Antimicrobial Use and Resistance in Pigs and Chickens

A review of the science, policy, and control practices from farm to slaughter

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November 2009

National Collaborating Centre for Infectious Diseases

Centre de collaboration nationale des maladies infectieuses

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Executive Summary

Over the course of a century, antimicrobials have evolved from the panacea for bacterial infections to an endangered tool because of rising resistance. Resistant bacteria in animals are one source of antimicrobial resistance (AMR) for people and Canadians' strongest connection to animals is our daily food. This report summarizes and evaluates the scientific knowledge and pertinent policy around the world on AMR and antimicrobial use (AMU) in pigs and chickens. This document will serve as a reference document for public heath, regulatory, and agricultural policy makers and will hopefully initiate intense discussion among and between these groups. Public health practitioners may find it informative regarding notable AMR risks to the Canadian population related to food and agriculture. Key research and policy gaps have been identified that compromise our ability to control this problem.

It is irrefutable that people can acquire antimicrobial resistant bacteria from animals through food. What remains unclear is the frequency that pathogenic and commensal bacteria are transmitted to humans and either cause disease or transfer resistance elements to bacteria in people. A related knowledge gap is the relative amounts that AMU in animals and humans each contribute to AMR in humans. Future research should expand on both ends of the current farm-to-fork research continuum. This could be achieved by studying health outcomes caused by resistant bacteria, and at the other end investigating why producers, nutritionists, and veterinarians use antimicrobials and the factors that influence their decisions. This research should be coupled with studies evaluating how various on farm practices, only one of which is AMU, affect the rate and severity of foodborne diseases in general and resistant bacterial infections in particular. By broadening on farm food safety knowledge, policy and interventions can expand from a specific focus on AMU and AMR to improving overall food safety.

Antimicrobials are a necessary tool for appropriate veterinary care of food animals. Certainly some AMU could be dispensed with while still humanely raising animals; but the data distinguishing why antimicrobials are used, and their range of effects, are limited so evaluating the appropriateness of agricultural AMU is contentious. For example, AMU to improve productivity is not necessary for good animal welfare yet prophylactic and metaphylactic use arguably could be. The agriculture industry and regulators must continue to address this problem together so that AMU policy can preserve appropriate AMU, thus ensuring the production of safe food and humane rearing of livestock, while eliminating inappropriate AMU practices but still ensuring economic viability of the industry.

Indisputably, AMU causes AMR. Yet, it is equally indisputable that the relationship is complex. Use of *some* antimicrobials in *some* species in *certain* situations clearly results in resistance in *some* bacteria. However, what happens with one animal species, bacterium, antimicrobial, or management system does not necessarily happen in others. Evidence showing increasing resistance with increasing AMU is far more consistent than evidence showing declining resistance with cessation of use. Our understanding is rudimentary of the complex selective pressures for resistance in livestock, the AMR transmission rates between animals and people, and the management practices that help or hinder the emergence and persistence of resistance on farms. Such knowledge gaps make evaluating the risk posed by AMU in livestock to public health a topic ripe for

debate. Consequently, evidence-based interventions are elusive and controversial.

Links through travel and trade make AMR in people and animals a global problem. International agencies are supporting national solutions to this worldwide issue through prudent AMU guidelines, surveillance strategies and standardized risk assessment techniques. At home, Canada operates an integrated AMU and AMR surveillance program in humans and the major meat commodities. This program, along with an active research community, is providing government and industry strategists with a scientific foundation for decisions. Scrutiny of our veterinary drug regulations has resulted in preliminary policy and regulatory changes to eliminate inappropriate antimicrobial access and use, but this process has been slow and leaves much to be done. The Canadian government is responsible for ensuring the health of Canadians through policies and regulations that ensure safe food production. It must carry this responsibility out while concurrently supporting a sustainable livestock industry that can produce food in a financially and environmentally sound manner. This task requires continued commitment to evidence-based policy and advocating that other nations do the same.

Without doubt, unnecessary and inappropriate AMU occurs in agriculture. There is currently no prudent AMU education for producers or nutritionists. Education is desperately needed for the people who initiate much of the agricultural AMU in Canada. Yet concerns over inappropriate AMU often overshadow the good-news stories in the industry. Agriculture and veterinary medicine have made advancements in animal health that decrease their reliance on antimicrobials. Livestock producers have embraced improvements in sanitation, nutrition, and vaccine technology. Biosecurity enables flocks and herds to remain negative for diseases that are endemic in the industry—diseases that were traditionally controlled with antimicrobials. Producer-organizations have taken a proactive stance on food safety. Both chicken and pork have self-mandated on-farm food safety programs. These programs support best management practices and appropriate AMU. In conjunction with these farm-based improvements, the slaughter and meat processing industry has made substantial advancements in reducing bacterial contamination of meat. In North America, the rate of foodborne diseases declined after slaughter and processing plants implementing hazard analysis and critical control point (HACCP) systems. These interventions undoubtedly decreased the burden of illness in people from resistant bacteria in food but have not been quantified as our surveillance ends at 'the fork,' rather with than health outcomes.

Although great progress has been made, there is still a great deal of work to be completed. We have advocated for continued research but also recognize that such research is ineffective without improvements in knowledge management. Novel techniques must be found to systematically assimilate the immense volume of discrepant research, ensure results are evaluated in context, and distribute the contextualized output to practitioners and policy makers. Beyond this, the main recommendations from this report are as follows:

- Seek and support research into the effectiveness of interventions, including but not limited to AMU withdrawal, to mitigate existing AMR
- Seek and support research that expands on the current 'farm-to-fork' approach to account for diverse human health outcomes
- Advocate for fair, transparent, veterinary drug regulations, AMR and AMU monitoring around the world based on scientific evidence, risk assessment, and appropriate precaution to ensure free and open trade of safe meat products
- Change Canada's veterinary drug regulations to ensure prudent and safe AMU while committing to transparent policy evaluation
- Deliver antimicrobial use education to producers and nutritionists
- Foster an innovative and collaborative relationship between regulators, public health officials and the agriculture industry

Table of Contents

Introduction

Arguably, one of the most important discoveries in medicine was the development of antimicrobials. It was not until the start of the 'antibiotic era' that human and veterinary medicine became equipped to deal successfully with various bacterial diseases. The introduction of antimicrobials had profound effects on patient morbidity and mortality and ushered in an era of great optimism. The optimism was so great that in 1967, the U.S. Surgeon General somewhat infamously declared that it was "time to close the book on infectious diseases" (1). In hindsight, statements such as that seem ludicrous as the pendulum has now swung in the opposite direction. Some people have expressed concern that the end of the 'antibiotic era' may be near and questioned whether continued emergence of multidrug-resistant pathogens represents an 'unwinnable war' (2,3). Like most situations, the reality likely lies between those two extremes, but concerns over the emergence and dissemination of antimicrobial resistant pathogens in human and veterinary medicine are significant.

It was not long after antimicrobials became widely used in human medicine that antimicrobial resistance emerged in human pathogens. Biologically, there was no reason to believe that the response of bacteria in livestock to antimicrobial exposure would be any different and, indeed, the use of antimicrobials in animals has certainly led to the development and dissemination of AMR in various animal pathogens and commensal organisms. It is irrefutable that antimicrobial use (AMU) is a key component for development of antimicrobial resistance (AMR). However, it is equally clear that there is not a simple and consistent relationship between AMU and AMR. Use of *some* antimicrobials in *some* species in *certain* situations clearly results in resistance in *some* bacteria. However, something that happens with one animal species, bacterium, drug, or management system does not necessarily happen in all others, illustrating the complexity of the field. Responses to previous efforts to control AMR based solely on AMU have met with variable responses because of this complex and rather unpredictable biological variation, indicating the need for detailed interdisciplinary (and international) study to optimally address this pressing issue.

Antimicrobial use in animals and subsequent identification of AMR in pathogens and commensal bacteria has raised numerous concerns. The greatest involves the potential for AMU in animals to be reflected in AMR in human pathogens. The closest contact that most people have with livestock is through handling and consumption of food, and that is generally the main route of exposure of humans to bacteria of food-animal origin. As AMR increases in animals, and resistant bacteria contaminate food, the potential for human exposure increases. However, just as AMU can lead to AMR, and the presence of resistant bacteria in animals can lead to infections in people, defining what 'can' represents in terms of true incidence and risk is problematic. More objective information is needed to quantify the health risk and to develop interventions for the transmission of resistant bacteria to humans through food.

A broad, multidisciplinary approach is needed to address the relationships between AMU and AMR because this topic is constantly evolving with respect to management practices, AMU, pathogen distribution, monitoring methods, and research techniques. This document provides an overview of issues, concerns, research, policies, regulations, strategies, and legislations pertaining to AMU and AMR in swine and poultry, as well as an assessment of critical gaps in knowledge and efforts. Using a review and critical assessment of the literature for relevant research, policies and strategies that can, or have been used to address AMR in swine and poultry, along with consultation of relevant experts, this document takes a stepwise approach to understanding and assessing this topic. The first step is a basic description of AMR ecology, including mechanisms of resistance and means of acquisition or transmission of resistance, in order to provide the scientific background required for assessment of this field. An understanding of zoonotic

pathogens of particular concern with respect to AMU and AMR in swine and in poultry is critical, but so is recognition of the role of the commensal microflora in AMR acquisition and transmission. Zoonotic bacteria of lesser concern are also discussed, as is the potential role of some emerging pathogens that may become a significant concern to the swine and poultry industries.

An understanding of current and historical antimicrobial use practices in livestock is provided, including why antimicrobials are used and how they are administered. The strength of evidence on the appropriateness of AMU in livestock production is evaluated. The logical extension of this is the merging of the two initial topics, and an assessment of links between AMU and AMR in poultry and swine. An important aspect of this topic is critical assessment of the current literature to address the contentious question of the extent that AMU in swine and poultry contributes to AMR in human pathogens.

Various policies, regulations, and legislations have been developed or enacted in an attempt to control AMU and AMR, with varying degrees of evidence and efficacy. The complexity of the field, differences in perspectives and absence of objective data in many key areas have led to much controversy regarding previous, current, and proposed control methods. The various organizations involved in AMU and AMR and their roles are described. This includes both international bodies and national systems. Additionally, the surveillance activities of these groups are compared. The varied approaches between groups underlie the differences in concern, perceptions, resources, and leadership in different countries and organizations. The structure and function of current systems are important for assessing current practices. There is particular focus on the Canadian perspective including surveillance, responsibility for AMU and AMR regulation, current regulatory practices and issues, and identified strengths and weaknesses within this system.

While objective data regarding the links between AMU and AMR are variable and information on effective interventions is sporadic and sometimes contradictory, both the livestock industry and regulatory bodies have developed various approaches to reduce the impact of AMR in chickens and pigs on human health. Key interventions are highlighted. Potential therapies such as prebiotics and probiotics are attractive but are largely unproven and under-investigated. Management measures to reduce the need for antimicrobials through improved animal health are an obvious area of interest. Management can have a tremendous effect on animal health, but research in many aspects is limited and a broad approach balancing animal health, animal welfare and production economics is needed, thereby complicating this area. Research on these topics is evaluated and presented with a description of the current production, management and marketing system differences between swine and poultry that can have an effect on feasibility, outcomes, and required measures. Additionally, current AMU guidelines that have been developed are discussed in terms of their content, practical application and potential consequences.

Zoonotic diseases will certainly remain as a pressing concern in human medicine, and animal diseases will be similarly important in veterinary medicine. Balancing human and animal health is a complex concept as measures taken in one group can have unpredictable or unknown effects in the other. Clearly, there are no straightforward answers for control of this issue. Cessation of all antimicrobial use is not practical for animal health, animal welfare, and production economics. Continuation of the status quo is similarly unacceptable because of evidence regarding the impact of agricultural AMU on some aspects of human disease. Identification of the appropriate middle ground is required to optimize human health while maintaining humane, safe, and economically viable swine and poultry production. Rhetoric, opinions and anecdotes are not acceptable foundations for wide-reaching policy, regulation, and legislation, but current knowledge gaps create an environment where such subjective information can have major impact. This document identifies important concepts and practices that should be considered for setting priorities, developing and implementing policy, regulation and legislation, and educating all relevant stakeholders.

Methods

This project reviews the literature on the ecology of antimicrobial resistance (AMR), AMR in zoonotic and commensal bacteria with the potential to cause foodborne disease, antimicrobial use (AMU) in pigs and chickens, and the surveillance of AMU and AMR in pigs and chickens. It evaluates the literature describing the relationships between AMU and AMR as well as the policies, strategies, and interventions to control AMR in bacteria carried by chickens and pigs. Throughout this document, the term 'antimicrobial' is defined as, "any substance of natural, semi-synthetic, or synthetic origin that kills or inhibits the growth of a microorganism but causes little or no damage to the host." (4) and is used in the context of inhibiting or killing bacteria.

Publications utilized in this project were obtained either through publication databases subscribed to by the University of Saskatchewan library or through known resources (both written and personal) that the authors were aware of, or in contact with, as a result of their expertise in this area. The authors preferentially utilized peer-reviewed scientific literature. When publications were unavailable or insufficient, the alternatives of reports, websites and personal communications were employed. Relevant databases were identified and prioritized to ensure the literature search was comprehensive but not redundant.

The study inclusion and exclusion criteria were defined (Table 1) and used to establish *a priori* search terms, search strings, and medical subject heading (MeSH) terms (Appendix 1). Searches were restricted to English publications and the years 1990 to 2009. A professional librarian conducted each search, recorded the identified citations, and acquired necessary full texts. Three databases were searched systematically using consistent search terms: CAB, Embase, and Medline. Two databases were searched systematically with terms tailored to the database content: Agricola and Scopus. These searches returned a large number of citations (> 3,500) and a high rate of duplicates (approximately 40%), thus further databases were only used to address specific inclusion criteria. The citations identified by the searches were evaluated using *a priori* relevance screening criteria that elaborated on the inclusion and exclusion criteria. All relevant publications were briefly summarized as to which inclusion criteria they addressed.

Because of the volume of publications identified, a supplementary search strategy was applied to ensure critical publications were prioritized. A series of searches was completed using only the Medline database to identify articles indexed by the following: i) terms similar to foodborne, food, animal origin, antimicrobial resistance, or antibiotic resistance; and terms similar to *Escherichia coli, Enterococcus, Salmonella, Campylobacter;* MRSA or *Clostridium difficile;* and terms similar to swine, pig, pork, poultry, broiler, or chicken; ii) terms related to antimicrobial use in the broiler or swine industry, microbial ecology, or relationship between AMU and AMR and; iii) terms designed to look at the potential association between AMR in chickens and pigs and AMR and health in humans. This search was limited to publications since 1999 to emphasize the most current developments in the field. Again, the search strings and number of citations identified were recorded. Duplicate articles were removed and the results were saved in an electronic reference manager software (Refworks ©). The resulting data files were exported into a custom designed database, using Microsoft Access, for reviewers to describe and search the content of each paper for relevance to the project.

Two veterinary epidemiologists independently reviewed citation titles and abstracts and excluded citations that did not meet the inclusion criteria. The title and abstract of the remaining citations were used to describe the publication by a series of dichotomous variables that included the bacterial species, livestock species, stage of production or processing, AMU data, and type(s) of AMR data

(prevalence, associations with human health, microbial ecology or relationship between AMU). The independent reviews were combined and discrepancies discussed and agreed upon. The full publications were acquired and reviewed by the author(s) of pertinent sections.

Following the conclusion of these searches, and while describing and evaluating the literature, authors occasionally conducted ad hoc searches of publication databases, the Internet, or known grey literature. These results of ad hoc searches were not recorded. All authors reviewed the entire document. An external review was graciously provided by veterinary epidemiologists specializing in swine, poultry, antimicrobial resistance, and surveillance techniques from the Public Health Agency of Canada.

As a result of this process this report cites 539 references. The majority of references (n = 360, 67%) are articles published in journals. Of these, 252 (47%) describe original research, 98 (18%) are reviews, and 10 (2%) describe government programs or research. These journal articles were published between 1977 and 2009; 13% were published prior to 2000, 65% were published between 2000 and 2007 and 22% were published in 2008 or 2009. The remaining 179 citations reference books (26, 5%), websites (44, 8%), conference proceedings (29, 5%) and reports or monographs on the internet (80, 15%).

In addition to the journal articles, original research was described in 21 conference proceedings, 19 reports, and 5 websites. Thus, 297 (55%) of the citations refer to original research. The remaining 242 citations (45%) were government reports (72, 33%), scientific reviews (136, 56%) and grey-literature (27, 11%). The proportion of citations that are original research is disproportionately low relative to the proportion of original research citations identified in the *a priori* literature searches because many of the grey literature citations and government reports were intentionally sought out to address the project objectives.

Table 1. Comprehensive review criteria for literature inclusion and exclusion.

Category	Inclusion	Exclusion
Exposure Route	**Foodborne**	Non-foodborne
	Exposure route	Direct contact with animals
	Direct consumption	Occupational exposure
	Exposure to raw meat	Environmental
Bacteria	**Zoonotic Pathogens**	Animal pathogens
	Salmonella	Environmental bacteria
	Campylobacter jejuni	Human pathogens infecting animals
	Campylobacter coli	Animal commensals beyond list
	Clostridium difficile	
	Yersinia enterocolitica	
	Listeria monocytogenes	
	MRSA	
	Commensal	
	Escherichia coli	
	Enterococcus	
Pharmaceuticals	**Antimicrobials**	Antifungals
	Antimicrobials used in humans or veterinary medicine	Antivirals
	Antimicrobial feed additives	Hormones
	Ionophores	Vaccines
Sectors	**Swine**	
	Farm	Boar studs
	Abattoir	Wild boar operations
	Commodity boards	Wild animals
	Broilers	
	Broiler breeder	Layers
	Hatchery	Eggs
	Growers	Turkeys
	Abattoir	Minor species (duck, quail)
	Commodity boards	Wild birds
		All other food or livestock sectors
	Governments	
	Agriculture departments	Governments below federal level
	Health departments	
	International agencies	
	Support Industries	
	Veterinary associations	
	Pharmaceutical industry	
	Nutrition/feed companies	

Chapter 1: The Hazard of Antimicrobial Resistance in Foodborne Bacteria

Introduction

People are increasingly affected by antimicrobial resistant bacteria; they can be exposed to bacteria through many environmental connections, one of which is the food they eat daily. Through food, people can be exposed to antimicrobial resistant zoonotic pathogens and commensal bacteria. Foodborne bacteria typically originate in the animal's gastrointestinal tract and reach people through fecal contamination of meat at slaughter. The gastrointestinal tract provides an ecological reservoir that supports a diverse bacterial population. Most bacteria in this population are beneficial or neutral to the animal host and are not the target of antimicrobial drug use. However, antimicrobial exposures intended to kill pathogens or improve growth also affect these bacteria. Resultantly, the normal flora becomes an unintended casualty or develops/acquires resistance. When people consume enteric bacteria through contaminated food, the bacteria are returned to a similar ecological niche and can either cause disease or share resistance elements with the diverse bacterial flora resident in people (5–7).

Scope and Objectives

The objective of this section is to provide sufficient knowledge of the hazards to public health, the source of bacteria, and the resistance types of greatest concern to facilitate the evaluation of the programs, policies, and strategies to control AMR that have been created by regulators and the agriculture industry. This chapter is limited to describing antimicrobial resistance (AMR) in bacteria that infect people through food. It is restricted to discussing resistance as it pertains to chicken and pigs/pork. This limitation in scope does not infer that these commodities pose a different risk to people than other meat commodities. After describing how people are exposed to resistant bacteria, the biology of AMR is explained. The main body of this chapter is devoted to describing concerning pathogen/resistance combinations. Some are well known, like *Campylobacter* and *Salmonella,* while others are either of secondary importance to Canadians, such as *Yersinia enterocolitica,* or emerging food safety concerns such as *Clostridium difficile.* The relevance of resistance in these foodborne resistant pathogens for Canadians is the focus of this chapter.

The Link Between AMR Bacteria in Animals and Humans

Bacteria are ubiquitous in our environment and often infect multiple animal species including humans. The probability that people will be infected or colonized by bacteria from animals is affected by the route of exposure, frequency of exposure, the exposure dose, and the host-adaptation of the bacteria. Bacteria carried by animals that can cause disease in humans are termed 'zoonotic.' The disease caused by these zoonotic bacteria can be exacerbated if the bacteria are resistant to antimicrobials (8–10). In addition, antimicrobial resistant bacteria can be a source of resistance elements for bacteria harboured by people (11–13). Direct physical contact, shared environments, and exposure through vectors and fomites are all routes for bacterial transmission between animal species. This review focuses on foodborne bacteria.

The dominant link between most Canadians and viable bacteria from animals is likely food consumption. Resistant bacteria undoubtedly spread into the environment through aerosol and animal wastes (14–16). However, modern livestock production has greatly reduced the frequency and extent of contact between humans and agricultural animals. Only 2.2% of Canadians live in the farm population; the remaining 97.8% have limited direct contact with animals and their environment is largely separate from the airspace and properly managed waste of livestock (17). People in direct contact with

animals face different hazards and have different risk factors for acquiring AMR bacteria and, while further study is required to clarify the extent that resistance spreads to people by these routes, these links are beyond the scope of this project.

Antimicrobial resistance directly affects human health when infection with pathogenic bacteria leads to illness that requires antimicrobial therapy and the selected therapeutic is ineffective due to resistance. This results in prolonged illness with potentially more severe symptoms. The worst case scenario is a bacterial infection that is refractory to all available treatments leading to death (9,10,18). Although treatment failure is the most obvious effect from AMR, other health hazards exist due to AMR in bacteria. Numerous studies show the risk of hospitalization and the severity of disease are greater in people acquiring a resistant bacterial infection compared to those with a similar but susceptible strain (19–23). This increases the cost of treatment and the burden on the health care sector, and places people at risk for exposure to other nosocomial infections (10,24). The increased disease severity may partially be related to ineffective treatment early in the disease and subsequent disease progression. But increased hospitalization and disease severity is reported even after accounting for ineffective therapy. This could be due to co-selection of virulence traits, up-regulation of virulence traits, or improved fitness of resistant strains (25,26). Antimicrobial resistance also increases the incidence of foodborne disease as people taking antimicrobials for any reason have an increased likelihood of being colonized by resistant bacteria because the therapeutic drug alters the body's normal flora and concurrently selects for resistant bacteria (9,18).

Two types of resistant foodborne bacteria contribute to health burdens. Resistant pathogenic bacteria directly contribute to all four effects described above: treatment failure, increased hospitalization rates, increased disease severity, and increased disease incidence. Resistant commensal bacteria indirectly contribute to the problem by harbouring and spreading resistance genes to bacteria pathogenic to people (27–30). Transmission can theoretically occur from commensal bacteria originating in livestock to pathogens in humans, or vice versa, from commensal bacteria in humans to pathogenic bacteria in livestock. Although this contribution is indirect, it may pose a risk to humans that is equal to or even greater than that posed by pathogenic bacteria. Commensal bacteria are ubiquitous in healthy animals and can contaminate carcasses at slaughter. The high prevalence of these bacteria drastically increases the probability of exposure compared to pathogenic bacteria.

Introduction to AMR Ecology

Resistance Development

Antimicrobials either kill (bacteriocidal) or inhibit (bacteriostatic) bacteria. Not all antimicrobials are effective against all bacteria: bacteria that are intrinsically resistant lack the structural or functional cellular mechanisms that are required for the antimicrobial to act (4,31). Intrinsic resistance is a genus or species-specific property of bacteria (32). While it is necessary to understand that some bacteria are inherently resistant to certain antimicrobials, this is not the focus of this review; therefore, the remainder of this report will pertain to acquired AMR. Acquired resistance occurs due to a change in the bacterial genome. The two major ways that susceptible bacteria acquire AMR are through mutation or horizontal acquisition of foreign genetic material (31,33).

Mutation

Mutation is a spontaneous change in the genome resulting in a susceptible bacterium becoming resistant, usually during replication (33). Chromosomal mutations often result in structural changes to the bacterial cell wall which subsequently confers resistance (4,31). Mutation may lead to dramatic resistance development or to slower more gradual resistance development depending on the antimicrobial agent affected (4,31). Mutants may be disadvantaged compared to the parent and, therefore, less able to survive in the population in the

absence of the selective pressure of an antimicrobial. Alternatively, mutants may be as or more viable than the original strain and may persist in the population with or without selective pressure from the antimicrobial (34). The emergence of resistance from mutational events happens at high frequencies for drugs such as streptomycin, nalidixic acid, and rifampin and has not been reported for others such as vancomycin and polymixin B (4).

Horizontal Transfer of Resistance

The horizontal transfer of resistance genes from donor to recipient bacteria is a second method through which bacteria can acquire resistance. Transformation, transduction, and conjugation are the three primary means for the horizontal transfer of resistance genes (35).

Transformation is the uptake of naked bacterial DNA from the environment by acceptor bacteria (4,35). It is an important method of gene transfer *in vitro* but less important *in vivo* (32). Transformation generally occurs between closely related genera and may result in gene recombination producing new forms of resistance genes. This method of resistance transfer is particularly important in bacteria species such as *Streptococcus* and *Neisseria* that have a high frequency of natural transformation (4).

Transduction is the transfer of resistant genes via a bacterial virus or phage (4,35). This is thought to be a relatively unimportant method of resistance transfer because bacteriophages are very specific to the bacterial host and can carry a limited amount of DNA; but occasionally, resistance plasmids can be accidentally packed into phage heads during phage assembly and subsequently be able to infect new cells by injecting plasmid DNA into a recipient cell (4,32). Neither transformation nor transduction requires a viable donor cell or a link between donor and recipient (31).

Conjugation is the transfer of resistance genes from a donor to a recipient bacteria through a temporary protein channel (4,35,36). Gene transfer in conjugation allows the spread of mobile genetic elements such as plasmids, transposons, or integrons/gene cassettes (35–37). These elements can possess multiple AMR genes and may be responsible for the rapid dissemination of resistance genes among different bacteria (28,38,39). Linked clusters of resistance genes on a single mobile element can aggregate in such a way that antimicrobials of a different class or even non-antimicrobial substances like heavy metals or disinfectants can select for AMR bacteria (40,41). Exchange of resistance genes between pathogens and non-pathogens or between gram-positive and gram-negative bacteria has been documented (40).

The Spread of Resistance: Mobile Genetic Elements

Of the three mechanisms for horizontal transfer of resistance genes: transformation, transduction, and conjugation, conjugation is undoubtedly the most important to understand. The acquisition of genetic elements such as plasmids, transposons, or integrons/gene cassettes is a critical part of horizontal transfer of AMR because these elements connect and re-assort resistance mechanism thus enabling the spread and establishment of resistance elements in bacteria populations. These elements vary considerably from each other in regard to their carriage of resistance, their replication, and transmission.

Plasmids are extra-chromosomal circular DNA that can replicate independently of chromosomal DNA. When resistance is transferred via plasmids, a copy of the plasmid is retained by the donor cell. Most plasmids carry the gene required for conjugation, but not all do. In these cases, plasmids can be mobilized by using the conjugal apparatus of other self-transmissible plasmids within the cell (4,32). Plasmids can harbour resistance genes for between one to ten different antimicrobials (multiple AMR) (4). Multi-resistant plasmids are often the result of interplasmidic recombination, integration of transposons, or insertion of gene cassettes (32). All resistance genes on a multi-resistant plasmid are transferred when the plasmid is transferred, whether there is selective

pressure for all of the resistance genes on the plasmid or for just one of the resistance genes (32). Plasmids can act as vectors for transposons and integrons/gene cassettes (36).

Transposons (jumping genes) are short sequences of DNA that can move from plasmid to plasmid, or from plasmid to chromosome, and vice versa. Transposons do not possess replication systems and must be incorporated into chromosomal DNA or plasmids (32). Unlike plasmids, no copy of the transposon remains within the original cell as the transposon moves between the donor and recipient. All transposons can move and integrate into foreign DNA by non-homologous recombination, which permits the same transposon to be found in the genome or plasmids of highly unrelated organisms (4).

Integrons are a mobile element often found on plasmids and are distinct from transposons. They are a site-specific recombination system consisting of an integrase enzyme, a gene-capture site, and a captured gene cassette(s). Each gene cassette encodes a single resistance gene and a specific recombination site (4,41,42). An integron's site-specific integrases recognizes gene cassettes and catalyzes their insertion at a specific site. Repetition of this sequence results in integrons linking together multiple resistance gene cassettes (4).

Gene expression of an integron is dependent on various factors including promoter strength, gene copy number, the relative distance of the gene cassette from the promoter, and the presence of additional internal promoters. Expression is usually mediated via a common promoter situated upstream (5'-end) of the gene cassettes, rather than through individual promoter copies. Higher levels of gene expression can be achieved if a second promoter is included adjacent to the first, or if the gene in question is included as multiple copies. The relative distance between a gene cassette and the promoter plays a significant role regarding expression; proximal genes tend to be expressed more effectively than distal genes. As a result, distal genes may have very little effect on the susceptibility of the host bacterium to relevant antimicrobials (43,44). Integron carriage of resistance gene cassettes by host bacterium can be dependent on the environment; host bacteria can potentially lose integron-borne resistance genes in the absence of antimicrobial selective pressure (45).

Resistance Selection: Direct Selection, Cross-resistance and Co-selection

As mentioned earlier, the development of AMR is a complex process and the speed with which it develops depends on the bacteria involved, the selective pressure, and the availability and transferability of resistance genes (32). Recent studies have shown that the majority of multi-resistant phenotypes are obtained by the acquisition of external genes that may provide resistance to an entire class of antimicrobials (46). Antimicrobial use can select for antimicrobial resistant bacteria in three ways: direct selection, cross-resistance, and co-selection.

Direct selection is the most simplistic form of selective pressure and occurs when a drug selects for bacteria resistant to it. For example, tetracycline is used and tetracycline resistant bacteria survive. Cross-resistance occurs when the expression of one antimicrobial resistance gene infers resistance to several related drugs that have similar targets or mechanisms of action. The *blaCMY-2* gene provides an example of cross-resistance. This gene confers resistance to many potentiated ß-lactams (ampicillin and amoxicillin-clavulanic acid) and cephalosporins (ceftiofur, cefoxitin, and ceftriaxone). Therefore, exposure to ceftiofur will both select for bacteria carrying this gene and indirectly increase the frequency of resistance to ampicillin. Cross-resistance is also common in the macrolide and fluoroquinolone classes (4).

Co-selection is the phenomenon of antimicrobial use selecting for resistance to completely unrelated drugs. Bacteria with multiple resistance genes can survive exposure to any drug affected by these genes. Therefore, the use of any of these drugs perpetuates resistance to all of the unrelated antimicrobials for which bacterium possesses resistance genes. (4).

Co-selection has important implications for policies designed to eliminate existing resistance and contributes to the complex relationship between antimicrobial use and resistance development (use of drug A can select for resistance to drug A; but because of co-selection, it may also be selecting for resistance to drugs B, C, and D).

Phenotype versus Genotype

Information on both the resistance genotype and phenotype are valuable in evaluating resistance. Genotype data illustrate the diversity and distribution of resistance, which improves our understanding of transmission and selection. Phenotype data provide an indication of the susceptibility of the organism and is of clinical relevance. Phenotype and genotype AMR results may not correlate completely. Bacteria can have a resistant phenotype and susceptible genotype if the active resistance genes were not considered in testing or are novel and not yet identified. Conversely, a susceptible phenotype and resistant genotype can arise if genes are incompletely expressed, confer resistance below the phenotypic threshold, or are non-functional (47,48). Because each provides different information, considering both the phenotype and genotype provides a more complete understanding of AMR.

AMR in Foodborne Bacteria

Zoonotic Bacteria

Campylobacter

Campylobacter is the leading reported cause of bacterial foodborne enteric infections in many developed nations. Most cases are mild, self-limiting and do not require antimicrobial therapy, but severe cases can be prolonged, progress to septicaemia or extra-intestinal infection, and require antimicrobial therapy (49). In Canada, approximately 12,000 cases are reported annually with an estimated 23 to 29 unreported illnesses per reported case (50,51). *Campylobacter* can be contracted from both food and water but warm blooded animals are the only site of amplification (52). Eighty to ninety percent of human infections are caused by *C. jejuni* while *C. coli* accounts for 5 to 10% (50,53). Although less significant than *C. jejuni, C. coli* can rank among the top four causes of enteric infection in people and should not be discounted as a food-safety concern (54).

Campylobacter primarily cause sporadic disease and to date, an effective typing system has not been developed. Together, these factors make source attribution difficult (55). Poultry is considered a leading source for foodborne infections while the role of pork is less clear (56–59). Chickens generally carry *C. jejuni* as part of their commensal flora and pigs typically harbour *C. coli* (56,60–63). Although both pigs and chickens commonly carry *Campylobacter* at slaughter, retail meat sampling consistently identifies much higher *Campylobacter* recovery rates from poultry than red meats (64–66), presumably because chicken intestines are more friable and prone to breaks that can cause carcass contamination. Case control studies routinely aggregate *C. jejuni* and *C. coli* cases. This may cause risk factors unique to *C. coli* to be missed because *C. coli* accounts for a smaller proportion of cases and could partially explain why pork is not consistently identified as a risk factor for *Campylobacter* infection (67).

It is believed that resistant cases of human campylobacteriosis generally arise from acquiring a resistant strain, versus human therapeutic drug use selecting for resistance (23,68). Initially, this seems counterintuitive given that resistance arises rapidly following therapy in people. However, AMU in uncomplicated cases is contraindicated so exposure in people should be limited. In contrast, antimicrobial consumption prior to an infection can increase the risk of a resistant infection in people (69), which suggests that human AMU provides a competitive advantage to already resistant strains. *Campylobacter* are ubiquitous in healthy pigs, so long durations of AMU can result in a prolonged and pervasive selective pressure on the *Campylobacter* population to acquire resistance and establish resistant strains. In reality, attributing resistance in *Campylobacter* to agricultural AMU is difficult as it requires data on AMR in isolates

prior to therapy and should account for AMU prior to infection. The resistance rates in *Campylobacter* isolated from Canadians are available from regional research projects (70–72), but not from national surveillance. The most recent results from American surveillance found over half of the *Campylobacter* tested were resistant to one or more Clinical Laboratory Standards Institute (CLSI) antimicrobial classes and 13.6% were resistant to two or more subclasses (73).

Campylobacter's most concerning resistance types are macrolides and fluoroquinolones. Ciprofloxacin is a frontline drug for undifferentiated gastroenteritis, so fluoroquinolone resistance may cause treatment failure. High level fluoroquinolone resistance occurs with a single-step chromosomal mutation in the *gryA* (60,74). Resistance to fluoroquinolones in *C. jejuni* from poultry and humans is very common in certain parts of the world, including Mediterranean Europe and South East Asia (53,75–77). In comparison, almost complete susceptibility to ciprofloxacin is reported in *C. jejuni* from Australia, Norway, and Sweden (78–81). In poultry, emerging resistance in *C. jejuni* correlates with the use of fluoroquinolones and this resistance does not appear to impose a fitness cost, and may even be advantageous (34,49,82). No fluoroquinolones are licensed for use in pigs or chickens in Canada although injectable enrofloxacin could be used in an extra-label manner. The rates of ciprofloxacin resistance in *C. jejuni* from retail chicken ranged from 0 to 2.3% from different parts of the country in 2006 (66). National surveillance for AMR in *Campylobacter* from pigs is not conducted in Canada, but Canadian research projects have reported 2.4 and 10% ciprofloxacin resistance in *Campylobacter* from pigs on farms (83,84).

Resistance to macrolides is also concerning in *Campylobacter* because this antimicrobial class is prescribed for severe cases of campylobacteriosis or immunocompromised cases. This has raised concern over widespread macrolide use in veterinary medicine (85,86). From American human cases, 1.6% of the *C. jejuni* and 3% of the *C. coli* were resistant to macrolides (87). The rate of resistance in human infections is highly variable world wide; rates as high as 51% in Singapore and 80% in Nigeria have been reported in *Campylobacter* isolates from children (88).

Macrolide resistant *Campylobacter* are relatively rare in chickens (generally <1%) but are prevalent in pigs (generally >50%) (66,79,89). This difference is partly due to the predominance of *C. coli* in pigs and *C. jejuni* in broilers; *C. coli* are genetically predisposed to macrolide resistance. This species characteristic is so strong that 40% of *Campylobacter* from antibiotic-free swine farms have been identified as macrolide resistant. This could reflect intrinsic resistance, persistence of acquired resistance from historical AMU, or transfer of resistance that arose from AMU in other groups of pigs on the farm (90,91). *Campylobacter* from pigs also demonstrate more macrolide resistance due to extensive macrolide use in swine (84). Comparing macrolide use between pigs and chickens is hampered by different drug-use metrics, but over half of Canadian swine producers use macrolides in feed while less than 5% of chickens in America are exposed to macrolides (66,85,92,93).

