Slaughtered Hogs with Discoloured Bones and the Relationship with Tetracycline Medication in the Grower-Finisher Stage

by

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A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Doctor of Philosophy
in
Population Medicine

Guelph, Ontario, Canada

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Bone discolouration of pig carcasses is a quality concern that has been observed in Ontario slaughter plants. The objectives of this study were to establish the prevalence of pig carcasses showing bone discolouration, its relationship with residues of tetracyclines in bones, and to investigate the use of tetracyclines in feeding programs for grower-finisher pigs as the main risk factor for discolouration.

Abattoir data were examined to determine the extent of the problem and the prevalence of bone discolouration during 2006, 2008, 2009, and 2010 was found to be 0.13%, 0.22%, 0.26%, and 0.28%, respectively, indicating that the issue of bone discolouration was present at low levels over the entire period of the study.

A controlled trial using feed, water, and injectable tetracycline products to investigate the effect of tetracyclines on residue and bone colour was conducted. Bones were assessed visually for signs of discolouration, and high performance liquid chromatography (HPLC) was used to measure the levels of tetracycline residues in the bones. Results from this trial demonstrated that discolouration could be produced with 660ppm of chlortetracycline (CTC) in feed for 12 weeks.
even when 33 days of withdrawal time was observed. It was also found that residues of tetracyclines can be present in bones in the absence of discolouration.

A retrospective study was conducted to investigate tetracycline use in herds identified as having discoloured bones at slaughter. Positive shipments were associated with dosage and duration of CTC use as well as with length of withdrawal.

In conclusion, discoloured bones of pig carcasses were identified at low levels in one large Ontario abattoir; however, further investigation is needed in order to determine the impact it may have on the swine industry.
DEDICATION

To the most important people in my life:

My mother Cecilia Cruz
My brother Michel Varela
My Husband Cesar Caballero
My lovely kids David and Matthew.
ACKNOWLEDGEMENTS

I would like to acknowledge and express my gratitude to all the people who made this dissertation possible.

First and foremost, I would like to thank my graduate advisory committee: Dr. Robert M. Friendship, Dr. Cate Dewey, and Dr. Jerome R. E. del Castillo. I thank them for their support and mentorship throughout my PhD program.

Dr. Friendship, I would like to offer my deepest thanks for your time, advice, and invaluable and unconditional support, always available to me.

This project was possible due to the financial support of Ontario Pork and the University of Guelph- Ontario Ministry of Agriculture (OMAFRA) Sustainable Production Systems Program.

I would like to thank the Department of Population Medicine at the Ontario Veterinary College for giving me the opportunity to perform my graduate studies. A special thanks goes to the faculty members, fellow graduate students, secretaries, and friends who created a stimulating environment making this a positive experience for me.
I would like to thank all members of the Laboratory Services Division – University of Guelph, particularly Louise Spilsbury, for her technical assistance with the High Performance Liquid Chromatography (HPLC).

I would like to recognize William Sears for his statistical support.

I would like to thank Eric Brandt for his unconditional support and friendship since Cesar and I arrived to this beautiful country “Canada”.

In particular, I would like to thank those special people who always supported and encouraged me to achieve my dreams. Thanks to my mother, Cecilia Cruz, my brother, Michel Varela, my husband, Cesar Caballero, and my sons, David and Matthew.

To my mother, thank you mom for always being there believing in me, your support, love and guidance have meant so much to me. I feel blessed to have a mom like you. “I love you mom”.

To my brother, thank you Michel, I know you are with God, but somehow you are also with us.

To my husband Cesar, your love, friendship, and support means more to me than you could ever imagine. I feel so lucky to have you in my life. “I love you”.

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To my kids, David and Matthew, I have no word to express how much you guys mean to me. “I love you”.
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CHAPTER 1: General Introduction and Literature Review

1.1. GENERAL INTRODUCTION

In October 2005, a shipment of pigs from one producer in Ontario caused alarm among meat inspectors because the bones of most of the pigs were strikingly yellow. This one incident triggered discussion and awareness among Canadian Food Inspection Agency (CFIA) inspectors in Ontario and possibly as a result, about 500 pig carcasses were identified with bone discolouration over the next two months. The original shipment resulted in a farm investigation and it was revealed that tetracyclines had been fed to these pigs at relatively high levels continuously for at least 12 weeks in the grower-finisher barn.

Tetracyclines have been used for more than 60 years in food animal production; with tetracycline, chlortetracycline and oxytetracycline being among the most widely used antimicrobial drugs in the Canadian swine industry (Health Canada, 2002). Tetracyclines are commonly used in feed, water, or as injectable products to improve growth rate and to prevent, treat or control disease (Riviere and Spoo, 1995). It has long been known that tetracyclines bind to calcium and can lead to bone discolouration (Albert and Rees, 1956; Benitz et al, 1967; Buyske et al, 1960; Weinberg, 1957). Bound tetracyclines in bone is an important issue in the swine industry regarding the safety and acceptability of pork products since residues of tetracyclines not only decrease the quality of pork carcasses, but also may affect food safety through contamination of both mechanically deboned meat and bone meal (Kühne and Körner, 2001; Kühne et al, 2000). Meat obtained from mechanically deboned carcasses may contain bone splinters
and thus be contaminated with tetracyclines; likewise bone meal from pigs fed high levels of tetracyclines used for preparation of animal feeds could be a concern given that complete destruction of tetracycline and chlortetracycline has not been demonstrated after treatment at 133°C for up to 45 min (Kühne et al., 2001). Despite widespread use of tetracyclines in the pig industry worldwide, there are few reports of problems with discoloured bones at slaughter. It is possible that because the Ontario pig industry was dealing with a disease outbreak in the Fall of 2005 (See Appendix A.1.1) that higher levels of antibiotics were being used on pig farms during this period.

Although muscle and organ tissue from pigs with discoloured bones in the October 2005 incident were tested and found to be acceptable for human consumption with residue levels of tetracyclines well below the targets set by regulatory agencies, the CFIA released a Meat Hygiene Directive ordering positive carcasses deboned (See Appendix A.1.2). Subsequently a second Meat Hygiene Directive was issued that described the problem as one of cosmetic concern rather than a food safety issue and gave the packers the responsibility of dealing with the problem (See Appendix A.1.3).

This remains an important issue in the Canadian pig industry because consumer confidence is essential to maintain good sales both domestically and internationally.
1.2. MEAT INSPECTION AND XENOBIOTIC RESIDUES

Meat inspection is a sanitary control process of slaughter animals and meat intended for human consumption. The main purpose of inspection is to assist in preventing the spread of food borne disease, or undesirable meat from entering the food supply and, thus ensuring safe and wholesome meat and meat products. This activity, which involves examination of the living animals awaiting slaughter and examination after slaughter of head, carcass, and viscera, is carried out by public health authorities represented by veterinary meat inspectors at the abattoir stage. In conducting an inspection, an inspector must apply the standards related to food safety and animal health under the Food and Drugs Act (Canada) and the Meat Inspection Act (Canada).

The presence of chemical residues in food accounts for a high level of concern among consumers because toxicity and allergic reactions may occur. Furthermore, exposure to low levels of antibiotics could possibly result in the development of antibiotic resistant strains of bacteria (Nisha, 2008).

Antibiotics are widely used in livestock production and therefore there is always a risk that residues may be present at slaughter. Residue testing of edible tissue is often carried out if inspectors suspect an animal has been treated with medication. Antibiotics used to treat livestock undergo an intensive approval process. In Canada, prior to being offered for sale, veterinary drugs must be tested and approved by Health Canada’s Health Products and Food Branch, Veterinary Drugs Directorate. Thus, when used according to label directions,
levels of drug or antibiotic residue will not violate the allowable limits established for the drug or antibiotic.

Veterinarians have the responsibility to extend withdrawal periods if necessary when a drug is prescribed in an extra-label manner in order to avoid drug residues. When tetracyclines or other drug residues are found in pork and pork products above allowable limits, there is a potential risk to human health and it is a violation of the Canada Food and Drugs Act and Regulations (FDAR). The FDAR lists the permitted feed additives and how they may be used; its implementation and enforcement with respect to foods is the responsibility of the Canadian Food Inspection Agency (CFIA). In swine, the tolerance levels for chlortetracycline, oxytetracycline and tetracycline residues are 0.2 ppm, 0.6 ppm, and 1.2 ppm in muscle, liver, and kidney tissue, respectively (Health Canada 2011). Maximum Residue Limits (MRLs), a residue considered to pose no adverse health effect if ingested daily by humans over a lifetime, are established for edible tissues such as skeletal muscle, liver, and kidney and not for non-edible tissues (bile, blood, bone, brain, diaphragm, gastrointestinal contents, gastrointestinal tract, heart, lung, skin, spleen, and thyroid gland). The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set standards for acceptable daily intake (ADI) and maximum residue limits (MRLs) in food of animal origin. The acceptable MRLs for chlortetracycline, oxytetracycline, and tetracycline as recommended by the Joint FAO/WHO Expert Committee on Food Additives is 200, 600, and 1200 µg/kg for muscle, liver, and
kidney, respectively (World Health Organization: Join FAO/WHO Expert Committee on Food Additives, 1998)

In most cases, antimicrobial residues in pork are caused by farmers’ failure to observe appropriate withdrawal times. In addition, on-farm feed preparation practices that allow medicated feed to mix with non-medicated feed, human errors in feeding or in the use of inaccurate scales, can all lead to residues in meat (Friendship, 2006).

When conducting a carcass inspection, meat inspectors should be able to identify any abnormality that may compromise the food safety and the wholesome image of Canadian pork products; this includes the identification of carcasses from animals that had been treated with antibiotics or any other chemical agent (Herenda et al., 1994). According to the Canadian Food Inspection Agency (Canadian Food Inspection Agency, 2010), antibiotic use should be suspected in any animal in which an injection site is found, and any animal affected with a septic condition, which might have been treated with antibiotics (Canadian Food Inspection Agency, 2010). It is important to consider, however, that the extent and severity of any condition differs among inspectors because judgement is based on subjective evaluation and then observational bias may happen. Variability in condemnation rates among slaughter plants with similar condemnation criteria has been documented (Tuovinen et al., 1994, Jackowiak et al., 2006, Elbers et al., 1992). Carcasses from animals which an inspector believes may have been exposed to a drug should be held until appropriate testing is conducted and results received, If a residue of a medication
normally administered to a group of animals is detected in one animal, all the animals in the same lot are suspected of the residue (Canadian Food Inspection Agency, 2010). Appropriate testing and regulations have been established by the legal authorities depending on the particular residue suspected and type of exposure (individual or lot), respectively. Overall, individual exposure testing is dependent on the particular compound suspected, and the held product can be treated as condemned material keeping samples for additional testing. On the other hand, if exposure of a lot is suspected, initial screening of 6 carcasses from the suspected lot is conducted using the Swab Test On Premises (STOP). If the test result is negative the carcasses are released; otherwise, the carcass remains under detention and will be condemned should the muscle sample contain violative levels (Canadian Food Inspection Agency, 2010).

To improve the quality and safety of pork, different programs have been instituted in different countries with the main objective to increase farmers’ awareness of antibiotic use and improve adherence to withdrawal times. In Canada, the Canadian Quality Assurance Program, CQA was developed for producers to encourage them to adhere to withdrawal times by keeping a complete drug record under a veterinarian’s supervision. The use of on-farm mixing medication is also examined under the CQA program.

1.2.1. Issues of Carcass Quality

Being recognized as one of the major world pork exporters, the Canadian pork industry depends on a reputation for a safe and high quality product. One
area associated with pork quality is colour. The colour of meat is a primary indicator of quality and has a huge impact on export sales, especially to Japan. Although generally darker pork is preferable, it may vary from bright reddish-pink to slightly darker; both pale and dark colour are unattractive pork colour to consumers and indicate possible issues of quality (Lammers et al., 2007). Besides muscle colour, there are other characteristics that are important when assessing cuts of meat such as bone content, fat trim, and marbling or intramuscular fat (Sebranek and Judge, 1990). Fat should be firm, greasy and white; fat and lean separation are undesirable (Wilson, 2005). Various feed ingredients can affect the appearance of fat and impact consumer acceptance; diets containing high saturated fatty acids such as soybean oil and poultry fat are known to produce soft fat, which results in difficult processing and pork cuts that are less firm and unattractive to the customer (Goodband et al., 2006).

Pork is often sold as cuts of meat with the bone included which is expected by consumers to be shiny and white; thus discolouration of the bones is a quality defect. Cuts of meat showing discoloured bones results in consumer rejection because of bone colour and for this reason the value of the carcasses with discoloured bones is reduced.

1.3. BONE GROWTH AND PHYSIOLOGY

Bone is a dynamic and rigid tissue that forms part of the endoskeleton of vertebrates. Its main function involves: mechanical support and protection, storage for body calcium, and a site for adult haematopoiesis (Salo et al., 1996).
1.3.1. Structure

Bone is composed of two types of osseous tissue mainly classified on the basis of porosity as: compact or cortical bone and trabecular bone also known as cancellous or spongy bone (Garner et al., 1996). Compact or cortical bone makes up to 80% of the total bone mass and is mainly responsible for the support function; it is a denser tissue with a high mineral content, which gives bones their smooth, white, and solid appearance. Trabecular bone is much more porous and accounts for the remaining 20% of the total bone mass; it is composed of bone plates filled with blood vessels and marrow, which makes trabecular tissue an important component for bone remodelling.

Regarding its cellular structure, skeletal tissue is composed of various types of cells grouped into two general types, bone-forming cells and bone-resorbing cells. Bone-forming cells or osteoblasts originate from the mesenchymal stem cells by a linear sequence that progresses from progenitor cells, restricted to osteoblast development and bone formation known as osteoprogenitor, to preosteoblasts, osteoblasts and then lining cells or osteocytes (Aubin, 1998; Aubin, 2001). Preosteoblasts are elongated cells with the unique ability to divide, and are found in the tissue layer near bone-forming surfaces. Osteoblasts secrete collagen and lay down new extracellular bone matrix known as osteoid, which mineralizes to become bone (Cool and Nurcombe, 2005). During the process of extracellular matrix formation and in the presence of tetracyclines in serum, some tetracycline may become incorporated with the matrix. Calcium ions at the site of mineralization may be chelated by the extracellular compounds.
such as tetracycline, following its incorporation into any hydroxyapatite forming crystal or into the collagenous matrix (Skinner and Nalbandian, 1975). Tetracyclines or any other compound previously incorporated into bone tissue can be released by dissolution of the bone during osteoclastic activity (bone resorption), and thus bone remodelling plays an important role in the permanence of tetracyclines in bone tissue (Skinner and Nalbandian, 1975).

Osteoblasts give rise to two cell types within bone: osteocytes and bone-lining cells which maintain some osteoblastic characteristics but no longer synthesize collagen for the formation of new bone (Garner et al., 1996). As extracellular matrix is secreted and calcified around osteoblasts, osteoblasts become trapped and differentiate further into osteocytes, which are in charge of homeostasis, exchanging wastes for nutrients from the blood, and regulating calcium release back into the blood stream (Horton, 1995). Bone-lining cells, also known as resting osteoblasts or surface osteocytes, are inactive osteoblasts which are essential for the maintenance of blood calcium levels by pumping Ca\(^+\) ions from the bone fluid compartment to the extracellular fluid compartment (Garner et al., 1996).

Different compounds present in the blood stream may diffuse from the extracellular fluid to the hydroxyapatite crystals of the bone mineral and be incorporated into new or remodelled bone.

Bone-resorbing cells also known as osteoclasts are derived from hematopoietic stem cells and are responsible for bone resorption by dissolving crystalline hydroxyapatite and degrading organic bone matrix rich in collagen.
fibr (Vaananen et al., 2000). Its promyeloid precursor can differentiate into either an osteoclast, a macrophage or a dendritic cell if it is exposed to osteoclast differentiation factor (ODF), macrophage colony-stimulating factor (M-CSF), or granulocyte-macrophage colony-stimulating factor (GM-CSF), respectively (Suda et al., 1999).

At the molecular level, bone consists of two fundamentally different components: the organic and inorganic. The organic or intercellular component is formed by approximately 90% of collagen, synthesized and secreted by osteoblasts, and 10% of a mixture of proteins that are either synthesized by osteoblasts or are serum-derived (Garner et al., 1996). The inorganic component is formed mainly by mineral salts and calcium present in the form of hydroxyapatite crystals deposited within an organic phase of cross-linked collagen fibres. The process of calcification occurs when these crystals are laid down between the collagen fibres of the bone matrix (Ham, 1974).

