *Salmonella* infection and peanut butter sandwich is a possible answer that is labeled unlikely, which is no longer true given a recent *Salmonella* Typhimurium outbreak in peanut butter. There will be a need for an administrator to be identified to ensure content is kept current either by themselves or through a team. Overall, I look forward to the day the tool is released for use by veterinary curricula, as I believe it will be an effective way for students to learn about the prudent use of antimicrobial agents.

# APPENDIX

# APPENDIX A

## Appropriate Use of Antimicrobial Agents in Veterinary Medicine

**Appropriate Use of Antimicrobial Agents in Veterinary Medicine**

This computer-aided learning module can be viewed at the following website: http://old.cvm.msu.edu/cdc. At this time the CAL is password protected as both the username and password are *cdcmsu*. In the near future the CAL will be moving to the following location: http://arls.cvm.msu.edu. If the CAL is not working feel free to contact Dr. Paul Bartlett at Michigan State University via phone or email. His contact information is: phone 517-432-3100 email bartlett@cvm.msu.edu

The following pages contain screen shots to show you what the CAL looks like. Modules that are included are: Principles and Dairy Cattle, which include medicated milk replacer, neonatal scours, contagious mastitis, and farm based mastitis program.

**Figure A.1** The Principles Module

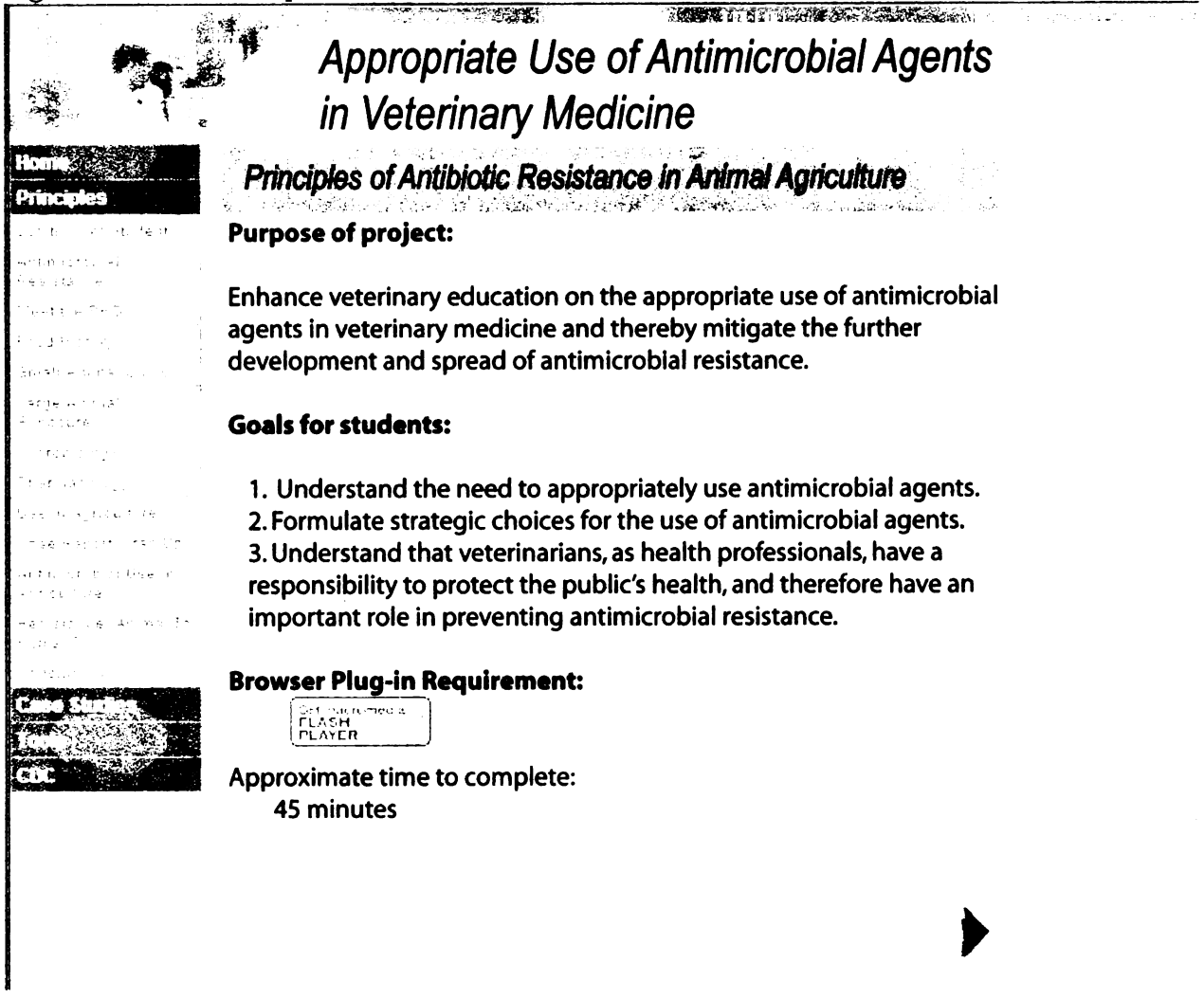

**Figure A.2** Medicated Milk Replacer Module

**Figure A.3** Neonatal Scours Module

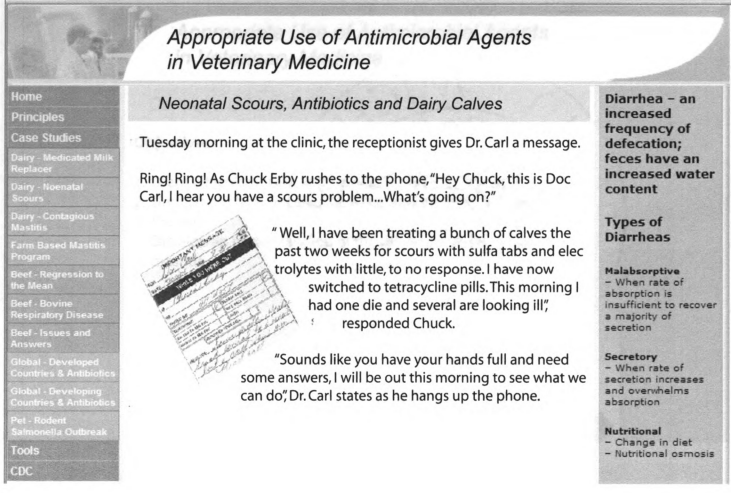

**Figure A.4** Contagious Mastitis Module

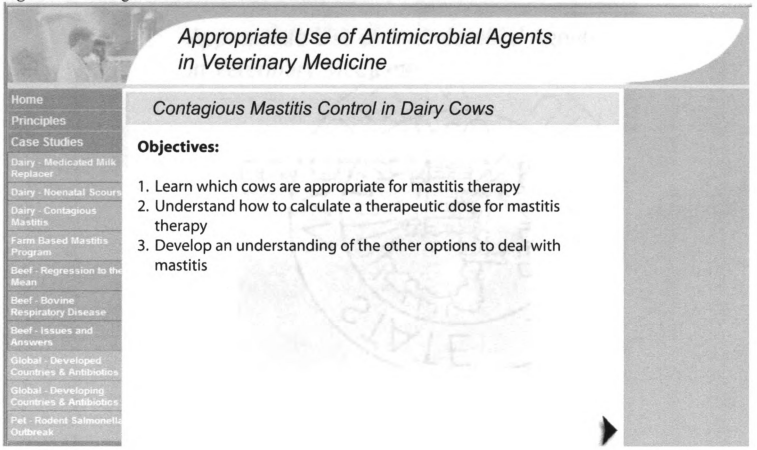

**Figure A.5** Farm Based Mastitis Program Module