The food safety hazard posed by macrolide resistant *Campylobacter* could plausibly increase in the future. Over 20 *erm* genes have been identified, which are the genes responsible for many of the mutations in the rRNA genes that cause cross-resistance between macrolides, lincosamide and spectinomycin. These *erm* genes exist in gram-positive and gram-negative bacteria and many are located on transmissible genetic elements. Acquisition of these genes, along with *Campylobacter's* ability to pick up heterologous DNA through transformation, makes the likelihood of macrolide resistance establishing in *C. jejuni* a concern (88). Because *C. jejuni* predominates in human disease, this could have a far greater human health impact than the current high rates of macrolide resistance found in *C. coli.*

Salmonella

Non-typhoid *Salmonella* are a leading cause of bacterial gastrointestinal disease worldwide. Most cases are self-limiting, but severe cases can become invasive and cause extra-intestinal infections. In many developed nations, the incidence of reported enteric infections from *Salmonella* is second only to *Campylobacter.* Canadians report 6,000 cases of *Salmonella* annually, and for each reported case, an estimated 13 to 37 cases go unreported (50,51). The global incidence of resistance in *Salmonella* infections appears to be rising; and in Canada, over one-third of human clinical isolates are resistant to at least one antimicrobial and 11% are resistant to five or more (66,94,95).

Ninety to ninety-five percent of non-typhoid *Salmonella* infections are foodborne (96–99). *Salmonella* causes both disease outbreaks and sporadic infections. Disease outbreaks are more likely to be detected by surveillance as the likelihood of medical involvement increases with the number of cases. However, outbreaks represent unusual events and can substantially bias source attribution given the large proportion of infections that go unreported (96). Surveillance often implicates meat, most commonly chicken, less frequently pork, and eggs as causes of *Salmonella* infections (97–99). Food surveillance most commonly isolates *Salmonella* from fresh meat, again more commonly from poultry than pork, and less frequently in eggs, beef, fishery products, vegetables and fruit, and milk (97). This shows that the food safety risk from certain sources, such as contaminated vegetables, can be overstated by disease outbreaks.

The source of *Salmonella* infections can vary depending on diet and geography, and over time, the prevalence and serovar distribution of *Salmonella* in food-animal populations can change. All of these factors affect human case attribution. Canadian attribution data are not currently available, but a surveillance program for enteric disease (C-Enternet) has been established in Waterloo, Ontario. As this program expands to its planned five or six sentinel sites, it will provide insight into the food sources of *Salmonella* for Canadians (96).

Not all *Salmonella* serovars cause disease in all hosts. For example, S. Cholerasuis and occasionally S. Typhimurium can cause clinical disease in pigs, and S. Gallinarium, S. Pullorum, and occasionally S. Enteriditis can cause disease in chickens (100,101). Chickens and pigs can carry many other serovars that rarely cause overt disease in their animal host but regularly cause disease in people. The most common serovars causing human disease worldwide are S. Enteriditis and S. Typhimurium (98,102). Along with these, S. Heidelberg is a predominant serovar in North America (66,103). Controlling zoonotic *Salmonella* serovars in food animals is the impetus for on-farm *Salmonella* control programs, as opposed to control of epizootic serovars.

The most direct threat posed by AMR in *Salmonella* is treatment failure following use of an antimicrobial to which the infecting strain is resistant. Accordingly, resistant infections are associated with higher case fatality ratios and increased hospitalization rates and durations (19,20,104,105). In addition to more severe disease, AMR may cause a higher disease incidence. A Danish model found that relative to their prevalence, quinolone-resistant *Salmonella* caused more disease than would be expected than quinolone-susceptible *Salmonella.* Also relative to their prevalence, multidrug-resistant *Salmonella* were associated with more illness than would be expected compared to pan-susceptible *Salmonella.* This trend was consistent across all serovars modelled. It was proposed that this trend could be due to enhanced ability to survive food processing or increased susceptibility in people consuming antimicrobials for other indications (98). Collectively, these studies demonstrate that antimicrobial resistance in *Salmonella* creates a health burden additional to baseline salmonellosis and that the increased burden is not limited to a particular serovar.

Public health officials recognize two resistance types in *Salmonella* pose an undue threat to human health. These include resistance to fluoroquinolones,

the main treatment for invasive salmonellosis, and resistance to newer generation cephalosporins, the indicated treatment for salmonellosis in pregnant women and children (86,106). Historically, fluoroquinolone resistance was conferred by two stepwise chromosomal mutations, and therefore, resistance only developed when bacteria were exposed to a fluoroquinolone and subsequently only transmitted vertically to progeny. Over the last few years, plasmid-mediated fluoroquinolone resistance genes have been reported in Europe, Asia, and the United States (107–111).

The emergence of horizontally transmissible fluoroquinolone resistance genes raises two concerns. First, these can be transmitted between *Salmonella* and *E. coli* in vitro, and presumably, in vivo (107). This exponentially increases the bacterial reservoir that can harbour these genes and eliminates species or geographical barriers to the dissemination of resistance. So far reports of plasmid-mediated quinolone resistance genes in animals are rare, but it appears inevitable that these genes will become established in livestock and poultry (74,107). This will further exacerbate control efforts regarding fluoroquinolone resistance. The second concern is that the emerging *qnr* genes that moderate fluoroquinolone resistance can be associated with mobile genetic elements with novel combinations of resistance genes including extended spectrum ß-lactam resistance genes (ESBL) (107,112,113). This drastically increases the likelihood of treatment failure as these are the two drug classes most commonly used to treat *Salmonella.* Potentially of equal concern is that use of drugs outside the fluoroquinolone class can now supply the selective pressure necessary to establish and disseminate fluoroquinolone resistance.

Public health authorities are also concerned about ESBL resistance in *Salmonella.* In the 1980s, resistance was predominantly due to broad spectrum ß-lactamases that hydrolyzed penicillins and older generation cephalosporins (TEM-, SHV-, CTX-M-). In the 1990s, resistance to newer cephalosporins emerged through two routes: one was via minor mutations of broad-spectrum ß-lactamases to become extended spectrum ß-lactamases and the other was through the emergence of the plasmid-mediated Ambler class C (AmpC) enzymes, predominantly mediated through CMY- genes. Each enzyme hydrolyzes a slightly different set of cephalosporins (114).

In humans, resistance types tend to cluster geographically, but extended spectrum ß-lactamases and AmpC resistance both occur in Europe and North America. In animals, extended spectrum ß-lactam resistance genes predominate in *Salmonella* in Europe with the few reported cases of AmpC-type resistance in European livestock linked to imported animals (111,114). In North American food animals, Ambler class C enzymes predominate and are generally mediated by the *blaCMY-2* gene. Across North America the major foodborne source of ESBL resistant *Salmonella* differs. In Mexico, multidrug-resistant S. Typhimurium carrying *blaCMY-2* are the primary cause of ESBL resistant *Salmonella* infections. The *blaCMY-2* gene has been identified in children with diarrhea and a foodborne link was made to pigs (19). In Canada and the United States, the most concerning serovar for ESBL resistance is S. Heidelberg and the main food-animal reservoir appears to be poultry, as determined from retail meat surveillance in these countries (66,73,115). In addition to sampling retail meat, the Public Health Agency of Canada tests isolates and reports AMR from clinical cases of *Salmonella* in people. In late 2003, 30% of the human S. Heidelberg infections from Quebec were resistant to ESBL. By 2004, this had risen to almost 50% of the cases. Concurrent with this rise, a sharp increase was observed in ceftiofur resistant S. Heidelberg in retail chicken sampled from eastern Canada (see Chapter 2 Figure 2) (116,117). The role of poultry as a main source of S. Heidelberg in Canadians has been supported by a case control study (118).

The epidemiology of AMR in *Salmonella* can relate to serovar. This was clearly recognized with the global dissemination of the infamous S. Typhimurium DT 104 clone carrying resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole,

and tetracycline (ACSSuT) (119,120). The ACSSuT resistance was carried on a chromosomal island, thus was vertically disseminated within this serovar (120). Yet, the predominance of *blaCMY-2* in S. Heidelberg demonstrates that certain *Salmonella* serovars can have an affinity for particular plasmid-mediated resistances or plasmids. *Salmonella* serovars and clones can shift independent of AMU, but shifts can also be influenced by AMU (121). This makes it difficult to understand how AMU is affecting *Salmonella* epidemiology in general and resistance in particular. The relationship between serovar and AMR also makes it difficult to generalize about the prevalence or patterns of resistance in *Salmonella.* This challenge is addressed by limiting discussions to a specific serovar or, as will be discussed, circumvented by using generic *E. coli* as an indicator for *Salmonella* in particular and gram-negative bacteria in general.

Commensal Bacteria

E. coli

Salmonella and *Campylobacter* clearly demonstrate that bacteria can be transferred from animals to people through food. These zoonotic pathogens cause disease in people, and if the strain is resistant, it can increase the disease burden. However, these bacteria represent a small fraction of the total possible spread of resistance elements to people through food. The normal bacterial flora responds to antimicrobial exposures by establishing resistant populations. Fecal contamination of meat at slaughter is one mechanism that can result in human exposure to these bacteria, and once consumed, genetic exchange with pathogenic or commensal bacteria in the human gastrointestinal tract can occur. While transmission rates remain unknown, the sheer volume of these bacteria makes many experts believe that the global threat from AMR may be more greatly impacted by this commensal reservoir than emerging resistance in pathogens (12,122).

Fecal contamination of food is generally monitored through recovery of *E. coli* (123). Expanding on the role as an indicator of meat contamination, *E. coli* from healthy animals have also been adopted as an indicator of AMR for gram-negative bacteria. *E. coli* are used to understand resistance in bacteria with complex ecologies such as *Salmonella.* Although a relationship does exist between serotype and resistance types in *E. coli,* this relationship is largely ignored in *E. coli* isolated from healthy animals (aka generic *E. coli*). This is valid because, as opposed to pathogen overgrowth, a diverse population of *E. coli* can concurrently exist in the gut which differs from the clonal populations seen with pathogen overgrowth. The ubiquity, simple isolation, and diversity of *E. coli* makes it as a good model for understanding AMR in *Salmonella* because the genes operating in *Salmonella* also operate in *E. coli* and can be transmitted between these bacterial species (27,107).

Although *E. coli* is a useful model for AMR in *Salmonella,* differences certainly exist. The same resistance phenotypes, and often genes, operate in these bacteria, but their behavior and transmission can differ. For example, the genes encoding the ACSSuT phenotype in S. Typhimurium are located on the bacterial chromosome yet when this resistance phenotype is identified in *E. coli,* the genes are typically located on plasmids. Furthermore, the value of *E. coli* for understanding AMR in *Salmonella* does not extend to predicting resistance in *Salmonella* based on resistance in *E. coli* on a farm or regional level (124). This limitation is at least partly due to differences in the location and transmission of these genes in each bacterium as well as an inability to account for the relationship between resistance and *Salmonella* serovar.

Escherichia coli are used to study the selective pressure on the gram-negative bacterial population to develop and retain resistance. The intestinal normal flora is not the target of antimicrobial treatments, but nevertheless these bacteria are exposed and become resistant—much like civilian casualties in war. The degree of resistance in *E. coli* mirrors this selective pressure on gut bacteria (48,125,126). Thus the greatest utility of studying AMR in *E. coli* is

improved understanding of the health risks posed by antimicrobial use.

As an indicator organism, the main resistance outcomes of concern in *E. coli* are those that are a concern in *Salmonella.* Resistance to fluoroquinolones and newer generation cephalosporins are monitored closely. In contrast to the relatively rare reports of plasmid-mediated fluoroquinolone resistance in *Salmonella* from the agri-food sector, several reports of plasmid-mediated resistance in *E. coli* exist (108,127–129). Detecting emerging resistance is much simpler in *E. coli* than *Salmonella* simply because large, representative isolate collections can be assembled quickly and economically. *E. coli* is also useful for understanding the reasons for changing AMR in *Salmonella.* Through retail chicken surveillance in 2004, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) noted the rise in ceftiofur resistance in S. Heidelberg was mirrored in *E. coli* (66). This provided supporting evidence that resistance was changing due to antimicrobial drug pressures rather than the emergence and dissemination of a resistant *Salmonella* clone.

Resistant gram-negative bacteria, including *E. coli,* are part of the resistance reservoir that can spread resistance elements from animals to people. *E. coli* from mice, chickens, and humans can exchange resistance genes (130). Integrons from multidrug-resistant *E. coli* from animals and human cases of urinary tract infection (UTI) had identical gene cassettes and configurations, and these gene cassettes were also identical to isolates sequenced from around the world. The authors raised the issue of food animals acting as an integron reservoir, with global and cross-species transmission, for AMR transmission to human commensal and pathogenic bacteria (30). Resistance gene transmission is not limited to pathogenic *E. coli.* Resistance elements can be transmitted to numerous pathogens, both within and beyond the Enterobacteriacia family (27,28). Multi-resistant gram-negative infections are increasingly important in human medicine (131). If these are acquiring resistance genes from gram-negative commensal bacteria, studying the prevalence and determinants of AMR in *E. coli* can improve our understanding of AMR epidemiology in a diverse set of human pathogens.

Of course, *E. coli* is not only important as an indicator organism. *E. coli* can be pathogenic. Verotoxigenic *E. coli* (VTEC) cause symptoms from diarrhea to haemorrhagic colitis and haemorrhagic uraemic syndrome. This virulence type is frequently expressed by the O:157 serotype but is not exclusive to that strain (25,132). VTEC infections are predominantly foodborne and associated with beef. Human illness from VTEC is largely independent of AMR. Antimicrobials are contraindicated in VTEC cases because this can induce release of the verotoxins, so treatment failure is a relatively minor concern (133). The attributable fraction (i.e. infection in people taking antimicrobials that select for a resistant foodborne pathogen) also seems small as studies that have compared AMR rates in VTEC and non-VTEC from healthy animals have found lower rates of resistance in the VTEC isolates (although higher rates of resistance may be reported in isolates from sick animals, these have likely been exposed to therapeutic AMU and are not representative of the background rate of bacteria that may contaminate food) (134,135). In pigs, the concern that antimicrobial use could select for virulent *E. coli* was raised because statistical relationships were identified between resistance and virulence genes (26,136). The virulent isolates were obtained from sick pigs and thus raised an animal health question. However, similar relationships between resistance and virulence genes in *E. coli* from healthy animals would be a food safety issue because this would suggest antimicrobial use could increase the prevalence of virulent isolates and increase foodborne disease risks. One study of E. coli from healthy pigs destined for the food chain found no relationship between virulence and resistance genes (48). Based on the main source of VTEC being beef and relatively minor human health concerns from AMR, pathogenic *E. coli* are not addressed further in this project.

Foodborne *E. coli* may also be related to human illness from extra-intestinal pathogenic *E. coli* (ExPEC). Clusters of urinary tract infections (UTI) caused by clones of uropathogenic *E. coli* raised the hypothesis that people shared a common infection source, which could be food (137,138). This hypothesis has been investigated using traditional and molecular epidemiology: a case-control study compared the dietary habits of women with susceptible and resistant infections and found consumption of pork and poultry were each risk factors for specific resistance phenotypes (139). Recent work on avian pathogenic *E. coli* and uropathogenic *E. coli* from human cases found that within a particular strain, O1:K1:H7, the genomes of avian and human isolates were highly similar. This was interpreted as supporting the hypothesis that some avian pathogenic *E. coli* may act as a foodborne source of uropathogenic *E. coli* for people. Johnson et al. concluded that there is "no convincing genetic evidence for host or syndrome-specific pathotypes of *E. col*i within ExPEC," and called for further investigation to determine the extent of the relationships between animal and human pathogens (140). These results have expanded the realm of food safety concerns from foodborne bacteria and will undoubtedly be investigated in future research.

When the potential for pathogenic *E. coli* to cause extra-intestinal disease is taken into consideration along with the potential for *E. coli* from animals to share AMR genes with a variety human commensal and pathogenic bacteria, it becomes clear that the effects of foodborne resistant bacteria are wide-reaching (27,30,130). Together, epidemiology and molecular genetics are elucidating ecological links between people and animals, and bacteria and their transmissible genes, which until now have been discussed but largely unsupported. Hence, what was plausible is increasingly appearing possible, although the prevalence of these connections remain unknown. These recent developments demonstrate that risks from resistant bacteria are unpredictable and that complete understanding of the relationship between AMR in animals and people will require thinking outside of the box. Our understanding of the epidemiology of foodborne *E. coli* infections is still emerging, and to our knowledge, no studies have evaluated the severity of disease or cost of treatment of resistant versus susceptible infections where resistance is directly or indirectly attributed to gene transfer from commensal bacteria. From this summary, it is obvious that much more remains unknown than is presently known.

Enterococcus

Antimicrobial resistance in enterococci is monitored for three purposes. First, enterococci are an indicator organism and serve as the gram-positive indicator organism. Like *E. coli,* enterococci are commensal bacteria that are ubiquitous in healthy animals including humans. They can survive in the environment after release from their animal host and can reflect the antimicrobial selective pressures experienced in their host (141–143). Secondly, enterococci have ready ability to accept and transfer AMR genes. Congruent transposons have been found in pigs, pork and people indicating resistance elements are mobile across animal species (144). Enterococci can also transfer resistance genes to other bacteria. In particular, concerns exist over the potential spread of vancomycin resistance from enterococci into multidrug-resistant *Staphylococcus aureus.* Such transfer has occurred experimentally and a limited number of human infections have been reported (145–148).

The third and arguably most important reason to study AMR in enterococci is because they are among the most important opportunistic pathogens in people and resistance can affect treatment protocols. Approximately 60% of enterococcal infections are nosocomial (149). Infections are predominantly caused by *E. faecalis* (80 to 90%) and to a lesser extent *E. faecium* (5 to 10%), although the proportion attributed to *E. faecium* is rising (149–151). Enterococci are intrinsically resistant to cephalosporins, fluoroquinolones, clindamycin, trimethoprim-sulfonamides, and low doses of aminoglycosides, and *E. faecium* also have variable

susceptibility to ß-lactams (106,150). Acquired resistance to a variety of drugs further limits available treatment options for these infections (150).

Resistance to vancomycin and quinupristin/dalfopristin (QD) are among the greatest current concerns in treating enterococci infections. These concerns are related because streptogramins have been used to address vancomycin resistance. This discussion largely applies to *E. faecium* because acquired resistance is less common in *E. faecalis* (150). Numerous genes confer vancomycin resistance: vanA concurrently infers teicoplanin resistance and is one of the most prevalent and concerning genes (150). Resistance to vancomycin emerged in the late 1980s and over the course of a decade rose to account for 25% of all enterococci blood-borne infections in the United States (24,152,153). Because nosocomial infections with E. faecium are less prevalent than *E. faecalis,* and vancomycin resistance occurs more commonly in *E. faecium,* by 2002 this translated to rates of 75% resistance in *E. faecium* in some American hospitals (152). The extent of this problem varies between countries. Over half of the European countries involved in nosocomial surveillance reported <1% vancomycin resistance in their already extremely low rates of invasive *E. faecium* infections. Yet Greece, Ireland, and Portugal reported more than 25% of the invasive *E. faecium* infections were vancomycin resistant (151). This puts substantial burden on the health care system as these infections cause more severe disease than vancomycin-susceptible infections (24).

In enterococci from pigs and chickens, vancomycin resistance is mediated by the *vanA* gene cluster carried on the horizontally transmissible Tn1546 transposon, which is the same transposon and gene found in many human vancomycin-resistant enterococci (VRE) (149,154). Vancomycin resistance is rare in enterococci from North American food animals. Canadian surveillance of retail chicken and pigs on farms has found no VRE (66). In the United States, none of over 6,700 enterococci obtained from chicken carcass rinsates between 2003 and 2006 were resistant to vancomycin (73,115). In contrast, VRE were prevalent in pigs and chickens in Europe prior to the ban on avoparcin, a related glycopeptide that was licensed for use in animal feeds (141–143, 155,156). The ban of avoparcin seemed to have an impact on VRE; for example, Denmark banned avoparcin in 1995 and reported 43% glycopeptide resistance in E. faecium from broilers and 21% in *E. faecium* from pigs in 1996. By 2000, resistance had declined to 6% of the *E. faecium* in both broilers and pigs (157).

In Europe, but not North America, there is a large community reservoir of VRE (149). Foodborne exposure to VRE or transmission of resistance genes from livestock to humans through food may have played a role in the establishment of VRE in the European community because experiments have shown transient colonization with animal-derived *E. faecium* (158) and human acquisition of the *vanA* gene from contaminated food (29). But the role of food as a source of human disease remains uncertain because illness is rarely caused by the same strains of *E. faecium* as are found in livestock, and community-acquired infections are rare (149,156,159,160). While ecological connections between VRE in pigs, chickens, community carriers, and ill people appear to exist, much remains to be learned about their relative importance. For example, *E. faecium* collected over a decade from hospitals, community infections, swine and poultry were compared, and some clonal groups contained isolates originating from more than one source, but overall the groups largely held isolates from one source (161). No risk assessments were identified describing the probability of avoparcin use in livestock and poultry resulting in treatment failure from vancomycin-resistant *E. faecium.* This is now an academic curiosity, as avoparcin has been removed from the world market (162).

In the late 1990s, a streptogramin antimicrobial, quinupristin/dalfopristin (QD) (Synercid®), was released to treat multidrug-resistant gram-positive infections. Primary indications included vancomycin-resistant *E. faecium* (*E. faecalis* are innately resistant) and MRSA infections (162). Shortly after the release

of QD, streptogramin-resistance was recognized in *E. faecium.* Once again, agricultural antimicrobial use was questioned because virginiamycin (a streptogramin) is used as a feed-grade antimicrobial. Virginiamycin use can select for the *vat(D)* or *vat(E)* genes which infer resistance to dalfopristin. These genes do not cause full resistance to virginiamycin or QD because they affect streptogramin A but not B. However, streptogramin B targets the same ribosomal subunit as macrolides and lincosamides and can be affected by resistance mechanisms in this antimicrobial class (163). Thus QD resistance may be selected for by virginiamycin and macrolide use in livestock. Prior to the release of QD, virginiamycin was not related to any antimicrobials used in humans.

European countries banned virginiamycin on the precaution that it could select for resistance to QD. The United States and Australia both conducted quantitative risk assessments evaluating the human health risk from virginiamycin use in food-producing animals (162,164). In 2004, Australia revoked the label for virginiamycin use for growth promotion and/or improved feed conversion and required modifications to the label for prophylactic and therapeutic claims. In contrast, neither the United States nor Canada changed the label indications or drug availability. In North America, virginiamycin continues to be extensively used in poultry and minimally used in pigs (66,92,93,165). Canadian surveillance (2006) of *E. faecium* from retail chicken found 50% to 75% resistance depending on the province of origin. In near-to-market pigs (2006), 24% of the 37 *E. faecium* isolates tested were resistant to quinupristin/dalfopristin (66). In people, linezolid is now often used preferentially over QD to treat multidrug-resistant enterococci because of fewer side effects. Canadian surveillance found no linezolid resistant enterococci in retail chicken or near-to-market pigs in 2006 (66).

Bacteria of Secondary Interest

Yersinia enterocolitica

Yersinia enterocolitica is often ranked as the third or fourth most common foodborne bacterial pathogen following *Campylobacter, Salmonella* and occasionally *E. coli* O:157. *Yersinia enterocolitica* causes acute gastritis, diarrhea, abdominal pain, and fever, and children have increased rates of infection (64,97). The reported incidence rates are similar in Ontario (2.3 cases per 100,000 person years) (national estimates not available) and Europe (2.8 cases per 100,000 person years) (96,97). Finland, Germany, Lithuania, and Sweden have reported higher rates (6 to 15 cases per 100,000 person years) while the incidence is lower in the United States (0.4 cases per 100,000 person years) (166). The dominant pathogenic strains differ slightly in Europe and North America (97,167,168).

Yersinia enterocolitica primarily causes sporadic infections so source attribution is difficult (97). Outbreak investigations and meat contamination rates suggest that pigs are the main livestock reservoir for foodborne infections (64,169,170). *Yersinia enterocolitica* is a commensal in pigs. Other species also carry *Y. enterocolitica,* but these belong to the non-pathogenic 1A biogroup. On a ranked basis, the reported prevalence in slaughter hogs tends to correlate with reported human incidence rates: Germany, 67%; Canada, 42%; United States, 13% (167,170,171). However, caution should be used when comparing these prevalence estimates because the sampling and laboratory methods differed between studies. Data were not available from many countries, making this comparison incomplete.

Extra-intestinal *Yersinia* infections can require antimicrobial therapy. Broad-spectrum cephalosporins with or without aminoglycosides are generally effective as are fluoroquinolones, trimethoprim-sulfonamide combinations and doxycycline. Antimicrobial susceptibility to ß-lactams is serogroup specific with some strains possessing chromosomally

mediated ß-lactamase genes (168). The resistance conferred by these genes to penicillins and cephalosporins is not entirely predictable but does not extend to newer generation cephalosporins such as ceftriaxone or cefoxitin.

German and Canadian surveys of *Yersinia* from pigs found that resistance to drugs other than ß-lactams or erythromycin is rare (167,170). Isolates from meat in Greece largely corroborated this, with no reported resistance to 3rd and 4th generation cephalosporins or fluoroquinolones. Low rates of resistance were noted to streptomycin, tetracycline, and co-trimoxazole, but isolates were obtained from a variety of sources and were not exclusively *Y. enterocolitica.* Statistical associations were identified between certain resistance phenotypes and virulence genes (172). Similar findings in *E. coli* have been interpreted as an increased potential for antimicrobial use to select for virulent strains (26,48,136). Therefore, interventions that minimize AMU in pigs could potentially decrease the morbidity attributed to *Yersinia.*

Listeria monocytogenes

The *Listeria* genus consists of six species, but only *L. monocytogenes* is an important foodborne pathogen. Mild infections typically present with flu-like symptoms. More severe cases can cause septicemia, meningitis, and miscarriage or stillbirth. The case fatality rate from *L. monocytogenes* can be as high as 30% (173). The incidence of *L. monocytogenes* infections is low relative to other foodborne bacterial pathogens. Europe and the United States report 0.3 cases per 100,000 people (97,166). People with impaired T-cell immunity are at increased risk for infection. This group includes young children, elderly people, pregnant women, and the immunocompromised (97,173).

Main reservoirs of *L. monocytogenes* include soil, forage, and water. Environmental contamination and cross-contamination of food in meat processing facilities are key factors in foodborne transmission of *L. monocytogenes.* The structure of modern food processing systems means that *L. monocytogenes* typically causes outbreaks rather than sporadic infections (173). Many domestic animals can harbour *L. monocytogenes,* which can lead to retail meat contamination. Retail meat testing in Ontario found 7% of pork chops and 28% of skin-on chicken breast were contaminated (96). Ready-to-eat foods are at increased risk of causing disease because this bacterium can multiply at refrigeration temperatures. Canada, the United States (US) and the European Union (EU) prohibit the sale of products containing ≥ 100 cfu/g of *L. monocytogenes* (106,174,175).

Effective treatment includes early diagnosis and antimicrobial therapy. Choice antimicrobials are typically a penicillin or ampicillin in combination with an aminoglycoside. Cases allergic to ß-lactams may be treated with a trimethoprim/sulfonamide combination, tetracycline, or chloramphenicol (176). Vancomycin and erythromycin are indicated for use in pregnant women (173). To date, AMR has not been a major concern with clinical *L. monocytogenes* infections. Studies that have examined *L. monocytogenes* from meat have found almost complete susceptibility to penicillin, ampicillin and aminoglycosides. Resistance to tetracycline and fluoroquinolones is more variable and high rates of resistance to sulfonamides, but not trimethoprim-sulfonamide combinations, have been reported (176–181). *Listeria monocytogenes* are intrinsically resistant to cephalosporins (106).

While resistance to clinically relevant drugs is currently very low, it is still of some concern given that not all patients can be treated with penicillins. Case reports of resistant infectious strains exist, and isolates with transposons and plasmids carrying antimicrobial resistant genes have been identified (176,182,183). From these, it has been determined that *L. monocytogenes* can acquire resistance elements from other gram-positive bacteria, particularly *Enterococcus* and *Streptococcus* while transiting the gastrointestinal tract (176,183). Enterococci carrying multi-resistance to ß-lactams and aminoglycosides exist which makes the potential for

resistant *Listeria monocytogenes* that are refractory to treatment a concern. Monitoring of this pathogen will be important to ensure human therapy is predictable and to allow interventions should resistance become a problem, which would be devastating in such a virulent pathogen.

Emerging Issues

The following section presents two bacteria that do not directly fit this project's inclusion criteria of foodborne resistant bacteria. Despite this, it is imperative to discuss methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* because concerns are escalating over the public health risk from these bacteria in food animals. For both bacteria, concerns are being fuelled by a lack of information on the hazard. These bacteria remind us of the importance of 'thinking outside-the-box.' Neither pathogen has been traditionally considered zoonotic. Due to unique concerns and limited information on these novel problems, the organization of the following sections differs from the preceding bacteria.

Methicillin-Resistant Staphylococcus Aureus

Staphylococcus aureus are gram-positive bacteria that can colonize the skin or nose and mouth of people and animals. *Staphylococcus aureus* is a common commensal in humans and can be found in the nasal passages of approximately 30% of healthy individuals. Colonization rates are variable in other species. Colonized individuals are a source of infection to others and are at greater risk for infection themselves. ßeta-lactamase-mediated penicillin resistance is very common in *S. aureus,* so ß-lactamase-resistant penicillins are a main treatment for community-onset *S. aureus* infections. There may be various other therapeutic options as a range of antimicrobials are potentially effective against *S. aureus.* However, *S. aureus* has a tendency to develop or acquire antimicrobial resistance and multidrug-resistant staphylococci are a leading health concern in human medicine. Much of this is focused on methicillin resistance. This resistance, conferred by the *mecA* gene, provides resistance to all ß-lactam antimicrobials including methicillin, penicillins, cephalosporins, and carbapenems. This gene is carried within the staphylococcal cassette chromosome mec (SCCmec) and, once acquired, is stable within the bacterial chromosome. *Staphylococcus aureus* carrying the *mecA* gene are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA) (184,185).

The 1960s saw the emergence of MRSA as a nosocomial pathogen (hospital-acquired MRSA or HA-MRSA). Over the last two decades, MRSA has also become established in people outside of hospital settings (community-acquired MRSA or CA-MRSA) (106). Studies have estimated that 1.5% to 3% of the North American population are colonized with MRSA (186–188). Infection with MRSA is associated with increased mortality and higher treatment costs compared to infection with methicillin-susceptible *S. aureus* (MSSA) (24). Serious infections lead to 19,000 American deaths annually with 84% attributed to HA-MRSA and 14% to CA-MRSA (189).

There are major lineages within *S. aureus* that can show host specificity for humans or animal species; this specificity extends to MRSA. Within a geographical area certain lineages or clones dominate, and these can differ between hospitals, the community, and agricultural settings. The clones associated with each setting tend to have somewhat predictable phenotypic characteristics and virulence traits (184). This discussion focuses on a specific clone relevant to food safety, hereafter referred to as livestock-associated MRSA (LA-MRSA). Other descriptions of MRSA are limited to the minimum necessary to provide context for understanding the role of LA-MRSA as a potential foodborne pathogen.

Staphylococcus aureus is an animal pathogen. Because of the clonal lineage, diseases in humans and animals were historically considered unrelated. This view has changed over the last decade with the recognition that dogs, cats, and horses can be colonized with and clinically affected by pathogenic

MRSA strains that are indistinguishable from human strains (190–192). Epidemiological evidence suggests that infection in these species resulted from a spill-over from humans; such transmission has been dubbed a 'humanosis.' These animal infections create a human health risk because people exposed to carrier animals can be infected by MRSA.

Concern over MRSA in animals expanded when a clonal strain, sequence type 398 (ST398), was identified in pigs (193). Further study identified a high prevalence of this clone in pigs, as well as some other livestock types, and an association between human infection or colonization with this strain and contact with food animals. Subsequently, MRSA associated with this clone has been referred to as LA-MRSA. In contrast to the situation in companion animals, the LA-MRSA clone is distinct from previously common human strains. Since its identification, LA-MRSA has been found in pigs in Austria, Belgium, Canada, Denmark, France, Germany, the Netherlands and Singapore. As summarized by the European Food Safety Authority (EFSA), the reported prevalence of LA-MRSA in pigs is high in these countries (184): Europe, 10% to 39%; United States, 70%; Canada, 25% (194–199). Caution should be exercised in comparing these prevalence estimates because sampling and laboratory methods were not standardized. To date most investigations have focused on swine, thus pigs are often considered a primary reservoir of LA-MRSA. However, veal calves, dairy cows, and broiler chickens can be positive and may be important in the epidemiology of this clone (184). In general, LA-MRSA does not cause clinical disease in colonized agricultural animals.

For people working directly with food animals, LA-MRSA is an occupational hazard. Direct contact with colonized livestock can result in human colonization or infection with LA-MRSA (193). Colonization risk increases with exposure: the highest rates occur in people that work directly with pigs (29%), followed by people entering barns but not working with pigs (12%), then people not working with pigs but living on positive farms (2%) (200). LA-MRSA has been associated with skin and soft tissue infection, pneumonia, and septicemia in people. Across the EU, LA-MRSA accounts for 0.7% of typed MRSA isolates, but the rate varies between countries; higher rates are reported in countries that have low overall rates of MRSA and high exposure to pig farming (184).

The zoonotic potential of LA-MRSA has raised concerns that LA-MRSA could be a food safety hazard. Food has served as a vector in a hospital outbreak of MRSA but food handlers, rather than animals, were considered the primary source (201). Theoretically, colonized animals could contaminate carcasses and meat during slaughter and create a source of exposure for people. MRSA has been detected in numerous foods of animal origin including chicken and pork (202–207). The majority of these studies found only human-related strains, which is suggestive of meat contamination from food handlers. The exceptions were a single pork sample by van Loo et al. and the study by de Boer et al. which found more than 90% of the MRSA from chicken, pork and a variety of other meats were LA-MRSA (204,205). Although a large proportion of tested meat samples were positive for LA-MRSA, the degree of contamination was below 10 cfu/g. Similar results have been found for retail meat in Canada, with MRSA being identified in retail pork, and beef, albeit at very low concentrations (208). Currently, beyond the 1995 report suggesting, but not proving, that food prepared by a colonized hospital worker was the source of an MRSA outbreak in a hospital (201), there are no reports of food as a source of human MRSA infection.

LA-MRSA is frequently multidrug-resistant, but the pattern differ from CA-MRSA and HA-MRSA. Resistance to tetracyclines is observed in almost 100% of LA-MRSA isolates, and resistance to trimethoprim, but not trimethoprim in combination with a sulfonamide, is common. Fluoroquinolone resistance has been reported from 0% to 35% (197,209). Based on this multi-resistance pattern, therapeutic treatment options are limited to valuable classes of drugs including the oxazolidinones and pleuromutilins. Resistance to these drugs can be

conferred by the *cfr* gene. This gene affects the binding sites of 23S RNA and infers resistance to antimicrobials with this target site including oxazolidinone, pleuromutilins, phenicols, lincosamide, and streptogramin A. Two *S. aureus* isolates, one of which was a LA-MRSA clone carrying the cfr gene, were identified in pigs in Germany. Although not an indication of its prevalence, this report heightens concerns over limited treatment options should LA-MRSA become more established in agricultural animals and subsequently people (210).

The European Food Safety Authority has determined that current evidence indicates the food safety risk from LA-MRSA is small and is much lower than the risk from exposure to colonized animals or humans (184). *Staphylococcus* are killed by heat and will be eliminated with cooking or pasteurization. This limits the food exposure risk to unpasteurized dairy products and meat that is consumed raw or minimally cooked. The risk of exposure from handling meat also appears low as reports of colonization in meat handlers are infrequent.

Clostridium difficile

Clostridium difficile is the most commonly identified cause of antimicrobial-associated diarrhea and health care-associated (HA) diarrhea in most areas worldwide and is responsible for virtually all cases of pseudomembranous colitis (211,212). *Clostridium difficile* infection (CDI, previously called *C. difficile*-associated diarrhea or CDAD) is also emerging as an important cause of community-associated (CA) diarrhea with dramatic increases in the incidence rate reported over the last decade (213,214). Severe CDI is now being reported in populations previously considered to be at low risk with unique features that had not described including close contact transmission, high recurrence rate, young patient age, bloody diarrhea, and lack of antimicrobial exposure (215–217).

The source of infection of people in the community is unclear. Various sources are possible, including colonized individuals, people with CDI, the environment, pets, food, and water (218–227). This section of the report focuses on the potential role of food in CA-CDI. The main factor that has led to suspicion of foodborne transmission is the similarity of food animal and human CA-CDI strains. Currently in people, PCR ribotype 027 is the most important clone while ribotype 078 is increasingly attracting attention. Both of these clones are found in HA and CA-CDI cases (228–235). Although still preliminary, toxinotype V strains, predominantly ribotype 078, may be overrepresented among CA-CDI isolates (228,229,236).