1.3.2. Bone Formation

The process of bone formation or osteogenesis involves four main steps: organic matrix synthesis by osteoblasts, mineralization of the matrix to form bone, bone resorption and bone remodelling.

1.3.2.1. Organic Matrix Synthesis (Osteoid)

The prenatal development of bone occurs by deposition of hydroxyapatite crystals within the collagen matrix; however, the process by which the collagen
matrix is laid down differs according to the two major types of bone formation: intramembranous and endochondral. These terms refer to the sites in which ossification occurs: endochondral means “in cartilage” and intramembranous means “within membrane”; in both cases, bone forms because osteoblasts evolve and secrete the organic intracellular substance of the bone matrix (Garner et al., 1996; Ham, 1974).

1.3.2.1.1. Intramembranous Bone Formation

Intramembranous ossification, in which cartilage is not present, mainly occurs during formation of most of the bones of the skull, the jaw, and the ribs (Garner et al., 1996; Ham, 1974). During this process, embryologic mesenchymal cells differentiate directly into preosteoblasts and then into osteoblasts in areas of highly vascular embryonic connective tissue to begin the process of ossification (Garner et al., 1996). Osteoblasts begin to secrete the organic matrix of the bone, known as “osteoid tissue” or uncalcified bone and those that completely surround themselves with osteoid tissue differentiate into osteocytes (Ham, 1974).

Cells that do not differentiate and osteoblasts, remain at the margin of the bone already formed, with some of the osteogenic cells continuing to proliferate and others differentiating and secreting intracellular substance around themselves to become osteocytes to form a beam of bone called a spicule (Ham, 1974). As spicules continue to grow, they join together to form the trabeculae. Osteoblasts continue to line up on the surface and as growth continues,
trabeculae become interconnected and immature or woven bone is formed (Ham, 1974). Differentiating mesenchymal cells surrounding the trabeculae will produce the periosteum richly supplied with blood vessels involved in the nutrition of the bone, and osteoblasts from the periosteum on the bone matrix will replace woven bone by lamellar or compact bone.

1.3.2.1.2. Endochondral Bone Formation (Bone Modeling)

Endochondral ossification, in which bone is formed by calcification of a cartilaginous structure that serves as a model for the new bone, occurs in long bones such as those of the limbs; and it is also an essential process during the growth of the length of bones and the natural healing of the bone fractures.

Young cartilage can grow by two different methods: interstitial and appositional growth.

The word interstitial refers to the cells in the lacunae known as chondrocytes. Cartilage is formed by chondroblast cells, which are derived from prechondroblasts arising from the same mesenchymal cells that form osteoblasts (Garner et al., 1996). Chondroblasts secrete intercellular collagen matrix, eventually enclosing themselves, to differentiate into chondrocytes characterized by their ability to divide until they become mature. Chondrocytes can continue to enlarge and divide expanding the lacunar spaces, and this continuous cell division with their subsequent formation of intercellular collagen matrix causes the cartilage to grow in length (Garner et al., 1996; Ham, 1974).
Appositional growth implies a mechanism whereby new layers of cartilage are apposed to one of its surfaces. During this process the deeper cells of the connective tissue that surrounds the cartilage of the developing bone, known as perichondrium, divide to increase their numbers. These cells differentiate into chondroblasts and then into chondrocytes surrounding themselves with intercellular substance which is applied to the surface of the cartilage. By this mechanism a new layer of cartilage is laid down under the perichondrium on the surface of the cartilage causing the cartilage to grow in width (Ham, 1974).

1.3.2.2. Mineralization of Organic Matrix to Form Bone

Since cartilage is an avascular tissue, cells receive their nutrients by diffusion through the fluid surrounding the collagen fibers; when the cartilage is invaded by blood vessels, the process of mineralization of the organic matrix begins (Garner et al., 1996). In the earliest bone formation either in endochondral formation, or in woven bone, mineralization is preceded by vesicle-driven-mineralization. Osteoclasts secrete matrix vesicles that provide a site for the hydroxyapatite crystal formation; these vesicles act as the foci for calcium and phosphate deposition and when crystallization produces a crystal larger than the original vesicle, the vesicle is destroyed and acts as a centre for crystals to grow on (Garner et al., 1996).

In lamellar bone formed during remodelling, mineralization occurs directly within the collagen matrix where mineral is deposited in association with the
tightly spaced collagen fibrils and associated proteins; vesicle formation is not involved in this process (Garner et al., 1996).

### 1.3.2.3. Bone Resorption

Bone resorption is defined as the removal of both the mineral and organic matrix to produce a cavity in the bone structure. When dead bone, calcified bone in which the lacunae are empty because the osteocytes they formerly contained have died and dissolved, is exposed to the contents of a Haversian canal (capillaries and osteogenic cells) cells of the monocyte-macrophage cell line differentiate into osteoclasts and the dead bone is resorbed (Chambers, 2010). During this process minerals are released and transfer of calcium from bone fluid to blood occurs (Garner et al., 1996; Ham, 1974).

In general, the process of bone resorption involves migration of osteoclasts to the resorption site, its attachment to bone matrix, polarization and formation of new membrane domains, dissolution of hydroxyapatite crystals, degradation of organic matrix, removal of degradation products from the resorption lacuna, and either apoptosis of the osteoclasts or their return to the non-resorbing stage (Vaananen et al., 2000).

When bone resorption initiates, osteoclasts migrate to a resorption site, and three distinct membrane domains are formed: the sealing zone which attach the osteoclastic plasmatic membrane to the bone matrix from its surroundings, the ruffled border with finger-like projections formed by fusion of intracellular acidic vesicles that penetrate the bone matrix, and the functional secretory domain.
used to transfer degradation products from the resorption lacuna to the extracellular space (Vaananen et al., 2000). Osteoclasts attach to bone matrix through the sealing zone and the ruffled border and dissolution of minerals/hydroxyapatite crystals occurs by targeted secretion of HCl through the ruffled border into the resorption lacuna, an extracellular space between the ruffled border and the bone matrix (Vaananen et al., 1990; Vaananen et al., 2000). After dissolution of minerals occurs, several proteolytic enzymes degrade the organic bone matrix which is rich in collagen, and degradation products are transferred from the resorption lacuna from the ruffled border to the functional secretory domain to be further liberated into the extracellular space (Vaananen et al., 2000).

1.3.2.4. Bone Remodelling

The remodelling of bone can only be achieved by bone being absorbed from a surface and added to other surfaces; it is an important process that occurs in healthy bones to maintain their optimal shape for support (Ham, 1974).

After bone resorption has occurred, the osteogenic cells line up around the inner surface of the cavity and differentiate into osteoblasts; osteoblasts which will line the surface of the cavity and lay down new bone until they have filled the excavation leaving space for blood vessels and nerves (Garner et al., 1996; Ham, 1974). In growing bone, remodeling (resorption and formation) takes place in separate regions of the bone with formation exceeding resorption; while in mature bone, osteoblastic bone formation occurs only at sites of osteoclastic
activity, resulting in replacement of the exact quantity of bone removed by resorption (Garner et al., 1996).

1.4. TETRACYCLINES

Tetracyclines compose a large family of antibiotics but only chlortetracycline, oxytetracycline and tetracycline are used in pig production in Canada and therefore the following discussion will primarily focus on these three forms of tetracyclines.

1.4.1. The Origin of Tetracyclines

Tetracyclines are a large family of antibiotics first described in 1948 as the fermentation product of a soil bacterium: *Streptomyces* spp (Wainwright, 1990). *Streptomyces* spp. produces metabolites known as polyketides, the key tetracycline precursors, which are used as defensive toxins. Chemical isolation and purification of *Streptomyces aureofaciens* polyketides produced the compound chlortetracycline, an antibiotic with many advantages over previously discovered penicillin, streptomycin and chloramphenicol, such as an increased spectrum of activity especially against Gram-negative organisms (Nelson, 1998). Examination of other strains of *Streptomyces* led to the production of other natural compounds. Oxytetracycline is obtained as a metabolic product of *Streptomyces rimosus* (Chambers, 2006). Tetracycline was first produced as a semi-synthetic derivate from chlortetracycline (Nelson, 1998). Nowadays,
tetracycline is produced by a mutant strain of *Streptomyces aureofaciens* (del Castillo, 2001).

### 1.4.2. Chemical Structure

Tetracyclines are a group of four-ring amphoteric substances slightly soluble in water at pH 7.0 (Prescott, 2000). They are available for use, mainly as hydrochloride (acid solutions) as tetracyclines are stable in this form; however, they rapidly lose their activity in solution (Bryskier, 2005).

In spite of diverse pharmacokinetic properties of tetracyclines, they have the same basic molecular structure required for bioactivity: the tetracyclic naphthacene carboxamide system conformed by two rings: A and DCB ring (Figure 1.1) (Nelson, 1998; Prescott, 2000). The DCBA ring structure is composed of two distinct regions, the upper and lower peripheral regions which contain different chemical functional groups and substituents. Tetracyclines with antibiotic activity must possess a functional group along the upper peripheral region responsible for the antibiotic properties: the dimethylamine group at carbon 4 (C4) in Ring A (Sapadin and Fleischmajer, 2006). Similarly, along the lower peripheral region bioactive tetracyclines must possess the following functional groups: a diketo-enol system across carbons C11 and C12 between rings C and B, a tertiary hydroxyl group at the lower bond between rings B and A, and the tricarbonyl-methane system spread across carbon C1, C2 and C3 (Nelson, 1998; del Castillo, 2001). Structural variations from the reference a a molecule tetracycline which has the empirical formula of C$_{22}$H$_{24}$N$_{2}$O$_{8}$ (Figure 1.1),
constitute the specific sequence of other tetracyclines. Chlortetracycline has a chlorine atom substituent at position C7 and thus its empirical formula is \(C_{22}H_{23}N_2O_8\) Cl. Oxytetracycline has a hydroxyl substituent at position C5 and its empirical formula is \(C_{22}H_{24}N_2O_9\) (del Castillo, 2001; Bryskier, 2005).

Removal of the dimethylamine group at C4 in Ring A enhances the activity of this compound against non-antibiotic targets but abolishes antibiotic potency (Nelson, 1998; Sapadin and Fleischmajer, 2006).

### 1.4.3. Mechanism of Action

Tetracyclines inhibit bacterial protein synthesis in susceptible microorganisms by binding to the 30S subunit of the ribosome. In Gram-negative bacteria, the more lipophilic molecules such as doxycycline and minocycline penetrate the outer membrane partly by passive diffusion, while the more hydrophilic molecules such as oxytetracycline and tetracycline cross the membrane by active transport through water-lined transmembrane protein porin routs as positive charged cations (Foye et al., 2008; Bryskier, 2005).

Once inside the cell, tetracyclines form a complex with \(Mg^{2+}\) and bind reversibly to the 30S and 16S subunits of the bacterial ribosome. Binding of the molecule to the 30S ribosomal subunit ensure inhibition of protein synthesis by interfering with the binding of aminoacyl-tRNA to the acceptor (A) site on the messenger RNA molecule/ribosome complex, needed for protein synthesis in growing organisms (Riviere and Spoo, 1995; Chambers, 2006; Prescott, 2000; Giguère, 2006). Although tetracyclines have less affinity for mammalian
ribosomes, interaction with the 16S rRNA occurs indirectly through primarily distortion of the ribosome (Bryskier, 2005). The binding of tetracyclines to their target is reversible, which would explain their purely bacteriostatic nature in prokaryotic cells, due to their capacity to accumulate in the cytoplasm at greater internal concentrations than the external ones (Bryskier, 2005).

1.4.4. Pharmacokinetics of Tetracyclines

1.4.4.1. Absorption

Unless given IV, a drug must cross several semi-permeable cell membranes or biologic barriers before it reaches the systemic circulation. These membranes are mainly composed of biomolecular lipid matrix which determines its permeability characteristic (Kahn, 2005). Efficient assimilation and enhanced distribution of oral administered tetracyclines in the body relies on their lipid solubility (Aronson, 1980). Tetracyclines are rapidly absorbed from the intestinal lumen into the blood stream and a maximum plasma concentration is reached within 2 – 4 hours in carnivores (Agwu and MacGowan, 2006; del Castillo, 2001). In pigs, the level of absorption of chlortetracycline (28%) has been found to be significantly greater than that of oxytetracycline (5%) when similar levels of the drug are fed (del Castillo et al., 2000). The low bioavailability of orally administered tetracyclines in pigs is probably due to the expression of P-glycoprotein, an energy-dependent efflux transporter found in the gastrointestinal tract, responsible for intestinal clearance of lipophilic drugs such as tetracyclines (del Castillo et al., 2000). As tetracyclines diffuse through the epithelial intestinal
cells known as enterocytes, the transporter protein “P-glycoprotein” dump back the drug molecule into the intestinal lumen preventing them from reaching the systemic circulation and therefore limiting its bioavailability (Horn et al, 2004). Furthermore, the oral bioavailability of tetracyclines may be impaired by different factors such as food particles and divalent cations found in dairy products, aluminum hydroxide gels, and bismuth subsalicylate (Chambers, 2006). Swiss researchers documented reduced chlortetracycline bioavailability, a parameter used to measure absorption, when high dietary calcium was used (Wanner et al., 1991). Binding of tetracyclines to organic macromolecules occurs in the presence of Ca\(^{++}\) and other cations present in the diet. The tetracyclines precipitate and become unable to cross the biological membranes, which in turn decreases their absorption markedly (Nelson, 1998; Agwu and MacGowan, 2006; Poiger and Schlatter, 1979; White and Pierce, 1982). In contrast, dietary citric acid positively affects the bioavailability and absorption of orally administered tetracyclines, especially chlortetracycline. The bioavailability of orally administered oxytetracycline and chlortetracycline in piglets was reported to be significantly increased by the citric acid content in feed, even when a basal calcium diet was administered (Wanner et al., 1991; Pollet et al., 1983; Wanner et al., 1990).

1.4.4.2. Distribution

Once tetracyclines are absorbed into the bloodstream, they bind to plasma proteins (Riviere and Spoo, 1995). When plasma-protein binding of antimicrobials is greater than 85%, it negatively affects drug distribution because
only free molecules are microbially active and able to diffuse into the target tissue (Craig and Suh, 1978). Plasma-protein binding of more lipid-soluble chlortetracycline and less lipid-soluble oxytetracycline are 70% and 20%, respectively (del Castillo et al., 1998). High lipid soluble tetracyclines have the advantage of penetrating tissues better than others with less soluble properties and thus an increased bacteriostatic efficacy. The fact that chlortetracycline distributes more easily into the site of infection is due to its increased lipid solubility when compared to oxytetracycline (del Castillo et al., 1998). One of the factors involved in deposition of tetracyclines in bone is the presence of chelate tetracyclines in the bloodstream. Although bone is a relatively inert tissue, deposits of crystals in its organic matrix expose a large surface area to extracellular fluids, which is important for the rapid exchange of ions and cellular activity (Barrére et al, 2006). Once in the bloodstream, tetracyclines are transported mainly as Ca\(^{++}\) and Mg\(^{++}\) chelates, and once chelated, act as ionophores capable of crossing lipophilic barriers delivering ions and tetracyclines into cellular compartments (Nelson, 1998). Tetracyclines distribute rapidly throughout the body entering almost all tissues and secretions. They accumulate mainly in the kidney, liver, lung, muscle, serum, spleen, bones, and enamel of unerupted teeth (Chambers, 2006; Black and Gentry, 1984); relatively high concentrations of tetracyclines have been found in umbilical cord plasma, amniotic fluid and breast milk (Chambers, 2006).

Persistence of fluorescence in bone, as compared with cartilage and other soft tissues has been related to either a direct binding of tetracyclines to matrix or
a more complex formation occurring between new bone matrix, calcium, and tetracyclines (Milch et al, 1957). Fluorescence has been observed in unmineralized osteoid, and thus some authors speculate that during the process of collagen synthesis concentrations of tetracyclines from the serum may occur resulting in a direct binding of tetracyclines to matrix per se (Skinner and Nalbandian, 1975; Milch et al, 1957). A more complex phenomenon occurs at the site of mineralization, calcium ions may be chelated by the extracellular tetracycline, and any crystallites forming on or within the collagenous matrix may be the foci for tetracycline incorporation (Skinner and Nalbandian, 1975). Incorporation of tetracyclines with crystals of calcium carbonate (CcCO\(_3\)) and hydroxyapatite (HA) crystals which are deposited in the organic matrix has been documented to be essential in the process by which tetracyclines are fixed in mineralized tissues (Skinner and Nalbandian, 1975). However, only a portion of the initially absorbed tetracycline is tightly bound in the bone; the rest is removed by diffusion into the blood (Buyske et al, 1960). A more abrupt decline in the amount of tetracycline deposited in bones was observed when compared to chlortetracycline after 24 hours following the last of two intraperitoneal injections in adult rats, which is explained by the fact that chlortetracycline is the stronger of the two chelate agents (Buyske et al, 1960).