The following section exclusively discusses *C. difficile* in pigs and chickens. Readers should be aware that parallel knowledge is emerging in other meat-animal commodities. Ribotype 078 has been reported in pigs from different countries (237,238). In an American study, 83% of *C. difficile* isolates from pigs were ribotype 078 (237). Although far less investigated, *C. difficile* has been identified in chickens (239,240). Reports of *C. difficile* in food animals led to concerns about the potential for retail meat contamination. An American study reported *C. difficile* contamination in 47% (21/45) of examined pork products (225). In that study, 67% of positive pork products harboured ribotype 078 while the remainder harboured ribotype 027. A study of retail pork using samples collected from four Canadian provinces only identified *C. difficile* in 1.4% (4/296) of ground pork and 3.1% (3/97) pork chops (241). The most common strain was ribotype 027 and ribotype 078 was not found. A second Canadian study of ground pork found *C. difficile* in 12% (14/115) of samples but most samples had low numbers of *C. difficile* and were only positive with enrichment culture (242). Where numbers could be determined, samples typically contained only 20–240 spores per gram. While the infectious dose of *C. difficile* is not known, it is assumed that low levels are of lesser concern than high levels. Further, one considers that *C. difficile* can be found in treated water, vegetables, and the household environment, so it is clear that

simple exposure to low levels of *C. difficile* is not the sole factor in the pathophysiology of disease (219,243,244).

Determining the relevance of *C. difficile* in food animals to CA-CDI will require much more investigation. Unanswered questions include the reasons for different ribotypes in American and Canadian livestock studies and the correlation between *C. difficile* types found in retail meat and those found in healthy food animals at slaughter. More work is also needed on the prevalence of contaminated meat and the degree of contamination. If meat is commonly contaminated with *C. difficile* at levels sufficient to cause disease, it could be important in the epidemiology and control of CA-CDI.

The concerns over antimicrobials and *C. difficile* differ from the other bacteria described in this report because therapeutic failure in humans is a minor concern. Instead, the concern with antimicrobial use is disruption of the normal intestinal flora and subsequent overgrowth of *C. difficile.* The recent recognition of a possible foodborne link and different concerns for AMR in *C. difficile* explain why studies to date have focused on elucidating potential transmission routes between food animals and people. Future studies may study AMR in *C. difficile* from food animals, with an application of understanding on-farm practices that affect the epidemiology in livestock populations.

Conclusion

This chapter has summarized the main foodborne hazards that humans face from antimicrobial resistant bacteria in food animals. It has focused on specific resistance types in individual bacterial species. This 'drug-bug' orientation is necessary to understand the issues that influenced many policies and regulations to control AMR. However, many scientists are evolving from a 'drug-bug' perspective to an ecological approach. This has been largely driven by the recognition that barriers to resistance exist only in our minds. Resistance elements move between bacterial populations and their animal hosts, between urban, rural and agricultural environments, and between bacterial hosts. Transmission and evolution can involve re-assortment of linked resistance genes, which consequently alters the selective pressures from co-selection. This has forced scientists and policy makers to seek solutions that extend beyond limiting the use of one antimicrobial in an attempt to mitigate a single resistance outcome.

This chapter also presented many foodborne links between AMR bacteria in pigs and chickens and humans. However, what is possible is not necessarily probable. Although experimental and observational studies have described many transmission routes, the probability of each occurring is often conditional on numerous sequential events. We felt it was important to describe these routes, but do not mean to leave the impression that every bacteria and resistance type described are in a crisis situation.

In conclusion, this chapter has presented numerous recent developments. These include the emergence of plasmid-mediated fluoroquinolone resistance, the description of the *cfr* gene, and an emerging connection to animals for the traditionally human-based diseases of MRSA and *C. difficile.* These discoveries are shaping our approach to food safety and zoonotic disease. They remind us of the need to be constantly alert and that what we think we know today will likely be questioned tomorrow.

Chapter 2: Antimicrobial Use

Introduction

Antimicrobial resistance (AMR) is an important issue facing both human and veterinary medicine. The increasing number of antimicrobial resistant pathogens in human medicine has raised both public and scientific interest. For human pathogens, most of the AMR development is considered attributable to the selection pressure from antimicrobial use (AMU) in people (245). However, the volume of antimicrobials used in food animal production has led to concerns in the public, regulatory, and scientific arenas that AMU in food animals is contributing to the AMR problem by creating a reservoir of resistant bacteria (134,246,247). For human health, the transfer of such resistance to zoonotic enteropathogens is of primary interest, but the development of antimicrobial resistance in animal pathogens, the associated subsequent loss of therapeutic options for veterinary medicine, and the potential need to use antimicrobials of greater importance in human medicine is also an important concern.

While AMR is an issue in veterinary medicine in Canada, its overall clinical impact is much less than in human medicine. However, the continued development of AMR in human medicine and the occurrence of AMR as a veterinary problem in other parts of the world indicate that AMR may become more of a concern in Canada with regard to continued therapeutic efficacy in veterinary medicine (248). The use of antimicrobials in animal agriculture is important for maintaining and improving animal health and welfare through disease prevention and treatment, and arguably for increasing carcass quality, as well as for enhancing the economic efficiency of growth and production. If the livestock industry loses efficacious antimicrobials because of resistance development, or experiences limited access because of tighter regulations, the consequences and costs to the industry are difficult to quantify but have the potential to be substantial.

The development and spread of AMR is a complex process involving interactions among antimicrobials, microorganisms, and the surrounding environment (249). There are multiple sources that may contribute to the creation and dissemination of AMR (Figure 1).

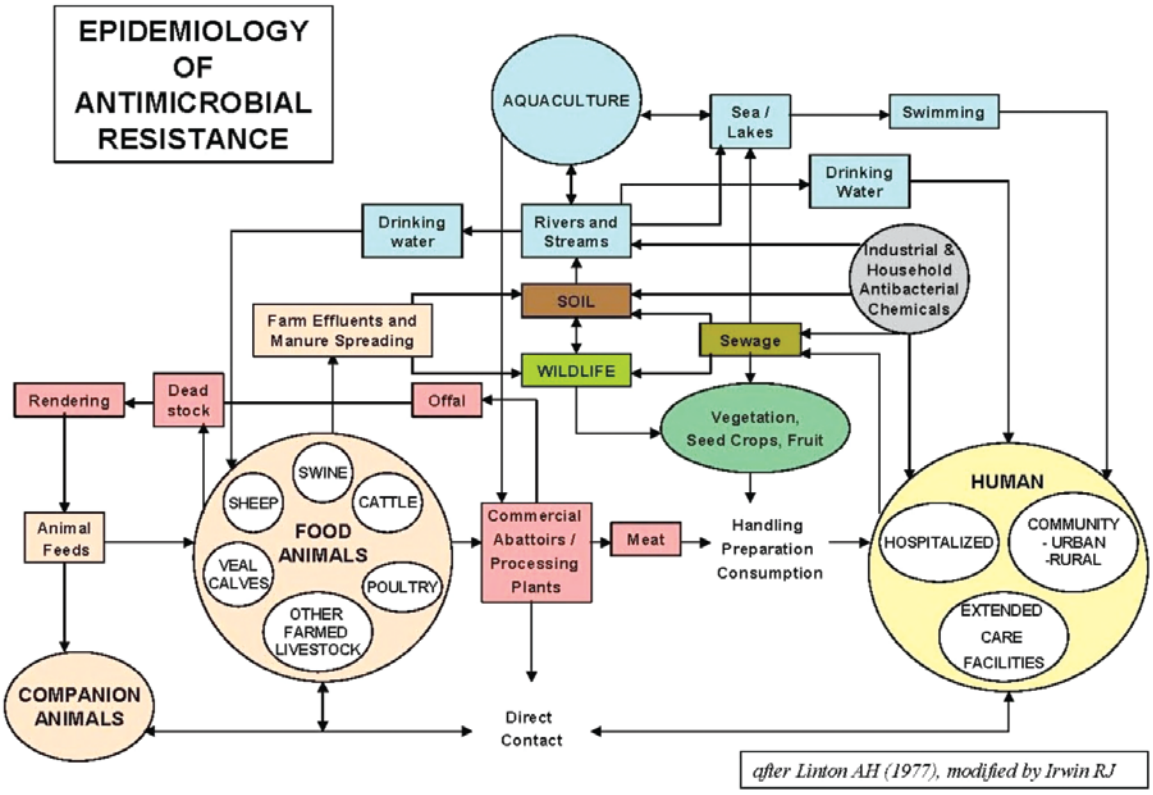
While there is evidence indicating each pathway exists, our knowledge of the importance of each remains incomplete (Figure 1) (12). There are many routes for AMR to spread within a local environment, including but not limited to, spread between humans, livestock, companion animals, wildlife, fish, water and soil, and vegetables (12), but the global aspect of AMR is also important to consider especially in the context of international travel, animal movement, and food trade.

The complex ecology of AMR development and dissemination can create considerable challenges in determining how much each potential source impacts AMR development in humans. As the issue of AMR is further explored and investigated, it becomes increasingly difficult to evaluate often conflicting results or statements with regard to the role of each of these potential sources. This point can be argued as being a fundamental question that needs to be addressed or a question of academic interest. It is the division between these two schools of thought that have lead countries to implementing either evidence-based AMR policy or a precautionary principle based-approach.

Scope and Objectives

In order to understand the potential impact that swine and poultry production may have on AMR development, the literature was examined and an overview was compiled. The objectives of this chapter are to: highlight the reasons for AMU in swine and broiler chickens, review the classes of antimicrobials used and the utility of continued availability of efficacious antimicrobials in Canadian

Figure 1. Epidemiology of the potential major pathways for antimicrobial resistance to transfer. After Linton (250), modified by R. Irwin, reproduced with permission.



swine and broiler chicken production, describe current known risk factors for AMR development in bacteria isolated from pigs and chickens and the potential relationship with AMR bacteria isolated from humans, and discuss the information gaps that still remain in our understanding of the effect of AMU in livestock on public health. This chapter provides examples rather than exhaustively listing all antimicrobial use practices, risk factors or proposed links between AMU in livestock and AMR development in humans. It is intended to provide a broad background for all readers regardless of their familiarity with agriculture and the issue of AMU in livestock and its impact on human health.

General Considerations, Terminology, and Reasons for Antimicrobial Use

Different AMU regimens can select for various resistance genes (251), and therefore, AMU patterns are expected to have some impact on the distribution of AMR phenotypes (252–254). Among the ramifications associated with resistance gene selection are the degree of resistance conferred and the carriage of linked resistance determinants (251). Sometimes only minimal antimicrobial exposure is necessary to select for continued persistence of resistance genes within enteric microflora (251).

Persistence of AMR in bacteria is generally related to the persistence of antimicrobial use/exposure, but AMR may also persist in the absence of known antimicrobial selective pressure. An example of this is provided by a study of the University of Kentucky swine farm. This farm ceased all antimicrobial use and then followed the animals housed on the farm over a period of 126 months. Resistance to tetracycline declined by less than 50% over this time, leading the authors to conclude that long-term withdrawal of antimicrobials failed to markedly reduce AMR and that long-term feeding of antimicrobials could lead to AMR that may not readily be reversed by withdrawal of antimicrobials (255). From this and other studies (256,257) it seems that some resistant bacteria can become stabilized in the intestinal tracts of animals and become the dominant intestinal flora potentially passing from one generation to the next (258). These findings therefore indicate the need to consider the long-term effects of AMU in any environment.

Antimicrobial Growth Promoter Terminology

Popular debate over AMU in agriculture often refers to drug use for growth promotion. While many deem 'growth-promotional AMU' inappropriate and potentially even unethical, what does this term mean? The phrase 'antimicrobial growth promotion/promoter (AGP)' was a descriptive term coined early in the history of agricultural AMU to describe the overt effects of including drugs in the feed of apparently healthy animals. It became ingrained in AMU terminology in the 1970s when the European Union separated agricultural AMU legislation into two classes (See Chapter 3). One class contained non-prescription feed additives that producers could access without veterinary involvement and all other AMU (i.e. prophylactic, metaphylactic and therapeutic use of feed-grade, water-soluble, and injectable antimicrobials) required a veterinary prescription (259). In Europe, these uses were mutually exclusive meaning that an antimicrobial could not be licensed in both categories. Thus European references to AGP AMU pertain to a very specific and distinguishable type of AMU. It was these classes of drugs that were revoked in the 1999 and 2006 European Union drug bans (259,260).

The terminology surrounding AMU for growth promotion is less precise in North America. Regulations have always permitted feed-antimicrobial labels to claim improved growth, disease prevention and therapy. In the 1970s and 1980s, the United States government investigated the potential implications to human health from feeding tetracyclines and penicillins to pigs and chickens (261,262). The evidence for potential human harm was deemed insufficient to justify action. As a result, neither Canada nor the United States changed the veterinary drug legislation to separate production and health indications and both countries have allowed continued access to these products without a veterinary prescription (263,264). So in North America, there are no drug use characteristics that reliably distinguish between AGP and other types of in-feed AMU. Describing in-feed AMU by the reported reason for inclusion is the only valid mechanism to proportion in-feed AMU into AGP versus other effects.

An outdated method of categorizing AMU as AGP is to consider all feed-grade antimicrobials included in diets at less than 200ppm for more than fourteen days to be AGP (261,265). Effectively, this means that in-feed AMU prevents sub-clinical disease. Throughout this document, the acronym AGP will exclusively refer to the regulated feed-grade antimicrobial additives within Europe prior to their prohibition. Outside of the European situation, all other in-feed AMU is either referred to by the reported purpose of use (including growth promotion) or, if that is not available, simply as in-feed or feed-grade AMU.

Reason for Antimicrobial Use in Livestock

Intensive livestock operations, such as swine or poultry operations, are typically confinement agricultural systems. These are defined as systems

where "the movement of animals is confined and they are raised in high density, usually with stimulated feeding, and weight gain optimized so as to decrease time to mature weight." (122). As demand for meat grows, so does the confined livestock production because it allows for economies of scale. Traditionally, raising large numbers of animals in a close proximity often required the use of the tools of prophylactic, metaphylactic, and growth promotional AMU in order to prevent morbidity and mortality, to ensure animal welfare, and for economic benefit.

It is not the need for AMU in livestock that is at question, but the extent to which antimicrobial use is necessary that is up for debate. Clearly animals can be raised without routine use of antimicrobials under some circumstances, but presumably there is always a need to access them if an animal or a group of animals requires treatment. How far the industry can go and needs to go in regularizing farming without antimicrobials is the question. The potential for increased mortality, days to market, feed costs, and overall increase in the cost of production may need to be passed on to the consumer resulting in higher food costs and precluding some individuals from accessing animal-based protein sources. Therefore, a balance between ensuring animal health and welfare, human health, and producing a reasonably priced safe and wholesome product needs to be achieved.

In swine, the majority of antimicrobial use is for treatment or prophylaxis of respiratory and enteric disease, while in poultry, antimicrobials are primarily used for intestinal infections, namely colibacillosis and necrotic enteritis (265,266). The method of administration and the volume of antimicrobial used will vary depending on the animal species, stage of production, and risk of disease. There are three primary reasons for AMU in food-producing animals: treatment of sick animals, prevention and control of disease, and improved productivity.

Prevention and control can be further divided into metaphylactic or prophylactic applications. Metaphylaxis is a disease control measure involving the mass medication of a group of animals to prevent the spread of disease when only a few individuals have been identified as infected. Prophylaxis is a preventative treatment of an animal or group of animals at a time when it may be more susceptible to infection (267). Antimicrobials are given at critical points in production to help prevent the development of disease. Prophylactic treatment may involve the entire group of animals or may be targeted toward specific high risk individuals depending on the animal species, the production system, and the disease condition.

Growth promotion is another reason for AMU in livestock and it generally involves the use of antimicrobials licensed for this purpose. Normally, antimicrobials labelled for growth promotion are included at a dose lower than those approved for therapeutic purposes and are fed for a longer duration than antimicrobials used for prevention and control (263). Long-term mass exposure to antimicrobials can increase the selection pressure for AMR development and persistence (268–271).

Until recently in North America, the focus has been on minimizing the use of drugs of critical/very high importance in human medicine for all purposes including therapy and prophylaxis because these uses could increase selective pressure and the pool of bacteria resistant to these important antimicrobials (272,273). Lately, in the United States there has been increasing lobby to eliminate the use of other antimicrobials, particularly AMU for improved productivity rather than health purposes (274). This more closely mirrors Europe's premise that the human health risk from AGP does not justify its continued use. In Europe, AGP AMU was of particular concern because it was not strictly necessary for health, and as it was responsible for the majority of antimicrobial exposure by mass, it was presumed that this use caused most of the selection pressure leading to the indirect effect on the bacterial biomass and the role of reservoirs in AMR transmission (275).

Antimicrobial Use Estimates in Livestock Production

Many countries are attempting to gain a better understanding of the volumes of antimicrobials used in livestock production, but the problem is that these estimates can be misleading if the context is not understood. The main issue is that AMU data are difficult to collect and report. National, regional, or even farm level data are scarce. On a national level in numerous European countries, since antimicrobials are by prescription only, a central pharmacy database can provide information on the volume of AMU in animals (35,79,89). Other countries like Canada and the United States are still working on the best methodology for collecting these data since they do not have any legislation compelling pharmaceutical companies to provide this information nor do they have centralized pharmaceutical databases.

At the national level, AMU is often reported in kilograms or tonnes of active ingredient sold but use can also be reported by divided daily doses (DDD) (276), animal daily doses (ADD) (276), or as animal-units per treatment days (277). While each of the above methods tries to capture the true exposure of an animal to a drug, they all are limited and much debate still surrounds the best approach to reporting AMU information. Jensen et al. (276) provides a good overview and highlights the major potential methods for reporting drug use and the associated limitations. In the case of reporting kilograms or tonnes of active ingredient, while these data provide the volume of drug used, they do not allow for assessment of how the drug was used and how this use may affect AMR. Data on the species drugs are delivered to, the number of animals exposed, the dose received, and the method of delivery are generally unavailable. Therefore, end-user data are often important to collect in order to gain a better appreciation of how and why antimicrobials are being used in livestock production.

While end user data can provide valuable information, it also has some serious limitations. Complete and accurate farm/hatchery-level AMU records are often difficult to obtain. In order to be able to generate useful national estimates, these data need to contain sufficient detail. Differences in record keeping methodologies and priorities result a lack of standardization and therefore summarizing information that is collected in multiple formats on individual farms is extremely challenging. Capture of use information can be expensive for the researcher and burdensome for the producer to accommodate especially during times of additional demands with limited resources. Under-reporting is potentially a problem since producers are busy with day-to-day operations on the farm and, therefore, record keeping may be relatively low on the priority list. Subsequently treatment records may be forgotten or incomplete. Dunlop et al. (277) reported a 35% under-reporting rate for AMU recorded by swine producers as compared to inventory and disappearance data collected by the research team. Reasons for under-reporting include misunderstandings between researchers and producers and lack of time during periods of increased work load such as in disease outbreak situations (278). Current Canadian producers may be less prone to under-reporting as Dunlop et al's. study was conducted before on-farm food safety programs made AMU record keeping mandatory (See Chapter 4). Current producers have adjusted to maintaining AMU records but the accuracy of these records has not been assessed.

Although there are several challenges associated with AMU data collection in livestock, several countries are attempting to capture some AMU information. The next several paragraphs provide some estimates of AMU in animals from various countries. While reviewing these numbers, it is important to keep in mind the limitations of reporting tonnes of antimicrobials used so as to not over or misinterpret what these numbers are really telling us. Also, comparing one country to another is difficult because information on the denominator is lacking, i.e. the number of animals, or the type of animal and/or the dose given. Without this information, it may appear like one country is using considerably more antimicrobials in their livestock than another. By not knowing how many animals were exposed and at what dose, etc., one cannot really compare

one country or region to another, since this may equate to comparing apples to oranges. The main goal of presenting these data are to familiarize the reader with how these data are often presented and how they might be difficult to interpret and compare between regions and within species.

Estimates of antimicrobial use in Europe in 1997 were 10,494 metric tonnes (MT) of active ingredients (35). Human use accounted for 52%, animal treatments for 33%, and growth promotion for 15% (35). In-feed use accounted for the majority of the animal use with 90% of the antimicrobials administered this way (35). The breakdown of the total antimicrobial volume per species was as follows: pigs 60%, poultry and rabbits 20%, ruminants 18%, and fish and pets at 1%. For therapy, prevention, and control, the antimicrobial classes used were tetracyclines (66%), macrolides (12%), penicillin (9%), and others (12%) (35).

In the United States in 1989, the Institute of Medicine estimated that 50 million pounds of antimicrobials were produced in the States annually and 50% of this was used in animals (279). The Union of Concerned Scientists re-evaluated this and suggested that there are 35 million pounds of antimicrobials used annually in the United States with 87% of that being used in animals (280). Non-therapeutic was the primary reason for use (280). In 1999, the Federal Drug Administration (FDA) and Center for Disease Control and Prevention (CDC) began gathering AMU data from the Animal Health Institute. From these data, therapeutic and preventative use made up 83% of the total (281). The drug classes used included aminoglycosides, fluoroquinolones, ionophores, penicillins, sulfonamides, and tetracyclines. Current estimates of the volumes of antimicrobials used in the United States are unavailable because at this time the US does not routinely report AMU in livestock.

In the most recently available Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) annual report, approximately 1.8 million kgs (~4 million lbs) of active antimicrobial ingredients were distributed for animal use in Canada (66). Tetracyclines were the most commonly used antimicrobial, followed by ionophores and macrolides (66). These estimates were provided by the Canadian Animal Health Institute (CAHI), which is the trade association representing the companies that manufacture and distribute drugs for administration to companion, sporting, and food animals in Canada (66). These data were aggregated to the class level and represent the distribution of these antimicrobials in 2006; they therefore do not represent what was actually used during this timeframe (66).

In order to gain a better understanding of AMU and to supplement this information, in 2006 CIPARS also began a pilot project to collect AMR and AMU information from Canadian swine herds. A preliminary report was released that includes the first animal agriculture AMU data collected by this national program (66). From these first few years of farm surveillance, AMU data collection challenges and limitations have been identified and data collection instruments have been refined to enable the team to gather the most useful AMU data available. While swine was the first commodity group that CIPARS began collecting data from, CIPARS has also been participating in large-scale research projects looking at AMR and AMU in feedlot cattle, dairy cattle, and sheep production, and is currently in discussions with the poultry industry with regard to developing a farm surveillance program in this industry. Canada is now facing challenges with how best to report their farm-based antimicrobial use data while acknowledging data limitations.

In order to substantially contribute to our understanding of AMU in animal production and its impact on AMR in people and/or animals, it is necessary to evaluate how antimicrobial use data are collected and reported so that it can be optimally utilized. While this may intuitively be easy to do, the reality is it is a challenge that still needs to be overcome. Even human antimicrobial use does not seem to be accurately estimated and reported (279,280), which leads one to surmise that there is additional work that needs to be done to not only understanding use in animals, but also in obtaining more consistent AMU estimates in human medicine.

Table 2. Antimicrobials licensed for use in pigs and chickens in Canada (2009).

		Pigs			Chickens		
Class	**Antimicrobial**	**Feed**	**Water**	**Injection**	**Feed**	**Water**	**Injectable**
Beta-lactam	Ampicillin	–	–	Pr	–	–	–
	Ceftiofur	–	–	Pr	–	–	Pr
	Penicillin G	OTC	OTC	OTC	OTC	OTC	OTC
	Amoxicillin	–	Pr	–	–	Pr	–
Sulfonamide	Sulfonamide	OTC	OTC	–	–	OTC	–
	Trimethoprimsulfonamide	–	–	Pr	–	–	–
Tetracycline	Hydrochloride	–	OTC	–	–	OTC	–
	Chlortetracycline	OTC	–	–	OTC	–	–
	Oxytetracycline	OTC	OTC	OTC	OTC	OTC	–
Macrolide	Erythromycin	–	–	OTC	OTC	OTC	–
	Lincomycin	OTC	OTC	OTC	OTC	OTC	–
	Tiamulin	OTC	OTC	Pr	–	–	–
	Tilmicosin	OTC	–	–	–	–	–
	Tylosin	OTC	OTC	OTC	–	OTC	–
	Tulathromycin	–	–	Pr	–	–	–
Aminoglycoside	Apramycin	–	OTC	–	–	–	–
	Gentamicin	–	–	Pr	–	–	Pr
	Neomycin	–	OTC	–	–	OTC	–
	Spectinomycin	OTC	OTC	–	–	OTC	–
	Streptomycin	–	OTC	OTC	–	OTC	–
Ionophores	Salinomycin	OTC	–	–	–	–	–
Streptogramins	Virginiamycin	OTC	–	–	OTC	–	–
Bacitracin		OTC	–	–	OTC	–	–
Flavomycin	Bambermycin	–	–	–	OTC	–	–
Phenicols	Florfenicol	–	–	Pr	–	–	–

OCT: available without a veterinary prescription; Pr: available with a veterinary prescription; –: no licensed products.

Antimicrobial Use: Routes, Antimicrobials and Reasons for Use

A background on the swine and poultry industries and common production practices is available in Chapter 4 and may assist those readers unfamiliar with these industries in considering the following information. As well, to further assist the reader, antimicrobials licensed for use in pigs and chickens in Canada are summarized by the labelled administration route(s) in Table 2.

Antimicrobial Use in Swine Production

Swine producers around the world use antimicrobials to treat and prevent disease, and depending on the country that they are being raised in swine, can also be exposed to antimicrobials to improve feed efficiency and daily gain. Antimicrobials can be administered to pigs through feed, water, and injection. Sows and suckling piglets are more often treated with injectable antimicrobials while nursery and grow-finish pigs more often are administered antimicrobials as groups through feed or water (93,277).

Overall, AMU is a common practice in Canadian swine production. Data from CIPARS indicated that 75% of the participating herds were exposed to at least one antimicrobial through feed, and 82% of the herds incorporated antimicrobials into either the feed or water (66). Assuming that reporting feed or water AMU means that all pigs in the batch were exposed, the majority of samples tested for antimicrobial susceptibility within the CIPARS Farm program were collected from pigs that had been exposed to an antimicrobial in the 20 weeks preceding sampling (66). These estimates relate only to grow-finish hogs but they do provide some estimates of conventional hog production antimicrobial use in this phase of production.

Injectable AMU is most important for treating clinically ill animals. The advantages of parenteral AMU are that the exposure to the appropriate dose can be ensured, treatment can be customized to each animal's condition, and only animals that require treatment need be exposed. The limitations of parenteral AMU are feasibility in treating large groups of animals and animal stress associated with handling and restraint. As pigs approach market weight, fewer herds use any injectable drugs and those continuing to use injectable antimicrobials report lower exposure rates (277). This is because of animal handling and disease factors. Market weight pigs are harder to handle and inject than small pigs. This increases the risk of injury to the staff or the pig and the risk of needle breakage. Some provinces allow pigs with a broken needle to be slaughtered if permanently marked while others require all pigs with a broken needle to be euthanized. The increased likelihood of a broken needle and greater economic loss from euthanizing older pigs both account for some of the decline in injectable AMU in near-to-market pigs. As well, there are decreasing infectious disease pressures as pigs age. However, when health problems occur in near-to-market pigs, producers may choose to inject individual animals to avoid having to hold groups of pigs until antimicrobial withdrawal times (period of time between the last administration of the drug and the collection of edible tissue or products from a treated animal that ensures the contents of the residues in food comply with the maximum residue limit for that drug) are observed. Additionally, producers may choose drugs with low or no withdrawal period; in particular, drugs like ceftiofur, a third-generation cephalosporin, are used close to market because of the zero day withdrawal.

In Chapter 1, the reasons for concern over resistance development to new-generation cephalosporins and fluoroquinolones were described. The use of these critically important drugs is discouraged by the World Health Organization (WHO) and Veterinary Drug Directorate (VDD) (for further information on this classification please refer to Chapter 3). Just under one-third of CIPARS sentinel herds used ceftiofur (66) in grow-finish hogs. To date, no Canadian study has reported quinolone exposure (66,92,93,282,283). Fluoroquinolones are not licensed for use in pigs or chickens in Canada, but injectable enrofloxacin (Baytril® 100, Bayer) is licensed in Canada for use in cattle (263) and extra-label use (see Chapter 3 for definition) in swine is legal (284–286).

In Canada, common injectable antimicrobials used include penicillin, trimethoprim-sulphadoxine, and oxytetracycline (92,93,93). Seventy-six percent of CIPARS sentinel swine herds reported injectable antimicrobial use in grow-finish pigs and the most commonly reported injectable antimicrobial used was penicillin (66).

Antimicrobials can also be administered through water. Water soluble antimicrobials can be administered to groups of animals without stress and are more practical for the treatment of infectious disease outbreaks than parenteral medications. Unlike injectable antimicrobials, exposure is not ensured because sick animals may stop drinking or may not drink sufficient water to achieve targeted blood antimicrobial levels. In this regard, water antimicrobials are often an intermediate between injectable and feed-grade antimicrobials because animals typically cease eating before they quit drinking. Water-soluble antimicrobials allow producers to rapidly respond to changing disease situations in groups of animals because treatment can be initiated and ceased virtually instantaneously with minimal labor. In contrast, medicated diets must be ordered or made in advance and then administered until the medicated feed is consumed. Despite these perceived advantages, fewer swine producers use water soluble antimicrobials than feed-grade drugs. This is likely because administration requires supervision in the barn and physical infrastructure to deliver the medication. Water AMU is most common in nursery pigs (92,93). When investigated, much shorter exposure times are reported for antimicrobials administered through water than feed (66,92,277,287).

In grow-finish pigs, 36% of Canadian producers reported use of water medication over a one year period (66). In general, water medication is used mostly for metaphylaxis and treatment (66,92). Penicillins, tetracyclines, and sulfonamides are the most common antimicrobial administered through water (66,92,93).

Feed-grade antimicrobials are most useful for managing static or predictable health situations. Medicated diets can be administered at high-risk times including weaning or transportation. The antimicrobials can be mixed in precise amounts and are stable, which makes it easier to achieve the desired concentration in feed than in water. Feed-grade medications are more cost effective than either water-soluble or injectable drugs. Feed-grade medications are the only medication route used to improve growth.

In Canada, most conventional swine producers use feed-grade antimicrobials and in many herds the exposure to antimicrobials is extensive. A survey of Alberta producers found 57% added feed medication to weaner, grower, and finisher rations more than 95% of the time (93). Of the remaining 43%, feed medication was more commonly administered to weaner and grower than finisher pigs (93). More extensive use of feed-grade antimicrobial use in younger pigs has been reported by many studies and is due to increased disease pressures and stressors in young pigs (92,93,282,283,287,288).

Tetracyclines, sulfonamides, and penicillin are commonly used in nursery pigs (93,287). Grow-finish pigs are most commonly exposed to macrolides for disease prevention or growth promotion followed by tetracyclines, which are predominantly used to prevent disease or treat respiratory disease (66). In-feed medication use is relatively static over time within herds. Dunlop et al. found few producers changed medication practices over eighteen months and Rajic et al. reported most producers used antimicrobials in diets more than 95% of the time in the previous twelve months (93,282). Un-medicated rations were more common during the latter part of the grower-finisher period (66). The use of un-medicated rations later in the feeding period most likely relates to decreased efficacy of growth promotion as animals age (289) and producers wanting to have flexibility in when they market these hogs and avoid any concerns over withdrawal times to prevent antimicrobial residues in the meat.

Antimicrobial Use in Broiler Chickens

Currently, no published data describe the types, amounts, or reasons for antimicrobial use in Canadian

broilers and limited data are available from the United States. Injectable antimicrobials can be used in eggs or day-old chicks. Hatchery use of injectable antimicrobials is predominantly to control omphalitis caused by gram-negative bacteria (predominantly *E. coli*). Canadian hatchery drug use data are only available from Ontario; approximately 30% of the chicks hatched in Ontario are treated with an antimicrobial and the most common drug used was ceftiofur followed by gentamicin (290).

Beyond the hatchery, broiler chickens only receive antimicrobials through feed or water. As described in pigs, antimicrobials can be included in feed to improve growth and/or control disease (264). Industry representatives report extensive use of in-feed bacitracin and virginiamycin combined with ionophores (personal communication: various Canadian nutritionists). These drugs are primarily used for the control of coccidiosis and necrotic enteritis. Numerous water-soluble antimicrobials are available to producers without a veterinary prescription (Table 2). The exception to this is amoxicillin (Paracillin® SP, Intervet) (263).

Agri-Stats is a private research company that collects data in the United States. Their analyses show a marked decrease in feed-grade antimicrobial use over the past decade (165). Medications in starter and grower diets (primarily bacitracin) fell from almost 100% of surveyed producers in 1995 to 65% in 2000. Antimicrobial use in withdrawal diets (primarily virginiamycin) fell from 75% to 48% over the same period. These declines may reflect tight profit margins. Producers will voluntarily remove inputs that do not increase production enough to offset their cost (165). Declining drug use may also reflect voluntarily restrictions on antimicrobial use to satisfy consumer concerns and target premium markets or satisfy production contracts (291,292). No data were found describing feed-grade antimicrobial use since 2001, nor were any publications identified on therapeutic antimicrobial exposures in American broilers.

European AMU data are also limited as few countries stratify the volume of antimicrobials sold by animal species. A good example of reporting by animal species comes from Denmark. Broiler chickens consumed 56 kg of the 121 tonnes (<0.001%) of the antimicrobials used in food-producing animals (89). The small proportion is because Denmark has a large export-based pig industry producing 1,957 million kg of pork and a domestic broiler industry producing 163 million kg of chicken (89). From 2006 to 2007, Danish producers shifted from amoxicillin use to macrolide and sulfonamide use due to increasing problems with amoxicillin resistance in *E. coli* from imported chickens (89). Over this same time, a 75% decrease in fluoroquinolone use was attributed to government instructions to veterinary practitioners about fluoroquinolone use (89). No cephalosporins were reported used in Danish poultry (89).

Relationship between Antimicrobial Use in Swine or Poultry and Antimicrobial Resistance

A challenge for any study is demonstrating causality. Cause is defined as an event, condition, or characteristic that preceded the disease or the disease event and without which the disease event either would not have occurred at all or would not have occurred until some later time (293). While the context of a single cause is a useful concept, the reality is there are often multiple causes working together to lead to an effect. In AMR, a more appropriate concept is sufficient cause. Sufficient cause is a set of minimal conditions and events that inevitably produce disease or in this case resistance. Minimal condition in this sense means that all conditions or events are necessary (293). It is in situations where a cause is multifaceted or has multiple contributing sources that proving causality becomes increasingly difficult.

Links between AMU in Swine and AMR in Bacteria Carried by Swine

There have been several articles describing associations between AMU and AMR in swine (84,92,125,283,294–300). Depending on the

organism and the resistance of interest, various risk factors for the development of AMR have been identified on swine farms. These include primarily AMU exposures, but they also reflect that stage of production may influence AMR development.

The expected relationship of exposure to an antimicrobial correlating to resistance to that antimicrobial and other antimicrobials within that class has been reported (125,295,298,299). But the use of an unrelated drug class being associated with increasing resistance to a particular antimicrobial has also been described. For example, tetracycline resistance is related to the use of sulfonamides or aminoglycosides (299) or the use of ceftiofur can select for tetracycline resistance (283).These associations demonstrate the likelihood of the presence of linked genetic elements and the co-selection of AMR (295,298,299).

Rosengren et al. (299) demonstrated possible co-selection when they reported that macrolide use was associated with resistance to chloramphenicol, streptomycin, sulfamethoxazole, and trimethoprim sulfamethoxazole in *E. coli* isolates from finishing hogs. This was surprising because *E. coli* is intrinsically resistant to macrolides and yet macrolide use appeared to select for resistance to at least four other antimicrobials. Akwar et al. (295) found similar results. Further investigation to describe any genetic linkages between macrolide and other resistance genes is warranted to more fully understand potential repercussions of macrolide use on diverse bacterial pathogens (299).

In addition to AMU selecting for AMR, some negative associations, where AMU lead to decreased resistance, have also been reported (283,299). A possible explanation for these negative associations could include gene incompatibility between resistances to certain antimicrobials. For example, resistance genes *catA1* and *tetB* are negatively associated with each other (136). Another explanation could be that AMU in this instance is a surrogate for some other risk factor that was not identified (283). A better understanding of why the use of a particular antimicrobial seems to lead to the reduction of AMR is needed and warrants additional research to attempt to identify if these negative associations are truly due to gene incompatibility or to try and identify 'unknown' factors that could be contributing to this phenomenon.

The impacts of exposure rates on AMR development have been investigated. Rosengren et al. (299) described the exposure rate of AMU and considered the relationship between use and resistance. Assuming a causal relationship, this study demonstrated that long-term use in pigs affects AMR more than targeted or short-term use. Similar results were also reported by Dunlop et al (277). Supporting this concept of increased use leading to increased resistance, the alternative has also been demonstrated where the removal of use has decreased resistance (296,301). Langlois et al. (255) demonstrated a reduction in AMR with the removal of AMU, but indicated that the reduction was less than what would have been anticipated over the study period, demonstrating that AMR can continue to persist in these herds without the selective pressure of AMU.