Deposition of tetracyclines in bones has been reported to be related to their capability to chelate cations but also to the route of administration. Parenteral administration of tetracyclines is followed by an immediate deposition and this deposition remains for a long time. A single intravenous dose of 50 mg/g of body
weight of tetracycline was enough to result in detectable levels of 24 to 34 mg/kg in the bone after 1 minute of the injection. Fluorescence was observed in the skeleton of rats 16 weeks after tetracycline was administered intraperitoneally (Buyske et al., 1960). Withdrawal times are based on detectable levels of drugs dropping below the level deemed to be safe and is measured in edible tissues. However, there is little information regarding appropriate withdrawal times that ensure bone tissues are free of drug residues after administration of tetracyclines. Guillot et al. (2011) reported that bone staining in pigs is reversible when the time from drug administration until marketing is quite long in the order of 12 – 16 weeks. According to these investigators feeding CTC for four weeks is sufficient to induce discolouration which becomes more intense as treatment duration and age at treatment onset increase, but can be reduced or even disappear when a withdrawal time according to age at treatment onset is followed (Guillot et al., 2011). Prolonged withdrawal times are needed in pigs treated at later stages because discolouration vanishes at a slower rate when treatment onset increases due to the fact that the bone turnover rate decreases as age increases (Guillot et al., 2011; Li et al., 1989).

1.4.4.3. Metabolism and Excretion

Natural tetracyclines (tetracycline, chlortetracycline, and oxytetracycline) are unstable under strong acidic and alkaline conditions. Epimerization of tetracyclines in weak (pH 3) and strong (below pH 2) acid conditions leads to the formation of 4-epitetracycline and anhydrotetracycline, respectively (Pena et al,
Tetracyclines are mainly excreted by renal filtration and bilary routes as unchanged, with only 5% excreted as the metabolite 4-epitetracycline (Agwu and MacGowan., 2006).

1.4.5. Properties of Tetracyclines

1.4.5.1. Antimicrobial

Tetracyclines are bacteriostatic antibiotics that may be used in the treatment of many common infections. They are characterized by their chemotherapeutic efficacy against a wide range of bacteria. Development of strains of microorganisms resistant to tetracyclines has reduced the effectiveness of this drug; however glycylcyclines, minocycline and tetracycline analogs have been identified with activity against tetracycline-resistant strains due to their ribosomal protection mechanism (Giguère, 2006). Differences in antimicrobial activity of tetracyclines in-vivo result from their differences in lipid solubility which influences their pharmacokinetic characteristics such as: absorption, distribution, metabolism / excretion, and concentration of a specific tetracyclines within the cell (del Castillo and Besner., 2001).

1.4.5.2. Non-Antimicrobial

Chemically modified tetracyclines have non-antimicrobial properties of potential value in preventive medicine such as anti-inflammatory properties. They have been reported to be potent inhibitors of mammalian collagenases and several other matrix metalloproteinases involved in inflammatory and
degenerative disorders (Nelson, 1998). Interstitial collagenases are considered to mediate tissue breakdown during the progression of inflammatory diseases such as periodontal diseases, rheumatoid arthritis, corneal ulceration and dystrophic epidermolysis bullosa (Golub et al., 1990; Paulus, 1995). Tetracyclines have the ability to inhibit the pathologically excessive collagenase activity produced during the inflammation process, as well as to enhance the collagen turnover (synthesis and degradation) production required to maintain the normal tissue integrity (Golub et al., 1998).

Chemically modified tetracyclines have been used in the treatment, not only of inflammatory diseases, but also in treatment of dermatologic and immunological diseases such as cardiomyopathies (Sapadin and Fleischmajer, 2006; Tsankov et al., 2003; Reddy et al., 2004).

1.4.5.3. Metal Chelation

Tetracyclines have been found to have powerful ionophoretic properties. As ionophore compounds, they are capable of forming lipid-soluble complexes with metal cations and transporting them across hydrophobic barriers (Nelson, 1998, White and Pearce, 1982). Tetracyclines circulate in blood plasma primarily as Ca\(^{++}\) and Mg\(^{++}\) chelates, which allow the delivery of ions and tetracyclines into the intracellular compartments (White and Pearce, 1982). After cellular incorporation, Ca\(^{++}\) acts as a secondary messenger affecting many biological functions / pathways such as metabolic reactions and cell division cycles among others (Nelson, 1998). It also has been suggested that this ionophoretic characteristic of
tetracyclines is the cause of their inhibitory effect on bone growth and
discoloration of growing teeth (Nelson, 1998).

Tetracyclines have been used as a bone label due to their ability to form
stable tetracycline-calcium chelates, which fluoresce under UV light.
Osteogenesis involves organic matrix synthesis by osteoblasts and its
subsequent mineralization to form bone, a process in which new matrix requires
a defined period of time to become calcifiable (Garner et al., 1996). Fluorescence
bone markers such as tetracyclines, label instantaneously and permanently the
zone of demarcation of osteoid tissue from mineralized bone (Tam et al., 1980).
The amount of bone growth is then calculated by measuring the distance
between fluorescence bands that have been formed at the time of the marker
being deposited.

1.4.6. Use of Tetracyclines in Swine Production

Since their discovery in 1945, tetracyclines have been used in veterinary
medicine as broad-spectrum antibiotics that may be used in the treatment and
prophylaxis of many common infectious diseases and as growth promoters. They
have been widely used in swine production for over six decades as additives in
feed or water to treat and prevent disease, to increase animal growth or weight,
and to reduce the feed required per pig (Giguère, 2006).

The Compendium of Medicated Ingredient Brochures (CMIB) is the
document that lists those medicating ingredients permitted by Canadian
regulation to be added to livestock feed. In swine, chlortetracycline and
Oxytetracycline are approved in feed rations (meal or pellet feed) as an aid in the treatment or prevention of bacterial enteritis, maintaining weight gains in the presence of atrophic rhinitis or stress, as well as in the prevention of porcine proliferative enteropathy and abortion caused by leptospirosis. When used as feed additives, levels of 5.5 mg/kg and 55 to 550 mg/kg of feed are permitted to promote growth and to treat specific diseases, respectively, if a mandatory withdrawal period of 7 days is completed before animals are slaughtered for food (Canadian Food Inspection Agency, 2008). Detailed information regarding approved brands, levels, doses, and withdrawal times are presented in Tables 1.1 and 1.2. In addition to the use of tetracyclines according to the label claims and dosages approved by the regulatory agencies, tetracyclines may be used in an off-label manner if prescribed by a veterinarian. In this case, plasma drug concentration can be predicted and correlated to the Minimum Inhibitory Concentration (MICs) for the targeted bacteria to ensure an effective treatment strategy. Researchers at the University of Montreal created a multidosage pharmacokinetic model to predict the minimum in-feed dosage of CTC and OTC (in ppm) that would maintain the minimum drug concentration in plasma over a 20-hour interval (del Castillo et al., 1998). Plasma concentrations of CTC and OTC predicted by the model demonstrated that in-feed medication would rarely exceed the sensitive MIC used to evaluate potential drug activity against swine respiratory pathogens (MIC ≤ 2 µg/ml) (del Castillo et al., 1998; Apley, 2010). Plasma concentrations equivalent to this MIC were not even reached when pigs
were treated with CTC and OTC levels above 1,000 ppm (del Castillo et al., 1998; Kilroy et al., 1990).

A comparative feed pharmacokinetic of CTC and OTC demonstrate an enhanced plasma and tissue diffusion for CTC with intake of medicated feed, which can be explained by the greater lipid solubility of CTC. Thus, higher concentrations of CTC are expected in plasma and lung (about 30% greater than that of OTC) (del Castillo et al., 1998). Another fact that makes CTC a better choice for the treatment of respiratory diseases when compared to OTC is its greater steady-state average plasma concentrations obtained from medicated feed (del Castillo et al., 2000).

In North America, most pigs receive antimicrobials in-feed after weaning (starter rations), when they are most vulnerable to infectious diseases (Dewey et al., 1997; McEwen and Fedorka-Cray, 2002; Dunlop et al., 1998). Over 95% of weanling pigs are fed medicated starter rations and tetracyclines are among the most commonly used antibiotics in Ontario swine operations (McEwen and Fedorka-Cray, 2002; Dunlop et al., 1998). Antimicrobial treatment practices of grower-finisher pigs have also been reported and this may be a concern regarding drug residues in pork. Dunlop et al (1998) reported the overall percentage of Ontario pigs exposed to antimicrobials from finisher rations, and water to be 48% and 37%, respectively. In Alberta, the use of in-feed antibiotics in grower and finisher rations were reported in 90.9% and 80% of the swine operations, respectively, while the use of antibiotics through water was less frequent (20.5% and 17.8% for grower and finisher pigs, respectively) (Rajić et al,
Chlortetracycline, lincomycin, tiamulin, and tylosin are reported to be the most common type of in-feed antibiotics administered in various production phases (Rajić et al, 200; Rosengren et al, 2008). According to Alberta records, 10.8% and 47.1% of antibiotics used in the grower-finisher phase are intended to prevent disease and promote growth, respectively (Rosengren et al, 2008). Although antimicrobials are mainly given by oral administration for convenience in the case of treating large herds of pigs, intramuscular therapy is also commonly used. Dunlop et al. (1998) reported that about 15% and 6% of the surveyed farms in Ontario indicated that they would market finisher pigs exposed to an injectable and in-feed antimicrobial, respectively, before the appropriate withholding time had lapsed. The practice of injectable antibiotics in swine operations is intended for the treatment of individual sick pigs, and more than 50% of farms in Alberta reported the use of injectable antibiotics in the finisher phase of production (Rajić et al, 2006). Penicillin and oxytetracycline were reported to be the most frequently used antibiotics during the finisher phase (Rajić et al, 200; Rosengren et al, 2008).

1.4.7. Bone Discolouration in Animals Intended for Human Consumption

Bone discolouration or location of pigments in skeletal tissues was first observed in 1763 by John Hunter, a young surgeon who after having noticed a red bone in his meal, found that feeding animals with madder, a vegetable from which a dye known as alizarin is extracted, coloured the bone red as it was being formed and becoming calcified. His host, who was a dye merchant, had fed his
pigs some of the leftover madder from which he extracted dyes (Ham, 1974). After using alizarin, as a vital staining technique to study bone growth, Hunter made a great contribution to bone biology; he was the first to realise that as bones increase in size, they undergo a complex pattern of remodelling and that resorption is a crucial part of bone growth (Meikle, 1997). Since then, vital staining methods for following the deposition and calcification of new bone to determine the amount of bone growth within a certain period of time has been acknowledged.

The incidence of tetracycline residues in bones of slaughtered animals has been examined. Kühne and Ebrecht (1993) advocate fluorescence as a technique to test for residues of tetracyclines in bones. This research group found the prevalence of tetracycline residues in bones of slaughtered piglets, fattening pigs, ducks, chickens, turkeys, and veal calves to be 73%, 70%, 68.6%, 30.3%, 26.8%, and 10.5%, respectively (Kühne et al., 2000; Kühne and Ebrecht, 1993). An additional experimental evaluation on chickens to detect tetracycline residues after different withdrawal periods was performed in the same study; the total amount of oxytetracycline concentration in bones of chickens fed low dosages of oxytetracycline decreased from 26.1 µg to 1.1 µg when the withdrawal time was extended from 1 to 15 days, and no residues were observed after 25 days (Kühne et al., 2000). Although the intensity of fluorescence in bones differed among species, the intensity of fluorescence was in accordance with tetracycline concentrations and furthermore, fluorescence was not detected in samples from chickens not fed tetracyclines, or meat and bone meal. Bound
tetracycline residues in bones represent a potential risk for meat contamination because mechanically deboned meat contains significant amounts of bone splinters (Kühne et al., 2000; Kühne and Körner, 2001).

The effect that chlortetracycline may have on bone colour was investigated in growing pigs fed 880 ppm of chlortetracycline, starting at 28 and 84 days of age for a period of 4 and 8 weeks. This study not only demonstrated the relationship between chronic administration of chlortetracycline and green bone discolouration, but also a higher probability of discolouration as dosing duration and treatment onset increase (del Castillo et al., 2011). Furthermore, the probability of green discolouration decreased with withdrawal time and age at treatment onset interaction; this is explained by the fact that tetracycline-induced discolouration may entirely vanish with observed withdrawal times greater than 8 weeks, but discolouration disappear at slower rate when the age at treatment onset increased (del Castillo et al., 2011).

1.4.8. Liquid Chromatography Method to Detect and Measure Tetracycline Residues in Bone Tissue of Food Producing Animals

Chromatography, a technique used to separate complex samples into their constituent parts, is the most important procedure for isolating and purifying chemicals (Miller, 2005). The word chromatography means “color writing” because it graphically shows the separation of mixtures into a series of coloured bands. The Russian botanist M.S. Tswett, credited with the discovery of chromatography, coined the name chromatography from the Greek colour
(chroma) and write (graphein) to describe the process (Poole, 2003). The method is based on differential migration and involves two phases, the stationary and the mobile phase; molecules (solute) in a sample mixture are transported by the mobile phase over the stationary phase, and as solutes move through the stationary phase, they separate because different components (solute) move at different rates through the stationary phase (Braithwaite and Smith, 1996). In all cases, the sample is dissolved in a liquid that is transported either into or onto the chromatographic device. Chromatography involves a large number of applied methods that are classified based on the phases employed for separation. When the mobile phase is a gas and the stationary phase a solid or liquid, the separation techniques are known as gas chromatography (GSC) or liquid chromatography (LC), respectively (Poole, 2003). The former is a sophisticated analytical technique used to analyze volatile mixtures rather than liquid samples (Poole, 2003). Liquid chromatography can be performed using thin layer, paper, and/or column chromatography. These are simple and low cost methods with high sample capacity and minimal equipment requirements but these methods only provide qualitative results in which the sample separation is represented by different colors or dyes that can be observed either by the naked eye or under UV light (Poole, 2003; Braithwaite and Smith, 1996). A more sophisticated liquid chromatographic method which is also one of the most powerful tools in analytical chemistry is known as high performance liquid chromatography (HPLC). It can be used for qualitative and quantitative analysis of any sample that can be dissolved in liquid including pharmaceuticals and industrial
chemicals, among others (Miller, 2005; Center for Drug Evaluation and Research (CDER), 1994).

High performance liquid chromatography has not only been the adopted method by the Association of Official Analytical Chemists (AOAC) International, but it has also been recommended for determination of chlortetracycline, oxytetracycline, and tetracycline levels in edible animal tissue (MacNeil et al., 1996). HPLC is a liquid chromatographic column technique that uses high performance to generate more accurate results. This is a more powerful method because it quantifies the residues of tetracycline, oxytetracycline, and chlortetracycline in edible tissues, at levels that can be compared with the MRLs set for the tissue and the tetracycline being studied. However, the high tendency of tetracyclines to form chelated complexes is one limitation because when tetracyclines chelate to metal ions, the complex binds with the silanol groups present in the silica sorbents. A collaborative study evaluating results from 13 laboratories reporting a complete set of results found a wide variation in tetracycline recoveries from different lot numbers of solid-phase extraction (SPE) cartridges from the same manufacturer (MacNeil et al., 1996). These differences were more frequent for the concentration of residues in pork kidney than in pork muscle samples leading to the conclusion that although recovery from SPE cartridges remains a problem, the method is reliable to detect tetracyclines in edible animal tissues (MacNeil et al., 1996). Although the method has not been proven to be effective for detection of tetracyclines in bones, the lack of a standardized one makes the HPLC as the first choice for researchers attempting
to measure tetracyclines in bones. Kühne and Ebrecht (1993) demonstrated that adding hydrochloric acid to the mobile phase would result in higher recovery concentration levels of tetracyclines.

German researchers (Körner et al., 2001) investigated tetracycline residues in bone meal by high performance liquid chromatography analysis using three different extraction procedures: succinate buffer, hydrochloric acid, and hydrochloric acid after sedimentation. Extraction with hydrochloric acid was proved to be the best method for detecting OTC, CTC, and the total tetracyclines, while extraction after sedimentation was needed for detecting the highest concentration of TC in bone meals (Körner et al., 2001).

Although tetracycline residues in bone can be detected using the HPLC technique, residues of tetracyclines can still be expected bound to the bone.

1.5. SUMMARY OF THE PROBLEM

Tetracyclines, including oxytetracycline, chlortetracycline and tetracycline have been used for many years in the swine industry to treat and prevent diseases, as well as to promote growth (Health Canada, 2002). Tetracyclines have the ability to form lipid-soluble complexes with metal cations such as Ca$$^{++}$$ forming a tetracycline-calcium complex and as complexes are deposited in growing bones (Buyske et al., 1960) which in some cases may result in green or yellow bone discolouration. The Canadian Food Inspection Agency (CFIA) has declared bone is a quality issue only, which can negatively affect consumer confidence in national and international pork products. However, food safety
concern arises from the possible contamination of meat with microscopic bone fragments during the mechanical deboning process (Körner et al., 2000). To our knowledge, studies reporting the prevalence of pig carcasses showing bone discolouration under regular lighting conditions at the time of slaughter has not been conducted, and thus there is a lack of knowledge regarding the prevalence of discoloured bones, and whether or not this prevalence has changed over time. German researchers evaluated the occurrence of tetracyclines in bones of slaughtered pigs, but evaluation was conducted under ultraviolet (UV) light in not illuminated chilling rooms (Kühne et al., 2000), rather than under regular lighting conditions.

In-growing pigs, chlortetracycline induce a reversible green bone discolouration when withdrawal times greater of 8 weeks are observed (del Castillo et al., 2011); according to this study, the probability of bone discolouration is dependent on dosing duration and treatment onset. However, bone discolouration has not been related to different dosing regimen, type of tetracycline administered, method of drug delivered, and the actual levels of tetracyclines that can be extracted from bone. In order to increase our knowledge regarding bone discolouration it would be important to establish the relationship between medicated programs regularly used in Ontario for grower-finisher pigs and the probability of yellow bones at slaughter.
1.6. **SPECIFIC RESEARCH OBJECTIVES**

a) To determine the prevalence of bone discolouration of pig carcasses at a large Ontario abattoir and to investigate whether or not the prevalence of carcasses with discoloured bones has changed over time.

b) To establish whether the discolouration in the bone of pig carcasses at slaughter is related to the dosage, duration of exposure, type of tetracycline, and method of drug delivery, and to determine the concentrations of tetracyclines in bone using HPLC.

c) To investigate the association between medicated feeding programs for grower-finisher pigs with the likelihood of a shipment of pigs to slaughter having carcasses with discoloured bones.
References


Table 1.1. Approved Brands, Claims, Levels, Directions and Withdrawal Times for Chlortetracycline Hydrochloride as Listed in the Compendium of Medicating Ingredient Brochures

<table>
<thead>
<tr>
<th>Brands</th>
<th>Claim</th>
<th>Level (Complete feed)</th>
<th>Directions</th>
<th>Withdrawal time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aureomycin 50</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Growth promoter</td>
<td>5.5 ppm</td>
<td>Sole ration (starter, grower and finisher)</td>
<td></td>
</tr>
<tr>
<td><strong>Aureomycin 110 G</strong></td>
<td>Prevention of porcine proliferative enteropathy (ileitis) caused by Lawsonia Intracellularis sensitive to chlortetracycline hydrochloride</td>
<td>22 mg/kg of body weight/day</td>
<td>Continuously as the sole ration for 14 days</td>
<td>7</td>
</tr>
<tr>
<td><strong>Aureomycin 220 G</strong></td>
<td>Prevention of porcine proliferative enteropathy (ileitis) caused by Lawsonia Intracellularis sensitive to chlortetracycline hydrochloride</td>
<td>5.5 ppm</td>
<td>Sole ration (starter, grower and finisher)</td>
<td></td>
</tr>
<tr>
<td><strong>Chlor 50</strong></td>
<td>Maintaining/gain weight &amp; appetite stimulant</td>
<td>55 ppm</td>
<td>Sole ration up to 32Kg</td>
<td>7</td>
</tr>
<tr>
<td><strong>Chlor 100</strong></td>
<td>Atrophic Rhinitis</td>
<td>110 ppm</td>
<td>Sole ration from appearance of symptoms up to 3 days after symptoms disappear</td>
<td>7</td>
</tr>
<tr>
<td><strong>Stress</strong></td>
<td></td>
<td>110 ppm</td>
<td>Sole ration during stress period up to 10 days after stress condition has been eliminated</td>
<td>7</td>
</tr>
</tbody>
</table>

<sup>a</sup>: not indicated for prevention of proliferative enteropathy.
Table 1.2. Approved Brands, Claims, Levels, Directions and Withdrawal Times for Oxytetracycline Hydrochloride as Listed in the Compendium of Medicating Ingredient Brochures

<table>
<thead>
<tr>
<th>Brands</th>
<th>Claim</th>
<th>Level (Complete feed)</th>
<th>Directions</th>
<th>Withdrawal time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terramycin-50</td>
<td>Bacterial enteritis</td>
<td></td>
<td>Sole ration: early growth up to 27-31 Kg</td>
<td>7</td>
</tr>
<tr>
<td>Terramycin-100</td>
<td>Prevention</td>
<td>55 ppm</td>
<td>Continuously as the sole ration since appearance of symptoms until 3 days after symptoms disappear.</td>
<td>7</td>
</tr>
<tr>
<td>Terramycin -200</td>
<td>Treatment</td>
<td>110 ppm</td>
<td>Sole ration until symptoms disappear.</td>
<td>7</td>
</tr>
<tr>
<td>Oxysol 110</td>
<td>Maintaining/gain weight &amp; appetite stimulant</td>
<td></td>
<td>Sole ration during stress period up to 10 days after stress condition has been eliminated</td>
<td>7</td>
</tr>
<tr>
<td>Oxysol 220</td>
<td>Atrophic Rhinitis</td>
<td>55 ppm</td>
<td>Sole ration to pregnant and affected sows for a 2 week period. Only when abortion due to Leptospirosis has been diagnosed.</td>
<td>7</td>
</tr>
<tr>
<td>Oxysol 440</td>
<td>Stress</td>
<td>110 ppm</td>
<td>Sole ration to pregnant and affected sows for a 2 week period. Only when abortion due to Leptospirosis has been diagnosed.</td>
<td>7</td>
</tr>
<tr>
<td>Oxytetracycline 50</td>
<td>Reduce incidence of abortion caused by Leptospirosis</td>
<td>550 ppm</td>
<td>Sole ration to pregnant and affected sows for a 2 week period. Only when abortion due to Leptospirosis has been diagnosed.</td>
<td>7</td>
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<tr>
<td>Oxytetracycline 100</td>
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<td>Oxytetracycline 200</td>
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<td>Oxy – 110</td>
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<td>Oxy – 220</td>
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<td>Oxy – 440</td>
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Figure 1.1. Basic Molecular Structure of the Main Tetracycline Antibiotics

Functional Groups of the lower peripheral region of bioactive tetracyclines:
- diketo-enol system across C11 and C12 between Rings C:B
- tertiary hydroxyl group at the lower bond between rings B:A
- tricarbonil-methane system, an amide bonded to the middle of a β-diketone system spread across C1 and C3.

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<tr>
<th>Congener</th>
<th>Substituent(s)</th>
<th>Position(s)</th>
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<tbody>
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<td>N(CH₃)₂</td>
<td>R</td>
</tr>
<tr>
<td>C₂₂H₂₃N₂O₈Cl</td>
<td>H</td>
<td>R₄</td>
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<td></td>
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<tr>
<td>Oxytetracycline</td>
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<td>C₂₂H₂₄N₂O₉</td>
<td>OH</td>
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<td>R₁</td>
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Source: del Castillo, 2001; Nelson, 1998
CHAPTER 2: The Prevalence of Carcasses with Discoloured Bones at an Ontario Abattoir

ABSTRACT

Bone discolouration in pig carcasses caused by exposure to tetracyclines during the growth phase is a quality issue faced by meat packers. The objectives of this preliminary study were to investigate the prevalence of carcasses with discoloured bones at one large abattoir in Ontario over several years, and to examine the proportion of positive carcasses per shipment lot. The study was conducted in order to verify the hypothesis that bone discolouration is a common problem in the Ontario pork industry. Swine carcasses were visually inspected one day after slaughter by plant personnel to identify those carcasses with discoloured bones. The prevalence of carcasses with bone discolouration ranged from 0.13% over a 9-month period in 2006 to 0.28% for an 11-month period in 2010. A wide variation in the proportion of carcasses with discoloured bones amongst shipment lots was observed. On one shipment from a single source, 84% of the carcasses had discoloured bones but a number of shipment lots had a single positive carcass. It was concluded that bone discolouration was present at one large Ontario abattoir over the 4-year period of the study and that the prevalence did not appear to be decreasing.
2.1. INTRODUCTION

Bone discolouration of pig carcasses is a quality concern for the meat packing industry. Carcasses with bone discolouration must be handled separately at the abattoir adding to the expense of processing and hence essentially lowering the economic returns to the swine industry. In Canada, it has been suggested that this problem might have increased in prevalence and severity in late 2005 as a result of a severe outbreak of porcine circovirus type 2 (PCV2) infection which caused increased medication of herds in an attempt to control secondary bacterial infection (Guillot et al., 2011). The severe losses associated with PCV2 infection suddenly stopped as a result of the introduction of effective vaccines in late 2006; it can be assumed that vaccination made a contribution to the well-being of the swine population, and thus the use of tetracycline-related medication in grower-finisher pigs to battle secondary infections commonly associated with PCV2 might have also decreased.

Tetracyclines are broad-spectrum antibiotics that are widely used for mass medication in feed or water to control secondary bacterial infection in grower-finisher pigs. The accumulation of tetracyclines within bone via chelation of calcium ions leading to discolouration has been long recognized (Nelson, 1998, Buyske et al., 1960). Researchers have shown that the discolouration of bone is reversible after the administration of tetracyclines is stopped but the process may require several weeks or months (Guillot et al., 2011). The presence of bone discolouration is not associated with residue problems in the meat or organ tissue (Kühne et al., 2000).
It is hypothesized for this study, that the prevalence of carcasses with discoloured bones is a common problem in Ontario pork. The primary objective of this preliminary research was to determine the prevalence of bone discolouration of pig carcasses at a large Ontario abattoir and to investigate whether or not the prevalence of carcasses with discoloured bones has changed over time. Carcass was identified as the unit of concern.

2.2. MATERIALS AND METHODS

Data for this study were obtained from one large federally inspected abattoir in Ontario. Records containing seller identification, date of shipment, the total number of pigs in a shipment lot, the total number of discoloured carcasses per shipment lot, and the total number of pigs inspected per month were provided for a 9-month period in 2006. The total number of pigs in a shipment lot and the total number of discoloured carcasses within the same shipment lot were obtained for various time periods during 2008, 2009, and 2010. Shipment lot is defined as the total number of pigs from a batch being shipped to the slaughter plant at the same time.

After slaughter, carcasses were placed in coolers immediately after being dressed and kept refrigerated for 24 h. Plant employees waited one day before inspection because it was believed that mild discolouration might disappear during the overnight cooling process. Assessment was based on subjective visual inspection by different plant personnel trained to identify discolouration. A carcass was considered to have discoloured bones if unacceptable yellow or
green spots on the ribs or vertebrae were visible under incandescent light. Inspection and recording of carcasses with discoloured bones was done at the plant’s discretion and there were periods of time from 2006 to 2010 when inspection of carcasses for discoloured bones was not performed or the results were not recorded. Information on bone discolouration was recorded in 2006 from March to November, in 2008 from October to December, in 2009 from January to December (but not July), and in 2010 from January to May.

2.2.1. DATA HANDLING AND ANALYSIS

Data were entered into an electronic spreadsheet program Excel™ (Microsoft) for editing and manipulation, and then transferred into STATA software (StataCorp, Texas, USA, version 9.2) for statistical analysis.

Prevalence on a monthly basis was estimated by dividing the total number of carcasses identified with discoloured bones in a month by the total number of pigs slaughtered within the same month. The prevalence of discoloured bones on shipment basis was estimated by dividing the total number of pigs within a shipment lot from which bone discolouration was observed at slaughter by the total number of pigs within the same lot. Prevalence, standard errors, and confidence intervals of prevalence were estimated using the “cii immediate command in STATA” used for variables distributed as binomial. A two-sample test of equality of proportions was used to compare prevalence between the period in which vaccine against PCV2 was not fully offered (2006), to the following years when data were available (2008, 2009, 2010).
2.3. RESULTS

The overall prevalence of yellow bones during the periods of 2006, 2008, 2009, and 2010 when data were available was 0.13% (1,920 of 1,505,945), 0.22% (1,180 of 535,228), 0.26% (5,355 of 2,027,904), and 0.28% (2,097 of 759,957), respectively, resulting in 95% confidence interval of 0.122% - 0.133% for 2006, 0.208% - 0.233% for 2008, 0.257% - 0.271% for 2009 and 0.264% - 0.288% for 2010. Carcasses with discoloured bones were identified through all the months included in the study. The prevalence of carcasses with discoloured bones differed by month showing different trends in the years where there were sufficient data to compare (Figure 2.1). Overall, an apparently significant increase in prevalence over time was observed. A comparison between the prevalence during March to May 2006 to the same period of time in 2009 and 2010 indicated a significant increase in the proportion of carcasses determined to have discoloured bones ($z = -15.17; P < 0.001$ and $z = -12.03; P < 0.001$, respectively). Likewise, the prevalence of discoloured carcasses significantly increased during October to November 2008 and August to November 2009 when compared to the same period in 2006 ($z = -16.73; P < 0.001$ and $z = -28.40; P < 0.001$).

In 2006, a total of 69 farms shipped 460 lots of pigs identified as having carcasses with bone discolouration at slaughter. A total of 52,153 pigs were shipped within the 460 lots. There was a wide variation in the prevalence of carcasses with discoloured bones amongst those shipment lots with one or more
positive carcasses (from 0.3 to 83.6%). A few positive lots had single animals showing discoloured bones.

2.4. DISCUSSION

According to the Canadian Food Inspection Agency (CFIA) discoloured areas greater than 1½” (3.8 cm) in diameter in bones of pork carcasses, and numerous stains in one sample are considered a major product quality defect (Canadian Food Inspection Agency, 2011). The major concern associated with this defect is that carcasses with bone discolouration must be handled separately adding to the expense of processing and lowering the economic returns to the swine industry. Results from this study indicated that the prevalence of discoloured bones in pig carcasses at this particular abattoir is a common issue that has been observed during the last 5 years. It has been suggested (Guillot et al., 2011) that the problem arose in the fall of 2005 because of the use of tetracyclines to treat secondary bacterial infections during the sudden emergence of Porcine Circovirus-Associated Disease (PCVAD) in Ontario and Quebec in 2005 and 2006 (Carman et al., 2008). However, although the prevalence of PCVAD dramatically decreased in 2007 due to the widespread use of effective porcine circovirus vaccines, the prevalence of carcasses with discoloured bones did not correspondingly decrease. It seems that the prevalence of carcasses exhibiting bone discolouration has not decreased over the last 4 years of this study.
Bone discolouration has been associated with the presence of tetracyclines in bones of treated animals (Guillot et al., 2011). In the present study, the proportion of discoloured bones varied widely among lots, and a few positive lots had single animals with discoloured bones. It has been reported that even small doses of injectable oxytetracycline results in bone deposition lasting for long periods of time (Milch et al., 1957). One can assume that often pigs in the same lot have had similar exposure to tetracycline when mass medication through feed or water is used. Thus, the presence of one or two animals with discoloured bones within a lot may indicate individual exposure to injectable oxytetracycline (OTC). In contrast, we hypothesize that the presence of a greater number of pigs with discoloured bones within a lot may be suggestive of mass medication and therefore one can expect that residues of tetracyclines may also be present in the bones of pigs from the same lots whether or not discolouration is present. However, further studies are needed to evaluate dosages we could expect tetracycline residue in bones, and the chemical form of tetracycline most likely to be deposited in bone.