Antibiotic free (ABF) herds have been investigated and compared to conventional herds to elucidate the contribution of AMU to resistance over baseline. From these comparisons, varying results have been reported. Some indicated that resistance was more frequent on conventional farms that use antimicrobials than on ABF farms (302,303). These findings support AMU driving resistance. But, in a different study comparing ABF herds to conventional herds, not only did the authors report that at slaughter significantly more *Salmonella* were isolated from the carcasses of swine from ABF farms but that more multidrug-resistant *Salmonella* were isolated from ABF farms (300). Since the move of some farms to ABF production is relatively recent, there is paucity of data in this area. More research is needed to improve our understanding of the ecology of zoonotic bacteria and AMR on ABF farms and how these compare to conventional farms.

Links between AMU in Poultry and AMR in Bacteria Carried by Broilers

When looking at the impact of AMU on AMR in poultry production, there have been several studies supporting that AMU leads to AMR (302,304,305). Experimental trials have demonstrated that after therapy with a variety of antimicrobials there was a significant increase in resistance levels (302,306). While these experimental trials are useful, extrapolating these trial results to estimate in-barn selection pressure is challenging because the dynamics of the resident flora and historical AMU on the farm may impact the study results. Ultimately being able to use a wide variety of commercial farms for research purposes would be ideal since that would provide a range in management practices and exposures, but this is not always feasible from a management, economic or practical standpoint.

Due to the limitations of experimental research, an alternative approach is to evaluate the impact of widespread AMU changes on an industry. The most notable examples are the 1997 avoparcin and 1999 broad-AGP bans in Europe. The withdrawal of growth promoters resulted in a significant decline in resistance to avilamycin, erythromycin, vancomycin and virginiamycin in *E. faecium* isolated from broilers and broiler meat (304). But most recent reports acknowledge that these initial declines have been followed by stabilized persistence of resistance. A study from the United Kingdom indicated that vancomycin-resistant enterococci have persisted on intensive broiler farms despite no avoparcin use for seven years (307). Similar results have also been shown in Norway, where three years after the ban of avoparcin, vancomycin-resistant enterococci were still being detected in poultry environments (308). These data demonstrate that in commercial barn settings resistant strains do persist on some farms for extended periods of time and support the arguments that husbandry practices and/or therapeutic AMU probably contribute to the persistence of AMR in these situations.

Studies looking at ABF poultry production and AMR are also available. One study looking at ampicillin-resistant *E. coli* in organically raised broilers found that older heavier birds were significantly more likely to carry ampicillin-resistant *E. coli* than younger birds, despite the fact that the total *E. coli* shedding was lower in older birds and that they had not been exposed to antimicrobials (309). This indicated that as birds age the proportion of ampicillin-resistant *E. coli* increased (309) even in the absence of any antimicrobial use. In another study, that examined barns that moved to ABF production that had previously used fluoroquinolones, it was determined that previous use increased the percentage of fluoroquinolone-resistant *Campylobacter* and that this resistance continued to persist in the poultry environment despite the ongoing absence of AMU selective pressure (310).

In a study comparing retail chicken from organicversus intensively reared poultry, all meat had *Campylobacter* isolates resistant to nalidixic acid and erythromycin, but the resistance levels for nalidixic acid were significantly higher in the conventional birds versus the organic (311). Also, all *Campylobacter* isolates from organically raised birds were susceptible to ciprofloxacin (311). The results from the above studies indicate that organic or ABF production does not guarantee that birds or retail meat will be free from AMR bacteria and that there are factors other than AMU that are impacting the presence of AMR.

Another example that demonstrates our limited understanding of the ecology of AMR comes from work examining the impact of enrofloxacin and tylosin treatment of broilers. Both enrofloxacin and tylosin are potential therapeutic agents for *E. coli* air sacculitis (8,263). A controlled experiment found erythromycin resistant *Campylobacter* did not emerge in broilers treated with tylosin (0.53g/liter for three days, once or multiple times) (312). In contrast, separate studies found fluoroquinolone treatment significantly increased the frequency of resistant *Campylobacter;* 10 mg of enrofloxacin or difloxacin per bird for five days in drinking water (313), 40 ppm of sarafloxacin or enrofloxacin for five days (314). In both fluoroquinolone studies, resistant *Campylobacter* dominated at slaughter. Therefore, although macrolides and fluoroquinolone-resistant *Campylobacter* each pose a public health

hazard, the consequences to humans of therapeutic fluoroquinolone use in broilers appears substantially greater than macrolide use. Greater consideration for the effects of AMU, not only on AMR in the treated birds, but on meat contamination with resistant bacteria, could strengthen therapeutic guidelines developed to mitigate AMR.

The threat to public health is demonstrated by the development of resistance to third-generation cephalosporins by *Salmonella.* As mentioned in Chapter 1, Canada, through its national surveillance efforts, has identified a link between AMU and AMR in chickens (66). *Salmonella* Heidelberg is among the top five serovars isolated from human salmonellosis cases in Canada and the United States. Resistance to ceftiofur (a third-generation cephalosporin used only in animals) among S. Heidelberg isolates is highly correlated with reduced susceptibility to ceftriaxone, a third-generation cephalosporin used exclusively in humans. Ceftiofur is used as a prophylactic agent in broiler chicken hatcheries to reduce losses associated with omphalitis. Shortly after the inception of the CIPARS retail component, a sharp rise in resistance to ceftiofur was detected in both retail meat and human S. Heidelberg isolates (Figure 2)(66). When first detected, CIPARS presented these data to its stakeholders. To address this public health concern, hatcheries in Québec voluntarily stopped the use of ceftiofur in hatching eggs and day-old chicks. This voluntary withdrawal was followed by an observed decrease in ceftiofur resistance in retail chicken and human S. Heidelberg isolates (Figure 2) (66). Subsequently, there has been anecdotal information that the use of ceftiofur in the hatcheries has been reinstituted at some level. According to CIPARS data, an increasing trend of ceftiofur resistance supports this return to ceftiofur use (Figure 2). These data do show support for the correlation in trends over time between human S. Heidelberg resistance to ceftiofur and retail chicken S. Heidelberg resistance to ceftiofur. While this cannot show definitive causality, it does warrant further investigation to gain a better understanding how ceftiofur use in poultry is impacting human salmonellosis cases.

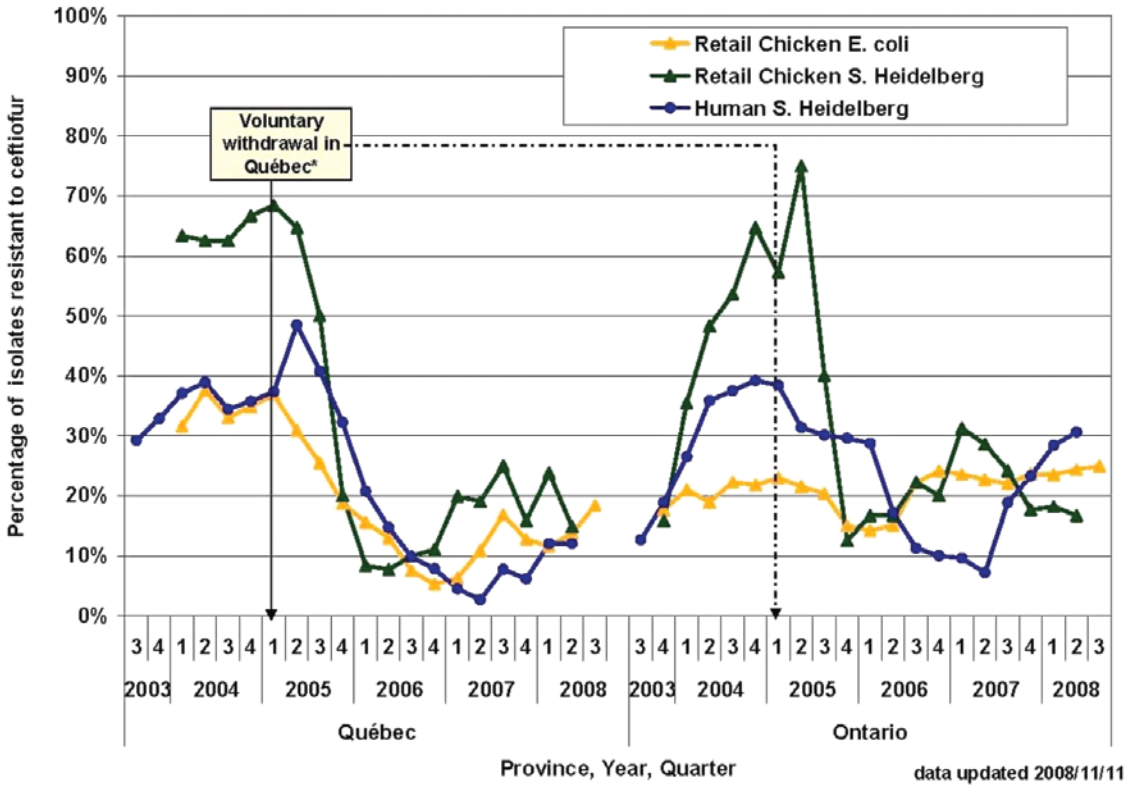
The above studies all support the fact that the ecology of AMR is not simplistic. There are biological factors, as well as exposure factors, that impact the prevalence and persistence of AMR. In some cases simply removing a drug exposure does not always lead to a reduction in AMR, and the lack of an exposure does not mean that AMR will not exist. There is still more research needed to try and identify what other factors may be impacting AMR development and/or persistence especially in the absence of AMU selective pressure.

Information Gaps and Challenges in Understanding the Effects of Antimicrobial Use in Livestock on Public Health

Most classes of antimicrobials used in livestock are also used in human medicine. One of the problems with using the same class of antimicrobials in both humans and animals is that as a result of cross-resistance the development of resistance to a particular drug within a class of antimicrobials can result in resistance to the entire class. One class of antimicrobials used in animals and not in humans is the ionophores such as monensin, narasin, salinomycin and lasalocid, the quinoxalines, and avilamycin (316). While these drugs are not used in people, their use could still potentially impact AMR development through the process of co-selection. Although, a particular drug may or may not be used in humans, the reality is that through gene linkage and transfer any antimicrobial use could ultimately have a negative impact on human health.

One way to try and understand the role of AMU on AMR is through risk assessments. Risk assessments (RA) can be used to try to evaluate the consequences to human health from the use of antimicrobials in animals. Risk assessments involve the estimation of risk for situations that cannot be measured or observed and provide an interface between science and policy (293). The goal of a RA is to determine a quantitative or qualitative value of risk related to a concrete situation and a recognized hazard. These

Figure 2. Resistance to ceftiofur for retail chicken *E. coli,* retail chicken, and human clinical S. Heidelberg isolates(315). Reproduced with permission.



assessments involve the calculation of the magnitude of loss and the probability it will occur based on assumptions and data estimates that are not directly testable (293). As result, RA are often inconclusive.

Several RA have been performed to assess the impact of AMU in animals on the development of AMR in humans (317–321). One of the identified limiting factors of these assessments in providing a precise estimate is the need for a better quantitative data on the rate of transmission of AMR bacteria and resistance genes between animals and humans (317,319). Other limiting factors of these assessments include limitation to one bacteria, antimicrobial, outcome, animal species, and antimicrobial use (246). These factors, along with limiting the scope of the analysis to what has already happened and to the bacteria the resistance was identified in, ignores the effects of continuing the practice and the ability for AMR to spread to other organisms. The other potential biasing effect of RA is that they are generally commissioned for a reason and may be either subject to bias in the interpretation and reporting of results, or prone to criticisms of bias. For these reasons RA may be limited in what information they can provide. However, in the absence of other methodologies that can provide insight on the potential impact of a particular hazard, risk assessments can be an important tool especially if the results are considered in light of the limitations of RA.

The alternatives to risk assessments are; reviews of the literature, primary research, observational studies, and interpretation of longitudinal data. Again, these

alternatives have potential limitations such as bias, misinterpretation, lack of power or study size, lack of ability to quantify certain critical data, and the inability to control for all potential confounders and risk factors. These methods are rarely able to put together the 'whole picture' as risk assessments attempt to do.

Regardless of the approach to summarizing data or to assessing the risk of AMU in animals on human health, there is still a great deal of controversy and conflicting evidence as to the actual risk. For example, in the case of invoking the precautionary principle there is a wide range of opinions as to the impact, not only on human health but to animal health and food safety. In the following paragraphs several examples have been provided in an attempt to demonstrate the divergence of opinion, with the ultimate goal of illustrating that this debate is far from being resolved.

A common position is that the bans were effective in achieving their goal and these proponents supported their position though evidence of decreased rates of AMR in bacteria from animals and food (13,304,322). But an opposing position is that policy that invoked the 'precautionary principle' to ban AGP lacked sufficient and conclusive scientific information. Concerns exist that these bans may ultimately have potentially dangerous and inconsistent effects on animal and human health (323). There is concern that the AGP bans have increased rates of animal illness which may impact human health by increasing the microbial load of zoonotic pathogens as well as increasing the need for therapeutic antimicrobial use (266,324,325)(325). In addition to increasing the shedding of zoonotic pathogens, concerns exist that diseased flocks can have a higher rate of processing errors, cuts, fecal contamination, and microbial loads (325,326).

There may be some merit in speculating that the removal of in-feed antimicrobials may have adversely affected public health. In the years after the European AGP ban, there was a rise in antimicrobial resistance of hospitalized patients (327), and in the case of *C. jejuni,* there was a rise in resistance of several hundred percent in human clinical isolates (89). This rise was beyond what was expected based on rates before the ban. Also, after the ban, human foodborne illness rates increased through most of Europe (328–330). When comparing the number of human campylobacteriosis cases after the ban in Europe to those in the United States (which did not have a ban), there was a significant rise in campylobacteriosis cases in Europeans while at the same time in the US, there was a drop in campylobacteriosis cases by 30% (87,331). While some of this rise in Europe may have been due to the increased consumption of fresh chicken during this time, the extent of the increase was in excess of what would have been predicted by a change in consumption practices (332). Also, between 1995 and 2001 in the United States, a 90% drop in *Campylobacter* loads in chickens was observed (333). The contrast between what was happening in Europe and in the United States is interesting because while Europe was banning AGP and seeing a rise in human health issues, the US was implementing hazard analysis and critical control points (HACCP), increasing public awareness through education and implementing prudent use guidelines (323), and as a result, the US subsequently saw a decrease in human illness. Whether these differences are like comparing apples and oranges or relate directly to the use of antimicrobials in livestock and poultry is impossible to determine, but they contribute to the scientific debate and rhetoric around AMU policy. Ideally if a similar phenomenon occurs in the future, national surveillance programs will be able to elucidate valid causes.

In addition to the impact on human health as a result of banning AGPs, there was also an impact on animal health. Some work has indicated that the ban of certain antimicrobials for animal use had a significant impact on animal health with an increase in necrotic enteritis in chickens and *Lawsonia intracellularis* in pigs (324,334). In the case of necrotic enteritis rates in broilers, it has been reported that morbidity rates went from approximately 0% to a transient high of 15% (323,335). In swine, Cox and Ricci indicated that it was difficult to find estimates for the impact of the ban on pig morbidity and mortality, but said that

trade journals reported that from the time of the ban in Denmark until 2005 there was increase in mortality rates from 17% prior to the ban to 21% after the ban (323,336). Probably, much of this mortality in swine was related to increased diarrhea and weight loss due to *E. coli* or *L. intracellularis* early in the post weaning phase of production (324). Although producers experienced increased losses as a result of disease and death immediately subsequent to the bans, after the initial surge, as a result of increasing the use of therapeutic antimicrobials, animal illness rates were controlled within two to five years depending on the animal species (334,337,338).

In contrast to these findings, Wegner states, "in broilers in Denmark, necrotic enteritis was at most a minor broiler health problem following the termination of antibiotic growth promotants" (339). Similarly, Emborg et al. indicated that there were no changes in weight gains or mortality in broilers and that the effects of antimicrobial growth promotant termination in poultry production were small and limited to decreased feed efficiency (340). The cost from lost feed efficiency was estimated to be offset by producers' savings on not needing to purchase antimicrobials for growth promotion (341). Callesen indicated that in swine there was a significant increase in treatments for diarrhea in nursery piglets and that there was some loss of productivity in weaners but no real effect in finishers (342). These conclusions are in sharp contrast to the interpretations of the effect of the ban on animal health and welfare described earlier, and they demonstrate the inconsistencies of this debate and the varied interpretation of the impact of the ban. Some of these inconsistencies arise from a lack of quality animal health monitoring prior to the ban, while others may reflect different health experiences in different regions (342).

Conflicting conclusions about the impact of AGP bans continue to be at the forefront of debate. In some instances, there has been a paradoxical increase in AMR after the ban of antimicrobial use. For example, ciprofloxacin/naladixic acid-resistant *Campylobacter coli* isolated from swine increased significantly from 3% in 2003 to 16% in 2004 in Denmark (89). The increase in resistance corresponded with a decreased use of fluoroquinolones as a result of legislation passed in 2001 (323). The reason for a rise in AMR despite decreased use supports the argument that the causal relationship between use and resistance is complex (323), and that other sources, including environment, water, pets, other humans, etc. may significantly influence the AMR profiles.

Banning Fluoroquinolone Use in Poultry in the USA

While the USA has not revoked labels for growth promotion, they have banned the use of fluoroquinolones in poultry as a result of concerns about AMR development. In the United States in 1995, the FDA approved fluoroquinolone use in poultry for treatment of *Escherichia coli* and *Pasteurella* spp. (343). Fluoroquinolones were used under veterinary prescription and delivered to the birds via water. After the launch of the product, the National Antimicrobial Resistance Monitoring System (NARMS), as a part of its regular monitoring, detected that resistance to fluoroquinolones increased from 12.9% in *Campylobacter* spp. to 17.6% in *Campylobacter jejuni* and 30% in *Campylobacter coli* between 1997 and 1999 (87). In 2000, because of the dramatic increase in AMR, the withdrawal of the approval for fluoroquinolone use in poultry was begun (344). A quantitative risk assessment was performed and it estimated that each year almost 10,000 Americans would be infected with fluoroquinolone-resistant *Campylobacter* from chicken, receive fluoroquinolone treatment, and experience a longer duration of illness because of decreased antimicrobial effectiveness (343). The Center for Veterinary Medicine cited the following as reasons for taking this action: use of fluoroquinolones in poultry causes the development of fluoroquinolone-resistant *Campylobacter* spp., this resistant *Campylobacter* spp. is transferred to humans and is the cause of the development of fluoroquinolone-resistant *Campylobacter* spp. infections in people, and that these infections are a hazard to human health (344). In several European and Asian countries a

similar situation was experienced (340), but at this time fluoroquinolones have not been banned in these countries.

Final Thoughts on the Link Between AMU in Animals and AMR in Humans

Cox and Ricci (323) suggest that the link between increased resistance in animals leading to increased resistance in humans is relatively weak, based on their work and the work of others (159,318,345,346). Others report that a link between AMU in livestock production and increasing AMR in people is virtually certain (275, 339,347–349). Turnidge (316) has a good summary of these opposing positions. He contends that the main reason that the debate continues is that there are virtually no studies that accurately quantify transmission rates of antimicrobial resistant organisms between humans and animals. Although the degree of transfer is unclear, both sides agree that animals can be a reservoir for AMR and that they can act as a source of amplification for AMR as a result of AMU. At this point, we need to decide if we move forward from the debate and start controlling antimicrobial use without indisputable evidence of the effects on AMR of such use (316), or if we continue to study the complex ecology and epidemiology of AMR before intervening on AMU.

Conclusion

AMR is a complex subject. The issue of AMU in livestock and its subsequent impact on human health will probably continue to be debated because there is conflicting evidence, personal bias, and potentially significant political impacts on all sides of the question. Conflicting conclusions between researchers about the impact of AMU in livestock on AMR development in humans may be the result of strong regional biases in the data since large amounts of data can come from specific regions. Differences in methodology and agriculture production must also be considered when these studies are evaluated to ensure that results are taken into context. Effects of interventions may not be identical in regions with different management systems, climate, and pathogen prevalence. Therefore, results of studies in specific regions should certainly be considered, but development of policy or legislation based on data from potentially incomparable systems should be approached with caution.

In light of data indicating that the elimination of AMU in feed can lead to increased bacterial contamination of carcasses, a variety of effects of banning antimicrobials on public health must be considered. Having food animal products that have minimal bacterial loads is critical to preventing foodborne illness. A part of reducing bacterial contamination on retail meat and poultry products may include ensuring good gut integrity and optimal animal health. Since there is still a great deal to learn about the role of antimicrobials in potentially reducing carcass contamination with bacteria, it is essential to evaluate what impact, if any, the removal of antimicrobial use would have.

Due to the wide distribution and multi-faceted nature of AMR, it is going to be very difficult to quantify of the amount of AMR in humans because of AMU in livestock production. Additional research examining the causal effect of AMU in livestock on AMR in people, along with improved quantification of the transmission dynamics of AMR between people and animals, are needed but these will not be simple tasks. When it comes to understanding and quantifying AMU in livestock, the knowledge gap grows exponentially. In order to be able to appreciate the true impact of livestock AMU on AMR, a much better system of collecting and reporting AMU needs to be developed. For most countries, and in the majority of species, the unfortunate reality is that good AMU data are truly limited at this time. With ever changing and evolving understanding of both the molecular and global aspects of AMR ecology, there will need to be continually committed to modifying previous interventions and strategies. It is also very likely that because of the complexity of AMR development and dissemination, there will never be an easy resolution to contain the future development of AMR.

Chapter 3: Overview of Antimicrobial Resistance Surveillance or Monitoring Programs and Subsequent Policy Outcomes

Introduction

Links through travel and trade make antimicrobial resistance an international problem requiring a mix of national and international solutions. Canada cannot address agri-food antimicrobial resistance (AMR) without first considering the history of other nations and their responses to AMR. An evaluation of how international activities relate to Canada is also required. For those readers new to the story of AMR control in food and food animals, sorting out the history of the policies and regulations can feel like a convoluted journey. Many actions occurred close in time, but only some were a related sequence. Overall, the strategies, regulations, and policies to date have predominantly focused on two particular aspects of antimicrobial use (AMU) and on surveillance of AMR. Regulations have aimed to improve the appropriateness of AMU. In Europe, this largely meant banning antimicrobial growth promoters. In contrast, North America has focused on policy and regulations to improve prudent AMU. Surveillance of AMR, and to a lesser degree AMU, is occurring in many regions of the world. Most countries appear to recognize that they lack sufficient data to make optimal decisions and are investing in long-term programs that provide consistent and comparable data over time and space.

Scope and Objectives

The objectives of this chapter are to familiarize readers with the history of policies and strategies to control AMR in other parts of the world to allow a sufficient evaluation of Canada's current approach and future options. Once again, the scope is limited to AMR as it pertains to bacteria in pigs and chickens from farm to abattoir and processing. This chapter does not investigate the effects of these policies because examples of these have been discussed in Chapter 2. This chapter begins by describing the actions and policies of four international organizations with mandates in human health, food safety, and animal health. Although these groups largely acted in the 21st century, which was after the major regulatory changes in Europe in the 1990s, they are presented first because they provide insight into the prevailing global attitudes about AMR and the recommendations for a cohesive policy approach. They are presented separately from the national regulations and legislations as these organizations can only issue recommendations which member countries may choose to implement.

The second segment describes regulations and policies pertaining to veterinary drug use in Europe, the United States, and finally Canada. Europe is presented first as it has the longest history with this issue, and many decisions there have shaped the international and North American responses. Canada is presented last to allow a comparison of our veterinary drug regulations with others. The final section describes international surveillance activities in AMR and AMU. Canada is presented first with a detailed description of our program followed by other noteworthy programs, which are compared to Canada.

International Organizations' Response to AMR in Agriculture

International organizations recognize that AMR transcends national boundaries. Several international organizations have, in accordance with their mandates, provided recommendations to foster a globally-cohesive response to this threat. Most of the international activity addressing AMR has occurred in the 21st century. The following discusses the mandates, positions, and recommendations of the United Nations' World Health Organization (WHO)

and Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE), and Codex Alimentarius.

Under the United Nations' system, the WHO is responsible for providing leadership on global health matters, shaping the health research agenda, setting standards, articulating evidence-based policy options, providing technical support to countries, and monitoring and assessing health trends (350). The WHO has acknowledged that the inappropriate use of antimicrobials has exacerbated the selection for antimicrobial resistant organisms and in response developed the first global strategy for the containment of antimicrobial resistance published in 2001 (270). This summarized six key recommendations regarding the use of antimicrobials in food-producing animals. These are as follows: i) obligatory prescriptions for all antimicrobials used for disease control in animals; ii) in the absence of a public health safety evaluation, terminate or rapidly phase out the use of antimicrobials for growth promotion if they are also used for treatment of humans; iii) create national systems to monitor antimicrobial usage in food animals; iv) introduce pre-licensing safety evaluation of antimicrobials with consideration of potential resistance to human drugs; v) monitor resistance to identify emerging health problems and take timely corrective actions to protect human health; and vi) develop guidelines for veterinarians to reduce overuse and misuse of antimicrobials in food animals. The WHO regularly works in conjunction with FAO and the OIE, which have food and animal oriented mandates, to address this issue.

The agricultural arm of the United Nations, the FAO, leads international efforts to achieve food security and aims to raise nutrition, improve agricultural productivity, improve the lives of rural populations, and contribute to the growth of the world economy. The FAO provides a neutral forum for developing and developed countries to negotiate agreements and debate policy (351). Jointly established by the WHO and OIE, the Codex Alimentarius is an international organization responsible for generating standards to protect the health of consumers while ensuring fair trade practices of food (352). In the case of antimicrobial use and resistance, Codex largely bases its recommendations on those issued by the WHO (353). The Codex Alimentarius Commission has released a code of practice to minimize and contain antimicrobial resistance in food. The aim of the code is to provide recommendations to prevent or reduce selections of antimicrobial resistant microorganisms in animals and humans through the responsible use of antimicrobial drugs in food-producing animals. The code provides guidance for the prudent use of antimicrobials in food-animals, addressing guidelines on the prescription, application, distribution and control of drugs used for treating animals, preserving animal health, and improving animal production (353).

While the mandate of Codex is limited to food, the OIE is an international organization that is responsible for improving animal health worldwide (354). The OIE has outlined in its terrestrial animal health code: i) criteria for development and harmonization of national AMR surveillance and monitoring programs; ii) recommendations for AMU monitoring in animals; iii) guidelines for responsible and prudent use of antimicrobials in veterinary medicine; and iv) risk assessment methodologies for assessing AMR arising from AMU in animals (355). The terrestrial code is published annually and is aimed to assure the sanitary safety of international trade in terrestrial animals and their products. The guidelines that are currently being developed for risk assessment aim to provide a transparent, objective, and scientifically defensible way for member countries to assess and manage the human and animal health risks of antimicrobial resistance from the use of antimicrobials in animals.

The World Trade Organization (WTO) recognizes both the OIE and CODEX as reference organizations. The WTO is the only global international organization that deals with the rules of trade between nations. The WTO agreement on sanitary and phytosanitary (SPS) measures was established in 1995. The SPS

agreement gives basic rules for food safety and animal and plant health standards. The agreement allows countries to set their own standards, but says regulations must be based on science and should only be applied to the extent necessary to protect humans, animal or plant life or health. They should not arbitrarily discriminate between countries with similar conditions (356). The SPS agreement encourages the use of transparent risk assessment practices where relevant for developing and applying standards for food in international trade (356,357). Hence, the risk assessment methodologies established by both Codex and the OIE are recognized tools for managing international trade and control of AMR.

These international organizations collectively hold a global mandate to address AMR from farm to fork. Each organization is responsible for a distinct segment of the supply chain. Thus inter-organizational co-operation has become the mainstay for most of the policies and recommendations released. All of these organizations have identified the need for national surveillance or monitoring systems for antimicrobial resistance (270,358–361). The global diversity of animal production systems, and veterinary and public health infrastructures, necessitates that these guidelines be general and overarching.

Ranking of Critically Important Antimicrobials

In 2003, the FAO, OIE, and WHO initiated a joint meeting to discuss issues related to antimicrobial use in agriculture and veterinary medicine, while understanding the essential role antimicrobials play in human and animal medicine (359). The outcome was a ranking system for critically important antimicrobials (CIA) (362). These were concluded as, "antimicrobial classes that provide specific treatment or one of a limited number of treatments for serious human diseases or pathogens that cause foodborne diseases." WHO developed a list of critically important antimicrobials based on the following two criteria: i) sole therapy or one of few alternatives to treat serious human disease and; ii) antibacterial used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases caused by organisms that may acquire resistance genes from non-human sources. Antimicrobial classes meeting criteria 1 and 2 were ranked as 'critically important;' antimicrobials meeting 1 or 2 were 'highly important;' and if neither criteria were met, then the antimicrobial class was deemed 'important' (86,362).

Following recommendations from this meeting, the OIE created a similar ranking of antimicrobials for their importance in treating animal diseases (362). The overlaps of both critical lists for human and veterinary medicine collectively provide insight to allow for a balance of what is needed in veterinary medicine while taking into account public health considerations. Australia, Canada, Japan, the United States, and possibly others have developed their own national ranking of antimicrobials for their importance in human medicine. Canada has outlined specific criteria for how it determines critically important antimicrobials (Table 3) (363). While the international list provides a global perspective and facilitates a consistent approach between countries, the national lists more precisely reflect the antimicrobial needs in each country. This makes them more applicable to regulatory decisions within those countries. For example, Canada and the United States use their respective lists for risk prioritization and assessment of which antimicrobials used in agriculture require risk management to avoid undue risk to human health, and then apply this information to reviews of new veterinary drug applications (272,364).

Table 3. Canada's criteria for categorization of antimicrobials by their importance for human medicine.

Category	Preferred option for treatment of serious human infections*	No or limited alternatives available
I – Very High Importance	Yes	Yes
II – High Importance	Yes	No
III – Medium Importance	No	No / Yes
IV – Low Importance	Not applicable	Not applicable

*Serious infections are considered those which if left untreated would lead to significant morbidity requiring emergency care including hospitalization and/or mortality. Table taken from Categorization of Antimicrobial Drugs Based on Importance in human Medicine, Veterinary Drugs Directorate (363).

Policy, Strategy, Regulation, and Legislation to Address AMR in Agri-Food Europe

In 1963, the United Kingdom experienced an epidemic of multidrug-resistant *Salmonella* Typhimurium that was linked to therapeutic antimicrobial use in veal calves. In response to the possibility that antimicrobial use in food-producing animals could be causing disease in people, the Joint Committee on the Use of Antimicrobials in Animal Husbandry and Veterinary Medicine was established and published a seminal report in 1969 (365). This report is widely referred to as the 'Swann report' after the lead author. In it, the following three recommendations stand out: i) antimicrobial growth promoters (AGPs) should be restricted to antimicrobials that are not used as therapeutics in animals or people and restricted to drugs that will not impair the efficacy of therapeutic drugs through the development of resistant bacteria; ii) antimicrobials used in animals should be divided into feed (i.e. growth promoting) and therapeutic classes; and iii) therapeutic antimicrobials should be available only by veterinary prescription (365).

Although the Swann report acknowledged gaps and uncertainties in the scientific evidence, European countries responded to its recommendations and separated the legislation for in-feed antimicrobial growth promoters from all other veterinary drugs. This resulted in prohibition of using tetracyclines and penicillins as AGP (259,260). Concurrently, European countries made all veterinary drugs available by prescription only. In 1986, Swedish producers opted to ban all AGP to assuage consumer concerns over AMR, while acknowledging that the scientific evidence was incomplete (337,366). Thus, it has been over forty years since Europe and North America initially diverged in their regulation of veterinary drugs, and over this time these differences have continued to grow.

The catalyst for the current global concern over AMR in livestock was the emergence of community-acquired vancomycin-resistant enterococci (VRE) in Europe. This raised concerns over the use of a related glycopeptide, avoparcin, as a growth promoter in animals (367-369). In 1995, countries began to independently ban avoparcin, and in 1997 an EU-wide ban was enacted (259). As described more fully in Chapter 1, the pharmaceutical industry responded to the need to treat VRE by releasing a new antimicrobial under the trade name Synercid® (162). The active pharmaceutical ingredient in Synercid® is quinupristin/dalfopristin which is related to a feed-grade antimicrobial, virginiamycin. Prior to the release of Synercid, the use of virginiamycin in animals was of minimal concern because there was no product from this class of antimicrobials that was being used in human medicine (162,164).

The premise of avoiding AGPs that are related to antimicrobials used in people is almost impossible to comply with in any system that permits this type of AMU. The lack of new antimicrobial discoveries has meant that the human pharmaceutical industry has remodelled old classes of antimicrobials to address emerging problems. This situation is not limited to virginiamycin. Bambermycins were historically limited to use in animals, but have recently been touted as the "holy grail for antibiotic drug discoveries" under the name moenomycin, and ionophores are being developed for topical therapy of bacterial and viral diseases under the name gramicidin (370–372). The limited discovery of truly novel antimicrobials means that drugs previously not used in human medicine are now being used to help combat resistance issues that have continued to emerge.

Following the ban on avoparcin in 1997, the EU banned four additional AGPs in 1999. These included spiramycin, virginiamycin, bacitracin, and tylosin (259). This ban did not target a specific bacterial pathogen or resistance outcome but was designed to decrease the ecological selective pressure from long-term, low-dose AMU. The EU's policy on AMU in agriculture was formally completed through the 2006 legislation that revoked the remaining four AGP feed additives: monensin sodium, salinomycin sodium, avilamycin, and flavophospholipol. The poultry industry was granted continued access to ionophores without prescription until 2013 (259,260,324). The broad-based European ban of all AGP is unique compared to the response of any other country. While other countries have banned individual drugs, to our knowledge, no others have made such sweeping changes. The European actions may be explained by the precautionary attitude to food safety, but the implementation of this action was almost certainly facilitated by the 1970s regulations that separated AGPs into a distinct class of antimicrobial inputs. Banning all AGP in North America would require changes to many aspects of veterinary drug regulations. In contrast, Europe did not have to open their veterinary drug regulations to change and eliminate access to AGPs.

The United States

The United Sates and Canada face many similar issues regarding veterinary drug regulations. Specifically relating to antimicrobials in food-producing animals, American concerns exist around the following: i) extra-label drug use (ELDU); ii) evidence-based risk assessment of human health risks from AMU in livestock; iii) non-prescription access to antimicrobials (commonly known as over-the-counter or OTC); and iv) use of in-feed antimicrobials for growth promotion or improved productivity. The first two points are being addressed through FDA regulations, no evidence of action was found on the third point, and the final point has fuelled numerous bills supporting more restrictive antimicrobial drug legislation.

Extra-label Drug Use

Extra-label drug use is the use of any drug in a way not indicated on the label. It is described in more detail under Canada, with only American specific legislation presented here. In 1994, the Animal Medicinal Drug Use Clarification Act (AMDUCA) gave American veterinarians the flexibility to use animal pharmaceuticals in situations not specifically listed on the label providing the use occurred in the context of a valid veterinary-client-patient relationship (VCPR). The AMDUCA specifically excluded certain ELDU practices. Feed-grade antimicrobials could not be used extra-label under any circumstance. As well, the FDA can impose a restriction of ELDU on a certain product or group of antimicrobials in food animals. This has been applied to chloramphenicol, fluoroquinolones, and glycopeptides and, as will be discussed, was recently considered for cephalosporins in food-producing animals (281).

Risk Assessment of Human Health from AMU in Food Animals

The FDA is addressing concerns of AMR in bacteria from food animals through a multifaceted regulatory framework that includes pre-approval safety evaluations, post-marketing surveillance, and risk management strategies. Evaluating new antimicrobial

drug submissions for both safety and microbial effects on bacteria of human health concern was embarked on in 1998 and rolled out to industry in 2002 (272,373). These regulations focus on enteric bacteria and require a risk assessment approach, which can be based on the OIE Ad Hoc Group on Antimicrobial Resistance recommendations (272,374,375).

The post-marketing requirements depend on the perceived risk to human and animal health and may include surveillance through the National Antimicrobial Resistance Monitoring System (NARMS). Risk management occurs via controlling drug access. Antimicrobials that require veterinary supervision to ensure appropriate use are licensed as prescription drugs. Other antimicrobials may be licensed as non-prescription drugs. The continued option to register new drugs as non-prescription is interesting, given the new regulations provided an opportunity to decrease the number of antimicrobials available without a veterinary prescription. The FDA may also prohibit the extra-label use of a drug or limit the extent of use. For example, drugs may be limited in exposure duration or to use in individual animals (272).