Assessment of pork carcasses in this study was based on subjective visual inspection completed under the light conditions present in the cooler and additional spotlighting or ultra-violet lighting was not used. Over time, different plant personnel conducted the inspection, and this may be a source of assessment bias because some of the employees may be better at detecting discolouration than others.
The presence of tetracyclines in normal appearing bone can be detected using ultraviolet light and observing fluorescence (Buyske et al., 1960). German researchers have used this technique in an attempt to monitor tetracycline residues in bones of slaughtered animals (Kühne et al., 2000; Kühne and Ebrecht, 1993; Körner et al., 2001; Kühne, 1998), and reported a relatively high prevalence of fluorescence of bones from carcasses which had passed meat inspection (70%, 10%, and 73% positive fluorescence for market hogs, sows, and young pigs, respectively) (Kühne et al., 2000).

Although the presence of tetracyclines in bones has not been associated with residues of these antimicrobials in muscle or organ tissue, toxicological implications emerge from the possible contamination of mechanically deboned meat with microscopic bone fragments and the use of inappropriate temperatures for the destruction of tetracyclines in the preparation of meat products (Körner et al., 2001; Kühne et al., 2001). Further investigation to establish the potential risk to human health of consumption of products containing particles of discoloured bones has been recommended (Guillot et al., 2011).

The lack of information about antimicrobial drug use on farms that shipped pigs with discoloured bones limits any conclusion about the use of tetracyclines and the prevalence of the condition. Further studies investigating drug management practices on farms identified as sources of pigs with bone discolouration at slaughter are needed in order to relate the use of tetracyclines as a risk factor for bone discolouration.
References


Figure 2.1. Monthly Prevalence of Pig Carcasses Detected with Discoloured Bones at One Ontario Abattoir from 2006 to 2010*.

* Data on discoloured bones was not collected for 2007.
Absence of columns represents months in which assessment of carcasses with discoloured bones was not performed or data not recorded.
CHAPTER 3: An Investigation of Residues of Tetracyclines in Pig Bones

ABSTRACT

A trial was conducted to investigate the effect of exposure to tetracyclines on residue and bone colour. Pigs were randomly allocated to one of six treatment groups and one pen served as an untreated control group. The first four groups received chlortetracycline (CTC) in the feed at either 110 ppm or 660 ppm and for either 3 wk or 12 wk. Group five was given oxytetracycline (OTC) IM for 3 d and slaughtered 54 d later. Group six received water medication containing tetracycline (TC) HCl for 5 d and slaughtered 56 d later, and Group seven served as untreated control. Muscle, liver, kidney, and bone samples were tested for tetracyclines using high performance liquid chromatography (HPLC).

Fluorescence was observable in bones from all groups, except those from the control group. Under normal light conditions, discolouration was observable only in bones from pigs treated with 660 ppm CTC for 12 wk. No residues of tetracyclines were found in muscle, liver, and kidney samples. Concentrations of CTC of 16±6 ppm in bone were recorded for pigs fed a ration containing 660 ppm for 3 weeks and shipped 54 d after the drug was withdrawn, compared to a mean level of 22±9 ppm CTC for discoloured bones from a packing plant. This work shows that there can be relatively high levels of tetracyclines still present in bone after withdrawal periods over 50 d, even when discolouration is not obvious.
3.1. INTRODUCTION

Tetracyclines, including chlortetracycline (CTC), oxytetracycline (OTC) and tetracycline (TC) are widely used in the swine industry and have been for many years. CTC and to a lesser extent OTC are often added to feed and used for growth promotion and prophylaxis (Dunlop et al., 1998). Such medicated feed is usually fed for several weeks. Tetracyclines are also commonly used in the treatment of disease, using high dosages in feed or water to medicate a pen or barn, and OTC may be used as an intramuscular injection for individual treatment.

For a long time it has been known that tetracyclines are active metal chelators that form complex compounds with calcium and are deposited in rapidly growing bones (Buyske et al., 1960).

Residues of tetracyclines have been reported to be commonly present in bones of pigs at the time of slaughter even though levels of these antibiotics in other tissues such as muscle and kidney are undetectable (Körner et al., 2001., Kühne et al., 2001., Kühne et al., 2000., Duong et al., 2006), or below the maximum residue limits (MRLs) defined by the Veterinary Drugs Directorate (VDD) of Health Products and Food Branch, Health Canada. The presence of residues of tetracyclines in bone at the time of slaughter raises two issues of concern. Firstly, there is a food safety concern because mechanical de-boning techniques can result in microscopic bone fragments being present in ground pork (Kühne et al., 2000., Kühne et al., 2001). Secondly, there is a cosmetic concern because tetracyclines can cause bone discolouration and this may lead
to consumer resistance to purchase certain cuts of meat (König and Tontis, 1995). However, tetracyclines may be present in bones that are not discoloured.

Measuring the level of tetracyclines in bone is challenging because these antibiotics are tightly bound and not easily extracted for analysis (Buyske et al., 1960, Milch et al., 1957, Milch et al., 1958). One simple screening method is the use of ultra-violet (UV) light. In order to quantify the levels of residue other procedures are necessary. Researchers have generally used high performance liquid chromatography (HPLC) (Körner et al., 2001, Kühne et al., 2001, Kühne et al., 2000, Sokol et al., 1994) although standardized procedures still need to be developed. Innovative non-invasive methods have also been used to estimate residue levels. Guillot et al. (2011) demonstrated the value of using quantitative computed tomography and dual energy x-ray absorptiometry to assess bone mineral density. It was shown that CTC caused a persistent increase of bone mineral density and this was dependent on dosing regimen.

The main objectives of this study were; to establish whether the yellow discolouration in the bone of pig carcasses is related to the dosage, duration of exposure, type of tetracycline, and method of drug delivery, and to determine the concentrations of tetracyclines in bone using HPLC for both experimentally medicated pigs and pigs sent to a commercial slaughter plant.

3.2. MATERIALS AND METHODS

This study was approved by the University of Guelph Animal Care Committee and conducted according to the guidelines and policies of the
Canadian Council of Animal Care. The study consisted of two parts. The first part relates to the administration of tetracyclines (OTC by injection, CTC as a feed medication and TC via water), slaughter of pigs, and bone assessment by visual inspection under natural light and ultraviolet (UV) light for signs of discoloration and fluorescence, respectively. The second part consists in the use of HPLC to estimate the levels of tetracyclines in edible tissue (muscle, liver and kidney) and the bones of the treated pigs, as well as in bones from pigs identified in the commercial slaughter plant with discoloration (case material).

3.2.1. Part 1 - Controlled Trial to Compare Tetracycline Exposure with Bone Discolouration and Fluorescence at Slaughter

Thirty-five weaned Yorkshire barrows were used in a trial conducted at the Arkell Research Station, University of Guelph, Ontario, Canada. Pigs were followed from birth until placed on the trial to ensure they received no tetracyclines during this period. Pigs were randomly assigned to seven treatment groups, including one control group (5 pigs per group) by using a phone book as a random number generator. Different dosages of tetracycline were administered using different treatment periods and different methods of drug delivery.

Group 1 (CTC in feed at high dose for long duration)

Pigs were fed a ration containing 660 ppm (26.4 mg/kg BW/day) of CTC (Aureomycin® 220 G, Alpharma, Mississauga, ON) for 12 weeks beginning when the pigs were approximately 8 weeks old and weighing approximately 20 kg (with
a feed consumption of about 3.2 kg/day). Pigs were slaughtered 33 days after medicated feed was stopped.

**Group 2 (CTC in feed at high dose for short duration)**

Pigs were fed a ration containing 660 ppm (26.4 mg/kg BW/day) of CTC (Aureomycin® 220 G, Alpharma, Mississauga, ON) for 3 weeks beginning when the pigs were about 11 weeks old and weighing approximately 33 kg (with a feed consumption of about 2.2 kg/day). Pigs were slaughtered 54 days after medicated feed was stopped.

**Group 3 (CTC in feed at low dose for long duration)**

Pigs were fed a ration containing 110 ppm (4.4 mg/kg BW/day) of CTC (Aureomycin® 220 G, Alpharma, Mississauga, ON) for 12 weeks beginning when the pigs were about 8 weeks old and weighing approximately 20 kg (with a feed consumption of about 3.2 kg/day). Pigs were slaughtered 33 days after medicated feed was stopped.

**Group 4 (CTC in feed at low dose for short duration)**

Pigs were fed a ration containing 110 ppm (4.4 mg/kg BW/day) of CTC (Aureomycin® 220 G, Alpharma, Mississauga, ON) for 3 weeks beginning when the pigs were about 11 weeks old and weighing approximately 33 kg (with a feed consumption of about 2.2 kg/day). Pigs were slaughtered 54 days after medicated feed was stopped.
Group 5 (Injectable OTC)

Pigs were injected with 6.67 mg per kg body weight of OTC hydrochloride (Oxy LP, Citadel Animal Health, Lavaltrie, QC) once a day for 3 days beginning when the pigs were about 14 weeks of age and weighing about 45 kg. Pigs were slaughtered 54 days after the last treatment.

Group 6 (Water medication with TC)

Pigs were provided with medicated water as the sole source of water for 5 days beginning when the pigs were about 14 weeks of age and weighing about 45 kg. TC HCL (Onycin 1000, Vetoquinol, Lavaltrie, QC) was mixed with water at the rate of 1 g of powder (containing 1000 mg of TC) to 8 L of water, that is, pigs received on average 12.5 mg/kg body weight of TC per day. Pigs were shipped 56 days after the end of treatment.

Group 7 (No medication – Control group)

Pigs were not exposed to tetracyclines in any form and housed in a separate pen so that manure and urine from treated pigs were not allowed to contaminate their environment.

Pigs were slaughtered at the University of Guelph abattoir in the Department of Animal and Poultry Science. At the time of slaughter the open carcass was visually inspected for evidence of discoloured bones. Muscle, liver, kidney, and bone (the right metatarsal bones) samples were collected and packed in individual plastic bags labelled with the corresponding pig identification number.
Bones were placed for approximately 1 month in steel containers holding dermestid beetles to ensure thorough cleaning of soft tissue from the bones. Clean bones were assessed for discolouration by subjective visual inspection and fluorescence under UV light (360 nm). Inspection was conducted by two individual observers who classified discolouration as high, intermediate, or moderate intensity.

3.2.2. Part 2 – Tetracycline Residues in Edible Tissue and Bones as Determined by HPLC

Muscle, liver, kidney, and bone samples from animals in part 1 of this study were tested for tetracycline residues (CTC, OTC, and TC), using High Performance Liquid Chromatography (HPLC) at the Laboratory Services Division (LSD), University of Guelph. Bone samples consisted of bones from some of the pigs treated with tetracyclines as well as non-treated controls (Part 1 of this study) and five bone samples from pigs identified at a commercial abattoir as having a high degree of discolouration (case material).

Bones were sawed into fragments and ground using a Retsch mortar grinder (Retsch Inc. Newtown, Pennsylvania, USA), and 5 g of bone fragments were weighed into 50-ml centrifuge tubes and stored frozen prior to analysis. Samples were taken from the freezer and 10 ml of 1 M hydrochloric acid (HCl) was added to each tube and vortexed for 10 seconds to mix well. All the tubes, were placed in a refrigerated water bath at 8°C (4:30 pm) and hydrolyzed overnight. After hydrolysis, samples were removed from the water bath and centrifugated at 2500
g for 10 min. The supernatant was passed through a paper filter, and its pH adjusted to 4.0 with sodium hydroxide (NaOH). The filtered extract was cleaned up on C\textsubscript{18} solid-phase extraction (SPE) column. Tetracyclines were separated using C\textsubscript{8} reversed-phase deactivated silica packing column, and detected with a UV detector set at 350 nm wavelengths. This procedure was based on the method adopted by the Association of Analytical Chemists (AOAC) International for extraction of CTC, OTC, and TC from edible animal tissue (MacNeil et al., 1996). For the calibration curves, predetermined concentrations (0.05, 0.1, 0.25, and 1 ppm) of CTC, OTC, and TC standard solutions were injected into the chromatography column and the sample detected at 350 nm wavelength. The calibration curve was produced by plotting the peak area against concentration, and the relevant tetracycline concentration was then calculated by measuring the peak area. Similarly, a negative bone sample (never exposed to any of the tetracyclines) was spiked to predetermined concentrations (0.05, 0.1, 0.25, and 1 ppm) of CTC, OTC, and TC standard solutions and determination of the recovery from hydrolyzed bone sub-sample was obtained.

3.3. DATA HANDLING AND ANALYSIS

Data were entered into Excel (Microsoft Corporation, One Microsoft Way Redmon, WA, USA) and transferred into SAS statistical software (SAS Institute Inc. 2005, Cary, North Carolina, USA, Version 9.1.3) for statistical analysis. Descriptive statistics, means and standard deviations were calculated for the amount of tetracyclines recovered in each group. Levels of tetracyclines (ppm)
recovered from pigs exposed to different tetracycline treatments were analyzed by the Kruskal-Wallis test, a non-parametric alternative to one-way ANOVA method for testing equality of population medians among groups.

3.4. RESULTS

Tetracyclines (CTC, OTC, and TC) were not present at detectable levels in muscle, liver and kidney tissue. All bone samples from study pigs treated with tetracyclines showed fluorescence under UV light, as well as all the samples from the packer. The degree of fluorescence varied across treatments. Intensity of fluorescence was highest in samples from pigs fed CTC, intermediate in samples from pigs treated with OTC by injection and minimal in samples from pigs treated with TC in the drinking water. Among pigs fed CTC, the highest degree of fluorescence was observed in samples from pigs in Group 1 (high dose, long duration), then Group 3 (low dose, long duration), followed in intensity by Groups 2 (high dose, short duration) and 4 (low dose, short duration). The degree of fluorescence was consistently high in all case material. In addition to having the highest level of fluorescence under UV light, the bones from pigs in Group 1 were also judged to be discoloured (yellowish-green) when viewed under natural lighting conditions.

Bones from two groups of pigs exposed to long periods of chlortetracycline consumption (660 and 110 ppm for 12 weeks) were not analyzed by HPLC because of an accident during processing. The levels of TC, CTC, and OTC detected by HPLC from bones are presented in Table 3.1.
The levels (ppm) of CTC in Group 2 (mean = 16.75; sd = 5.19) (660 ppm for 3 weeks) did not differ from the levels (ppm) of CTC of discoloured bones from the packing plant (mean = 22.5; sd = 9.87) ($\chi^2 = 0.74$; $P = 0.39$). Likewise TC (ppm) measured in bones from pigs in Group 6 (mean = 2.54; sd = 0.78) (water medication with TC) did not differ from those found in discoloured bones from the packing plant (mean = 3.2; sd = 1.93) ($\chi^2 = 0.18$; $P = 0.68$).

3.5. DISCUSSION

Discoloured bones in slaughtered pigs have been reported sporadically from various countries (Körner et al., 2001; Kühne et al., 2000; Sokol and Matisova, 1994; Guillot et al., 2011; Kühne and Ebrecht, 1993). At present the Canadian Food Inspection Agency (CFIA) considers this a cosmetic issue (Meat Hygiene Directive 2006 – 12, March 10, 2006). However, there is still an economic cost to the swine industry because pigs identified with abnormal appearance of the bones are generally processed differently, with the bone being removed (Kühne et al., 2000). One significant finding of this trial was that pigs fed a diet containing 660 ppm of CTC for 12 weeks had a greenish-yellow discolouration of bones at the time of slaughter 33 days after cessation of medicated feed. Quebec researchers (Guillot et al., 2011) have shown discolouration produced by feeding CTC at 880 ppm to pigs beginning at 28 d or 84 d and for either 28 d or 56 d. They showed that discolouration increased with dosing duration and age at treatment onset, but was reversible if an extended withdrawal time was observed.
Possibly a more serious issue than the appearance of the bone is the presence of tetracycline residues in bone of pigs at the time of slaughter. At present there are maximum limits set for the presence of tetracyclines in muscle, liver and kidney (0.2, 0.6, and 1.2 ppm, respectively) (World Health Organization: Joint FAO/WHO Expert Committee on Food Additives., 1998). The levels recorded in the bones of pigs examined in this trial far exceeded these levels. Bone is not regarded as an edible part of the pig and therefore the presence of residues has been ignored. However, concern has been raised regarding the presence of microscopic pieces of bone that are found in ground pork when mechanical de-boning techniques are used (Kühne et al., 2000., Varnam et al., 1995). Similarly, there have been concerns about the use of bone meal, a common ingredient in animal and poultry feeds. Studies that have examined the rendering process have raised concerns that the heating process may not destroy tetracyclines and that there is a risk that bone meal from pigs may pose a risk of contaminating livestock and poultry feed with low levels of this antimicrobial drug.