Through these regulations, the FDA has revoked, evaluated, and considered relabelling drugs to address risks to public health from AMU in food animals. The only antimicrobial that has been removed from the market is water-soluble enrofloxacin licensed for use in poultry. Enrofloxacin and another fluoroquinolone, sarafloxacin, were licensed in 1995 and 1996 respectively (376). As described in Chapter 2, rising rates of ciprofloxacin resistant Campylobacter were identified in poultry and human clinical isolates (68,262,376). Together, surveillance and quantitative risk assessment results were used to scientifically justify the removal of water soluble enrofloxacin from the American market in September 2005 (8,343,376). This legislation did not apply to sarafloxacin because its manufacturer Abbott Laboratories voluntarily removed it from the American market prior to 2000 (377).

Virginiamycin was the second antimicrobial evaluated by the FDA for AMR risks in humans. The human health risk from virginiamycin use in animals and the FDA-CVM risk assessment has been described in Chapter 1 (164). Following release of this risk assessment, no regulatory action has been taken (164); possibly because other antimicrobials have since replaced QDA as the most important treatment for vancomycin-resistant enterococci.

Most recently, the FDA proposed prohibiting extra-label cephalosporin use in food-producing animals (378). Ceftiofur is a third-generation cephalosporin available as an injectable product for use in chickens, pigs, and other food-animals (379). NARMS surveillance identified rising resistance to third-generation cephalosporins in *Salmonella* from cattle, pigs, chickens, and turkeys and these findings were corroborated by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) surveillance data from retail chicken (66,115). The proposed prohibition cited concerns that hatcheries were using ceftiofur extra-label, a practice also noted by CIPARS (378,380). Following an extended public comment period, with response from many agricultural sectors that restricting ELDU of cephalosporins would cause an animal welfare and disease crisis, the FDA revoked the extra-label cephalosporin prohibition in November 2008. Evaluation of this concern continues, but if the FDA wishes to prohibit extra-label cephalosporin use, it must re-initiate the entire regulatory processes (381).

Antimicrobial Use in Food Animals to Improve Growth and Productivity

In the United States, legislative bills are regularly introduced to federal and state governments in support of restricting or banning AMU in livestock. For example, bills entitled 'Preservation of Antibiotics for Medical Treatment Act of 2009' were submitted to the United States Senate (Senator Edward Kennedy S.169) and House of Representatives (Rep. Louise Slaughter H.R. 1549) on March 17, 2009 (382). These bills propose that new antimicrobial drug

applications be denied unless there is a reasonable certainty of no harm to humans due to AMR from non-therapeutic use of the drug. They also propose that approvals for non-therapeutic drug use in food-producing animals be withdrawn two years after enactment of the Act unless safety requirements are met. To date, such bills have not been passed, but the persistent submissions demonstrate a strong support base and the potential for AMU to become managed by legislation rather than regulations. If passed, these changes would move the United States closer to the precautionary principle approach of the EU.

Canada

In Canada, the role of food safety policy and regulation is mandated to Health Canada and enforcement to the Canadian Food Inspection Agency (CFIA). Within Health Canada, the Veterinary Drugs Directorate (VDD) has the directive to protect human and animal health and the safety of Canada's food supply with a focus on veterinary drugs administered to food-producing animals (383). Hence, licensing of veterinary pharmaceuticals is a federal responsibility that relates to the provincial responsibility for regulating the sale and distribution of veterinary drugs (248). Although there are is substantial differences between the Canadian provinces in their regulation of veterinary drugs, this document is limited to discussing federal regulations.

In 1997, Health Canada hosted a national consensus conference entitled 'Agriculture's Role in Managing Antimicrobial Resistance.' Delegates attending this conference called for Health Canada to address AMR and AMU in the agri-food and aquaculture sectors (384). This conference supported initiating AMR surveillance and influenced changes to Canada's veterinary drug use regulations.

Three years after this conference, the European Commission (EC) audited Canada's control of chemical residues in animals and meat. This audit was conducted to ensure Canada continued to meet the consumer needs of the EU and was sweepingly critical of Canada's veterinary drug regulations (385). Two concerns that were raised in this report, and Health Canada's subsequent response, are pertinent to the current discussion. The first concern was that Canada address ELDU. The second concern was over Canada's lack of action to ban the sale of drugs that, based on scientific evidence, pose a risk to human health. This concern pertained specifically to a non-antimicrobial drug, diethylstilbesteral, but was also applicable to in-feed non-therapeutic AMU which had been banned in Europe the previous year (385). The changes in Canada's veterinary drug regulations subsequent to the 1997 consensus conference and the 2000 EC audit are the focus of the following section.

In 2002, an Expert Advisory Committee that was convened by the Veterinary Drug Directorate (VDD), Health Canada, submitted an extensive report on "Animal Uses of Antimicrobials and the Impact on Resistance and Human Health" (248). This Committee was established in 1999 as a result of the 1997 consensus conference. In total, the Committee made thirty-eight recommendations, twelve of which specifically pertained to veterinary drug regulations and five of which directed specific actions. These five recommendations are as follows: i) develop methods and criteria for human health safety assessment of veterinary drugs with respect to antimicrobial resistance; ii) define threshold levels of resistance for post-approval surveillance and provide for appropriate remedial action if thresholds are surpassed, up to and including modification of approval or suspension of marketing; iii) develop an extra-label use policy that ensures this practice does not endanger human health. Such a policy should include the ability to prohibit the extra-label use of specific drugs of critical importance to human health; iv) evaluate, register, and assign a drug identification number (DIN) to all antimicrobials used in food animals in Canada, whether manufactured domestically or imported. This includes bulk active pharmaceutical ingredient (API) products with intent to stop the direct use of APIs in food animals and; v) make all antimicrobials used for disease treatment and control available by prescription only.

In addition to these five suggestions, a recommendation was made to conduct risk assessments of currently approved antimicrobials, including but not limited to those with a growth promotion claim, for their potential effects on human health. This recommendation echoed the concerns of the European Commission in 2000. In response to the recommended strategies from this expert Committee, Health Canada has made efforts to address the first four of the above recommendations of the Advisory Committee. We did not identify any activities pertaining to changing veterinary drug access to prescription only or to conducting risk assessments for currently approved antimicrobials.

Criteria for Human Health Safety Assessment of Veterinary Drugs with Respect to AMR

The VDD has established a multi-disciplinary Expert Advisory Committee on Antimicrobial Resistance Risk Assessment with a mandate to provide advice on scientific methods to evaluate the human health risk from antimicrobial resistance (386). In particular, this Committee is focused on the validity of various risk assessment methodologies for establishing thresholds and application to drug monitoring. Over a three-year history, a major contribution from this Committee has been guidance on a classification scheme for antimicrobials according to their importance in human medicine (as described above) (363,387,388). This classification is used by the VDD in new antimicrobial drug applications (364).

The VDD requires new antimicrobial drug applications to include information pertaining to AMR, including applicant submitted risk assessments. These include data on the phenotypic and genotypic resistance mechanisms in the target bacteria and the resistance patterns expected in non-target bacteria. Applicants must consider AMR development and transmission including cross-resistance and co-selection in target pathogens, relevant foodborne pathogens, and commensal bacteria. There is specific focus on the possible effects of the antimicrobial on the flora of the intestinal and colonic microbiota with emphasis on *Salmonella, Campylobacter,* commensal *E. coli* and enterococci. (364).

The VDD has post-approval marketing requirements. Guidance documents for industry emphasize surveillance for resistance to antimicrobial classes considered critical to human medicine (364,388). This surveillance may be conducted through a combination of federal programs, the most applicable of which is CIPARS (364,383). However, given that critical thresholds triggering action have not been established due to their complexity, post-approval surveillance is in truth actually monitoring (389). A role of the Expert Advisory Committee on Antimicrobial Risk Assessment is to consider how risk assessments may contribute to the development of thresholds for action.

Extra-label Drug Use

Extra-label drug use (ELDU) is the actual or intended use of a drug in animals in a manner that is not in accordance with the approved label (285,390). For example, any use that is in a species, for indications, at dosages, frequencies, or administration routes that are not on specifically stated on a product's label are ELDU. Deviation from label use creates concerns about drug residues as the appropriate withdrawal time has not been determined for the circumstances of use. Antimicrobial resistance may be a concern if the use alters the selective pressure on bacteria.

Extra-label drug use is an important tool for veterinary medicine. Minor or exotic species have few drugs licensed for use in them, which limits labeled treatment options (286). Likewise, less common diseases may have limited or no label treatment options. Pharmaceutical companies typically seek label claims for major commodities and prevalent pathogens; demonstrating safety and efficacy of drugs is costly and time consuming so labels for minor uses often lack sufficient return on investment.

In Canada, extra-label use occurs in broilers and pigs (personal communication; Dr. Patricia Dowling, Director of Canadian Global Food Animal Residue Avoidance Databank (CgFARAD)). The CgFARAD has

extensive data on possible ELDU in poultry because the CFIA requires that veterinarians obtain a CgFARAD issued drug withdrawal time recommendation for all poultry exposed to ELDU drugs. Producers report the ELDU and withdrawal time when they ship birds to slaughter. In chickens, most inquiries pertain to in-feed unlicensed drug combinations. Inquiries regarding AMU in-feed at doses that are not on the label or in age categories of birds that are not on the label is less common. Swine data are less complete because abattoirs do not require a CgFARAD withdrawal recommendation. Thus the inquiries are incomplete and more varied. To date, they have largely pertained to therapeutic AMU to control an endemic pathogen, *Streptococcus suis.*

Currently, extra-label use of non-prescription drugs is not limited to veterinarians but can be practiced by pharmacists, trainers, producers, animal owners, and other non-health care professionals. Untrained users may not appreciate the risks of ELDU to public health and food safety (285,286,391). In 2002, the VDD established a committee to provide expert advice and guidance on a commissioned study entitled 'Drug Use in Animals Study.' This committee became the ELDU Advisory Committee and released an issues identification paper in 2004. Since then, Health Canada has released a policy on ELDU, recognizing its importance but also recommending against ELDU outside of a VCPR and for antimicrobials considered of very high importance to human health (388). The federal government's jurisdiction of ELDU is limited because of the provincial regulation of veterinary drug sales. This seems to have limited the VDD to making a recommendation, which is less enforceable than the American prohibition of ELDU for certain critically important antimicrobials, but does match the approach and tone of the United States. Overall, this policy aims to promote the prudent use of drugs in food producing animals in order to minimize the risks to public, animal, and environmental health and addresses the concerns raised by the Expert Committee and Advisory Committee (285).

Active Pharmaceutical Ingredients

The Health Products and Food Branch (HPFB) have been addressing concerns over the use of active pharmaceutical ingredients (API) in animals. Licensed establishments (i.e. pharmaceutical companies or research institutions), veterinarians, and pharmacists can import API. Some API are in dosage form, have demonstrated manufacturing standards that meet Canadian requirements, and are issued a Canadian DIN and permitted for sale. Others are imported as bulk products for further compounding and do not receive a DIN until they have been modified to dosage form using processes regulated by the government. Health Canada's greatest concern is with bulk API that are being sold as finished products for veterinary use thus bypassing manufacturing standards and controls. If importers lack good manufacturing practices, these products could include impurities leading to human or animal toxicity or inaccurate active ingredient concentrations. This could potentially result in residues in excess of maximum limits or inadequate levels resulting in inappropriate dosing and the potential for further resistance-pressure. Inappropriate bulk API use puts public health and export markets at risk. Within the HPFB, stakeholder consultation has occurred and a policy statement was released in 2007. The policy specifically pertains to concerns over the sale and distribution of bulk API to farmers, pharmacists, feed mill operators, and veterinarians for use as dosage drugs (392). The HPFB policy outlines how API should be handled and the appropriate regulation that governs importation and use of API.

Own use Importation

Importation of antimicrobials without a Canadian DIN by producers is another concern in Canada's veterinary drug regulations. The 'own-use loophole,' as it has become known, is a regulatory omission that permits ninety days of worth of a pharmaceutical drug that is not licensed in Canada to be imported for personal use. The intent of this regulation was to allow people receiving medical

care internationally to return to Canada without interruption in therapy (393). However, it is used by producers to import pharmaceuticals that are either not licensed in Canada or are available more economically elsewhere. The ability to import drugs for 'own-use' purposes raises concerns about the impact that these drugs may have on antimicrobial resistance development and meat residues and toxic contaminants.

Health Canada has faced opposition to changing this regulation. Stakeholders argue that out-of-country access to pharmaceuticals is the only mechanism that ensures pharmaceutical prices remain competitive in Canada (393,394). Others have raised concerns about the VDD backlog in approving new-drug submissions. They argue that closing the own-use loophole puts industry at a competitive disadvantage due to delayed product access. An estimate from the Canadian Animal Health Institute suggests that about one-third of all veterinary drugs (not only antimicrobials) used in Canada are drugs imported by producers for their own use (380), but to date there are limited data on the effects that these importations may have on food safety or the economic effects on producers and the pharmaceutical industry. This issue remains at the consultation level. The task force that was put in place to examine this issue has called for further study before drawing any conclusions (394).

Limiting Antimicrobial Access through Prescription-Only Licenses

Continued access to non-prescription antimicrobials is counterproductive to prudent AMU guidelines, which are based on the premise that AMU should occur within the confines of a VCPR to ensure evidence-based decisions (395). It also could have a negative impact on animal health based on the lack of a veterinarian in the decision-making process and the potential for inadequate or contraindicated therapies. Over-the-counter (OTC) access to antimicrobials also complicates AMU data collection (281,396). The sale of veterinary pharmaceuticals is under provincial jurisdiction and Quebec has a prescription only system (248). Other provinces have struggled to address this issue because it requires changes to the pharmaceutical distribution system. Ethical debate over veterinarians concurrently prescribing and selling pharmaceuticals exists, and restricting antimicrobial access to prescription could be perceived as supporting a veterinary monopoly on drug sales (397,398). In some jurisdictions, this has been addressed by limiting veterinary profit on antimicrobial sales to a pre-defined handling fee. Another challenge for limiting OTC pharmaceuticals has been ensuring alternative access. In some rural areas competitor veterinarians may be distant and licensed pharmacists may not be trained in veterinary science. Our understanding is the only avenues open to the VDD are to re-license all antimicrobials as prescription drugs, effectively passing the distribution problem on to the provinces, or to continue supporting its policy statements that ELDU should be limited to VCPR and limit approvals of new applications for non-prescription antimicrobials.

Comparison of International Veterinary Drug Regulations and Policy

In summary, Canada has some distinct differences in veterinary drug regulations from Europe and the United States. First, in Europe all veterinary AMU must be accompanied by a prescription. In contrast, both Canada (excluding Quebec) and the United States permit non-prescription sales and use of antimicrobials. Second, European authorities do not allow antimicrobials to be included in animal feeds without a veterinary prescription and then use is only to address a disease indication rather than improve growth or productivity. In contrast, there are currently no feed-grade antimicrobials licensed for use in pigs or chickens in Canada that require a veterinary prescription when used according to the label (263,264). Label indications can include a list of specific diseases as well as a growth promotion claim. In Canada, feed-grade antimicrobials may be included in diets in an extra-label manner if accompanied by a veterinary prescription. Although non-prescription in-feed AMU is permitted in the United States, extra-label use is prohibited (281).

Superficially, the differences between Europe and North America may appear minor; but they belie a fundamentally different approach to food safety policy. Canada and the United States have committed to evidence-based policy while Europe has employed the precautionary principle in its legal statutes. The European Environmental Agency has defined and clarified the precautionary principle as, "the Precautionary Principle provides justification for public policy actions in situations of scientific uncertainty and ignorance, where there may be a need to act in order to avoid, or reduce, potentially serious or irreversible threats to health or the environment, using an appropriate level of scientific evidence, and taking into account the likely pros and cons of action and inaction" (399). The disparate approaches to regulating AMU in livestock mean that North America and Europe tend to interpret risk prioritization, assessment, and management differently. Ideally, risk assessment, evidence-based science, cost-benefit and precautionary approaches should be combined to provide a balanced decision-making process (400). As scientific research and observational studies help clarify the links between people, food, and animals, our approaches to managing AMR and veterinary AMU may become increasingly similar.

Surveillance and Monitoring of Antimicrobial Resistance and Use

There is international consensus that countries should conduct AMR surveillance in animals and/or food to monitor trends, provide a basis for the development of national policies, and assess interventions. Antimicrobial use data are essential to understand the causes of AMR. The WHO, FAO, and OIE recommend that countries collect veterinary AMU data (359). At a minimum, these data should include the national use of antimicrobial agents in kilograms of active ingredient on an annual basis and be reported using the Anatomical Therapeutic Classification system (ATC). When possible, data should be stratified by animal species (359,401). Many countries have made great progress in conducting AMR surveillance and most have faced challenges in responding to recommendations for AMU data collection.

Basic Premises of AMR Surveillance

Surveillance is a continuous and systematic process of collecting, analyzing, interpreting, and disseminating descriptive information for the purposes of understanding and describing health issues (402). Over time, surveillance can be used to identify trends and emerging situations. Surveillance can detect and report on the incidence or prevalence of health outcomes and identify at-risk groups. Surveillance data may be useful for assessing the effects of interventions applied to the issue of concern. There is often a balance between the desire for the best information and feasible data collection (402). The ideal national system should include monitoring both animal and human use as well as resistance levels in bacterial species from animals, animal derived foods, and humans. Several factors are important to consider when designing an integrated monitoring system, including: the main purpose of the system, the reservoir of interest, the bacterial species, and the antimicrobial agents to include.

As a result of needing long-term cooperation from participants, and due to the breadth of surveillance in time and geography, surveillance is generally less detailed and precise than research projects. However, surveillance and research can complement each other. If through surveillance a particular area of interest or concern is detected, then research can be used to investigate the issue in more depth and detail. Conversely, research studies can identify new issues that may benefit from formal ongoing surveillance.

Surveillance can be active or passive (403). Active surveillance employs statistically valid sample collection to obtain unbiased data from a targeted area or group. It must be implemented consistently across groups and over time. There is a need for ongoing contact with the participants in order to be able to collect specific data from the identified population in a consistent manner. It may involve

the use of sentinel sites that act on behalf of the surveillance system to provide the required information. Active surveillance requires substantial financial and human resources. Passive surveillance is less demanding on human and financial resources. In passive surveillance, the data or samples often arise from submissions to a lab, clinic, hospital, etc., and are subsequently provided to the surveillance system using a standardized protocol. In passive surveillance, the surveillance program has no influence on sample collection.

Both active and passive surveillance can provide useful information. Passive surveillance is more prone to bias than active surveillance (402,403). People seeking treatment could have differences in their infecting pathogens compared to people with mild symptoms that do not seek treatment. In food animal AMR, passive data typically represent clinical isolates while active data often represent healthy individuals. These clinical isolates may have different rates and patterns of resistance than isolates of the same bacterial species obtained from healthy animals. This may be due to linkages between virulence and resistance gene, differences between commensal and pathogenic strains of a bacterial species, and therapeutic drug exposure (26,48). This bias is amplified in passive surveillance of clinical isolates from animals because producers incur laboratory expenses. Diagnostic submissions are often only made for severe or non-responsive disease outbreaks, thereby creating a tendency to receive more highly resistant bacteria (404). Conversely, utilizing samples from unrelated mandatory monitoring programs can artificially increase the number of submission from a certain species or region.

Passive surveillance of AMR in bacteria from clinically ill animals increases the likelihood of detecting emerging resistance, as this is a form of targeted surveillance. It does not reflect the probability of human exposure to resistant bacteria because only healthy animals enter the food chain. While both healthy and sick animals harbour resistant bacteria, the patterns, frequencies and genetic basis for resistance differ (26,136). For these reasons, passive surveillance of clinical isolates is used as only one component of an AMR agri-food surveillance system. Surveillance programs must take all of the relative strengths and weaknesses of passive and active data collection from animals, food, and people into consideration, along with the objectives and resources available, to determine the most appropriate program design. Each piece can provide information on different populations, and therefore, studying both presents the most complete picture.

Surveillance from farm to fork is useful for assessing the impact of AMU in livestock and poultry production on AMR throughout the food production chain. Farm level AMU and AMR surveillance allows considerations of what drug are used and how they impact the AMR patterns on the farm. Collecting samples at abattoir can provide information about changes in AMR patterns between what is detected on farm and what is being detected in the processing plant. Depending on the stage of processing that these samples are taken at and how they are taken, this may allow for assessment of the effectiveness of HACCP programs, cross contamination, or changes in intestinal microbial flora as a result of transport and/or stress in the animals. Abattoir surveillance is the easiest point to ensure samples are representative of the livestock population and unbiased. Between slaughter and retail, the microbial load, resistance rates, and resistance patterns can again change. So, retail surveillance provides the best estimate of human exposure to resistant organisms. A challenge of interpreting retail surveillance is incomplete information identifying a product's source. Knowing if a product is domestic and where it came from within the country or if it is imported would obviously assist with the interpretation of data collected at all points of the surveillance program.

In summary, the 'farm-to-fork' approach is useful for understanding the transmission of resistance through the food chain and identifying potential areas for interventions and areas requiring further investigation. In future research and surveillance, the farm-to-fork concept should be expanded to foodborne disease in humans. This is currently being attempted outside

of AMR surveillance programs via source attribution projects (96,405) but to our knowledge, no country is attempting to monitor the effect of AMR on clinical outcomes of foodborne disease or the proportion of resistant foodborne infections that occur in individuals taking antimicrobials prior to infection.

National AMR and AMU Monitoring and Surveillance Programs

There are many national AMR monitoring and surveillance programs. These programs are challenging to catalogue and many have not published their methodologies in peer reviewed literature. Websites and annual reports were reviewed for methodological information, but were often incomplete. Summarizing all of the identified programs would have been fraught with errors by omission and somewhat redundant (406). The following section highlights surveillance programs as they relate to pigs and chicken and their meat and evaluates their relevance to Canada. We have selected the programs from the United States because Canada was designed to harmonize with this program, Europe because they have been instrumental in establishing methodologies and ensuring ongoing prioritization of surveillance, and Japan because it was the only program identified outside of Canada that uses on-farm sampling, and the only well described program outside North America and Europe.

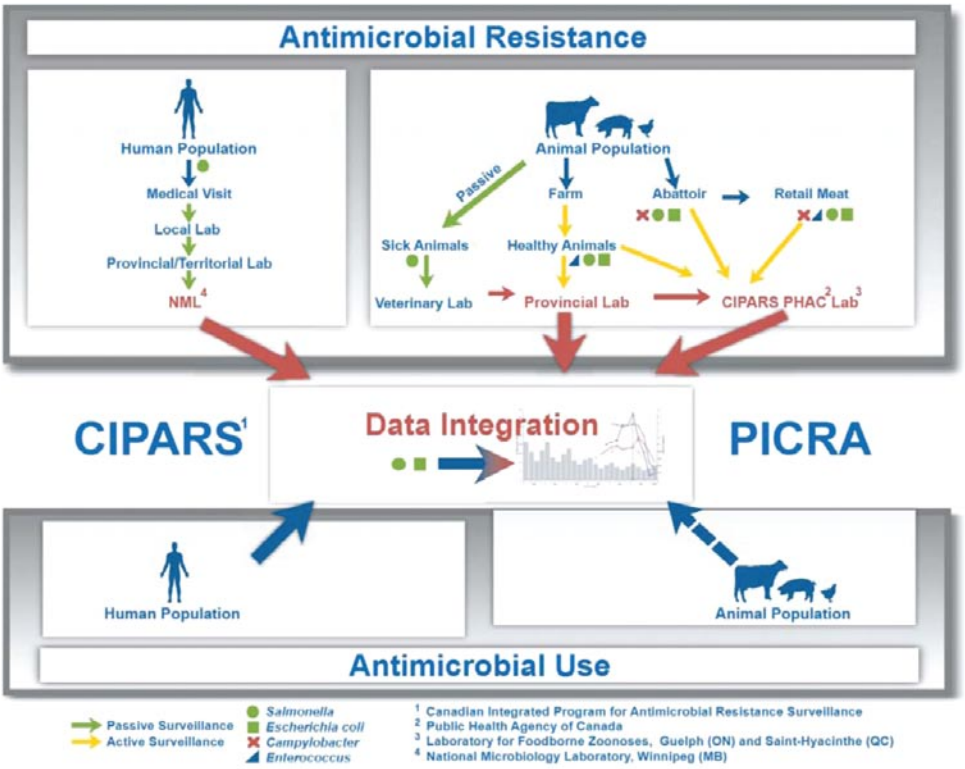
Canada

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was established in 2002 by Health Canada (66). This is a national program that collects, integrates, analyzes, and communicates trends in antimicrobial use and resistance in select species of enteric bacteria from humans, animals, and meat across Canada. The goals of CIPARS are to: i) use a unified approach to monitor trends in AMR and AMU in humans and animals; ii) generate timely reports; iii) generate data to facilitate the assessment of the public health impact of AMU in people and livestock; and iv) allow accurate international comparisons with other countries that use similar surveillance systems. The several components of CIPARS (Figure 3) are harmonized to monitor temporal and regional trends in the prevalence and patterns of AMR across regions, bacterial species, and hosts. Results of CIPARS surveillance are published in annual reports, and results of detailed studies are published in peer reviewed journals.

The human component of CIPARS involves passive surveillance of *Salmonella* isolates from clinical cases. *Salmonella* isolates are sent from provincial public health laboratories and reference laboratories across the country to the National Microbiology Laboratory for phage typing and antimicrobial susceptibility testing. As *Salmonella* is a reportable disease in humans, these isolates should represent the vast majority of clinically diagnosed cases. Clinical *Salmonella* isolates from animals are also tested. These originate from veterinarian or producer submissions to veterinary diagnostic laboratories. In animals, *Salmonella* is not reportable and coverage between provinces is variable.

In agri-food, surveillance is conducted in beef, chicken, and pork, but only chicken and pork are described. The retail surveillance component was initiated in 2002 and provides data on antimicrobial resistance in *Salmonella, Campylobacter,* and *Enterococcus* from raw chicken (skin on wings and legs), and generic *E. coli* from pork (chops) (Figure 2, Table 4). Weekly samples are submitted from retail stores. Stores are selected by a stratified sampling scheme which randomly selects census divisions, weighted by the population of each of the provinces. The abattoir component isolates bacteria from swine and poultry cecal contents. The unit of concern is the bacterial isolate. *Salmonella* and generic *E. coli* are isolated. The sampling design is a two-stage sampling. Each commodity is handled separately. The first stage randomly selects slaughterhouses by a probability that is proportional to their annual slaughter volume. The second stage is a systematic selection of animals on the slaughter line, where the number of samples taken per plant is proportional to their slaughter

Figure 3. Components of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)



volume and evenly distributed over the year. The farm surveillance component was initiated in 2003 and the national sample collection in swine began in 2006. Resistance is monitored in *E. coli, Salmonella,* and *Enterococcus* from near market weight pigs. The farm program relies on voluntary participation of veterinarians and producers across the five major pork producing provinces in Canada. This component involves approximately 100 swine farms under the supervision of twenty-six veterinary clinics.

Antimicrobial use data are currently collected in people and pigs. Human drug use data are obtained through Intercontinental Medical Statistics (IMS) data and describe oral human antimicrobial consumption at the community pharmacy level. Data are computed using DDD from dispensed prescription data. As more fully described in Chapter 2, animal drug use data are not readily available from a single source in Canada or the United States. Use data for pigs are obtained through farm surveillance. Insight into AMU in other commodities currently occurs through collaborative research projects. Recently, antimicrobial distribution data have been provided by the Canadian Animal Health Institute. These data will provide a context for interpreting livestock AMU data generated through research and farm surveillance (66).

CIPARS has consistently, and continues, to expand to include more commodities, bacterial species, and geographical regions within Canada (Figure 4). As CIPARS expands, it will continue to act as a research platform to investigate AMR and as a critical source for gaining insight into emerging trends in AMR and AMU over time.

Figure 4. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) Expansion Timeline.

2002	• Established CIPARS • Commenced Passive Animal Component
2003	• Commenced Human Clinical Component • Commenced Retail Component (Ontario, Quebec)
2004	• Expanded retail component to include Saskatchewan
2005	• Commenced Farm Component
2006	• Expanded retail component to include British Columbia
2007	
2008	• Expanded retail component to include the Maritimes
2009	

United States

In 1996, the National Antimicrobial Resistance Monitoring System (NARMS) was established by the Food and Drug Administration's Center for Veterinary Medicine (FDACVM) in collaboration with the United States Department of Agriculture (USDA), and the Center for Disease Control and Prevention (CDC). Within the CDC, NARMS's primary purpose is to monitor antimicrobial resistance among foodborne enteric bacteria isolated from humans. All fifty states forward samples of *Salmonella, Listeria,* and *E. coli* O157 to NARMS for antimicrobial susceptibility testing, and ten FoodNet states have been participating in *Campylobacter* surveillance (87). *Salmonella* are collected from clinical cases in sick animals. In agri-food, *Salmonella, Campylobacter, E. Coli,* and *Enterococcus* are monitored. Retail samples are collected from meat obtained from grocery stores, and abattoir specimens are collected from carcass rinsates, carcass swabs, and ground products through the USDA's Food Safety and Inspection Service (FSIS) Pathogen Reduction: Hazard Analysis Critical Control Point (PR/HACCP) testing program (87,407) (Table 4). NARMS differs from CIPARS by rolling its sampling focus through the livestock commodities so evaluating results for trends is more difficult.

The United States does not currently collect any AMR data from animals on farms. A pilot program called Collaboration in Animal Health and Food Safety Epidemiology (CAHFSE) was run in swine from 2003 to 2005 (73). From the beginning, this program was designed to only run a few years followed by an evaluation period. American studies have recognized that an on-farm surveillance component is one of the few feasible ways to collect AMU data (281,396,408). No information was found pertaining to the potential re-initiation in swine or expansion of farm surveillance or pilot projects in

other commodities. The United States describes AMU through surveys conducted by the National Animal Health Monitoring System (NAHMS) program. These surveys rotate between commodities and target appropriate regions of the country to achieve representative data for that industry. Commercial swine production was last targeted in 2006 and commercial poultry 2004. A small enterprise study that covered both poultry and swine was conducted in 2007 (409).

Europe

Antimicrobial resistance is monitored in most countries within Europe. A survey performed in 1998 outlined the methodologies of 12 countries in the European Union (EU). At that time, the most frequent bacterial group tested was *Salmonella* (410). The European Antimicrobial Resistance Surveillance System (EARSS) monitors resistance in human isolates for a variety of bacterium including *E. coli* and *Enterococcus* (151). Results from these bacteria in particular are of importance due to their potential foodborne connection. Participating EARSS laboratories within each country collect data obtained from routine susceptibility testing from invasive isolates of these bacterial species along with patient information such as age, sex, birth date, and level of care. These data are then provided to EARSS from the participatory nations. There are currently thirty-one European nations who participate in EARSS. Data provided to EARSS are reviewed by experts before publication in annual reports. This allows for a more thorough representation of the current status of antimicrobial resistance within Europe given resistance does not obey national boundaries.

The new Zoonoses Monitoring Directive was adopted by the European Food Safety Authority (EFSA) in 2003 to improve the comparability of data of member countries and to consistently monitor AMR (97). Data from 2005 onward are reported based on the new directive. These data include AMR in *Salmonella, Campylobacter, Enterococcus,* and *E. coli.* Bacterial isolates are obtained from humans, animals, and foods for susceptibility testing. Considering that extensive harmonization in surveillance has occurred across European countries, and that a single report summarizes both the results and methodologies of individual country's programs for AMR data from food and animals, we felt a detailed description of each European country's surveillance program was redundant (97,360,361). Instead, select national programs are described to highlight similarities and differences from Canada. Denmark was selected as the longest-running and most comprehensive AMR and AMU surveillance program in the world. Norway was selected as an example of a comprehensive program that is representative of Northern Europe. Both of these programs have been in place for over a decade. Many of the more recently developed European programs are less comprehensive but may still be in the development stages.

Denmark

Denmark established the world's first systematic and continuous monitoring program in 1995 (89,322,411). The Danish Antimicrobial Resistance Monitoring and Research Program (DANMAP) examines representative bacterial isolates from animals, foods, and humans in Denmark. Isolates include zoonotic and indicator bacteria (Table 4). Sampling occurs in food animals at abattoirs and through diagnostic submissions. Cecal contents of pigs and cloacal swabs of broilers are obtained and susceptibility testing is carried out for *Salmonella, Campylobacter, Escherichia coli,* and *Enterococcus faecium/faecalis. Salmonella* isolates collected from systematic random sampling from diagnostic submissions to the Danish Veterinary Laboratory and the laboratory run by the Federation of Danish Pig Producers and Slaughterhouses are also tested. Isolates are also included from submissions to the National Food Institute. Sampling of predetermined foodstuffs from retail outlets and wholesalers also occurs. The foods sampled include both Danish and imported foods, and *Salmonella, Campylobacter, E. coli,* and *Enterococcus* are tested.

Antimicrobial use data in people are available electronically from pharmacy sales (89). Data on animal use are also available electronically through pharmacy sales data. Veterinarians can sell medicines to farmers but profits are limited to 5%. Therefore, most antimicrobials are sold on prescriptions. The Danish Plant Directorate monitors the consumption of in-feed antimicrobials which can only be sold at feed mills in the form of premixes. Producers of premixes are required to report quantities of active ingredients used. This provides consumption data but no information on the recipient animal species (89). These limitations were addressed by the VETSTAT program, which was established in 2001 to continuously monitor the use of all prescription medicines in animals at the individual herd level (412). Veterinarians are required by law to report the sale of medicines to a central database. Information submitted includes the identity of the farm, receiving medicine, species of animal, age group, and reason for prescription.

Norway

The Norwegian AMR surveillance program (NORM) was established in 1999 to monitor resistance in human pathogens and the veterinary arm (NORM-VET) was established in 2000. An action plan developed by the Norwegian Ministry of Health and Social Affairs described the need to address antimicrobial resistance at a national level and emphasized the importance of ongoing surveillance and monitoring of both human and animal sectors to aid in controlling antimicrobial resistance. The NORM-VET program rotates through the food-animal commodities with a particular emphasis on one species per year. In the target commodity, commensal *E. coli* and *Enterococcus* spp. are described. Chickens were last targeted in 2006 and pigs in 2004. Four enteropathogenic bacteria are monitored in animals and people annually: *Salmonella, Campylobacter, Yersinia,* and *Shigella* (79). *Salmonella* from all food-animal species are obtained during clinical examinations or necropsies at the National Veterinary Institute. *Campylobacter* is cultured from samples collected at slaughter plants. One isolate per farm is included. The NORM program obtains human isolates from clinical specimens.

In Norway, all antimicrobials intended for human use are prescription and sold through pharmacies. Therefore, the human use data presented include total sales of antimicrobials for humans in Norway. The unit of measurement for human antimicrobial use is defined daily doses (DDD), reflecting the usage of the Anatomical Therapeutic Chemical (ATC)/ DDD system. In Norway veterinary antimicrobials intended for domestic animals and farmed fish for therapeutic use are available through prescription only and only dispensed through pharmacies. Medicated feeds have to be prescribed by veterinarians and produced by feed mills and are only available for farmed fish. Given the small size of herds/flocks in Norway, livestock are treated with antimicrobial agents, prescribed by veterinarians, through drinking water or injection. The reporting of sales of veterinary drugs is mandatory and, therefore, the number of items sold per year, drug formulations, strengths, and package sizes are able to be obtained. Drugs are classified using the Anatomical Therapeutic Chemical for Veterinary medicinal products (ATCvet) classification system and presented as the kg of active substance sold in the country. Data are not stratified by species (79).

Japan

The Japanese Veterinary Antimicrobial Resistance Monitoring Program (JVARM) was established in 1999. The objectives of JVARM are to monitor antimicrobial use and resistance amongst zoonotic and indicator bacteria from healthy animals and pathogens amongst diseased animals. Zoonotic (*Salmonella* and *Campylobacter*) and indicator (*Escherichia coli* and *Enterococcus*) bacterial isolates are obtained from healthy animal (cattle, pigs, broilers, and layers) fecal samples. The JVARM was the only program identified that collected samples

from farms. Six samples are collected per prefecture (region) annually with a limit of one sample per farm. Antimicrobial use data are acquired from pharmaceutical companies which produce and import antimicrobials for animals. The annual weight in kilograms of the active ingredient of approved antimicrobials used in animals is collected. This only includes therapeutic AMU in animals (413–418).

Studies and Pilot Projects

Many countries have not met WHO and OIE recommendations to implement national AMR surveillance or monitoring programs. In some, studies or pilot projects have evaluated AMR. A surveillance system pilot was implemented for *Salmonella* spp. in Mexico in 2002 through to August 2005. Active surveillance began in 2002 integrating samples from ill and asymptomatic persons and retail pork, chicken, and beef. In 2003, intestines of chicken, swine, and cattle were collected from slaughter (419). A study from South Africa was conducted to aid in the establishment of an integrated AMR monitoring program. The study was performed to make recommendations for a practical and sustainable monitoring program that could provide information on a national scale (420). Australia currently does not have a national AMR monitoring or surveillance program. However, the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR) has made a recommendation to establish an AMR surveillance program which will follow OIE recommendations (421). In other countries, research studies have been conducted. The following references provide evidence that research in AMR in food animals is occurring on every continent except Antarctica (66,77,97,115,419,422–427).