We used two methods to evaluate the presence of tetracyclines in bones. Firstly, we examined bone by UV light and subjectively noted the presence or absence of fluorescence. This technique has been used for many years and is considered a useful screening test (Kühne et al., 2000).

We also attempted to quantify the concentrations of tetracyclines and to determine the chemical form of the tetracyclines. For this we used HPLC. The bone needed to be cleaned of all muscle, ground to a powder to maximize
surface area and then treated with acid to break the tetracycline-calcium bounds (Kühne and Körner, 2001). Although HPLC is a reliable method for determination of CTC, OTC, and TC in edible animal tissue (MacNeil et al., 1996), it has not been proven to be an effective method for detection of tetracycline in bone. The presence of residual silanol groups and metals on the surface of the silica packing often interact with tetracyclines resulting in imperfect recoveries or chromatograms (Anderson et al., 2005). To overcome this issue, Kühne and Ebrecht (1993) added hydrochloric acid to the mobile phase and demonstrated that bound chlortetracycline residues in bones can be released under acidic conditions. The ability of the HPLC for detecting tetracycline residues in meat and bone meal was further investigated using three different extraction procedures: succinate buffer, hydrochloric acid, and hydrochloric acid after sedimentation (Körner et al., 2001). The extraction using hydrochloric acid was reported to be the most effective extraction procedure for OTC and CTC. Extraction after sedimentation was the best method for detecting TC (Körner et al., 2001). HPLC using hydrochloric acid was used in this study to recover tetracycline residues from bones of pigs treated with tetracyclines. The method did distinguish between the three different molecules of tetracyclines used in this trial in that pigs given CTC, TC, and OTC recorded high levels of CTC, TC, and OTC, respectively; however, it may be possible that the levels reported in this study are low because some of the tetracyclines may have remained bound to the bone.

The discoloured bones from the packing plant had levels of CTC comparable
to the group in our trial fed 660 ppm for 3 weeks and slaughtered 54 days after the CTC was stopped. This duration of treatment and level of drug has been quite commonly used in the grower-finisher period, particularly during outbreaks of porcine circovirus associated disease (PCVAD) that occurred between 2005 and 2007 in Ontario. During that period mass medication of tetracyclines were commonly used to control secondary bacterial disease in grower pigs. This time period also corresponded to reports of discoloured bones at packing plants and triggered the release of two directives on the issue by the CFIA (Meat Hygiene Directive 2006 – 01 dated January 4, 2006 and Meat Hygiene Directive 2006 – 12 dated March 10, 2006) (see Appendix).

Although pigs fed CTC were exposed to lower daily levels than those treated via water with TC (4.3 mg/kg BW and 12.5 mg/kg BW, respectively), the levels of CTC recovered from bones from pigs fed CTC were higher than those of TC in bones from pigs treated orally (5.46 ppm and 2.54 ppm, respectively). However, exposure to CTC was longer than exposure to TC (21 d and 5 d, respectively).

Previous studies have reported higher lipid solubility of CTC when compared to OTC and TC (del Castillo et al., 1998; Nielsen and Gyrd-Hansen, 1996). Furthermore, Mason et al (2009) studied the pharmacology of tetracycline water medication in swine and demonstrated that TC oral bioavailability is low. The higher lipid solubility of CTC allows it to cross the blood vessel wall and reach the target tissue resulting in a higher bioavailability when compared to TC. Although absorption of CTC can be improved under dietary acidic conditions (Wanner et al., 1990; Wanner et al., 1991; Pollet et al., 1983), its bioavailability is low when
compared to OTC (Milch et al., 1957). Thus, in all likelihood most cases of discoloured bones in Ontario are related to CTC in feed rather than water medication. The comparatively high levels of OTC found in bones of pigs receiving just 3 injections at the label dose over 50 days before slaughter reflects the high concentrations that are achieved by parenteral administration. In shipments of pigs where only 1 or 2 animals are affected with discoloured bones injectable OTC might be a reasonable suspect as to the source of tetracycline.

Oxytetracycline deposition in bones occurs at a rapid rate after parenteral administration; even when very small amounts of the compound are given (Milch et al., 1957). On the other hand, OTC molecules have lower affinity to calcium ions when compared to TC and CTC (Körner et al., 2001). The fact that OTC can be more easily released from the tetracycline-calcium complex due to its lower affinity to calcium ions provides another possible explanation for the high recovery levels of OTC observed in bones from pigs treated by parenteral administration of this antimicrobial.

3.6. Conclusion

- Bone discolouration due to tetracycline deposition is visible as yellow or green spots which fluoresce under UV light.
- Discolouration under natural light is a good indication of high levels of tetracyclines; however, significant amounts of tetracyclines can be found in bones in the absence of discolouration.
- Residues of tetracyclines are present in bone of slaughter pigs when
tetracyclines are given to grower pigs regardless of form and method of administration.

- HPLC is a useful tool to differentiate between the three forms of tetracyclines (CTC OTC and TC); however, results may underestimate the actual amount in the bone due to a very high affinity of tetracyclines for bone mineral.
- Better recovery levels by HPLC are expected for OTC because the OTC-calcium bonds can be easily broken under acidic conditions due to lower affinity of OTC molecules to calcium ions when compared to CTC and TC.


Table 3.1. Levels of Tetracycline HCL, Chlortetracycline, and Oxytetracycline (ppm) in Bone as Measured by High Performance Liquid Chromatography

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Group 2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group 4&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Group 5&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Group 6&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Group 7&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Abattoir Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High-Short (660ppm/3w)</td>
<td>Low-Short (110ppm/3w)</td>
<td>Injectable (300mg/45k)</td>
<td>Water (1g/8L)</td>
<td>No medication</td>
<td></td>
</tr>
<tr>
<td>CTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>1.4</td>
<td>0.47</td>
<td>0.24</td>
<td>2.0</td>
<td>ND</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
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<td>2.5</td>
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<tr>
<td></td>
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<td>1.8</td>
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<tr>
<td></td>
<td>1.4</td>
<td>0.48</td>
<td>0.42</td>
<td>2.6</td>
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<td>3.8</td>
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<td>± 0.08</td>
<td>± 0.78</td>
<td>ND</td>
<td>± 1.93</td>
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<tr>
<td>Std Dev</td>
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<td></td>
<td></td>
<td>ND</td>
<td>± 0.47</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
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<tr>
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<td>ND</td>
<td>ND</td>
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<td>0.94</td>
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<tr>
<td>Std Dev</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
<td>± 0.47</td>
</tr>
</tbody>
</table>

ND: No detectable levels of tetracyclines. N/A: Missing sample.

Bones from groups 1 and 3 were lost prior to analysis.  
<sup>a</sup> In-feed medication: 26 mg CTC/kg BW/day (withdrawal time: 54d)  
<sup>b</sup> In-feed medication: 4.4 mg CTC/kg BW/day (withdrawal time: 54d)  
<sup>c</sup> Injectable medication: 6.7 mg OTC/kg BW/day (withdrawal time: 54 d)  
<sup>d</sup> Water medication: 12.5 mg TC/kg BW/day (withdrawal time: 56 d)  
<sup>e</sup> Pigs were not exposed to tetracycline in any form.
CHAPTER 4: The Relationship between Discoloured Bones in Hog Carcasses and Medicated Feeding Programs for Grower-Finisher Pigs

ABSTRACT

The objectives of this study were to investigate the relationship between bone discolouration and the type of tetracycline used in grower-finisher production in field cases, and to determine the association of discoloured bones with dosing regimen and tetracycline treatment duration. Shipment lots identified with having at least one carcass with discoloured bones were traced to barns of origin, and information regarding medication was obtained dependent on dosage and duration of CTC consumption. However, there was no evidence of tetracyclines above 660 ppm being used or withdrawal times ignored.

Chlortetracycline consumption during the pig’s life was estimated to range from 0 to 66 g, with an average of 30 g. The proportion of carcasses with discoloured bones among pigs fed less than 30 g during the grower period was low (0.4%). In contrast, pigs fed larger amounts of chlortetracycline (45 to 66 g) were more likely to be identified with discoloured bones. Results from this study demonstrated that discolouration of bones in pig carcasses is a problem of cumulative deposition of chlortetracycline in bones associated with the use of this antimicrobial in feed rations for long durations during the grower-finisher stage of production. It also appears that withdrawal times greater than 8 weeks are needed to ensure pig carcasses have no discoloured bones.
4.1. Introduction

Bone discolouration of pig carcasses is a quality concern faced by the meat packing industry. There are reports of discolouration causing meat packers to debone carcasses in order to preserve a wholesome appearance and thus resulting in considerable economic losses (Kühne et al., 2000). It is well known that tetracyclines are active metal chelators that form yellow complex compounds with calcium (Oxford, 1953). These yellow compounds are deposited in areas of bone formation and become part of the mineralized bone leading to yellow discolouration of the bone (Buyske et al., 1960; Nelson, 1998; Albert and Rees, 1956). Tetracyclines are widely used in the pig industry and reports of bone discolouration are relatively rare. Therefore, when the problem does arise, it is important to investigate and determine the reason.

Researchers have recently demonstrated that yellow discolouration in bone of pig carcasses is reversible (Guillot et al., 2011). These scientists discovered in a controlled trial that discolouration increased with duration of exposure and age at treatment onset, and decreased as withdrawal time was increased. Therefore, it was hypothesized that discolouration of bone in pork carcasses at a commercial abattoir were likely associated with tetracycline medication of grower-finisher pigs in particular to dosage, duration of exposure and length of withdrawal period. The objective of this study was to investigate the relationship between bone discolouration and tetracycline use by means of a retrospective study attempting to correlate carcass bone staining data from a slaughter plant.
with medication use records from a pig producing organization that supplied a significant number of discoloured carcasses.

4.2. Materials and Methods

4.2.1. Source of Data

Data used in this retrospective analysis were obtained from two sources: a large processing plant that recorded information on discoloured bones and a company that supplied pigs to this particular packer; both located in Ontario. The large processing plant provided a summary of the records stating the actual counts of animals slaughtered per lot between March and November 2006, and the number of pigs declared as having discoloured bones within each lot. A lot is defined as a group of pigs shipped in one truck from one barn. Evaluation of the bones was based on visual subjective inspection of the bones that become visible when the carcasses were split into two longitudinally halves (vertebrae and rib bones). This task was not performed by meat inspectors but the lay staffs who judge the carcasses were trained by veterinary inspectors and used a series of coloured photographs as guidelines. The plant personnel decided to conduct the assessment one day after slaughter because, in their opinion, allowing the carcasses to cool for 24 hours could reduce the brightness of the discolouration and thus bone colour might become acceptable. The wait time is thought to allow the amount of blood pigments in the bone that interfere with visual subjective inspection of tetracycline staining, to decrease. A carcass was considered to have discoloured bones if areas of yellowish-green colour were visually detected
in the carcass bones by the inspector. A total of 64,232 pork carcasses were inspected for bone discolouration at the abattoir in 2006.

The summary provided by the abattoir, also contained date of shipment, lot number, barn and producer's identification.

Shipment lots containing one or more positive animals with discoloured bones were made available for this research, and the barn selling the pigs was identified. One single company listed as the source of the greatest number of pigs with discoloured bones (12.6%) was contacted and asked to participate in the study. This swine services company which owned some of the pigs and acted as an agent for other independent farmers, agreed to cooperate in the study. These grower-finisher barns operated on an all-in/all-out basis so that they were completely emptied and then a new batch of pigs filled the barn. Therefore, during the March-November 2006 study period, some barns have the time to be filled twice and sent two (exceptionally 3) batches of pigs to market. However, not all pigs from each batch were included in the analysis. They were excluded if they were shipped to a different abattoir or if they were in a lot that did not have a pig with yellow bones. In this study, the records from 69 barns and 143 batches were used. From each batch (or fill) there were multiple shipment lots of pigs sent to the packing plant because there was a premium paid for pigs of a specific weight so that the biggest and fastest growing pigs were shipped first and the slowest growing pigs were shipped several weeks later. Information collected from these records included producer, barn and batch identification, date when the barn was filled, feed program, total number of pigs that entered the barn, lot
identification, number of pigs shipped per lot, and commercial abattoir where the pigs were shipped.

All the barns included in this study had shipped at least one pig that had been detected with discoloured bones at the abattoir. The barns were emptied between batches allowing for the calculation of feed consumption per batch. Total medication administered to a batch of pigs and an estimate of withdrawal time was calculated. Pigs that were approximately 9-weeks-old were moved to these grower-finisher facilities and marketed at approximately 22 weeks of age.

With regard to exposure to tetracyclines, it was routine to provide a starter ration medicated with chlortetracycline (CTC) at 660 ppm in the nursery stage for approximately 20 days beginning when the pigs were 4 weeks old. Some pigs were medicated with rations containing tylosin phosphate at 22 ppm for approximately 21 days beginning when the pigs were about 6 weeks old. The management company provided assurance that to their knowledge water and injectable tetracyclines were not used at any of the grower-finisher barns. The exposure of CTC in the grower-finisher period varied. There were barns that did not receive medicated feed, but the packer did not identify any of these barns as having sent a shipment lot with a positive pig (a carcass with discoloured bones) and therefore batches from these barns were not included in the study. The dosage level of CTC in the feed for the grower-finisher pigs and the duration of feeding the medication were recorded. The age of the pigs at the beginning and at the end of the medication was calculated based on the production records of the pig flow system. In order to estimate the amount of CTC consumed per pig,
the tonnes of feed multiplied by the level of CTC (g/T) was divided by the number of pigs in the barn.

The barn data and the processing plant information were entered into two different electronic spreadsheets Excel™ (Microsoft Corporation One Microsoft Way Redmon, WA, USA) for editing and manipulation. Subsequently both datasets were transferred into STATA statistical software (StataCorp, 2007. College Station, TX, USA, version 9.2) and then merged by batch identification across all the observations to make a single record for the analysis.

4.2.2. Descriptive Statistics

Descriptive statistics were conducted to assess the frequency of discoloured bones by CTC consumption, treatment duration, withdrawal time, month, and shipment number for the batch. Shipment number was considered as one, two, three, and four for the first, second, third, and fourth group of pigs shipped out of the barn, respectively. Shipment five was considered as the group of pigs shipped afterwards because few numbers of pigs were identified with discoloured bones after the fourth shipment. The proportion of carcasses with discoloured bones according to withdrawal time and shipment was calculated using only those batches for which complete information regarding shipment dates was available.
4.2.3. Logistic Regression Analysis

The odds of a pig having discoloured bones at the time of slaughter were first modeled using binomial logistic regression analysis. This approach was selected as opposed to the rate of discoloured bones or Poisson distribution because of the low prevalence of observing discoloured bones in our data. Binomial distribution is used when the probability of an event occurring can be within a wide range; while Poisson distribution can be applied only when the probability of an event occurring is relatively large (Heldt, 1999). Barn was used as random effect to adjust for possible clustering of bone discolouration in pigs within a barn.

Unconditional associations between the independent variables (CTC consumption, treatment duration, dosing regimen, withdrawal time, month, and shipment) and the dependent variable yellow bones were assessed using Wald's test $P < 0.05$ level (2-sided). Mixed models were fit to the data by adding barn as a random effect and the logistic regression analysis was conducted using likelihood estimation via the General Linear Mixed Effects Model (GLIMMIX) procedure in SAS statistical software (SAS Institute Inc. 2005, Cary, NC, USA, Version 9.1.3). This procedure is a class of generalized linear mixed models (GLMMs) for predictors of mixed types including discrete and continuous data; these models assume normal (Gaussian) random effects, and conditional on these random effects, data can have any distribution in the exponential family (SAS, 2006).

Independent variables showing a significant unconditional association with the dependent variable (discoloured bone) at $P \leq 0.05$ were added as fixed
effects in the model. Polynomials associated to predictor variables, as well as all possible first-order interactions were assessed with a threshold of $P < 0.05$.

Significance of the random effects was assessed based on the covariance parameter estimates ($P < 0.05$). Barm Intra-class Correlation Coefficient (ICC) was calculated by using the null model without fixed effects.

The final multivariable random effects model was developed using a backward process of eliminating the least significantly associated variables until all the remaining variables were significant. The full multivariate mixed logistic model was:

\[
\text{Logit (Y)} = \beta_0 + \text{withdrawal time} + \text{shipment} + \text{CTC} + \text{CTC}^*\text{withdrawal time} + \nu_{(\text{barn}(\text{pig}))} \\
(Y) = \text{Odds of one lot of pigs having discoloured bones at the time of slaughter}
\]

4.3. Results

4.3.1. Descriptive Statistics

This study included records from 64,232 grower-finisher pigs produced and marketed via one company to a large packing plant in Ontario between March and November, 2006. The prevalence of carcasses showing discoloured bones in March, April, May, June, July, August, September, October and November was 4.10%, 0.08%, 8.46%, 4.20%, 1.49%, 0.83%, 0.51%, 0.55%, 0.45%, respectively, resulting in 95% confidence interval of 3.664% - 4.570% for March,
0.031% - 0.183% for April, 7.735% - 9.226% for May, 3.750% - 4.682% for June, 1.234% - 1.778% for July, 0.628% - 1.071% for August, 0.357% - 0.716% for September, 0.406% - 0.738% for October, and 0.300% - 0.643% for November. A single barn produced from 1 to 3 batches, and from each batch there were multiple lots (1 to 5 shipments). Overall, 69 barns were represented and 143 batches resulting in a total of 489 individual shipments.

The most common medication protocol was: 660 ppm of CTC in feed for the first 21 d (about 21 g/pig) in the grower barn, followed by 330 ppm of CTC in feed for 14 d (about 9 g/pig), resulting in a total exposure to CTC of approximately 30 g/pig/day. The lowest level of exposure was a feeding regime of 330 ppm of CTC for the first 21 days (approximately 10 g/pig). The highest exposure was 660 ppm for the first 35 days (about 39 g/pig) followed by 21 days of CTC at 330 ppm (about 10 g/pig), resulting in a total exposure to CTC of approximately 49 g/pig.

Estimation of CTC consumption on a pig average basis during the grower-finisher stage ranged from 0 to 66 g. The most common level of consumption was about 30g. The medication protocols from the barns on this study did not vary from month to month during the duration of the study. The proportion of discoloured bones based on CTC consumption, and the proportion of discoloured bones according to treatment duration is presented in Figure 4.1 and Figure 4.2, respectively. The proportion of discoloured bones among pigs fed only 330 g/T of CTC was 0.015%. Most of the pigs receiving feed medicated at this dosage were exposed to CTC for 5 or more weeks and completed a withdrawal time of 39 days or more.
From each batch, there were multiple shipments. There were only 43 batches of pigs marketed in 4 shipments, and therefore only these batches were used to estimate the proportion of carcasses with discoloured bones based to withdrawal time and shipment. The relationship between the estimated withdrawal time and the proportion of positive pigs (discoloured bones) is presented in Figure 4.3. The shortest withdrawal period was one shipment at 2 weeks post-medication and the proportion of positive pigs was almost 76%. The proportion of positive pigs decreased as withdrawal time increased. Similarly, the proportion of pig carcasses showing bone discolouration decreased among pigs marketed after the first shipment (Figure 4.4).

4.3.2. Logistic Regression Analysis

Table 4.1 lists all the significant independent/predictor variables and the significant first-order interaction included in the final model. The study population was defined as all the barns that had shipped at least one pig detected with discoloured bones at the abattoir from which information on bone discolouration was obtained. The measure of association used to assess the outcome for the fixed effects in the multivariable model was the odds ratio (OR); the effect that each predictor had in the odds of a lot of pigs having carcasses with bone discolouration. Therefore, the unit of analysis was lot. Barn cluster explained 31% of the random variation in bone discolouration after accounting for fixed effects.
The increase in the log odds of discoloured bones as a function of CTC consumption was linear. There was an increase in discoloured bones as consumption of CTC increased, as measured in g per pig. The odds of a lot of pigs showing discoloured bones increased as CTC consumption increased (OR = 1.195; 95% CI = 1.109-1.288). A significant interaction between CTC consumption and withdrawal time was found (CTC*Wthd). The odds ratio for discoloured bones at different concentrations of CTC significantly varies with withdrawal time. The relationship between discoloured bones and CTC in accordance with withdrawal time is presented in Figure 4.5. As withdrawal time increases, the probability of yellow bones decreases but a longer time is required for the highest levels of CTC.

Shipment number was associated with bone discolouration. Pigs sent to market in the first shipment were 1.88 (CI: 1.18-3.01), 2.11 (CI: 1.24-3.58), and 2.19 (CI: 1.34-3.58) times more likely to show discoloured bones than those on the 3\textsuperscript{rd}, 4\textsuperscript{th}, and 5\textsuperscript{th} shipment, respectively. No statistically significant differences were observed between other shipment comparisons. Month was not associated with bone discolouration, either as a main effect or as part of an interaction effect with CTC consumption.

4.4. Discussion

The effect of CTC on bone discolouration varied as a function of the withdrawal time. The frequency of discolouration increased as the total amount of CTC consumed in the grower period increased, particularly when levels
consumed exceeded 30g. It was rare (0.01% of pigs) for the packing plant to identify a carcass with discoloured bones in a shipment of pigs if those pigs had received less than 30 g of CTC during the grower period. In contrast, pigs that were estimated to consume relatively large amounts (45 to 66g) of CTC in the grower-finisher stage were more likely to produce a positive carcass (about 5% of pigs). This is similar to the findings reported in chapter 3, where bone discolouration was noted in pigs that consumed the most CTC. This finding that discolouration is associated with dosage is in agreement with other reports in the literature (Guillot et al., 2011, Benitz et al., 1967).

It is important to note that the most common concentration of CTC used in this group of barns was only 660ppm for 3 weeks which has been demonstrated to be a relatively moderate dose (Opriessinig et al., 2006), and thus, the level expected to be used by veterinary practitioners to combat respiratory diseases in grower-finisher pigs. According to the pharmacokinetic model created to predict plasma concentration in pigs offered in-feed tetracyclines (del Castillo et al., 1998) a daily steady-state average plasma concentration of 0.4 µg/ml is achieved in pigs exposed to 660ppm CTC, a much lower antibiotic concentration than the MIC indicate for CTC (MIC ≤ 2 µg/ml) (del Castillo et al., 1998; Apley, 2010). Furthermore, CTC when included in feed at various levels carries a warning that the drug must be withdrawn for 7 days prior to shipping to avoid tetracycline residues above the minimum allowable concentration in edible tissues. The shortest withdrawal period in this study was two weeks and there was only a single shipment with such a short withdrawal. The next shortest period was 4
weeks. It is possible that the withdrawal period might be slightly shorter than the estimates used in this study. Withdrawal was calculated using feed delivery records and it must be assumed that one or two days medicated feed would be still present in the feeding system when the feed was delivered, but it would be impossible to know the exact day all the medicated feed was out of the system and only non-medicated feed was being consumed. In other studies of discoloured bones, the CTC residue levels in edible tissues such as muscle, liver and kidney have not exceeded allowable limits. The production company that participated in this study did not receive a positive test for tetracycline residues in edible tissue during the period of the study. Based on the withdrawal times observed this is not surprising, the shortest period was 2 weeks and in most of the cases the withdrawal period last over a month.

A higher proportion of pigs with discoloured bones were found as treatment duration increased. A long treatment duration generally corresponded with a higher consumption of tetracyclines overall and a shorter withdrawal time. In fact, an abrupt increase in the prevalence of carcasses with discoloured bones from pigs fed for at least seven weeks was observed when compared to pigs fed CTC for shorter time. A large proportion of pigs (68.76%) fed CTC for at least seven weeks were exposed to 660 ppm and 330 ppm CTC for five and at least 2 weeks, respectively; an overall exposure of at least 39 g/pig. In addition pigs fed medicated feed for a longer period of time would be consuming CTC at an older age. Bone remodelling slows with age and it takes longer to remove tetracycline as the pig ages. All the pigs in this production system received CTC in the
nursery but some of these barns where pigs were not fed CTC in the grower-finisher stage produced pigs that did not have discoloured bones. During the process of bone resorption, molecules of tetracycline deposited in bone can be released and thus, tetracycline-induced bone discolouration is reversible (Guillot et al., 2011). It has been reported that release of tetracyclines may take 2 months in rats (DeMoss and Wright, 1997), which basal metabolic rate is higher than pigs. In the current study of commercially raised pigs, the probability of a pig being identified as having discoloured bones at slaughter was greatly reduced if tetracyclines had been withdrawn for more than 8 weeks. Guillot et al. (2011) suggest that to be certain discolouration will not be present at slaughter, it would be best to not feed CTC beyond the nursery stage, but if grower pigs are to be medicated, then duration of medication should be limited to 4 weeks or less. The data from the current study are in agreement with the work by Guillot et al. (2011). It was possible to identify pigs based on whether they were the first group to be shipped from a barn or a later group. It was noted that the first shipments were more likely to contain pigs that displayed discoloured bones at slaughter than the last shipments. One explanation for this finding is that the medication was stopped at the same time for all pigs in the barn so that the first pigs shipped would have a shorter withdrawal time compared to later shipments. In addition the first pigs shipped would have been the fastest growing and/or the largest pigs at the time of entry into the barn. One can assume that in a grower barn not all the pigs will have consumed equal amounts of feed and that the pigs on the first shipments likely ate more feed during the period of medication. It is a weakness
of this study in that the level of CTC consumption could only be roughly estimated as an average of the group.

In addition to a variation in CTC intake from one pig to another, other factors such as disease status and individual metabolism characteristics would have contributed to considerable variation and might help explain why one pig in a shipment may have been identified as positive and the rest were not despite similar husbandry and management.

Finally, it is also important to consider that evaluation of pig carcasses at the slaughter plant was based on subjective visual inspection, and more than one person was involved in scoring the carcasses over the period of the study. It is possible that bones that were mildly discoloured might be accepted as normal one day and classified as discoloured another day. The carcass was split down the midline so that the bones that were inspected tended to be primarily the vertebrae. It was useful that the same bones, cut the same way were inspected for each carcass because there would be variation in discolouration from one type of bone to another in the same carcass. Cortical bone is more dense and likely to display a more intense discolouration than trabecular bone, like vertebrae (Guillot et al., 2011). Therefore, it is possible that the numbers of carcasses with discolouration identified at slaughter is an under-estimate of the problem, in that, inspectors may have decided to allow mildly discoloured bone to pass, but also if the cortical bones had been more closely inspected, then discolouration may have been discovered in carcasses where the vertebrae were white.
Overall, this study helps to confirm that discoloured bone in pig carcasses is related to the use of tetracyclines during the grower-finisher stage of production. There was no evidence that tetracyclines were being fed at extremely high levels or that withdrawal times were being ignored. The problem of bone discolouration was associated with feeding CTC for a relatively long duration or in other words a problem of a cumulative deposition. A withdrawal time of greater than 8 weeks appeared to be necessary to insure all the pigs would be free of discolouration. The pigs at greatest risk appeared to be the fastest growing animals that were assumed to consume the most feed and medication and also reach market weight the quickest and therefore have the shortest withdrawal time.
References


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Table 4.1. Significant Independent Variables Included in the Final Model of Discoloured Bones of Hogs Marketed by One Commercial Swine Company in Ontario – 2006.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratios</th>
<th>Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline (g)</td>
<td>1.195</td>
<td>1.109 – 1.288</td>
</tr>
<tr>
<td>Withdrawal Time (days)</td>
<td>1.240</td>
<td>0.963 – 1.598</td>
</tr>
<tr>
<td>Chlortetracycline* Withdrawal Time</td>
<td>0.990</td>
<td>0.983 – 0.997</td>
</tr>
<tr>
<td>Shipment Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.191</td>
<td>1.343 – 3.574</td>
</tr>
<tr>
<td>2</td>
<td>1.520</td>
<td>0.907 – 2.545</td>
</tr>
<tr>
<td>3</td>
<td>1.164</td>
<td>0.646 – 2.095</td>
</tr>
<tr>
<td>4</td>
<td>1.040</td>
<td>0.560 – 1.931</td>
</tr>
<tr>
<td>5 (referent)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Shipment Comparisons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 vs 2</td>
<td>1.442</td>
<td>0.989 – 2.102</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>1.883</td>
<td>1.178 – 3.011</td>
</tr>
<tr>
<td>1 vs 4</td>
<td>2.107</td>
<td>1.241 – 3.577</td>
</tr>
<tr>
<td>1 vs 5</td>
<td>2.191</td>
<td>1.343 – 3.575</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>1.306</td>
<td>0.792 – 2.155</td>
</tr>
<tr>
<td>2 vs 4</td>
<td>1.462</td>
<td>0.834 – 2.561</td>
</tr>
<tr>
<td>2 vs 5</td>
<td>1.520</td>
<td>0.907 – 2.545</td>
</tr>
<tr>
<td>3 vs 4</td>
<td>1.119</td>
<td>0.604 – 2.074</td>
</tr>
<tr>
<td>3 vs 5</td>
<td>1.164</td>
<td>0.646 – 2.094</td>
</tr>
<tr>
<td>4 vs 5</td>
<td>1.040</td>
<td>0.560 – 1.931</td>
</tr>
</tbody>
</table>
Figure 4.1. Proportion of Pig Carcasses with Discoloured Bones at Slaughter Based on the Estimated Cumulative Chlortetracycline (CTC) Consumption of Hogs Marketed by a Commercial Swine Company in Ontario – 2006

\( n = \) Number of batches (a batch is the group of pigs that fills a barn at one time)

\(^a\) number of pigs declared as having discoloured bones

\(^b\) total number of pigs inspected for bone discolouration from that particular category of CTC consumption
Figure 4.2. Proportion of Pig Carcasses with Discoloured Bones at Slaughter, Based on Duration of Chlortetracycline (CTC) Treatment

Number of batches = 65 (a batch is the group of pigs that fills a barn at one time)

\[ n = \text{Number of batches} \] (a batch is the group of pigs that fills a barn at one time)

\[^a\text{number of pigs declared as having discoloured bones}\]

\[^b\text{total number of pigs inspected for bone discolouration from that particular category of CTC consumption duration}\]
Figure 4.3. Proportion of Pig Carcasses with Discoloured Bones Based on Withdrawal Times of Hogs Marketed by a Commercial Swine Company in Ontario – 2006

Number of batches = 43 (a batch is the group of pigs that fills a barn at one time)

\[ a \] number of pigs declared as having discoloured bones

\[ b \] total number of pigs inspected for bone discolouration from that particular category of withdrawal
Figure 4.4. Proportion of Pigs Carcasses with Discoloured Bones at Slaughter based on Shipment from the Grower-Finisher Barn

Number of batches = 43 (a batch refers to a group of pigs filling a barn)

\(^a\) number of pigs declared as having discoloured bones

\(^b\) total number of pigs inspected for bone discolouration
Figure 4.5. Probability of Pig Carcasses Showing Bone Discolouration According to Chlortetracycline (CTC) and Withdrawal Time Interaction*

*Data shown at the logit scale
CTC = Chlortetracycline
Chapter 5:  General Discussion

In Ontario, the discolouration of bones can be detected in hog carcasses at the time of slaughter in a small number of pigs. Data collected during this study, in 2006 and at various interval in the following 4 years, indicated that at one large Ontario swine abattoir approximately 1 or 2 out of every 1,000 hogs slaughtered are identified with bone discolouration. The discolouration appears to be related to medication of pigs with tetracyclines, and this aspect was investigated using feeding trials as well as examining the medication use of farms that were identified as sources of the hogs identified at the abattoir in 2006.

Tetracyclines are widely used in pig production the world-over and have been for over 60 years. Because modern pig production generally involves large groups of animals under intensive confinement management, the use of individual animal treatment is less commonly used as compared to mass medication with tetracyclines in feed or water. However individual treatment using an intramuscular injection of oxytetracycline might be used on occasion. However labour requirements and the concerns with issues like broken needles make this option less appealing than feed medication for many producers. The 3 molecular forms of tetracyclines used in pig production in Canada are tetracycline, oxytetracycline and chlortetracycline. Pijpers and Verheijden (1992) have reported that oxytetracycline is the most common form used in the Dutch swine industry for medicating feed and that oxytetracycline is the molecule found at the highest concentration in bones of pigs at slaughter. On the other hand
German researchers reported chlortetracycline as the most common form used and the molecule most likely found in bone (Körner et al., 2001). In Canada chlortetracycline has been reported to be more commonly used (Dunlop et al., 1998) and was the tetracycline molecule identified at the highest concentration in bones from cases of discoloured bones identified at slaughter that were analyzed in the current study. In the current study, herds that were identified because they had shipped a pig with discoloured bones used only the chlortetracycline form of the antibiotic. The use of chlortetracycline in Canadian herds for feed medication may reflect the reports that suggest the bioavailability of chlortetracycline is much higher than oxytetracycline and tetracycline (del Castillo et al., 2000). In the current study the rationale given by practitioners for using levels as high as 660 ppm in the feed for 3 or 6 weeks at the beginning of the grower-finisher phase was to combat respiratory disease. Studies show that levels of over 500 ppm are required to achieve serum and lung levels that will meet the MIC values for common respiratory bacterial pathogens (del Castillo et al., 1998). Possibly the preference for oxytetracycline in some studies may indicate that veterinarians are using the antibiotic to manipulate gut flora or prevent clinical enteric diseases such as porcine proliferative enteropathy caused by *Lawsonia intracellularis* and not systemic or respiratory pathogens.