Table 4. Comparison of National Integrated AMR and AMU surveillance programs in Canada, United States, Denmark, Norway, and Japan.

		Human		Agri-food		Pig			Chicken		
Surveillance Programs	**Initiation Year**	**Diagnostic Samples**	**AMU**	**Diagnostic Samples**	**AMU**	**Farm**	**Abattoir**	**Retail**	**Farm**	**Abattoir**	**Retail**
Canada	2002	*S*	Yes	*S*	†	*S, Ec,En*	*S,Ec*	*Ec*	–	*S,Ec*	*S,C,Ec,En*
USA*	1996	*S,C,En*	Yes	*S*	†	–	*S,C,Ec,En*	*S,C,Ec,En*	–	*S,C,Ec,En*	*S,C,Ec,En*
Denmark	1995	*S,C*	Yes	*S*	Yes	–	*S,C,Ec,En*	*S,C,Ec,En*	–	*S,C,Ec,En*	*S,C,Ec,En*
Norway *	1999	*S,C*	Yes	*S,C*	Yes	–	*C,Ec,En*	–	–	*C,Ec,En*	–
Japan	1999	**	Yes	*S,C*	Yes	*S,C,Ec,En*	–	–	*S,C,Ec,En*	–	–

(C) Campylobacter (S) Salmonella (Ec) Escherichia coli (En) Enterococcus

* Surveillance or Monitoring Program rotates between species annually for data collection.

** Information unavailable. Does not imply the program does not perform type of monitoring but rather information has not been found within the scope of this paper.

† Agricultural AMU data are obtained through several different methods, such as studies and national distribution data.

– Monitor AMR in animals and humans but methodologies are unclear. No differentiation made between active monitoring from a retail or abattoir program or diagnostic submissions.

Note: Information lacking within this chart does not mean the program does not perform such monitoring but rather information has not been found within the scope of the literature reviewed for this paper.

International Comparisons

International surveillance programs have become increasingly harmonized over the last decade. Many of the differences in sample collection and susceptibility testing are being overcome, which makes data comparisons more valid. The described programs differ in the regularity of their sampling schemes (Table 4). The CIPARS collects a predetermined number of isolates, for each studied bacterium, from each commodity every year (66). Most other programs rotate their focus to a particular commodity each year with or without a base level of sampling across all commodities (97,407). The CIPARS approach helps to identify changing trends in resistance over time. It also makes random variation due to chance appear less important. For example, CIPARS presents data trends and the 'noise' around resistance estimates is obvious. However, if data were collected sporadically, this noise could be considered representative of reality. Although the CIPARS approach of regular sampling is more expensive from a laboratory perspective, it may allow efficiencies in labour and program management. Effective relationships are established with sample collectors and once trained, minimal interaction is required.

Most integrated programs collect human clinical isolates along with food-animal isolates from abattoirs. Retail sampling is relatively common while only Canada and Japan collect data and samples from farms (415). The merits of on-farm AMR data have been discussed and largely relate to avoiding ecological bias between farm-level AMU data and abattoir-level AMR data, as well as the ability to look at management factors beyond AMU for their influence on AMR. There are currently no other viable sources of valid AMU data in Canada, although the possibilities of using surveys or data extrapolation continue to be evaluated. The main benefit of such alternative AMU methods would be cost and labour savings.

Other countries have also struggled to collect valid and useful AMU data. Many European countries report sales volumes which are not stratified by species. These data are of minimal use and are not available in Canada because of privacy regulations for pharmaceutical corporations (281,396,408). The Danish VETSTAT program is a census style farm-level data collection system that inventories the national AMU (412). This program is detailed and world-renowned. However, it cannot be applied to Canada for at least three reasons. First, in Denmark, all AMU is prescription only and largely occurs through pharmacies. This facilitates drug tracking and allows validation of the farm-level data. Second, the VETSTAT system is mandatory while Canada would require a voluntary program. Denmark appears to have different privacy regulations than Canada which facilitate regulated data collection. But even beyond these, operating the VETSTAT program is simplified by the structure of the livestock industry. The beef, dairy, and poultry sectors are relatively small and although the pork sector is large, it is integrated with producer co-operatives as the owners (428). This makes implementing and enforcing farm-to-fork food safety systems in Demark much easier because producers have a unified and influential voice in negotiations. The final reason the Danish VETSTAT system is not applicable to Canada is that Canada does not possess the pharmaceutical industry infrastructure that enables electronic data collection.

Canada's AMU data collection system most closely mirrors the pilot program run by CAHFSE in the United States, but the CAHFSE pilot program ceased operations in 2005 (73). As evidenced by the discussed veterinary drug regulations, substantial differences exist between North America and Europe. We expect that a North America AMU surveillance approach will emerge with minor modifications to account for Canadian and American differences (408). This will benefit both countries as harmonized AMR surveillance already occurs. In contrast, harmonization with existing European AMU surveillance is not realistic. The greatest issue that we foresee for AMU and AMR surveillance in Canada is financial sustainability. Continued commitment from the federal government is needed to ensure the long-term viability of this world-class program.

A weakness of this report is an inability to describe or compare the financial costs of national surveillance programs. An economic estimation of the costs to the Danish livestock industry from the AGP ban has been published, but did not extend to considering the cost of DANMAP or VETSTAT (341). The lack of information of cost efficiency of surveillance systems is an important information gap for decision-makers.

The surveillance programs described would be more appropriately referred to as monitoring programs. A monitoring system is an ongoing effort to describe disease in a population. Surveillance is distinguished from monitoring by having predetermined actions that occur when data indicate disease prevalence or incidence has risen above a threshold (403). The importance of antimicrobials for human medicine is only one component required to establish thresholds beyond which the risk to public health from AMR in food animals is deemed unacceptable. The second piece is the likelihood of humans being affected by the resistance elements in animals. In 1999, the FDA proposed to categorize antimicrobials according to the likelihood of human exposure to resistant bacteria from food animals that are either human pathogens or may transfer resistance elements to human pathogens. This would involve considering four likelihoods: i) that use in food animals will induce resistance in bacteria; ii) that food-producing animals will promote such resistance; iii) that resistant bacteria will be transmitted to people; and iv) that transfer will result in the loss of available human antimicrobial therapies (429). To our knowledge this categorization has not been released by the FDA or any other regulatory body.

In this same document, the FDA proposed that these two categorization schemes could be combined to establish and monitor thresholds for AMR. Such thresholds would be based on two premises. First, that a regulatory agency could determine a threshold of resistance that would adequately protect public health, and second, that the regulatory agency has the ability to detect when that threshold is reached. Numerous concerns were raised about the validity of these premises (429,430). These included: What determines a safe threshold? Should thresholds identify resistance frequencies where public health is initially affected, based on the premise that interventions will reduce resistance, or should the threshold be lowered to ensure that limiting further increases is sufficient to protect public health? Should resistance be monitored in food animals, food, or human cases? And should it account for resistance in pathogenic bacteria or sentinel bacteria? Who is responsible for the surveillance and its costs? And what actions should be taken when a threshold is exceeded: increased monitoring, restricted use, or complete cessation of use?

These are a few of the questions that have hindered regulators' abilities to establish action thresholds for AMR and that require consensus from scientists and stakeholders for progress. These concerns and questions are by no means limited to the United States but pertain to all countries attempting to understand and address AMR and AMU in food animals.

Conclusion

Even though North America has based, and continues to base, regulatory changes on scientific evidence, determining the appropriate time, sufficient evidence and appropriate response to scientific evidence is influenced by political pressures from public health authorities, the agriculture industry, the pharmaceutical industry, trading partners, and the public. What seems insufficient to some often seems too drastic to others. Establishing action thresholds for regulations is extremely difficult and potentially fraught with significant resistance from either side of the debate. There is a continued need for surveillance activities that can monitor trends over time in both AMU and AMR for humans and animals to help provide the critical data that are needed to assist policy makers in their decision-making process.

Chapter 4: Agricultural and Agri-Food Interventions to Reduce the Impact of AMR Bacteria in Pigs and Chickens on Human Health

Introduction

Pressures to decrease antimicrobial use (AMU) in meat animal production are being prompted by public health concerns, consumer demands, and narrowing profit margins (11,291,431). Canada's regulatory agencies are addressing international recommendations and standards (269,359). The livestock industry is also facing recommendations from national and international groups to eliminate unnecessary AMU and to decrease their reliance on antimicrobials by improving management and increasing the use of antimicrobial alternatives. Some recommendations are specific, such as the requirements imposed by fast food chains on their suppliers, while others give generic guidelines such as emphasizing prevention of infectious diseases to avoid AMU (122,291,395,432,433).

Scope and Objectives

This chapter is written for professionals in human medicine and public health who have minimal to no background in agriculture. It aims to inform these professionals about industry-led initiatives in pigs and chickens that mitigate foodborne AMR risks. It also describes alternative inputs or management practices that are available to producers to improve animal health including vaccines, competitive exclusion strategies, and biosecurity practices. It provides examples rather than an exhaustive catalogue of all alternatives and does not quantify the extent that each are used. Practices that control pathogens on-farm or bacterial contamination of meat during slaughter and processing with no plausible influence on the prevalence of resistant bacteria are excluded.

Chicken and Pig Production and the Broiler and Swine Industries

Basics of Chicken and Pig Production

The term 'broiler chicken' refers to birds raised for meat. Broiler producers receive day-old chicks from the hatchery. These birds are raised as a flock using all-in-all-out management which means that all chicks arrive together, are raised together and are marketed together. Depending on the target market, birds are finished between 1.75 and 2.2 kg and generally range from 34 and 42 days of age. Although the birds are managed as a single group and remain in the same barn for their lifespan, they are managed in phases. Each phase lasts approximately 14 days and progresses from the starter phase, through the grower and finisher phase, to the withdrawal phase. Disease pressures vary between phases and are greatest early in life. At each phase change, the birds' diets are altered and feed medications or coccidiostats may also be changed (personal communication: Dr. Tom Ingles, Poultry Health Services, Airdrie, Alberta).

In North America, pigs are also managed in phases, but progression between phases typically entails physical movement of the pigs. The suckling phase extends from birth until weaning. Pigs are weaned between 15 and 30 days, with most herds weaning pigs slightly less than three weeks (126,409). Weaning marks the beginning of the nursery phase. Piglets may be moved to a separate pen in the same room as sows but are more commonly moved to separate rooms or even sites. The nursery phase is associated with increased disease risk because stressors, including mixing and diet changes, occur concurrently with declining maternal immunity. Pigs generally remain in the nursery phase until they are

6 to 10 weeks of age (average, 61.8 days; S, 0.6) (409). Movement to the grow-finish phase completes the production cycle. Pigs remain in this phase until marketed or selected for the breeding herd. In Canada, pigs are typically marketed at 110 to 115 kg live weight and are generally between 24 and 30 weeks old (66).

Industry Background

Canada produces over a million metric tonnes of chicken on 2,000 farms annually. Most of this chicken is retained for domestic consumption while 15% is exported. This makes Canada the 13th largest global chicken producer and the 7th largest exporter (although our exports amount to less than 5% of the global leaders; Brazil and the United States) (434–436). In contrast, Canada produces a considerable proportion of the world's pork; ranking among the five largest producers in the world and accounting for 17% of the world's exports. In 2007, 31 million pigs were produced with one-third exported live. The remaining 21 million pigs resulted in slightly less than 2 million tonnes of pork, of which 55% was exported (435,437,438). Despite Canadians consuming slightly more chicken than pork (2008 average per capita consumption: Chicken, 11.2 kg; Pork, 9.7 kg (439), our pork industry is far larger than our chicken industry because it is export based.

Canada's poultry industry is supply managed. This is a marketing system that regulates domestic chicken production and imports. It matches demand to supply thereby ensuring that there is a consistent national market with stable commodity prices. Production is allocated to farms based on the amount of quota they own. Only producers that own quota can raise more than 300 birds annually and sell them to processors at the industry set price (440–442). This has protected the chicken industry from much of the market volatility experienced in the pork sector. Vertical integration is the term describing a single corporation owning all, or most, of the steps in a production chain. This business model is pervasive in much of the poultry industry worldwide (443). In Canada, the poultry industry has been described as vertically coordinated. While few corporations control the majority of slaughter, processing and distribution of chicken, and these corporations typically own or have strategic alliances with hatcheries, independent farms raise the broilers on a production contract with the processor. Feed mills are generally independent of both the producers and processors (441) (personal communications: Dr. Stewart Ritchie, Canadian Poultry Consultants and Dr. Agnes Agunos, Public Health Agency of Canada).

In Canada, the swine industry is not supply managed. Although the last three years have seen a contraction in the industry, this contraction follows twenty years of substantial growth. The expansion of the Canadian swine industry was accompanied by industry consolidation to capture economies of scale. The number of herds decreased, while herd size increased and large corporate producers became established. In some instances, there is vertical integration with single corporations owning multiple or all aspects of production including feed mills, pigs and slaughtering facilities. But much of the industry remains segmented, particularly in comparison with the American industry (444).

Vertical integration, or the lack of it, has implications for AMU decisions. Integrated companies can consider decisions from numerous perspectives, and the costs incurred in one division may be sufficiently offset in another to justify their incurrence (445,446). These operations may have better production data, ability to track the effect of decisions, and specialized staff, which could all influence the scientific and economic rigour applied to AMU decisions. The lack of vertical integration in the Canadian industry, particularly when animals change production phases and ownership, also affects AMU decisions. Hatcheries may treat eggs differently from breeder flocks with a known versus unknown health status, and may make different AMU decisions for domestic versus imported eggs. In the swine industry, some producers purchase weanling pigs and raise them to market weight. Again, AMU may differ in herds purchasing from a single supplier with a known-health status versus multiple suppliers.

Large producers and corporate farms that own multiple barns make AMU decisions that affect many animals. For example, in 2006, 40% of American farms used pre-weaning antimicrobials in feed but only 10% of pigs produced were exposed (409). Thus, 60% did not use pre-weaning antimicrobials and 90% of pigs were not exposed at weaning. This example shows that industry consolidation can positively affect AMU when large producers decide to limit or discontinue AMU. Supply chains can also affect AMU decisions. For example, numerous international fast-food chains impose the National Chicken Council Animal Welfare Guidelines on their poultry suppliers across North America (The Chicken Farmers of Canada's recently released Animal Care Guidelines may supersede these in Canada in the future). Under this and the related Canadian programs, diseases indicative of poor welfare, including mortality and lameness, require immediate euthanasia, veterinary advice, and appropriate therapy (447–449).

Supply management may have implications for AMU decisions. Supply management provides financial stability to chicken producers, relative to producers of export-based commodities (namely pork and beef) (441). This may contribute to an industry stance on antimicrobial use that has financial consequences but consumer and/or political appeal. Examples include an industry-mandated recommendation against active pharmaceutical ingredient (API) use and the own-use importation of drugs. In contrast, the Canadian pork industry is export-based and drug-use decisions may be influenced by importing nations' and customers' standards for antimicrobial use or resistance (450,451). As such, the Canadian Pork Council has stated in its Canadian Quality Assurance Policy on Drug use that own-use importation of drugs is not permitted, but that API use may occur providing that producers do not use bulk API and that the final dosage form is accompanied by a veterinary prescription and has been identity tested (452).

HACCP: On the Farm and At Slaughter

Hazard Analysis Critical Control Point (HACCP) is a food safety management system that enables the food industry to pre-empt problems rather than rely on end product testing. HACCP consists of six generic steps. These systematically apply science to control and document the safety of a food product. Since inception in the 1960s, this generic system has been effectively applied to a wide-range of food processing facilities (453–455).

The HACCP system begins with a hazard analysis: hazards are characterized as physical, chemical, or microbiological. Critical control points (CCP) are then identified for each hazard, which are points where an action could prevent, eliminate, or reduce the hazard to an acceptable level. The third step establishes a critical limit which, if exceeded, triggers a response. The final three steps of HACCP are establish a monitoring system, establish and implement corrective actions when monitoring identifies a problem with a CCP, and establish verification procedures that ensure you do what you say and say what you do. Records and documentation are crucial components of the HACCP program and are legal documents (454).

The HACCP programs are often implemented in processing plants to reduce carcass contamination and improve food safety (455,456). We found no evidence that AMR is an identified hazard in abattoirs. This is intuitive because controlling resistance inherently means controlling the bacterial host. Furthermore, critical limits for a 'safe' prevalence of resistance do not exist. National authorities and surveillance programs have struggled to establish thresholds and subsequent appropriate responses to exceeding such thresholds (See Chapter 3). Hence, any private HACCP plan developed to control AMR would struggle to identify sufficient scientific evidence for setting a ceiling for AMR levels. We speculate that if AMR became a trade issue, abattoirs might attempt to modify HACCP programs to address AMR hazards,

but we would expect control would be targeted at further minimizing bacterial contamination rather than attempting to control resistance elements.

The HACCP approach can be applied to systems beyond food processing. The Canadian Food Inspection Agency (CFIA) has released a Food Safety Enhancement Program that requires a HACCP plan for all federally registered food establishments, including hatcheries (457). Twelve hatcheries are currently registered across Canada. This plan must address hatching egg quality, and the CCP addresses risks from imported and domestic eggs, as well as the handling and quality of the hatched chicks. Antimicrobial use can be considered as a chemical and biological hazard (personal communications, Dr. Agnes Agunos, Public Health Agency of Canada).

On-farm food safety programs (OFFS) can be HACCP-based (284,458). They are not true HACCP systems because biological processes are inherently more variable and less controllable than industrial processes. The Canadian programs described next provide a general HACCP plan that is applied to all producers. In contrast, a true HACCP program is tailored to each process. Rather than using CCP, OFFS programs identify good production practices (GPP) that might minimize the risk of the hazard at any point in production, not just at a specific point. Finally, OFFS programs are not HACCP programs because while the OFFS requires a plan, with compliance ensured through external audits and verification, they lack monitored thresholds at CCP (284,458). This prevents processors from knowing the entry contamination levels from which they must act to achieve their CCP levels.

Canadian OFFS programs cannot yet feasibly address AMR because intervention points and thresholds must be science-based. More research is needed on how and where resistance elements enter flocks/herds, the transmission dynamics once they are present, and the factors that allow persistence. These same questions also require investigation at the industry level. However, it is foreseeable that OFFS programs could identify batches or flocks with AMR frequencies exceeding a threshold, which could subsequently be moved into low-risk product streams, such as further processed and pre-cooked meats. Such an approach has been taken with Salmonella and Campylobacter control in Europe (64,459,460).

On-Farm Food Safety Programs

The Canadian Pork Council's Quality Assurance (CQA) and the Chicken Farmers of Canada's Safe, Safer, Safest Programs

A lack of data describing AMU in agriculture has been a major stumbling block for understanding AMR. In Canada, producer-led organizations in swine and poultry administer on-farm food safety programs that collect data on antimicrobial drug use (284,458). The 'Safe, Safer, Safest' program was initiated by the Chicken Farmers of Canada in 1998, and the Canadian Pork Council implemented the Canadian Quality Assurance (CQA) program in 2001. These programs are designed to ensure best management practices in biosecurity, disease control, sanitation, drug handling, and residue avoidance.

Producers are required to record AMU data. The AMU data are not compiled or analyzed across the industry. The primary objective of recording AMU data is to mitigate risk from antimicrobial drug residues, and so these programs focus their description of AMU near to market. In pigs, AMU through feed is described through the entire production cycle and through injection and water after weaning. In poultry the AMU data are limited to the two weeks closest to slaughter. The AMU data do not accurately capture exposure dose, duration or drug combinations. In our opinion, the most valuable data from these programs for addressing AMR is the information on producer decisions and attitudes towards AMU. If compiled, these answers could facilitate the development of effective programs by describing producer knowledge uptake and compliance with OFFS programs. Although Canada's OFFS programs were not designed specifically to control AMR, they do provide an on-farm link to inform producers about

risks from AMU. The existence and survival of these programs demonstrates an industry-wide willingness to respond to regulatory and consumer concerns over food safety.

Prudent Antimicrobial Use Guidelines

Prudent use, judicious use, or antimicrobial stewardship involves reducing inappropriate AMU and selecting the optimal drug, dose, and duration when use is necessary to ultimately reduce the emergence of AMR (461). A necessary tool to ensure prudent use is education, thus the standardized AMR education for Canadian veterinary students that was jointly developed by the Canadian Veterinary Medical Association (CVMA) and the Canadian Committee on Antibiotic Resistance (CCAR) in 2007 should improve AMU by future veterinarians (462). In veterinary medicine, several organizations have designed prudent use guidelines which have been summarized by Weese (395). These consistently recommend avoiding unnecessary AMU. They also emphasize the importance of basing necessary use decisions on susceptibility testing, and they suggest that when an antimicrobial is required that the narrowest spectrum drug is administered for the shortest time possible. These guidelines all support the notion that AMU should involve veterinary advice (268). For example, the CVMA has stated that all antimicrobials, including non-prescription antimicrobials, should be used within the confines of a veterinarian-client-patient relationship (VCPR) (390).

Published AMU recommendations target veterinarians because of the premise that AMU should occur within a VCPR. Again, using the CVMA as an example, the recently released Prudent Use Guidelines 2008 for beef cattle, dairy cattle, poultry, and swine recommend that veterinarians: i) take efforts to design a health management program to prevent and reduce disease; ii) look for antimicrobial alternatives; iii) prescribe all antimicrobials within a valid VCPR; and iv) select and use antimicrobials appropriately (390). Detailed recommendations are accompanied by treatment protocols. These guidelines should be a valuable resource for veterinarians, but their influence is unknown because their uptake and use is not tracked. More specifically, the uptake of treatment protocols is unmeasured and may change over time if these guidelines are not flexible and kept current. Critics have raised concerns that, although these protocols are written for veterinarians, they may serve as a 'therapeutic recipe book' for producers, thereby undermining efforts to bolster VCPR. This, too, would be interesting to monitor.

Focusing guidelines on veterinarians may be valuable for initiating discussions about prudent AMU amongst veterinarians and between veterinarians and clients, but does not address the fact that most antimicrobials in Canada are available without a veterinary prescription. Administering antimicrobials within a VCPR is ideal because the veterinarian understands animal health as well as the herd's disease status and so can design treatment regimens to maximize efficacy while minimizing bacterial resistance. Veterinarians are trained to account for complex interactions between microbe pharmacodynamics and antimicrobial pharmacokinetics through their knowledge of physiology, anatomy, and disease pathology (463,464). However, non-prescription antimicrobials are available in Canada and the challenges involved in limiting antimicrobials to prescription only were discussed in Chapter 3. So, failing to target non-veterinarians in prudent AMU guidelines is an important oversight in Canada.

Only one education program for producers pertaining to appropriate antimicrobial drug use was identified. Ten years ago, the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) developed a swine medications course as a proactive response to address the possibility that certification would be required to purchase non-prescription antimicrobials. Veterinarians delivered this program to producers. Such certification was never required and the program was discontinued after Ontario Pork took over its operation. In the initial five years, producer uptake of the program was good, but it is now available only on an ad hoc basis through OMAFRA swine

experts (personal communication: Dr. Janet Alsop, OMAFRA). No other programs for producers, and no programs for nutritionists, were identified in Canada. Considering that no feed-grade antimicrobials, all but one water antimicrobial, and over one-third of the injectable antimicrobials licensed for use in pigs and chickens in Canada are non-prescription drugs (263), the number of prudent use guidelines targeted at veterinarians versus producers seems unbalanced. Producers and animal nutritionists should have access to training on which practices elevate the risk of antimicrobial residues at slaughter, antimicrobial resistance in bacteria carried by food animals, and approaches to prudent antimicrobial use. Such training should emphasize the value of seeking veterinary advice for antimicrobial use decisions rather than attempt to train the producer or nutritionist in the optimal antimicrobial decision.

Therapeutic recommendations within AMU guidelines often overshadow the crucial point that prudent AMU demands that animal health be optimized through husbandry (much like the reduce component of reduce-reuse-recycle is often overlooked). Veterinarians can play an important role in designing health programs. In order to do this effectively, veterinarians must continually update their knowledge of disease prevention, therapeutics, and AMR trends to ensure the most appropriate use of antimicrobials (390). Effective health programs that include vaccination, biosecurity, good hygiene, and improved management should prevent disease and thereby reduce the need for antimicrobials. Veterinarians must also seek current continuing education to ensure their ability to design and implement evidence-based health programs (390). Unless Canada licenses all antimicrobials as prescription drugs, building and establishing trust in VCPR is the only insurance that producers will seek veterinary advice for all AMU, regardless of availability. Continuing to build such relationships may ultimately foster efforts to capture people's imagination that through AMU stewardship they can refuse (do not use), reduce, and potentially affect AMR development and dissemination (268). The following sections describe examples of interventions that improve health and resultantly minimize AMU.

Alternatives to Antimicrobials

Vaccines

Antimicrobial resistance is most likely to emerge and persist when AMU involves low doses, mass medication, and long exposure durations (268–271). Some antimicrobial classes that are critically important in human therapy are also used as feed additives to control disease in pigs and chickens. The following examples relate to macrolide use to control ileitis (causative agent, *Lawsonia intracellularis*) in pigs and virginiamycin use to control bacterial enteritis (causative agent, *Clostridium perfringens*) in chickens (86,100,465,466). If expert opinion is correct and AMR can in part be mitigated by addressing long-term, low-dose antimicrobial exposures, reducing the use of these drugs should alleviate selective pressures for AMR in bacteria carried by pigs and chickens. Three recently developed vaccines allow non-antimicrobial control of these endemic conditions. These vaccines were selected as examples of advancements in animal health that positively affect public health.

Ileitis causes chronic diarrhea in pigs between 6 and 20 weeks of age. It is endemic in pigs worldwide, cannot be eradicated from herds, and until recently was extremely difficult to diagnosis. The combination of diagnostic limitations and financial losses has led to widespread AMU in feed for disease control. The most common prophylactic regimens include tiamulin, tylosin, chlortetracycline, or lincomycin fed continuously (100,465). In 2002, an avirulent live vaccine (Enterosol®, Boehringer Ingelheim Vetmedica, Inc.) was released on the Canadian market (467). This vaccine is administered through drinking water and significantly reduces disease prevalence and severity (465,468). For financial reasons, this product is used more extensively in grow-finish pigs in Europe and more commonly in breeding animals in Canada (468). The CIPARS sentinel herds in 2007 reported 76% of sow herds

supplying sentinel grow-finish sites were positive for ileitis and 26% vaccinated. In the grow-finish herds, 66% reported a positive status and 17% reported vaccinating (469).

In broilers, bacterial enteritis is among the most prevalent and financially costly diseases. An overgrowth of *C. perfringens* causes disease symptoms ranging from wet litter to death. In feed, bacitracin or virginiamycin, combined with a coccidiostat, is used to control this syndrome and these products account for much of the AMU in broilers (165). The coccidiostat is necessary because coccidia damage the gut and predispose birds to more severe disease (466,466,470). Both coccidia and *C. perfringens* are endemic in poultry. Two vaccines have been developed to control these pathogens.

Coccidiosis vaccines are licensed in Canada (263). These live oocyst vaccines are sprayed on chicks at the hatchery and stimulate a mucosal immune response (471). Vaccine efficacy depends on establishing high levels of vaccine-strain oocysts in the barn early in the birds' lives combined with good litter management. Coccidiosis vaccines alone are often insufficient to manage gut-health. Prophylactic in-feed antimicrobials cannot be used in conjunction with this live vaccine so, if vaccine failure occurs, the subsequent coccidiosis and bacterial enteritis outbreaks are severe. This can be resolved by concurrent use of a bacterial enteritis vaccine in the broiler breeders (466,472). A conditionally licensed vaccine (NetVax®, Intervet Schering-Plough Animal Health) is available in the United States and can be accessed in Canada via an emergency biological release permit (473). These products are important advancements because they prevent disease, the coccidiosis vaccine can diminish the virulence of field strains, and both are compatible with competitive exclusion strategies. These characteristics should speed industry adoption resulting in public health spin offs.

Canada has three bacterial and ten viral vaccines licensed for use in chickens and thirteen bacterial and five viral vaccines licensed for use in pigs (263). Describing these products would be redundant, but we want to emphasize that the selected examples are not the only vaccines with great effects on animal health. All vaccine use should decrease AMU, either by directly preventing bacterial disease or by preventing viral disease and secondary bacterial infections. The National Veterinary Institute in Denmark described the top 12 diseases diagnosed in pigs in 2007 (474). Canada has licensed vaccines to control all of these except swine dysentery (causative agents *Brachyspira hyodysenteriae* and *B. pilosicoli*) (263). Thus, advancements in vaccine technology have undoubtedly affected AMU, and therefore, AMR. Future advances will likely include new vaccines to control food safety hazards, such as the licensed *Salmonella* vaccines in chickens and pigs (263). Conversely, it would be amiss to leave the impression that all important disease can be controlled with currently available vaccines. Pathogens such as avian pathogenic *E. coli, Staphylococcus,* and *Pseudomonas* are important in broilers and have no effective vaccines. Other non-antimicrobial interventions are also not available. This demonstrates the industry's continued need for efficacious antimicrobials, and their continued reliance on antimicrobial availability.

Competitive Exclusion Strategies

Competitive exclusion strategies (CES) encompass a broad range of products that manipulate gut bacteria. As categories, these include competitive exclusion products, probiotics, and prebiotics. Most are oral products. Competitive exclusion (CE) and probiotics are live bacterial or yeast preparations that moderate the normal flora, while prebiotics are inert non-digestible additives that support beneficial bacteria. Competitive exclusion strategies are so named because CE or probiotics can be used synergistically with prebiotics (268,475,476). The objective of these products is to shift the gut ecology away from pathogens by displacing them with beneficial commensal bacteria (475).

Neonatal animals are born with an immature intestinal flora. Competitive exclusion products are

live obligate and facultative anaerobic bacteria, typically originating from the intestinal contents of healthy adults. They are administered in the first days of life. This expedites establishment of the climax population; a stable intestinal microflora that is more resistant to invasion from pathogens (335,475). Competitive exclusion technology has particular application in chickens. While pigs are exposed to maternal flora, chickens are hatched in clean environments and raised separate from breeder flocks. Establishing the climax population takes six weeks, which is the majority of a modern broiler's life, and is predominantly influenced by the environment (477,478).

Probiotics are live bacterial cultures that differ from CE products because they are fed continuously (478). They can be monostrain, multistrain (of one species), or multispecies combinations and are primarily composed of gram-positive bacteria (479). Unlike competitive exclusion products, probiotics are not always derived from the animal species they are administered to (475). The mechanisms of probiotics are not completely understood and likely vary with different organisms. Factors such as organic acid production, bacteriocin production, and stimulation of the immune system have been demonstrated, with an impact on colonization or infection by pathogenic bacteria or parasites (480).

Undifferentiated CE and probiotic products are derived from intestinal content. These are essentially crude preparations of bacteria from healthy animals. The bacterial content is not identified or quantified. Early studies suggested these may be more efficacious than defined cultures, but this may simply reflect the infancy of this field's ability to identify optimal mixtures of beneficial bacteria (481) Undifferentiated but quality-controlled and pathogen-free products are available in Europe but prohibited in Canada and the United States (364,479,482). In North America, cultures must be composed of identified bacteria demonstrated to be beneficial (478). This limits the range of products available in North America.

There are at least two reasons for the regulatory restrictions on undifferentiated CE and probiotic products. Health Canada's Veterinary Drugs Directorate (VDD) uses the same registration process for pharmaceuticals and natural health products. Although this is being reviewed, it is difficult to register natural health products because many requirements are difficult to achieve with biological processes (483). Delays in licensing veterinary pharmaceuticals are a serious concern for producers and the pharmaceutical industry, and natural health products are only one area requiring immediate attention to maintain the Canadian livestock industry's competitiveness internationally (484). Yet, amendments to VDD regulation may not result in market approval for undifferentiated bacterial products. Undifferentiated cultures may pose an AMR risk if they possess resistance elements. These could be rapidly disseminated from a single source to many commercial flocks/herds (482). An experimental study with a porcine-derived undifferentiated CE culture found higher levels of tetracycline and streptomycin resistance in *E. coli* from exposed pigs compared to controls (485). Undifferentiated CE products are derived from mature animals, which generally carry resistant bacteria, so these results were not unexpected. Ideally, future preparations will distinguish between bacteria that are non-therapeutic and commonly carry resistance elements, such as *E. coli,* and therapeutic bacteria that rarely carry resistance genes, such as lactic acid bacteria. If achieved with minimal impact on efficacy, the risk of AMR from these products could be diminished.

The European Union requires antimicrobial resistance susceptibility testing of undifferentiated products (479). This approach is not foolproof. Traditional culture-based techniques cannot demonstrate freedom of resistance because resistance genes can be silent (carried but not expressed) and culture-based techniques test a small, and potentially unrepresentative, sample of the bacteria in the product. Molecular testing for resistance genes addresses these problems but will not detect novel resistance mechanisms. But together phenotype and

genotype testing can provide some assurance that concerning resistance types are not being widely disseminated through undifferentiated cultures.

Studies of CE and probiotics products have had wide-ranging results, both beneficial and detrimental. Although this promising research field may some day help address the pharmaceutical void created by removing in-feed antimicrobials, advancements in basic knowledge along with improved study designs and more precise outcomes are needed before inconsistent findings are overcome. Serious concerns exist over the use of enterococci in CE products due to their propensity to carry resistance genes. As discussed above, other bacterial species may be more promising. A cautious regulatory approach should help to ensure that these products ameliorate rather than contribute to agri-food AMR.

Prebiotics are a dietary supplement that are available to microbes and provide limiting nutrients to the intestinal mucosa but provide no direct nutritional benefit to the animal host (335,475,486). Prebiotics are commonly used in people, while uptake in agriculture has been limited due to expense (475). Research from humans and animals suggest various prebiotics can modulate the immune system, neutralize pathogenic enterotoxins, and bind *E. coli* and *Salmonella* (471,475,487). Prebiotics are not restricted to antimicrobial-free production because the effects complement in-feed antimicrobials (488,489). Therefore, these can be considered an antimicrobial alternative or simply an advancement in human and animal intestinal management.

Studies of CES have shown variable results on animal productivity and health (489,490), which along with expense, has limited commercial uptake. Early studies sought to improve growth and did not evaluate the microbial ecosystem. But Europe's experience following in-feed antimicrobial bans made it clear that in-feed antimicrobials concurrently suppressed disease while improving productivity (491–494). Thus, recent efforts have focused on more specific objectives such as manipulating the microbial population to optimize niche dominance of beneficial bacteria and exclude pathogens. Neonatal animals with naïve flora are now commonly studied to minimize uncontrolled confounding and decrease variability. Beyond study design, disappointing results with live bacterial products have been attributed to challenges in maintaining an exogenous population of bacteria without continuous administration. This may be difficult to overcome as the balance of intestinal microflora appears to not only be species and gut location specific but to have important variability between individuals (454,475,478,481,495).

Food safety may be improved by CES if these prevent the colonization of animals with foodborne pathogens. A variety of studies in pigs and chickens have found these products can affect colonization or shedding of *Salmonella* and *Campylobacter,* but results are preliminary, variable, and are preparation and pathogen specific. (496,497). Commercial application will require continued research. However, this research field holds great potential because these products can concurrently address animal and human health challenges.

Bacteriophages

Another area that may have promise in the future is the use of bacteriophages, viruses that infect bacterial cells. There are two forms of bacteriophage, temperate and lytic. Temperate bacteriophages infect, but do not damage, bacterial cells and are a potential mechanism for transmission of virulence and antimicrobial resistance genes. Accordingly, they are not useful therapeutically. Lytic bacteriophages, in contrast, kill infected cells. Some bacteriophages have broad-spectrum activity while others only infect a narrow range of bacterial species, or even strains within a species. In addition to the potential for efficacy, lytic bacteriophages are appealing because of their safety. Bacteriophages are unable to infect mammalian cells and therefore are non-pathogenic to animals. This field is relatively young and ongoing safety evaluations are needed. However, temperate bacteriophages are classified as 'generally regarded as safe' (GRAS) by the FDA, are classified as Biosafety Level 1 microorganisms (as with *Lactobacillus* spp.)

and have fewer regulatory hurdles to clear, and have minimal to no evidence of ability to convert to temperate forms. The potential efficacy of lytic bacteriophages is of great interest and has been discussed for decades, however objective data has been lacking until recently.

Bacteriophages could be used for the elimination of bacteria in or on animals, or in the environment. Intensive research on bacteriophages in swine and poultry has been fairly recent, but preliminary data are promising. Bacteriophages with *in vitro* efficacy against swine pathogens such as ETEC, *Salmonella,* and *Streptococcus suis* have been identified (498–502). *In vivo* efficacy has been reported as well, with certain bacteriophages being effective at moderating the course of experimentally-induced ETEC (*E. coli* F4) diarrhea (503). Bacteriophages with *in vitro* efficacy against important pathogens in poultry such as Salmonella and *E. coli* have been identified (504,505). Bacteriophage therapy has been shown to reduce *Salmonella* and *Campylobacter* shedding in broiler chickens (506–509). Research is still needed on the rate and extent that bacteria will develop resistance to lytic phages.

While not acting directly against AMR, bacteriophages could have an indirect impact by reducing disease and concurrent need for antimicrobials. Further, bacteriophages could be used against multidrug-resistant pathogens such as MRSA. This is a field that is receiving increasing attention and might constitute a useful adjunctive therapeutic option in future years.

Best Management Practices

There is no 'magic bullet' that eliminates the need for AMU in food-animal production. European Union producers identified a return to 'good management' as the most effective intervention following antimicrobial growth promoter bans. Likewise, the North American experience in antimicrobial-free production emphasizes barn hygiene, stocking density, ventilation, and for chickens lighting, and litter management. Particular attention must be paid when animals are immunologically compromised. In broilers, this occurs during brooding, and in pigs, post-weaning (100,471,478,510).

Commercial operations almost exclusively raise a single commodity, of one genetic background, that are similar in age (note: single-site swine operations still have multiple ages in a barn) in a common airspace. This elevates the risk of infectious disease. Risk is determined by both the severity of the outcome and the probability of occurrence (374). Because of their size, both are magnified in intensive livestock operations. This risk is addressed by practices that control enzootic disease and block epizootic outbreaks. Good management practices to control endemic disease are relevant because they decrease the need for antimicrobials and may be extrapolated to controlling AMR within barns. Nutritional advancements are presented as an example. Disease exclusion strategies are relevant for preventing devastating disease and resultant therapeutic drug use. Biosecurity principles are presented as an example and may have application for precluding entrance of novel resistance determinants.

Nutrition and Feed

Animal nutrition is constantly advancing to optimize genetic potential and protect health. In both chickens and pigs, advancements in high quality starter diets have eased the transition to solid food. Anorexic animals can experience gastric stasis which places them at increased risk for pathogen colonization (100,101,476). Ensuring chicks and piglets get onto feed as soon as possible after brooding/weaning supports the gastrointestinal flora through this challenging period for the immune system.

In the late 1990s, the North American swine industry initiated a management system known as segregated early weaning. Pigs were weaned as young as fourteen days and moved to ultra-clean off-site nurseries. Separating piglets and sows was effective in containing many respiratory pathogens to the breeding herd, but it created nutritional challenges for feeding immature pigs. The transition from milk to

a plant-based diet in young animals is facilitated by high quality animal protein, which in North America is typically derived from milk or blood (spray-dried plasma) products. Using quality ingredients, palatable diet forms (i.e. crumble), managing feeders and waterers, and occasionally offering creep feed before weaning have all worked to get weaned pigs onto feed quickly. By eating and minimizing gastric stasis, a healthy gut flora is supported and enteric diseases are prevented. The standard industry weaning age has increased since the 1990s, but these advances continue to influence nutrition in the nursery (100). This is significant since nursery production uses more antimicrobials than the other production phases. (92,93,282,409).

Poultry nutritionists have modified diets to minimize the risk of bacterial enteritis (337,366,511). This was stimulated by European experience following the antimicrobial growth promoter bans when wheat-based diets fuelled necrotic enteritis outbreaks (493). Along with adjustments to the carbohydrate source, feed grind is now balanced for health (larger) and productivity (smaller), and whole grains are occasionally included to stimulate gizzard development. Other advancements have included increased use of synthetic amino acids, a focus on fat quality, and inclusion of enzymes to facilitate digestion of non-starch polysaccharides. Canadian nutritionists employ these techniques resulting in an industry with decreased risk of enteric disease.

Nutritional advances are relevant to AMR because they support health and decrease reliance on AMU. These advances also support the healthy intestinal flora, and like CES, help preclude colonization with enteric pathogens. Finally, this section has relevance for addressing endemic resistance problems by emphasizing the benefits that can arise when new technologies are applied to old husbandry questions.

Biosecurity

Biosecurity is the collective measures taken to ensure security from exposure to harmful biological agents. Thus biosecurity is both the plan and the actions to implement that plan. Biosecurity is a risk management tool, much like insurance. In the case of on-farm biosecurity, producers incur costs up-front to minimize future risks. Many biosecurity protocols also apply to bio-containment, which in this context is the control, eradication, or prevention, of new incurrence of infectious agents.

Biosecurity can be applied to farms, regions, or nations. Protocols are implemented to control pathogens with sufficient consequences or probability of incursion (i.e. risk) to justify the expense and effort. These protocols address animal, human, fomite, and vector borne risks. Sanitation is not truly biosecurity, but is often included because it is a barrier to disease between groups of animals in a flock/herd. In Canada, on-farm biosecurity protocols vary from non existent to extremely stringent (476,512-514).

Control of animal movement is essential for disease control. Many swine barns and all broiler operations are closed to live animals after they fill. Many swine and broiler operations prefer to receive animals from a single source. Within barns, an animal movement pattern known as 'all-in-all-out' is commonly used. This management approach batches animals by age, moves them as a group, and prevents contact between batches. When combined with sanitation, this helps to prevent aerosol and fecal-oral disease transmission between groups of animals (100). All-in-all-out is inherently more effective in poultry than pigs because hatcheries supply chicks that have never had physical contact with the breeder herd. Additionally, the entire poultry barn is emptied between groups, while swine barns often employ all-in-all-out by room within a barn. All-in-all-out is less effective without sanitation. Although it prevents the spread of disease between animals through direct contact, barns should be emptied of litter/manure, washed with detergent, disinfected, and allowed a dry down time between groups for optimal efficacy (515). Roughly three-quarters of American swine producers use all-in-all-out management and the uptake of this practice has increased from approximately half of producers in 2000 (409).

In theory, preventing bird/pig contact between groups could help to control AMR. A study investigating AMR in *Campylobacter* found pig herds with all-in-all-out management had lower odds of multiple drug resistance than herds with partial all-in-all-out or continuous flow management (516). Although western Canadian field studies examining *E. coli* and *Campylobacter* from grow-finish pigs failed to find a relationship between pig flow and AMR, this could be attributed to insufficient power. Conversely, a lack of relationship might exist in the grow-finish production phase because pigs enter the phase with high resistance levels (84,299).

In broilers, fluoroquinolone resistance in *E. coli* has been traced to vertical transmission from the breeder flock, most likely through hatchery contamination and re-infection (517). (Note: vertical disease transmission is the spread of disease from one generation to its progeny, typically from mother to offspring. It is used to differentiate from disease transmission within a group of contemporary animals which is termed horizontal transmission.) Hence, transmission dynamics are important to the question of AMR control through animal segregation. Demonstrating common transmission from breeder flocks to chicks (via the hatchery) or from sows to suckling pigs before weaning would mean that animal flow does not break resistance and an industry-wide control effort is needed. Alternatively, rare vertical transmission would provide an opportunity for individual operations to address resistance levels. These dynamics are likely to be bacterial species-specific and are likely to differ between pigs and chickens. In pigs, we would expect that industry-wide programs will be needed because piglets are exposed to the sow's feces.

Pigs and chickens can acquire disease from humans. To address this risk, visitors may be required to have no chicken/pig contact for a certain duration before entering the barn (i.e. swine barns often require 48–72 pig-free hours prior to entry). The entry requirements for people are more stringent in breeding flocks/herds than grow-out operations because of the continuous presence of valuable animals. Upon entry, visitors may be required to shower and/or change clothing. This minimizes the risk of people acting as a fomite. An experimental study demonstrated that enterotoxigenic *E. coli* can be transmitted between groups of pigs by handlers despite hand washing and outer-clothing change between groups. Transmission did not occur when handlers showered between groups (518). Downtime and showering between farms is important, as a British study found flocks using 'thinning' (split marketing heavy birds and leaving lighter birds to continue growing) are at increased risk of *Campylobacter* infection. The risk is attributed to the catchers who move from flock to flock (519).

People can act as a source of infectious disease to birds/pigs, as presumably occurred in the recent H1N1 (pandemic strain) infected swine farm in Alberta (520). No studies were identified that investigated the rate of transmission of resistant bacteria from healthy people to chickens/pigs under normal contact conditions. This is another area of transmission that needs study before AMR control programs can be applied on-farm. Drugs critically important for human medicine are used most commonly in people and thus novel resistance is most likely to emerge in people. If people subsequently transmit these resistance elements into otherwise isolated livestock or poultry operations, and amplification, re-assortment, and re-transmission to humans occurs, it exponentially increases the reservoir of resistance. This concern mirrors those over the role of pigs and poultry in influenza and has been demonstrated to occur with methicillin-resistant *Staphylococcus aureus* (MRSA) in horses and companion animals (184). 'Humanosis' control is an understudied area of AMR control. This area of study could be expanded to evaluate the risks of resistant bacteria derived from humans entering barns through alternative routes, such as water contaminated by urban effluent (161).

Biosecurity can be an effective tool to keep herds/flocks negative for diseases that are enzootic for the industry. A study of broiler farms found the odds of being *Campylobacter*-positive at slaughter

were nine times higher in control flocks than in flocks implementing a biosecurity program. Control flocks were also infected earlier than the 'biosecure' flocks that broke with disease (521). More research quantifying the effect of biosecurity practices or systems on animal and zoonotic pathogens is desperately needed. Such work could be expanded to evaluating associations between biosecurity practices and AMR, as has been done to a degree in studies that evaluated management practices as risk factors for AMR in bacteria from healthy pigs (299,516).

The 'art' of biosecurity has traditionally been empiric with minimal scientific rigor applied to recommendations (514). Many practices have not been proven to improve productivity or health, and despite increased uptake of biosecurity practices in the United States, pig health worsened from 2000 to 2006 (409). While this may reflect changes in endemic disease or management factors other than biosecurity, it reminds us that biosecurity and husbandry practices require rigorous scientific scrutiny to ensure they actually are an improvement.

Biosecurity decisions are made on an individual flock/herd basis, and producers implement systems based on the perceived costs and benefits (522,523). A variable system places the Canadian livestock industry at risk for foreign animal disease. A review of biosecurity practices in broilers was conducted after the avian influenza outbreaks in British Columbia in 2004. Lapses in biosecurity were deemed to contribute to disease spread between farms. Data do not exist to determine if the practices in British Columbia are representative of those across Canada (524). Addressing the reasons for breaches in biosecurity, along with the reasons for differences in biosecurity between flocks, could be relevant for understanding what changes are needed in biosecurity before programs could consider attempting to prevent novel resistance elements from becoming established in swine and poultry populations.

The CIPARS farm swine surveillance collects data describing biosecurity practices on sentinel farms (66). To date, these have not been published and are, to our knowledge, the only national data describing biosecurity practices in the Canadian swine industry. Biosecurity in American swine herds has been described but should not be extrapolated to Canada as half of the herds reported outdoor exposure of sows (409). Biosecurity requirements for indoor closed herds differ from free ranging pigs. In Canada, maintaining sows outdoors would be a relatively uncommon practice. Outdoor rearing of sows would impact sow exposures and disease, thus making any comparisons virtually impossible.

The swine and broiler industries are embarking on a new chapter of on-farm programs by developing on national biosecurity standards. These efforts are at the conceptual stage but will provide an outlet for scientific, Canadian-based research. This is a valuable opportunity to investigate potential controls for AMR dynamics and transmission. The resultant findings could be incorporated into these national standards. Without a concerted effort from researchers and policy makers, there is a risk that antimicrobial resistance issues being delegated to one of the many public-good issues will provide insufficient incentive for producers to act voluntarily. If this is the case, spin-off benefits will continue to be the main source of AMR control in Canadian livestock operations.

Slaughter and Processing

It is widely held that the best way to mitigate foodborne bacterial risk is to reduce the prevalence of pathogens in animals pre-harvest (7,455). Yet, post-harvest interventions have been responsible for most of the advancements in food-safety from bacterial disease (321). A growing burden could be placed on the slaughter and processing industries if producers change AMU practices. Decreased in-feed AMU could result in a subsequent decline in carcass uniformity, an increase in sub-clinical disease (and therefore more friable intestines), and an increase in adhesions from past clinical disease. An increase in these carcass and intestinal characteristics would lead to an increased risk of intestinal perforations and fecal contamination (325,326,525–527), and therefore, a reduction in food safety.

The meat industries have been proactive in identifying and implementing technologies to control foodborne bacteria on meat. Interventions have included: processes (i.e. separating infected and clean flocks/herds, application of HACCP systems), physical interventions (i.e. carcass decontamination with hot water sprays and dips, air chilling, plant design to control air flow, automated machine cleaning), and biological and chemical interventions (i.e. bacteriophages to control *E. coli* O157 on hides, microbial chemicals) (455,456,528,529). A summary of the advancements in poultry processing is provided by Bolder et al. (Table 5) (455). Since these interventions control the bacterial host contamination, they indirectly affect resistance elements on meat and control AMR dissemination. Further innovation to directly address the risk of contamination of carcasses or meat with AMR bacteria currently lacks obvious or simple controls. If the reduction of foodborne AMR bacteria becomes a goal, basic scientific research will be needed to address this challenge.

Table 5. Improvements in poultry processing plants over the past 15 years.

1.	Containers have replaced small plastic coops
2.	Gas stunning is becoming more popular, with less infection of the air sac, less physical damage to the birds and less defecation while being stunned
3.	Counter current and multi-stage scalding systems, where carcasses are washed during scalding
4.	Pluckers can be easily turned inside out, allowing cleaning during breaks and more efficient cleaning and disinfection after production, so more efficient hygiene is achievable
5.	Re-hanging is fully automated, so cross-contamination during the piling up of carcasses no longer occurs
6.	'Cleaning In Place' is installed on modern equipment
7.	Modern equipment can be easily adjusted, avoiding damage to intestines from opening machines and during evisceration
8.	During carcass opening, a vacuum system removes rectal contents and the cloaca is positioned at the back of the carcass, avoiding contamination of the carcass with intestinal contents
9.	At evisceration, intestines are physically separated from the carcasses for inspection
10.	Final washing and inspection of carcasses is fully automated and reliable, so no human checks are necessary
11.	Air chilling
12.	Introduction and application of HACCP
13.	Introduction of automatic portioning lines
14.	Vision systems introduced
15.	Electro-stimulation or maturation at different stages of the process allowing in-line processing including chilling, portioning, and deboning
16.	In-line processing with minimal contact between carcasses and with improved tracking and tracing

Reproduced with permission from World's Poultry Science Association (455).

Only three aspects of slaughter and meat processing were found that specifically pertained to food safety risks from AMR. Interestingly, all were concerns raised in Europe, and all evaluated the potential for introducing risk rather than risk mitigation.

Microbial control agents (also referred to as antimicrobial treatment substances in Europe) are licensed in Canada, the United States, and Australia. These include hydrogen peroxide, peroxyacetic acid (POAA), octanoic acid, peroxyoctanoic acid (POOA), and 1-hyroxyethylidene-1, 1-diphosphonic (HEDP) acid (528,530). In these countries, microbial control agents are registered for use on fruits, vegetables, meat, and poultry. Most microbial activity is attributed to POAA with reports of 2- to 9-fold reductions in total microbial loads and activity against *Listeria monocytogenes, E. coli* O157 and *Salmonella* (530). The safety of these compounds was reviewed by the FAO/WHO Expert Joint Committee of Food Additives, which ruled that the residual quantities of these acids on foods at the time of consumption posed no food safety risk (531). In a 2005 report, the European Food Safety Commission echoed this finding (532), but further assessed the issue in 2007, when potential effects on antimicrobial resistance were considered (533). Resistance was defined as both a decreased effectiveness for reducing microbial load and resistance to therapeutic antimicrobials. The panel concluded that there was no evidence to indicate that use will lead to either type of resistance but did recommend research on the likelihood of emergence of resistance (533).

The second and third concerns regarding processing interventions leading to AMR were summarized by the European Food Safety Authority (106). One concern was that laboratory studies showed that industrial non-thermal processing and preservation techniques can damage cell membranes, enzymes, and DNA. This could "promote the generation or transfer of resistance" (106). The other concern was that bacteria intentionally added to the food chain to assist in preservation, or fermentation could serve as a source of resistance elements. Much like the concern over commensal bacteria from animals, these bacteria could theoretically pass resistance elements on to commensal or pathogenic bacteria in humans. Although these concerns are theoretical, they deserve investigation to determine the likelihood and repercussions of such events.

Conclusion

This chapter summarized the industry-wide and producer-level activities that reduce the need for antimicrobials in chickens and pigs. From this summary, it is evident that the effect of individual interventions on AMU is unmeasured and a cohesive approach is lacking. The two notable gaps in activity were the lack of education programs on appropriate AMU for producers and animal nutritionists and a lack of publications related to AMR control at slaughter and processing. In contrast, the advancements in animal husbandry, management, and health that have occurred concurrently with the intensification of our livestock production have helped to mitigate the reliance on antimicrobials that is inherent with large confinement operations.

There are two impediments that we believe would speed industry-led action to control AMR if they were removed. The first is a lack of scientific evidence regarding the efficacy of interventions. Most veterinarians and producers recognize that decreasing AMU is the goal, but the best approach remains elusive because rigorously-tested, evidence-based research has largely not occurred. A cohesive industry-led approach requires a strategy based on science. Appropriately designed observational studies must be able to attribute AMR changes to an intervention, measure confounding effects, evaluate challenges and costs that may lead to non-compliance, and ensure that the AMU alternatives or therapeutic drug use shift in response to the intervention are beneficial. This is a massive undertaking especially since AMR ecology is so complex and to date not completely understood. However, it is absolutely necessary to begin to take these steps in order to scientifically address this problem while maintaining a viable agriculture industry.

Economics are the second impediment to industry-led AMR interventions. Producers are financially rewarded for healthy, uniform animals. Antimicrobials currently help to achieve that. Currently, beyond the niche market for antibiotic-free pork/chicken, there are no tangible incentives to offset production risk and losses from interventions to control AMR. At present there are also many industry challenges, such as market access, foreign animal diseases, and extensive financial losses, especially in the pork industry, that are directly jeopardizing producers' viability. It is very difficult to inspire producers to address AMR issues when their livelihood is threatened. Meat production is how these individuals make their living. Hence, they must see a demonstrable benefit from making changes and implementing new practices, especially to address an issue that is not negatively impacting their production. This would involve producer and industry education, but it would also need to include a component that would help the livestock industry to mitigate risks to animal health, welfare, product quality, and return on investment for making changes to address AMR. Such action is required as incentive to increase the priority of this issue. Widespread industry change will also need innovative ways to ensure cost-sharing between society and industry.

Chapter 5: Conclusion

AMR Bacteria in Agri-food

The emergence, dissemination, and maintenance of antimicrobial resistance (AMR) are complex processes that cannot be explained or addressed using simplistic methods. Factors such as co-selection of resistance genes, interactions between bacterial populations, and variable changes in bacterial fitness after resistance acquisition are but a few complex issues that require equally complex investigations and interventions. Research regarding AMR must be adequately broad to be able to identify true causal relationships. This knowledge can expedite implementing effective interventions and minimize trial and error in policy changes.

Innumerable connections link antimicrobial resistant bacteria in humans, food-producing animals, and the environment, and believing that we understand all of the possible links would be presumptuous. The transmission rates for these links are unknown, but food is a universal and direct connection between people and animals. It is irrefutable that people can acquire antimicrobial resistant bacteria from animals through food. What remains disputed is the frequency that pathogenic and commensal bacteria are transmitted to humans and either cause disease or transfer resistance elements to bacteria in people. A related knowledge gap is the relative amounts that AMU in animals and humans each contribute to AMR in humans. Answers to these questions would allow policy makers to evaluate the magnitude of risk imposed on the population by contaminated food and would allow appropriately aggressive intervention strategies.

Resistant pathogenic bacteria are the most obvious food safety concern. Within these bacteria, new threats are constantly emerging, such as the novel plasmid-mediated fluoroquinolone resistance genes. It is likely, and perhaps even inevitable, that fluoroquinolone resistance genes will become established in commensal and pathogenic bacteria in swine and poultry (74,107). Equally concerning is the potential for fluoroquinolone resistance to be encoded on mobile genetic elements together with other critically important resistance determinants such as extended-spectrum ß-lactamase genes (112,113). If fluoroquinolone resistance is linked to other resistance genes, then the use of any of the linked antimicrobials could increase fluoroquinolone resistance. This emerging issue emphasizes the need for research into transmission dynamics and possible interventions that could prevent human AMR problems from becoming established in food-animal populations.

While the potential role of the commensal gut microflora is frequently discussed, investigation of this diverse and poorly understood bacterial population is quite superficial. A large percentage, if not the majority of bacteria present in the intestinal tracts of livestock, have likely never been cultured and identified. So, current assessments of the commensal microflora are presumably being based on a small, and not necessarily representative, component of the bacterial population. Considering that this bacterial population may be of greater relevance to AMR than pathogenic bacteria, greater study, in quantity and depth, of the commensal microflora is required. This should include broader study of the true nature of the microflora, the effects of antimicrobial use (AMU) on population dynamics and on AMR, and the interaction between the commensal microflora and pathogens. Furthermore, more research is needed on environmental bacteria to investigate connections between AMR in soil bacteria and human commensal and pathogenic bacteria (534). Our limited understanding of commensal bacterial ecology is illustrated by the much studied relationship between *E. faecium* in livestock and people. This commensal bacterium is ubiquitous in humans, swine, and poultry. But determining the extent to which these bacterial populations are host adapted, interact, and share resistance elements has challenged the limits of science (156,159–161). Molecular epidemiology is constantly responding to this and similar challenges through new tools. Ultimately, we hope this will enable scientists to quantify these relationships, which

would allow interventions to focus on the greatest risks to human health.

The public health community has seen resistant nosocomial pathogens expand their niche into communities. Concurrently, health and veterinary authorities are acknowledging the porousity of the human/animal boundary to many bacteria. Controlling the 'infectious traffic' between nosocomial, community and animal bacterial populations will be an increasingly important task for AMR control. Future challenges are highly unpredictable, as already demonstrated by the potential for methicillin-resistant *S. aureus* (MRSA) and *C. difficile* to be zoonotic. The identification of MRSA in food animals and food has raised concerns about the risk of foodborne human MRSA infection (202–207). Basic food handling and cooking practices should greatly reduce the risk of food acting as a mode of transmission, but the risks cannot (and should not) be assumed to be negligible in the absence of evidence. More intensive research in this field is required, particularly characterization of strains found in pigs in Canada, mechanisms of foodborne contamination, and epidemiological investigation of food contact as a risk factor for community-associated MRSA infection in people. *Clostridium difficile* is also a high profile pathogen with recent concerns about foodborne transmission (225,241,242). The role of food in human *C. difficile* infection is unclear and is coupled with a rather superficial understanding of the role of *C. difficile* in community-onset diarrhea in humans. Study of the role of *C. difficile* in community-onset diarrhea and food as a risk factor for community-associated *C. difficile* infection is needed.

Despite the abundance of data regarding AMR and livestock, some significant deficiencies exist. While large volumes of data are present, there is tremendous repetition of the same methodologies in the same regions or between regions. This can provide information about inter or intra-region differences or changes, but it can also result in generation of data that contribute little to the overall understanding of AMR and AMU. While continuation of such studies is relatively easy, expansion of research to encompass a broader understanding of AMU and AMR is required. If this field is to truly develop, new methodologies and populations need to be used, and better collaboration between the fields of epidemiology, microbiology, molecular biology, ecology, food safety, and animal management are required. This type of integrated study can be technically challenging and expensive, but has great potential to generate true and accurate understanding of causal determinants of AMR. Funding agencies need to be open to supporting broader and novel methods as, in the long run, these may be a more efficient use of resources.

Active research is exponentially increasing the information available about AMR in food animals. Each study adds a piece to the puzzle. But the sheer volume of scientific publications is becoming unwieldy and is presenting challenges for concise and current knowledge translation to researchers and practitioners who need to apply these findings. We face the risk of data overload, where policy and practice is unable to assimilate new advancements and contextualize novel discoveries. The rapid expansion of information, often with conflicting results, means literature reviews quickly become obsolete and are prone to biased interpretations. In the health sciences, meta-analyses and systematic reviews are used to compile multiple research findings. These techniques can evaluate specific and extensively-studied questions, but are insufficient for understanding and communicating knowledge on many issues because the topic and concerns with AMR are broad, and understanding of diverse but inter-related aspects is necessary. Yet, we believe that analogous approaches must be developed through multidisciplinary teams involving information technology specialists, librarians, and scientists to assimilate the vast scope of AMR knowledge and reliably evaluate conflicting findings. Without such tools, information paralysis may undermine the value of current and future research. In a related vein, decision analyses have been used to model health decisions at individual and population levels. We suggest it would be useful to investigate whether similar techniques could assist in incorporating new information into intervention or policy decisions.

Antimicrobial use in Food Animals

Antimicrobials are used in food-animal production around the world. The food safety hazards arising from this vary based on the types and amounts of antimicrobials used. But despite the concerns about AMU on farms and the interventions that are recommended or implemented, our understanding of AMU practices remains limited. This is problematic because assumptions and weak data are being used to determine policy, strategy, and even legislation. Antimicrobial volumes collected through sales data may be reliable, but are of limited value by themselves for understanding AMR. Many products are licensed in numerous species for multiple indications and may also be used in an extra-label manner. Antimicrobial volumes cannot reflect selective pressure without accounting for the recipient population (535). In contrast, end-user data hold great potential for identifying risk factors for AMR but are more prone to bias and are more costly to collect. We have seen global progress in standardizing antimicrobial susceptibility testing and surveillance techniques (281,408). Ideally, advancements in AMU data collection will soon follow. This will allow for valid analysis of the selective pressures for resistance and will bolster the validity of ecological level studies.

In the meantime, it is widely stated that antimicrobials are necessary for animal health and welfare. We agree that AMU is vital for humane animal production but AMU can also compensate for sub-optimal management. The true role of antimicrobials in animal health and welfare needs to be objectively quantified. The AMU that is unnecessary or unreasonable must be eliminated while preserving use that is required to produce safe food, humanely rear livestock, and ensure economic viability of the industry. Research should broadly consider animal health, economics, and AMR. Answering these questions could be facilitated by evaluating differences between conventional, antibiotic-free, and organic production systems.

It was traditionally believed that ceasing AMU would inevitably and promptly lead to reductions in AMR. But data supporting an increase in AMR with increasing AMU are stronger and more consistent than studies showing a decrease in AMR following withdrawal of AMU. Declining resistance after drug cessation has been demonstrated for some pathogen/drug combinations, but it is certainly not universal and has often been less substantial and sustained than was expected. This is important because it suggests that established AMR is not consistently resolved by removing the inciting cause. Our ability to contain or decrease specific AMR trends may be limited. Many factors, including co-selection, bacterial fitness, and linkages with virulence and other important genes, means that a direct relationship between AMU and AMR is not always present. Therefore, efforts focused simply on one organism or one antimicrobial could be inadequate. Research demonstrating the effectiveness of a variety of interventions to mitigate existing AMR, only one of which should be drug withdrawal, will be necessary for future policy. This should include interventions through sanitation, animal movement, and husbandry.

The apparent relationship between in ovo ceftiofur use and resistance in human *Salmonella* isolates illustrates how agricultural AMU can affect humans. The correlation between decreasing AMR in *Salmonella* and *E. coli* from chicken following reports of voluntary cessation of the practice and return of resistance after use was reportedly reinstituted are one of the clearest demonstrations of the link between AMU and AMR (117). However, these surveillance findings need additional research to clarify the total effect of AMR/AMU interventions on human health. For example, research is needed on human health repercussions from the rise in ESBL resistant *E. coli* in chicken that paralleled the rise in *Salmonella* Heidelberg (Chapter 2; Figure 2). Food safety research is also needed, such as comparing pathogen loads on the carcasses derived from treated and untreated eggs, and comparing the health and subsequent AMU in chicks that did and did not receive ceftiofur in ovo. Surveillance should be used as a time sensitive tool to instigate research activities that expand beyond the farm-to-fork continuum to measure a wide range of effects on human health.

Quantitative risk assessments have examined the magnitude of risk imposed on people from foodborne resistant bacteria. Several American publications estimated substantially lower risks than were intuitive based on a qualitative evaluation of the literature (8,85,526). These models can provide a tool to investigate the relative importance of transmission pathways, but are limited in scope to known and studied connections. Therefore, our confidence in the input parameters determines their validity. To date, quantitative risk assessments have been confined to single pathogen/antimicrobial combinations. Perhaps in the future it will be possible to expand the scope of risk assessments to account for a wide variety of repercussions including environmental contamination from AMU, environmental pollution from lack of AMU, animal health and carcass characteristics, pathogen loads on meat, and nutritional repercussion from changing meat prices. This would be a colossal undertaking but would help ensure policy results in the greatest benefits for society.

Legislation, Regulation, and Policy to Address Antimicrobial Resistance in Agri-Food

The WHO, OIE, and FAO have led international co-operation to address foodborne resistant bacteria. These agencies have released prudent use guidelines and collaborated on risk assessment techniques. The effects of these actions on AMU and AMR are difficult to judge because countries that have implemented regulations and policies may have done so in the absence of these mandates, while others may be preparing to act but appear unresponsive. Some of the countries that have not developed formalized systems to address AMR have either piloted surveillance programs or conducted research into AMR in foodborne bacteria. This indicates that concern is widespread.

National differences in veterinary drug regulations and AMU/AMR monitoring raise concerns about the international movement of animals and food. Importing nations are concerned about the safety of imported products and exporting nations are concerned about open and transparent trade. International standards, such as the draft methods released by Codex to conduct risk assessment, can help to smooth trade but are not enforceable regulations (536–538). Thus, member countries can still set their own requirements. For example, risk prioritization relies on the ranking of antimicrobials for their importance to human medicine. But countries may rank antimicrobials using a list developed through international consensus or one reflecting the concerns of the member country (86,388). Trade barriers to control animal or foodborne resistant bacteria have not yet occurred but are a realistic concern given the increasing divergence in regulations and controls between countries. Until such a barrier is invoked, challenged, and ruled on by the World Trade Organization, the ability of countries to block importation of products based on antimicrobial use or resistance remains speculative.

Canada has played an active international role in AMR policy. We applaud the efforts to support evidence-based regulations. It is important that Canada continue to push other nations to address high-risk drug use practices because the problem of AMR is truly global. Movement of animals, people, and food means that Canadians are affected by AMR in distant countries. The Canadian government also has a responsibility to the agriculture sector to hold our competitors to similar standards as are imposed at home. Much remains to be achieved on this front as many competitors have permissive veterinary drug regulations and scant to non-existent reporting. The reverse of this statement is also true. Canada has more permissive veterinary drug regulations than Europe or the United States. If we expect to hold other countries accountable to our standards, we will be expected to respond to the concerns raised over our inability to ensure prudent antimicrobial use (248,385).

Canada is one of several countries that has established an agri-food AMR surveillance system. Amongst these, Canada stands out as one of the most comprehensive. Unlike many other programs, Canada monitors three steps of the production chain—the farm, abattoir, and retail—and annually collects samples for a range of bacteria in every

major food commodity (66). Advancements in collecting AMU data from pigs, with parallel initiatives in other commodities, have made this a robust and comprehensive program. From the swine component, positive news has emerged including the absence of fluoroquinolone use and close to 10% of sentinel farms voluntarily using no antimicrobials. Concerning practices have also been identified including ceftiofur use and long-term macrolide use. The swine and broiler industries have recognized the value of third-party reporting to demonstrate transparency. Ideally, this system should enhance the effectiveness of policy addressing AMU in Canada.

Collecting multi-source AMR data allow 'farm-to-fork' risks to be studied. We recommend continued strengthening of the CIPARS program design to draw causal links from AMU in animals to AMR in animals to AMR in humans. Surveillance programs are most effective when the data collected are consistent over time. Canada has developed a comprehensive program, but due to budgetary limitations, there are some concerns about long-term sustainability of the farm component in its current form. Continued research into alternative AMU data collection methods, such as surveys and data extrapolation, may identify valid and cost-effective alternatives. Preparing for the worst is an unfortunate reality of shifting budget priorities. However, we advocate continued support for Canada's CIPARS program and its expansion to other provinces and commodities.

The Canadian regulatory system is committed to evidence-based policy. This has led to risk assessments being applied to new drug applications and potentially to future post-approval evaluations. Canada has been criticized for its veterinary drug regulations by national and international experts. Clearly, the VDD acknowledges these criticisms and is striving to address them as shown by the extensive stakeholder consultations that have occurred since 2002 (285,364,391,394). From an outside perspective, it appears that stakeholder paralysis may be an issue. This will be challenging for the VDD to address, as science will not be able to provide definitive solutions to many of the current concerns before regulatory changes are needed. We recommend the VDD commit to evaluating policy for efficacy with established targets to ensure it is working. In the case of ineffectiveness or (even worse) harm, the VDD must be committed to revoking or changing such policy. We urge the VDD to work in conjunction with the appropriate provincial regulators to address the thoroughly investigated recommendations of the 2002 Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance in Human Health before they are obsolete (248).

Agri-food Industry Actions to Address AMR

The broiler, swine, veterinary, and meat industries continue to make advancements that improve animal health and decrease their reliance on AMU. Excluding the prudent AMU guidelines for veterinarians, these actions are not undertaken specifically to address AMU and so their effects on AMR are presumed but unconfirmed. Viable on-farm food safety (OFFS) programs in the chicken and pork sectors are consistently implemented across Canada (284,458). When sufficient knowledge pertaining to on-farm AMR is available, these could provide a foundation for creating and delivering an AMR control program. Beyond these, we have limited knowledge of how extensively the other activities are used across the industry. Prudent AMU guidelines were the only identified activity that specifically addresses AMR. These guidelines tend to be broad-based and provide little clear information about how to incorporate prudent use into current practices (395). Of the numerous guidelines that exist, we found none that were monitoring their influence on AMU. This information void is unfortunate because, without evidence of their effect, it is difficult to sustain the necessary funding to promote, update, and modify the standards in order to keep them viable. Also it is impossible to identify the ineffective areas for improvement. The Canadian Veterinary Medical Association (CVMA) generated the most recent guidelines for Canadian swine and poultry veterinarians (390). We urge the CVMA to survey its membership on AMU practices and attitudes immediately to provide a baseline for the recently

released guidelines, with follow-up surveys on the use of these guidelines. Compared to the efforts that have gone into the creation of this document, this seems like a small and invaluable undertaking. In conjunction, we support a collaborative relationship between the CVMA, the Chicken Farmers of Canada (CFC), the Canadian Pork Council (CPC), and the Animal Nutrition Association of Canada (ANAC) to develop and deliver prudent use education for producers and animal nutritionists. These groups are the greatest users of non-prescription antimicrobials, thus this may counterbalance Canada's controversial regulations. Data describing how and why producers make AMU decisions exist but have not been utilized. This could strengthen the creation of prudent use programs for producers and shape effective policy. Thus, our final recommendation to the CFC and CPC is to analyze and disseminate the valuable information they possess describing AMU attitudes, OFFS program sustainability, and the implementation of best management practices.

Vaccines, competitive exclusion strategies, nutritional advancements, and biosecurity were presented as examples of industry-led advancements to decrease reliance on antimicrobials. Research to develop new products and to identify new biosecurity techniques will continue to improve animal health. Indirect support for prudent AMU must not be undervalued. Funding agencies should continue to invest in health-building research. Data on the extent that these alternative management practices are implemented nationally, and their alleviation of AMU dependence, could be studied.

Empirically, it appears that the implementation of interventions by industry correlates with financial reward and/or risk mitigation. Perhaps Canada's public health and agriculture sectors can learn from two recent American experiences. A positive example was set when the United States' Waxman-Markey clean energy bill was strongly supported by corporate America because it fostered innovation in the corporate sector without stifling financial gain from innovation and early adoption (539). In contrast, a negative example has been set by the repeated introduction of bills that threaten to revoke antimicrobials, which has fostered distrust, fear, and non-cooperation between corporate agriculture and health activists. Canada should strive for an innovative and collaborative relationship between the medical, veterinary, and agriculture communities. If public health authorities explored the risks of inaction in conjunction with the agriculture industry, and funding agencies concurrently provided resources for applied innovations, while regulators minimized impediments to novel alternatives, we could collectively enable the agriculture industry to drive, rather than drag, solutions for AMU and AMR.