At the start of the current study involving investigating the tetracycline use of pig farms that were identified as shipping pigs with discoloured bones, it was thought that possibly the research would identify unusually high levels of drug use or a disregard for withdrawal times. Previous work has identified that
tetracycline-related bone discolouration is influenced by the dosage and duration of treatment, as well as the length of time from when medication stopped until the animal is slaughtered. Guillot et al. (2010) have shown that the discolouration is reversible if sufficient time is allowed to pass. Our study did reveal that the likelihood of positive pigs (carcasses with discoloured bones) was reduced if withdrawal time was 8 weeks or longer and if chlortetracycline was used for a shorter duration (3 weeks versus 6 weeks) or at a lower dosage (330 ppm versus 660 ppm). The conclusion drawn from this study is that in order for bone discolouration to be minimized, the use of chlortetracycline at therapeutic levels should be restricted to the very early grower period.

The relationship between bone discolouration and tetracyclines was noted as early as the 1950’s (Albert and Rees, 1956). Likewise, it was recognized that tetracyclines form a tight bond with calcium ions and that during the process of bone absorption and reformation, the calcium bound tetracycline was reincorporated into bone, so that tetracycline residues persisted in bone long after tetracyclines could no longer be detected in edible tissue (Körner et al., 2001). In a controlled exposure trial in the current research, pigs were administered tetracyclines via injection (oxytetracycline), and in water (tetracycline) and in feed at various dosage levels and for different durations. After lengthy withdrawal periods of more than 50 days, tetracyclines could be detected in bone in all cases where pigs were exposed to the antibiotic regardless of molecule used or method of administration. It is reported that the calcium-tetracycline bond varies depending on the molecule with oxytetracycline
being the weakest bound and chlortetracycline the strongest and tetracycline having an intermediate bond (Körner et al., 2001). The presence of residues of tetracyclines in bone of animals at slaughter has been reported to be very common, although the measurement of residues is difficult. The phenomenon of tetracyclines in bone fluorescing under ultraviolet light has been used as a convenient screening test for many years. Unfortunately tests to determine accurate concentrations of tetracyclines present in bone are difficult to perform. In the current study a high performance liquid chromatography test was employed. The bones were ground and treated with acid to break the tetracycline-calcium ion bonds. It is likely that only a portion of the bound tetracyclines were released and the measurement may have been low, particularly for chlortetracycline. One interesting finding, despite the limitations of this analytic technique, is that bones with normal colour may contain high levels of tetracyclines. The presence of residues of tetracyclines in bone of animals passed by the meat inspection process for human consumption raises concerns regarding food safety. Under Canadian law, bone is considered to be meat; however the minimum allowable limits set for tetracyclines are based on analysis of muscle, liver and kidney tissue. Bone is not included, possibly because of the difficulty in measuring the levels or possibly because authorities don’t consider bone to pose a risk. Jones et al. in a paper in the Canadian Veterinary Journal in 1977 raised the concern of oxytetracycline residues and food safety and their conclusion was that bone residues need to be further investigated. German researchers (Kühne et al., 2000) have demonstrated that microscopic particles of
bone are present in ground meat when mechanical deboning is used. Further research (Kühne and Körner, 2001) has noted that gastric acidity can release biologically active tetracyclines so that it is possible low levels of tetracyclines present in ground meat when ingested may contribute to antimicrobial resistance in gut flora. In addition when bone and meat meal derived from animals that were medicated with tetracyclines, were fed to chickens, tetracycline residues were found in the bones of the chickens. It was not within the scope of the current study to investigate the aspect of tetracycline residues as a food safety concern but clearly this is an area where further work should be performed.

There is a substantial economic cost of discoloured bones to the pig industry if it results in the packer having to handle the processing of the carcasses in a special manner such as removing the bones to make the cuts of meat visually acceptable. Swine practitioners and producers need to be mindful of the risk of bone discolouration when choosing medication regimens for the grower-finisher period. In addition, there is the potential cost associated with negative publicity regarding a food safety issue of antibiotic residue in pork, and this could be of far greater concern. Further work is required to determine if residues of tetracyclines in bone poses a health risk to consumers or to other livestock via bone meal.
References


Appendices
A.1.1. Letter from Animal Health Laboratory (AHL) - University of Guelph reporting an outbreak of disease in Fall 2005.

Porcine circovirus type 2 (PCV2)-associated disease is increasing

Josepha Delay, Beverly McEwen, Susy Carman, Tony van Dreumel, Jim Fairles

Porcine circovirus type 2 (PCV2)-associated disease has increased markedly in 2005 compared to the previous 7 years (Fig. 1). Although many of the cases have had other pathogens identified (e.g., PRRSV, Streptococcus suis), many cases have had PCV2 identified as the only agent. Most necropsy cases of PCV2-associated disease seen at the AHL during 2005 have some of the typical lesions of this disease, including poor body condition, firm lungs that fail to collapse (implying interstitial pneumonia), and multiple pale enlarged lymph nodes.

Several additional lesions are ‘new’ to Ontario pigs with PCV2 infections:

- Prominent pulmonary interlobular edema has been observed in many pigs. Histologically, interlobular septa are widely expanded by edema, and alveoli are flooded by proteinaceous edema fluid, but infiltration of mononuclear inflammatory cells in alveolar septa is generally much less pronounced than in traditional PCV2 cases.

- Thickening of the walls of ileum and colon, sometimes accompanied by mucosal erosion or ulceration and reminiscent of porcine proliferative enteritis due to Lawsonia intracellularis, has been identified in many pigs with diarrhea. Histologically, these animals have granulomatous enteritis and colitis, with extensive infiltration of histiocytes and fewer lymphocytes throughout the lamina propria.

- In lymph node, histologic lesions of lymphoid depletion and histiocytic infiltration are more severe and extensive than previously seen, with large numbers of typical circoviral inclusions in histiocytes and multinucleated giant cells. In recent cases, spleen and tonsil are also frequently affected, and sometimes have evidence of acute lymphoid necrosis.

Typically, all manifestations of PCV2-associated disease in recent cases have much larger viral antigen loads, as demonstrated by immunohistochemistry, than we have seen routinely in cases prior to 2005.

Concurrent with these changes in the pathology of PCV2-associated disease, cases of suspected PCV2-related vasculitis and immune-complex disease (porcine dermatopathy and nephropathy syndrome) have increased. Lesions commonly involve kidney, lymph node, and spleen, with variable lung involvement, although cutaneous lesions have not been present in affected animals. Vasculitis (including glomerulonephritis) is present consistently, sometimes with obvious splenic infarcts, and these lesions make it necessary to consider classical swine fever as a differential diagnosis. PCV2 antigen is rarely evident in association with vascular lesions, as expected in immune-complex disease, although variable amounts of antigen are present in other tissues such as lung in some cases. We have also seen several cases of pigs with clinical neurologic disease in which PCV2 antigen was demonstrated in association with endothelial or inflammatory cells in brain, as well as in other tissues.

Compared to 1998, a swine submission to the AHL in 2005 is now 14 times more likely to have PCV2-associated disease on histopathology. PCV2 PCR-RFLP typing for all PCR testing requests shows that a significant change from RFLP type 422 to type 321 also occurred in 2005 (Fig. 2). These changes in RFLP typing are the result of a consistent change in gene sequence, recognized by two restriction enzymes.\*\*\*

Reference


![Figure 1. Number of PCV2 pathology cases and percentage of PCV2 pathology cases of total swine submissions.](#)

![Figure 2. PCR-RFLP typing of PCV2.](#)

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Ottawa, Ontario
K1A 0Y9

January 4, 2006

MEAT HYGIENE DIRECTIVE:
2006 - 01

SUBJECT:
Disposition of Carcasses with Bones Discoloured by Tetracyclines

In the last couple of years, some pork slaughter plants have seen carcasses in which some or all of the bones show a distinct yellow discoloration. Often, an entire production lot is affected. The yellow areas fluoresce strongly under ultraviolet light, such as the lights used for reading Sulfia on Site tests.

The most common reason for this discoloration is exposure to one of the tetracyclines (tetracycline, oxytetracycline, chlortetracycline) at some point during the animal's life. Tetracyclines form an insoluble complex with calcium, so will deposit in active areas of bone deposition, where they will remain for much of the animal's life.

Ottawa (Ontario)
K1A 0Y9

Le 4 janvier 2006

DIRECTIVE DE L'HYGIÈNE DES VIandes :
2006 - 01

OBJET :
Disposition des carcasses aux os décolorés par les tétracyclines

Depuis quelques années, certains établissements d'abattage de porc ont eu à traiter des carcasses de porcs dont les os, ou une partie des os, présentaient une décoloration jaunâtre particulière. Souvent, un lot entier est affecté. Les zones décolorées présentant une fluorescence marquée sous la lumière ultraviolette, comme celle émise par les lampes utilisées pour lire les épreuves de Sulfamidés sur Place.

La cause la plus fréquente de cette décoloration est une exposition aux tétracyclines (tétracycline, oxytétracycline, chlortétracycline) à un moment quelconque de la vie de l'animal. Les tétracyclines formant un complexe insoluble avec le calcium, elles se dépôsent dans les zones d'ossification active où elles demeurent pendant une bonne partie de la vie de l'animal.
The presence of the discoloration in bones is not an indicator of tetracycline residues in muscle or organs. Because the deposits are essentially permanent, the medication may have long since been cleared from other tissues. Meat from hogs with yellow bones does not appear to be at an increased risk of having unacceptable tetracycline residues, and residue testing is not warranted unless there are indications of recent treatment.

However, it has been determined that tetracycline can be released from the bones under some conditions. For this reason, the yellow discoloration is both a quality defect and a potential residue hazard.

Hog carcasses showing a yellow discoloration of the bones must be detained and remain under inspectional control until the discoloured areas are boned out.

Bones removed from carcasses may not be used for mechanically separated meat or any other edible purpose. These bones must be disposed of in a manner that meets the requirements of the Meat Inspection Regulations, section 54.

La présence de cette décoloration dans les os n’est pas un indice qu’il y a des résidus de tétracyclines dans les muscles ou les organes. Puisque les dépôts sont essentiellement permanents, le médicament peut avoir été éliminé depuis longtemps des autres tissus. La viande des porcs qui ont les os décolorés ne semble pas présenter de risque accru de présenter des résidus de tétracycline et ce n’est pas justifié de la tester à moins qu’il y ait des indices d’un traitement récent.

Toutefois, il a été déterminé que la tétracycline peut être relâchée des os sous certaines conditions. Pour cette raison, cette décoloration pose à la fois un problème esthétique et un problème de risque potentiel pour la santé.

Les carcasses de porc montrant une décoloration jaune des os doivent être retenues et demeurer sous le contrôle de l’inspection jusqu’à ce que les portions affectées par la décoloration aient été désossées.

Les os issus de ce désossage ne peuvent pas être utilisés pour la production de viande séparée mécaniquement ou à d’autres fins comestibles. On doit disposer de ces os d’une façon qui rencontre les exigences de l’article 54 du Règlement sur l’inspection des viandes.

Le Directeur
Division des aliments d’origine animale

ORIGINAL SIGNED BY/COPIE ORIGINALE SIGNÉE PAR

Dr. William R. Anderson
Director
Food of Animal Origin Division

Att./p.j.

Canada

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Ottawa, Ontario
K1A 0Y9

March 10, 2006

MEAT HYGIENE DIRECTIVE:
2006 - 12

SUBJECT:
Disposition of Carcasses with Bones Discoloured by Tetracyclines


In the last couple of years, some pork slaughter plants have seen carcasses in which some or all of the bones show a distinct yellow discolouration. Often, an entire production lot is affected. The yellow areas fluoresce strongly under ultraviolet light, such as the lights used for reading Sulfam on Site tests.

The most common reason for this discolouration is exposure to one of the tetracyclines (tetracycline, oxytetracycline, chlortetracycline) at some point during the animal's life. Tetracyclines form an insoluble complex with calcium, so will deposit in active areas of bone deposition, where they will remain for much of the animal's life.

Ottawa (Ontario)
K1A 0Y9

Le 10 mars 2006

DIRECTIVE DE L'HYGIÈNE DES VIandes :
2006 - 12

OBJET :
Disposition des carcasses aux os décolorés par les tétracyclines


Depuis quelques années, certains établissements d'abattage de porc ont eu à traiter des carcasses de porcs dont les os, ou une partie des os, présentent une décoloration jaunâtre particulière. Souvent, un lot entier est affecté. Les zones décolorées présentent une fluorescence marquée sous la lumière ultraviolette, comme celle émise par les lampes utilisées pour lire les épreuves de Sulfamidés sur Place.

La cause la plus fréquente de cette décoloration est une exposition aux tétracyclines (tetracycline, oxytetracycline, chlortetracycline) à un moment quelconque de la vie de l'animal. Les tétracyclines formant un complexe insoluble avec le calcium, elles se déposent dans les zones d'ossification active où elles demeurent pendant une bonne partie de la vie de l'animal.
The presence of the discolouration in bones is not an indicator of tetracycline residues in muscle or organs. Because the deposits are essentially permanent, the medication may have long since been cleared from other tissues. Meat from hogs with yellow bones does not appear to be at an increased risk of having unacceptable tetracycline residues, and residue testing is not warranted unless there are indications of recent treatment.

For the time being, the yellow discolouration is to be solely considered a quality defect.

Operators of hog slaughter and processing establishments are responsible for ensuring that discoloured products, including yellow bones, are not offered for sale to consumers. No action or special inspection activity is to be undertaken by CFIA during post-mortem procedures as this is, for the time being, an operator quality managed defect. Furthermore, considering that the yellow colouring of bones may disappear after the carcass has remained a certain time in the cooler, operators can decide that the removal of parts of bones that showed a yellow discoloration at the time the carcass was dressed, is no longer justified once the carcass is ready to be boned at the establishment or shipped.

Yellow bones may not be used for mechanically separated meat or any other edible purpose. These bones must be disposed of in a manner that meets the requirements of the Meat Inspection Regulations, section 54.

La présence de cette décoloration dans les os n'est pas un indice qu'il y a des résidus de tétracyclines dans les muscles ou les organes. Puisque les dépôts sont essentiellement permanents, le médicament peut avoir été éliminé depuis longtemps des autres tissus. La viande des porcs qui ont les os décolorés ne semble pas présenter de risque accru de présenter des résidus de tetracycline et ce n'est pas justifié de la tester à moins qu'il y ait des indices d'un traitement récent.

Pour le moment, le jaunissement est considéré simplement comme un défaut de qualité.

Il est de la responsabilité des exploitants des abattoirs de porcs et des établissements de transformation de veiller à ce que les produits de couleur altérée, dont les os jaunis, ne soient pas offerts en vente aux consommateurs. L'ACIA ne prendra aucune mesure et ne s'engagera dans aucune activité d'inspection spéciale durant les procédures post-mortem, car, pour le moment, le jaunissement des os est considéré comme faisant partie des défauts de qualité dont la détection relève de la responsabilité de l'exploitant. De plus, étant donné que le jaunissement des os peut disparaître lorsque la carcasse a séjourné un certain temps dans la chambre froide, les exploitants peuvent décider que l'enlèvement des parties de la carcasse dont les os étaient jaunis au moment de l'habillage n'est plus justifié au moment où la carcasse est prête pour le désossage à l'établissement ou pour l'expédition.

Les os jaunis ne peuvent être utilisés pour la fabrication de viande mécaniquement séparée ou d'autres produits destinés à l'alimentation. Le sort réservé à ces os doit être déterminé suivant les exigences du de l'article 54 du Règlement sur l'inspection des viandes.

Le Directeur  
Division des aliments d'origine animale

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Dr. William R. Anderson  
Director  
Food of Animal Origin Division

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