Main Recommendations and Knowledge Needs

- Initiate investigations into techniques for knowledge assimilation, evaluation, and utilization
- Seek and support research into the effectiveness of interventions, including but not limited to AMU withdrawal, to mitigate existing AMR and apply such findings to future policies and actions
- Seek and support innovative research that expands on the current 'farm-to-fork' approach to truly account for diverse human health outcomes
- Continue to advocate for fair, transparent, veterinary drug regulations, AMR and AMU monitoring around the world based on scientific evidence, risk assessment, and appropriate precaution to ensure free and open trade of safe meat products
- Change Canada's veterinary drug regulations to ensure prudent and safe antimicrobial use in animals and commit to transparent policy evaluation and action in the absence of efficacy or demonstrable harm
- Deliver antimicrobial use education to producers and nutritionists
- Foster an innovative and collaborative relationship between regulators, public health officials and the agriculture industry

References

1. Garrett L. Betrayal of trust: The collapse of global public health. New York: Hyperion; 2000.
2. Perez F, Hujer A, Hujer K, Decker B, Rather P, Bonomo R. Global challenge of multidrug-resistant *Acinetobacter baumannii.* Antimicrob Agents Chemother. 2007;51:3471-3484.
3. Newton G. Antibiotic resistance: An unwinnable war? [webpage on the Internet]. United Kingdom: Wellcome Trust; [updated July 1, 2005; cited July 31, 2009]. Available at: http://www.wellcome.ac.uk/stellent/groups/corporatesite/@msh_publishing_group/documents/web_document/wtx026231.pdf.
4. Giguère S, Prescott JF, Baggot JD, Walker RD, Dowling PM editors. Antimicrobial therapy. 4th ed. Ames, Iowa: Blackwell Publishing; 2006.
5. O'Brien T. Emergence, spread, and environmental effect of antimicrobial resistance: How use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clin Infect Dis. 2002;34 S3:S78-84.
6. Summers AO. Generally overlooked fundamentals of bacterial genetics and ecology. Clin Infect Dis. 2002;34 S3:S85-92.
7. Callaway TR, Anderson RC, Edrington TS, Bischoff KM, Genovese KJ, Poole TL, et al. Microbial ecological principles underlying preharvest intervention strategies. In: Beier RC, Pillai SD, Phillips TD, editors. Preharvest and postharvest food safety. Ames, Iowa: Blackwell Publishing; 2004. p. 129-40.
8. Cox LAJ, Popken DA. Quantifying potential human health impact of animal antibiotic use: Enrofloxacin and macrolides in chickens. Risk Anal. 2006;26(1):135-146.
9. Barza M. Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals. Clin Infect Dis. 2002;34 S3:S123-5.
10. Travers K, Barza M. Morbidity of infections caused by antimicrobial-resistant bacteria. Clin Infect Dis. 2002;34(S3):131-134.
11. van den Bogaard, A E, Stobberingh EE. Epidemiology of resistance to antibiotics: Links between animals and humans. Int J Antimicrob Agents. 2000;14:327-335.
12. Boerlin P, Reid-Smith RJ. Antimicrobial resistance: Its emergence and transmission. Anim Health Res Rev. 2008;9(S2):115.
13. Aarestrup FM, Wegener HC, Collignon P. Resistance in bacteria of the food chain: Epidemiology and control strategies. Expert Rev Anti Infect Ther. 2008;6(5):733-750.
14. Binh CTT, Heuer H, Kaupenjohann M, Smalla K. Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids. FEMS Microbiol Ecol. 2008;66(1):25-37.
15. Duriez P, Topp E. Temporal dynamics and impact of manure storage on antibiotic resistance patterns and population structure of *Escherichia coli* isolates from a commercial swine farm. Appl Environ Microbiol. 2007;73(17):5486-5493.
16. Yao M, Gao Y, Chai T, Cai Y,Duan H. Antibiotic resistance of airborne *Escherichia coli* from hen house and rabbitry and their spreading to surroundings. Proceedings of 13th International Congress in Animal Hygiene; 17-21 June, 2007: Tartu Estonia: Estonian University of Life Sciences; 2007. p. 578-83.
17. Government of Canada. 2006 Census of agriculture: Agriculture-population data tables. 95-633-X. Ottawa, Ontario: Statistics Canada; 2008. [cited July 23, 2009]. Available from: www.statcan.gc.ca.
18. Barza M, Travers K. Excess infections due to antimicrobial resistance: The "attributable fraction." Clin Infect Dis. 2002;34 S3:S126-130.
19. Zaidi MB, Leon V, Canche C, Perez C, Zhao S, Hubert SK, et al. Rapid and widespread dissemination of multidrug-resistant *blaCMY-2 Salmonella* Typhimurium in Mexico. J Antimicrob Chemother. 2007;60(2):398-401.
20. Martin LJ, Fyfe M, Doré K, Buxton JA, Pollari F, Henry B, et al. Increased burden of illness associated with antimicrobial-resistant *Salmonella enterica* serotype Typhimurium infections. J Infect Dis. 2004;189:377-384.
21. Varma JK, Mølbak K, Barrett TJ, Beebe JL, Jones TF, Rabatsky-Ehr T, et al. Antimicrobial-resistant nontyphoidal Salmonella is associated with excess bloodstream infections and hospitalizations. J Infect Dis. 2005;191(-):554-561.

22. Nelson JM, Smith KE, Vugia DJ, Rabatsky-Ehr T, Segler SD, Kassenborg HD, et al. Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. Clin Infect Dis. 2004;190:1150-1157.

23. Engberg J, Neimann J, Nielsen EM, Aarestrup FM, Fussing V. Quinolone-resistant *Campylobacter* infections in Denmark: Risk factors and clinical consequences. Emerg Infect Dis. 2004;10:1056-1063.

24. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: Mortality, length of hospital stay, and health care costs. Clin Infect Dis. 2006;42 Suppl 2:S82-9.

25. Boerlin P. Associations between virulence factors of shiga toxin-producing *Escherichia coli* and disease in humans. J Clin Microbiol. 1999;37(-):497-503.

26. Boerlin P, Travis R, Gyles CL, Reid-Smith R, Janecko N, Lim H, et al. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. Appl Environ Microbiol. 2005;71(11):6753-6761.

27. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob Agents Chemother. 2001;45(10):2716-2722.

28. Kruse H, Sorum H. Transfer of multiple drug resistance in animals. Clin Infect Dis. 1994;60:4015-4021.

29. Lester CH, Frimodt-Moller N, Sorensen TL, Monnet DL, Hammerum AM. In vivo transfer of the vanA resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. Faecium* isolate of human origin in the intestines of human volunteers. Antimicrob Agents Chemother. 2006;50(2):596-599.

30. Ajiboye R, Solberg O, Lee B, Raphael E, DebRoy C, Riley L. Global spread of mobile antimicrobial drug resistance determinants in human and animal *Escherichia coli* and *Salmonella* strains causing Community-Acquired infections. Clin Infect Dis. 2009;49(3):365-371.

31. Guardabassi L, Courvalin P. Modes of antimicrobial action and mechanisms of bacteria resistance. In: Aarestrup F, editor. Antimicrobial resistance in bacteria of animal origin Washington, DC: ASM Press; 2006. p. 1-18.

32. Schwarz S, Cloeckaert A, Roberts M. Mechanisms and spread of bacteria resistance to antimicrobial agents. In: Aarestrup FM, editor. Antimicrobial resistance in bacteria of animal origin. Washington, DC: ASM Press; 2006. p. 73-98.

33. Catry B, Laevens H, Devriese LA, Opsomer G, De Kruif A. Antimicrobial resistance in livestock. J Vet Pharmacol Ther. 2003;26:81-93.

34. Luo N, Pereira S, Sahin O, Lin J, Huang S, Michel L, et al. Enhanced in vivo fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. Proc Nat Acad Sci. 2005;102(3):541-546.

35. Schwarz S, Chaslus-Dancla E. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet Res. 2001;21:201-225.

36. Bennett PM. The spread of drug resistance. In: Baumberg S, Young JPW, Wellington EMH, Saunders JR, editors. Population genetics in bacteria. Cambridge, United Kingdom: Cambridge University Press; 1995. p. 317-44.

37. Hall RM, Collins CM. Mobile gene cassettes and integrons: Capture and spread of genes by site-specific recombination. Mol Microbiol. 1995;15:593-600.

38. Salyers AA, Amiable Cuevas CF. Why are antibiotic resistance genes so resistant to elimination? Antimicrob Chemother. 1997;41(2321):2325.

39. Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol Lett. 1998;160(1):37-41.

40. Salyers AA, Gupta A, Wang Y. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. 2004;12(-):412-416.

41. Recchia GD, Hall RM. Origins of mobile gene cassettes found in integrons Trends Microbiol. 1997;10:389-394.

42. Nandi S, Maurer JJ, Hofacre C, Summers AO. Gram-positive bacteria are a major reservoir of class 1 antibiotic resistance integrons in poultry litter. Proc Nat Acad Sci. 2004;101:7118-7122.

43. Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VSC, Verhoef J, Jones ME. Class 1 integrons in gram-negative isolates and association with decreased susceptibility to multiple antibiotic compounds J Antimicrob Chemother. 1998;42:689-696.

44. Martinez-Freijo P, Fluit AC, Schmitz FJ, Verhoef J, Jones ME. Many class 1 integrons comprise distinct stable structures occurring in different species of Enterobacteriaceae isolated from widespread geographic regions in Europe. Antimicrob Agents Chemother. 1999;43:686-689.

45. Rosser SJ, Young HK. Identification and characterization of class 1 integrons in bacteria from an aquatic environment. J Antimicrob Chemother. 1999;44:11-18.

46. White DG, McDermott PF. Emergence and transfer of antimicrobial resistance. J Dairy Sci. 2001;84:E151-E155.

47. Aarts HJM, Guerra B, Malorny B. Molecular methods for detection of antimicrobial resistance. In: Aaerstrup FM, editor. Antimicrobial resistance in bacteria of animal origin. Washington, DC: ASM Press; 2006. p. 37-48.

48. Rosengren LB, Waldner CL, Reid-Smith RJ. Associations between antimicrobial resistance phenotypes, antimicrobial resistance genes, and virulence genes of fecal *Escherichia coli* isolates from healthy grow-finish pigs. Appl Environ Microbiol. 2009;75(5):1373-1380.

49. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli:* Resistance mechanisms and trends in human isolates. Emerg Infect Dis. 2001;7(1):24-34.

50. Government of Canada. Canadian integrated surveillance report *Salmonella, Campylobacter,* pathogenic *E. coli* and *Shigella,* from 1996 to 1999. Canada Communicable Disease Report. Ottawa, Ontario: Division of Enteric, Foodborne and Waterborne Diseases, Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada, Health Canada.2003.

51. Thomas MK, Majowicz SE, Sockett PN, Fazil A, Pollari F, Doré K, et al. Estimated numbers of community cases of illness due to *Salmonella, Campylobacter* and verotoxigenic *Escherichia coli:* Pathogen-specific community rates. Can J Infect Dis Med Microbiol. 2006;17:227-234.

52. Wagenaar JA, Mevius DJ, Havelaar AH. *Campylobacter* in primary animal production and control strategies to reduce the burden of human *Campylobacteriosis.* Rev Sci Tech. 2006;25(2):581-594.

53. Nachamkin I, Blaser MJ editors. *Campylobacter.* Second ed. Washington, D.C.: ASM Press; 2000.

54. Tam CC, O'Brien SJ, Adak GK, Meakins SM, Frost JA. *Campylobacter coli* – an important foodborne pathogen. J Infect. 2003;47(1):28-32.

55. Siemer BL, Nielsen EM, On SLW. Identification and molecular epidemiology of *Campylobacter coli* isolates from human gastroenteritis, food, and animal sources by amplified fragment length polymorphism analysis and penner serotyping. Appl Environ Microbiol. 2005;71(-):1953-1958.

56. Nielsen EM. Most *Campylobacter* subtypes from sporadic infections can be found in retail poultry products and food animals. Epidemiol Infect. 2006;134(4):758-767.

57. Kapperud G, Espeland G, Wahl E, Walde A, Herikstad H, Gustavsen S, et al. Factors associated with increased and decreased risk of *Campylobacter* Infection: A prospective case-control study in Norway. Am J Epidemiol. 2003;158(-):234-242.

58. Neimann J, Engberg J, Mølbak K, Wegener HC. A case-control study of risk factors for sporadic *Campylobacter* infections in Denmark. Epidemiol Infect. 2003;130(-):353-366.

59. Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender JB, Shiferaw B, et al. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin Infect Dis. 2004;38(-):S285-96.

60. Zhang Q, Lin J, Pereira S. Fluoroquinolone-resistant *Campylobacter* in animal reservoirs: Dynamics of development, resistance mechanisms and ecological fitness. Anim Health Res Rev. 2003;4(2):63-71.

61. Aarestrup FM, Engberg J. Antimicrobial resistance of thermophilic *Campylobacter.* Vet Res. 2001;32(3-4):311-321.

62. Nielsen EM, Engberg J, Madsen M. Distribution serotypes of *Campylobacter jejuni* and *C. coli* from Danish patients, poultry, cattle and swine. FEMS Immun Med Microbiol. 1997;19(1):47-56.

63. Harvey RB, Young CR, Ziprin RL, Hume ME, Genovese KJ, Anderson RC, et al. Prevalence of *Campylobacter* spp. isolated from the intestinal tract of pigs raised in an integrated swine production system. J Am Vet Med Assoc. 1999;215:1601-1604.

64. Trends and sources: Report on zoonotic agents in Belgium in 2006: Working group on foodborne infections and intoxications, trends and sources. D/2008/10.413/1. Brussels, Belgium: FAVV-AFSCA; 2006. [cited August 4, 2009]. Available from: http://www.afsca.be/publicationsen/_documents/2006_Report-on-zoonotic-agents_en.pdf.

65. Hong J, Kim JM, Jung WK, Kim SH, Bae W, Koo HC, et al. Prevalence and antibiotic resistance of *Campylobacter* spp. isolated from chicken meat, pork, and beef in Korea, from 2001 to 2006. J Food Prot. 2007;70(4):860-866.

66. Government of Canada. Canadian integrated program for antimicrobial resistance surveillance (CIPARS) (a combined reference of 5 CIPARS reports, 2002-2006, and 1 preliminary report, 2007). Guelph, Ontario: Public Health Agency of Canada; 2009. [cited March 24, 2009]. Available from: http://www.phac-aspc.gc.ca/cipars-picra/pubs-eng.php.

67. Gillespie IA, O'Brien SJ, Frost JA, Adak GK, Horby P, Swan AV, et al. A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: A tool for generating hypotheses. Emerg Infect Dis. 2002;8(9):937-942.

68. Gupta A, Nelson JM, Barrett T, Tauxe R, Rossiter S, Friedman CR, et al. Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. Emerg Infect Dis. 2004;10:1102-1109.

69. Effler P, Ieong MC, Kimura A, Nakata M, Burr R, Cremer E, et al. Sporadic *Campylobacter jejuni* infections in Hawaii: Associations with prior antibiotic use and commercially prepared chicken. J Infect Dis. 2001;183(7):1152-1155.

70. Guévremont E, Higgins R, Quessy S. Characterization of *Campylobacter* isolates recovered from clinically healthy pigs and from sporadic cases of Campylobacteriosis in humans. J Food Prot. 2004;67(-):228-234.

71. Guévremont E, Nadeau É, Sirois M, Quessy S. Antimicrobial susceptibilities of thermophilic *Campylobacter* from humans, swine and chicken broilers. Can J Vet Res. 2006;70:81-86.

72. Levesque S, Frost E, Michaud S. Comparison of antimicrobial resistance of *Campylobacter jejuni* isolated from humans, chickens, raw milk, and environmental water in Québec. J Food Prot. 2007;70(3):729-735.

73. United States Department of Agriculture. Bacterial Epidemiology Antimicrobial Resistance Research Unit [webpage on the Internet]. [updated 04/20/2009; cited 07/21]. Available at: http://www.ars.usda.gov/Main/site_main.htm?modecode=66-12-05-08.

74. Committee for Medicinal Products for Veterinary Medicine (CVMP). Public statement on the use of fluoroquinolones in food-producing animals in the European Union: Development of resistance and impact on human and animal health. EMEA/CVMP/SAGAM/184651/2005. London, England: European Medicines Agency; 2007. p. 1-24.

75. Boonmar S, Morita Y, Fujita M, Sangsuk L, Suthivarakom K, Padungtod P, et al. Serotypes, antimicrobial susceptibility, and *gyrA* gene mutation of *Campylobacter jejuni* isolates from humans and chickens in Thailand. Microbiol Immunol. 2007;51(5):531-537.

76. Saenz Y, Zarazaga M, Brinas L, Lantero M, Ruiz-Larrea F, Torres C. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. Int J Antimicrob Agents. 2001;18(4):353-358.

77. Kang YS, Cho YS, Yoon SK, Yu MA, Kim CM, Lee JO, et al. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw chicken meat and human stools in Korea. J Food Prot. 2006;69(12):2915-2923.

78. Miflin JK, Templeton JM, Blackall PJ. Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in the south-east Queensland region. J Antimicrob Chemother. 2007;59(4):775-778.

79. NORM/NORM-VET 2006. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromso. Oslo: National Veterinary Institute; 2007. [cited May 6, 2009]. Available from: http://www.vetinst.no/eng/Research/Publications/Norm-Norm-Vet-Report.

80. SVARM. Swedish veterinary antimicrobial resistance monitoring. Uppsala, Sweden: The National Veterinary Institute (SVA); 2008. [cited August 1, 2009]. Available from: www.sve.se.

81. Norstrom M, Johnsen G, Hofshagen M, Tharaldsen H, Kruse H. Antimicrobial resistance in *Campylobacter jejuni* from broilers and broiler house environments in Norway. J Food Prot. 2007;70(3):736-738.

82. Gyles CL. Antimicrobial resistance in selected bacteria from poultry. Anim Health Res Rev. 2008;9(S2):149.

83. Varela NP, Friendship R, Dewey C. Prevalence of resistance to 11 antimicrobials among *Campylobacter coli* isolated from pigs on 80 grower-finisher farms in Ontario. Can J Vet Res. 2007;71(3):189-194.

84. Rosengren L, Waldner C, Reid-Smith R, Valdivieso-Garcia A. Associations between antimicrobial exposure and resistance in fecal *Campylobacter* spp. from grow-finish pigs on-farm in Alberta and Saskatchewan, Canada. J Food Protect. 2009;72(3):482-489.

85. Hurd HS, Malladi S. A stochastic assessment of the public health risks of the use of macrolide antibiotics in food animals. Risk Anal. 2008 Jun;28(3):695-710.

86. World Health Organization. Critically important antimicrobials for human medicine: Categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use, report of the second WHO expert meeting, Copenhagen, 29-31 May 2007. [cited July 7, 2009]. Available from: www.who.int/foodborne_disease/resistance/antimicrobials_human.pdf.

87. Centers for Disease Control and Prevention. National antimicrobial resistance monitoring system for enteric bacteria (NARMS): Human isolates final Reports 1997 to 2005. Atlanta, Georgia: U. S. Department of Health and Human Resources; 2008. [cited August 1, 2009]. Available from: http://www.cdc.gov/narms/reports.htm.

88. Gibreel A, Taylor DE. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli.* J Antimicrob Chemother. 2006;58(2):243-255.

89. DANMAP. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark, a combined reference for 11 annual reports (1996 to 2007). Denmark: Danish National Food Institute; Danish National Veterinary Institute; 2009. [cited August 1, 2009]. Available from: www.danmap.org.

90. Gebreyes WA, Thakur S, Morrow WE. *Campylobacter coli:* Prevalence and antimicrobial resistance in antimicrobial-free (ABF) swine production systems. J Antimicrob Chemother. 2005;56(4):765-768.

91. Thakur S, Gebreyes WA. Prevalence and antimicrobial resistance of *Campylobacter* in antimicrobial-free and conventional pig production systems. J Food Prot. 2005;68(11):2402-2410.

92. Rosengren LB, Waldner CL, Reid-Smith RJ, Harding JCS, Gow SP, Wilkins W. Antimicrobial use through feed, water and injection in 20 swine farms in Alberta and Saskatchewan. Can J Vet Res. 2008;72(2):143-150.

93. Rajic A, Reid-Smith R, Deckert AE, Dewey CE, McEwen SA. Reported antibiotic use in 90 swine farms in Alberta. Can Vet J. 2006;47(5):446-452.

94. Cailhol J, Lailler R, Bouvet P, La Vieille S, Gauchard F, Sanders P, et al. Trends in antimicrobial resistance phenotypes in non-typhoid *Salmonellae* from human and poultry origins in France. Epidemiol Infect. 2006;134(1):171-178.

95. Futagawa-Saito K, Hiratsuka S, Kamibeppu M, Hirosawa T, Oyabu K, Fukuyasu T. *Salmonella* in healthy pigs: Prevalence, serotype diversity and antimicrobial resistance observed during 1998-1999 and 2004-2005 in Japan. Epidemiol Infect. 2008;136(8):1118-1123.

96. Government of Canada. National integrated enteric pathogen surveillance program (CEnterNet) 2005-2006. Guelph, Ontario: Public Health Agency of Canada; 2006.

97. Community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. European Food Safety Authority; 2009. [cited May 15, 2009]. Available from: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902269834.htm.

98. Hald T, Lo Fo Wong DMA, Aarestrup FM. The attribution of human infections with antimicrobial resistant *Salmonella* bacteria in Denmark to sources of animal origin. Foodborne Pathog Dis. 2007;4(3):313-326.

99. Hald T, Vose D, Wegener HC, Koupeev T. A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. Risk Anal. 2004;24(1):255-269.

100. Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ editors. Diseases of swine. 9th ed. Ames, Iowa: Blackwell Publishing; 2006.

101. Saif YM, Barns HJ, Glisson JR, Fadly JR, McDougald LR, Swayne DE editors. Diseases of poultry. 11th ed. Ames, Iowa: Iowa State Press; 2003.

102. Galanis E, Lo Fo Wong, D. M. A., Patrick ME, Binsztein N, Cieslik A, Chalermchaikit T, et al. Web-based surveillance and global *Salmonella* Distribution, 2000-2002. Emerg Infect Dis. 2006;12(3):381-388.

103. Zhao S, White DG, Friedman SL, Glenn A, Blickenstaff K, Ayers SL, et al. Antimicrobial resistance in *Salmonella enterica* serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Appl Environ Microbiol. 2008 Nov;74(21):6656-6662.

104. Helms M, Vastrup P, Gerner-Smidt P, Molbak K. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. Emerg Infect Dis. 2002;8(5):490-495.

105. Lee LA, Puhr ND, Maloney EK, Bean NH, Tauxe RV. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. J Infect Dis. 1994;170:128-134.

106. Panel on Biological Hazards. Foodborne antimicrobial resistance as a biological hazard. EFSA J. 2008;765:1-87.

107. Garcia-Fernandez A, Fortini D, Veldman K, Mevius D, Carattoli A. Characterization of plasmids harbouring *qnrS1, qnrB2* and *qnrB19* genes in *Salmonella.* J Antimicrob Chemother. 2009 Feb;63(2):274-281.

108. Wu C-, Wang Y, Cao X-, Lin J-, Qin S-, Mi T-, et al. Emergence of plasmid-mediated quinolone resistance genes in Enterobacteriaceae isolated from chickens in China. J Antimicrob Chemother. 2009;63(2):408-411.

109. Cavaco LM, Hendriksen RS, Aarestrup FM. Plasmid-mediated quinolone resistance determinant *qnrS1* detected in *Salmonella enterica* serovar Corvallis strains isolated in Denmark and Thailand. J Antimicrob Chemother. 2007;60(3):704-706.

110. Cavaco LM, Korsgaard H, Sorensen G, Aarestrup FM. Plasmid-mediated quinolone resistance due to *qnrB5* and *qnrS1* genes in *Salmonella enterica* serovars Newport, Hadar and Saintpaul isolated from turkey meat in Denmark. J Antimicrob Chemother. 2008;62(3):632-634.

111. Committee for Medicinal Products for Veterinary Use (CVMP). Reflection paper on the use of 3rd and 4th generation cephalosporins in food-producing animals in the European Union: Development of resistance and impact on human and animal health. EMEA/CVMP/SAGAM/81730/2006-Consultation. London, England: European Medicines Agency; 2008. p. 1-37.

112. Avsaroglu MD, Helmuth R, Junker E, Hertwig S, Schroeter A, Akcelik M, et al. Plasmidmediated quinolone resistance conferred by *qnrS1* in *Salmonella enterica* serovar Virchow isolated from Turkish food of avian origin. J Antimicrob Chemother. 2007;60(5):1146-1150.

113. Hopkins KL, Day M, Threlfall EJ. Plasmid-mediated quinolone resistance in *Salmonella enterica,* United Kingdom. Emerg Infect Dis. 2008;14(2):340-342.

114. Arlet G, Barrett T'B, P., Cloeckaert A, Mulvey MR, White DG. *Salmonella* resistant to extended-spectrum cephalosporin: Prevalence and epidemiology. Microb Infect. 2006;8:1945-1954.

115. United States Department of Agriculture. National Antimicrobial Resistance Monitoring System (NARMS) – Enteric Bacteria Veterinary Isolates – Interactive Data Query Page [webpage on the Internet]. [updated 01/21/2009; cited 07/11/2009]. Available at: http://www.ars.usda.gov/Main/docs.htm?docid=6750&page=4.

116. Andrysiak AK, Olson AB, Tracz DM, Dore K, Irwin R, Ng LK, et al. Genetic characterization of clinical and agri-food isolates of multi drug resistant *Salmonella enterica* serovar Heidelberg from Canada. BMC Microbiol. 2008;8:89.

117. Government of Canada. *Salmonella* Heidelberg – ceftiofur-related resistance in human and retail chicken isolates. Guelph, Ontario: Public Health Agency of Canada; 2007. [cited June 19, 2009]. Available from: http://www.phac-aspc.gc.ca/cipars-picra/heidelberg/pdf/heidelberg_e.pdf.

118. Currie A, MacDougall L, Aramini J, Gaulin C, Ahmed R, Isaacs S. Frozen chicken nuggets and strips and eggs are leading risk factors for *Salmonella* Heidelberg infections in Canada. Epidemiol Infect. 2005;133:809-816.

119. Glynn MK, Dabney P, Bopp C, Angulo FJ,the NARMS Working Group. Emergence of multiresistant *Salmonella* serotype Typhimurium DT104 R-type ACSSuT in the United States. Proceedings of 46th Annual Epidemic Intelligence Service Conference; 18 Apr 1997: Atlanta, GA: Center for Disease Control and Prevention; 1997.

120. Cloeckaert A, Schwarz S. Molecular characterization, spread and evolution of multidrug resistance in *Salmonella enterica* Typhimurium DT104. Vet Res. 2001;32:301-310.

121. Emborg HD, Baggesen DL, Aarestrup FM. Ten years of antimicrobial susceptibility testing of *Salmonella* from Danish pig farms. J Antimicrob Chemother. 2008 Aug;62(2):360-363.

122. Silbergeld EK, Price L, Graham J. Antimicrobial resistance and human health. Industrial Farm Animal Production.: PEW Commission on Industrial Farm Animal Production; 2008. [cited July 5, 2009]. Available from: www.ncifap.org/reports/.

123. Beerens H. *Bifidobacteria* as indicators of faecal contamination in meat and meat products: Detection, determination of origin and comparison with *Escherichia coli.* Int J Food Microbiol. 1998;40(3):203-207.

124. Varga C, Rajic A, McFall ME, Reid-Smith RJ, Deckert AE, Pearl DL, et al. Comparison of antimicrobial resistance in generic *Escherichia coli* and *Salmonella* spp. cultured from identical fecal samples in finishing swine. Can J Vet Res. 2008;72(2).

125. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev Vet Med. 1998;34(-):283-305.

126. Abatih EN, Ersboll AK, Lo Fo Wong, D. M. A., Emborg HD. Space – time clustering of ampicillin resistant *Escherichia coli* isolated from Danish pigs at slaughter between 1997 and 2005. Prev Vet Med. 2009;89(1-2):90-101.

127. Cavaco LM, Frimodt-Moller N, Hasman H, Guardabassi L, Nielsen L, Aarestrup FM. Prevalence of quinolone resistance mechanisms and associations to minimum inhibitory concentrations in quinolone-resistant *Escherichia coli* isolated from humans and swine in Denmark. Microb Drug Resist. 2008;14(2):163-169.

128. Liu JH, Deng YT, Zeng ZL, Gao JH, Chen L, Arakawa Y, et al. Coprevalence of plasmid-mediated quinolone resistance determinants QepA, qnr, and AAC(6')-ib-cr among 16S rRNA methylase RmtB-producing *Escherichia coli* isolates from pigs. Antimicrob Agents Chemother. 2008 Aug;52(8):2992-2993.

129. Yue L, Jiang H, Liao X, Liu J, Li S, Chen X, et al. Prevalence of plasmid-mediated quinolone resistance *qnr* genes in poultry and swine clinical isolates of *Escherichia coli.* Vet Microbiol. 2008;132(3):414-420.

130. Hart WS, Heuzenroeder MW, Barton MD. A study of the transfer of tetracycline resistance genes between *Escherichia coli* in the intestinal tract of a mouse and a chicken model. J Vet Med B Infect Dis Vet Public Health. 2006;53(7):333-340.

131. Livermore DM. Minimising antibiotic resistance. Lancet Infect Dis. 2005;5:450-459.

132. Boerlin P. Evolution of virulence factors in shiga-toxin-producing *Escherichia coli.* Cell Mol Life Sci. 1999;56(9-10):735-741.

133. Ateba CN, Bezuidenhout CC. Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the north-west province, South Africa. Int J Food Microbiol. 2008;128(2):181-188.

134. Schroeder CM, Zhao C, DebRoy C, Torcolini J, Zhao S, White DG, et al. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. Appl Environ Microbiol. 2002;68(2):576-581.

135. Bettelheim KA, Hornitzky MA, Djordjevic SP, Kuzevski A. Antibiotic resistance among verocytotoxigenic *Escherichia coli* (VTEC) and non-VTEC isolated from domestic animals and humans. J Med Microbiol. 2003;52:155-162.

136. Travis RM, Gyles CL, Reid-Smith R, Poppe C, McEwen SA, Friendship R, et al. Chloramphenicol and kanamycin resistance among porcine *Escherichia coli* in Ontario. J Antimicrob Chemother. 2006;58(1):173-177.

137. Ramchandani M, Manges AR, DebRoy C, Smith SP, Johnson JR, Riley LW. Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli.* Clin Infect Dis. 2005;40(2):251-257.

138. Prats G, Navarro F, Mirelis B, Dalmau D, Margall N, Coll P, et al. *Escherichia coli* serotype O15:K52:H1 as a uropathogenic clone. J Clin Microbiol. 2000;38(1):201-209.

139. Manges AR, Smith SP, Lau BJ, Nuval CJ, Eisenberg JN, Dietrich PS, et al. Retail meat consumption and the acquisition of antimicrobial resistant *Escherichia coli* causing urinary tract infections: A case-control study. Foodborne Pathog Dis. 2007;4(4):419-431.

140. Johnson TJ, Kariyawasam S, Wannemuehler Y, Mangiamele P, Johnson SJ, Doetkott C, et al. The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. J Bacteriol. 2007;189(8):3228-3236.

141. Pantosti A, Del Grosso M, Tagliabue S, Macri A, Caprioli A. Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. Lancet. 1999;354(9180):741-742.

142. Bager F, Aarestrup FM, Madsen M, Wegener HC. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. Microb Drug Resist. 1999;5(1):53-56.

143. Manero A, Vilanova X, Cerda-Cuellar M, Blanch AR. Vancomycin- and erythromycin-resistant enterococci in a pig farm and its environment. Environ Microbiol. 2006;8(4):667-674.

144. De Leener E, Martel A, Decostere A, Haesebrouck F. Distribution of the *erm(B)* gene, tetracycline resistance genes, and Tn1545-like transposons in macrolide- and lincosamideresistant enterococci from pigs and humans. Microb Drug Resist. 2004;10(4):341-345.

145. Centers for Disease Control and Prevention (CDC). Public health dispatch: Vancomycin-resistant *Staphylococcus aureus* – Pennsylvania, 2002. MMWR. 2002;51(40):902.

146. Noble WC, Virani K, Cree RGA. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NT12201 to *Staphylococcus aureus.* FEMS Microbiol Lett. 1992;93:195-198.

147. Tenover FC, Weigel LM, Appelbaum PC, McDougal LK, Chaitram J, McAllister S, et al. Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. Antimicrob Agents Chemother. 2004;48(1):275-280.

148. Finks J, Wells E, Dyke TL, Husain N, Plizga L, Heddurshetti R, et al. Vancomycin-resistant *Staphylococcus aureus,* Michigan, USA, 2007. Emerg Infect Dis. 2009;15(6):943-945.

149. Mascini EM, Bonten MJ. Vancomycin-resistant enterococci: Consequences for therapy and infection control. Clin Microbiol Infect. 2005;11(S4):43-56.

150. Huycke MM, Sahm DF, Gilmore MS. Multiple-drug resistant enterococci: The nature of the problem and an agenda for the future. Emerg Infect Dis. 1998;4(2):239-249.

151. European antimicrobial resistance surveillance system (EARSS) annual report 2007. The Netherlands2009. [cited July 15, 2009]. Available from: http://www.rivm.nl/earss/.

152. Treitman AN, Yarnold PR, Warren J, Noskin GA. Emerging incidence of *Enterococcus faecium* among hospital isolates (1993 to 2002). J Clin Microbiol. 2005;43(1):462-463.

153. National nosocomial infections surveillance (NNIS) system report, data summary from January 1990 – May 1999. Am J Infect Control. 1999;27(6):520-532.

154. Biavasco F, Foglia G, Paoletti C, Zandri G, Magi G, Guaglianone E, et al. *VanA*-type enterococci from humans, animals, and food: Species distribution, population structure, Tn1546 typing and location, and virulence determinants. Appl Environ Microbiol. 2007;73(10):3307-3319.

155. Klare I, Badstubner D, Konstabel C. Decreased incidence of *VanA*-type vancomycin-resistant enterococci isolated from poultry meat and from faecal samples of humans in the community after discontinuation of avoparcin in animal husbandry. Microb Drug Resist. 1999;5:45-52.

156. Sorum M, Johnsen PJ, Aasnes B, Rosvoll T, Kruse H, Sundsfjord A, et al. Prevalence, persistence, and molecular characterization of glycopeptide-resistant enterococci in Norwegian poultry and poultry farmers 3 to 8 years after the ban on avoparcin. Appl Environ Microbiol. 2006;72(1):516-521.

157. Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother. 2001;45(7):2054-2059.

158. Sorensen TL, Blom M, Monnet DL, Frimodt-Moller N, Poulsen RL, Espersen F. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. N Engl J Med. 2001;345(16):1161-1166.

159. Kuhn I, Iversen A, Finn M, Greko C, Burman LG, Blanch AR, et al. Occurrence and relatedness of vancomycin-resistant enterococci in animals, humans, and the environment in different European regions. Appl Environ Microbiol. 2005;71(9):5383-5390.

160. Willems RJ, Top J, van Den Braak N, van Belkum A, Endtz H, Mevius D, et al. Host specificity of vancomycin-resistant *Enterococcus faecium.* J Infect Dis. 2000;182(3):816-823.

161. Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L. Dispersion of multidrug-resistant *Enterococcus faecium* isolates belonging to major clonal complexes in different Portuguese settings. Appl Environ Microbiol. 2009;75(14):4904-4908.

162. Australia Pesticides & Veterinary Medicines Authority. Findings of the reconsideration of the registration of products containing virginiamycin and their labels. Canberra, Australia: National Registration Authority for Agricultural and Veterinary Chemicals; 2004. [cited July 20, 2009]. Available from: www.apvma.gov.au.

163. Giguère S. Lincosamides, pleuromutilins, and streptogramins. In: Giguère S, Prescott JF, Baggot JD, Walker RD, Dowling PM, editors. Antimicrobial therapy in veterinary medicine. Fourth ed. Ames, Iowa: Blackwell Publishers; 2006. p. 179-90.

164. Center for Veterinary Medicine. Risk assessment of streptogramin resistance in *Enterococcus faecium* Attributable to the use of streptogramins in animals *"virginiamycin risk assessment."* Rockville MD: United States Food and Drug Administration; 2004. [cited May 15, 2009]. Available from: http://www.fda.gov/cvm/Documents/SREF_RA_FinalDraft.pdf.

165. Chapman HD, Johnson ZB. Use of antibiotics and roxarsone in broiler chickens in the USA: Analysis for the years 1995 to 2000. Poult Sci. 2002;81:356-364.

166. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 states, United States, 2005. MMWR. 2006;55(14):392-395.

167. Hariharan H, Giles JS, Heaney SB, Leclerc SM, Schurman RD. Isolation, serotypes, and virulence-associated properties of *Yersinia enterocolitica* from the tonsils of slaughter hogs. Can J Vet Res. 1995;59(3):161-166.

168. Bottone EJ. *Yersinia enterocolitica:* The charisma continues. Clin Microbiol Rev. 1997;10(2):257-276.

169. Fosse J, Seegers H, Magras C. Foodborne zoonoses due to meat: A quantitative approach for a comparative risk assessment applied to pig slaughtering in Europe. Vet Res. 2008;39(01).

170. Bucher M, Meyer C, Grotzbach B, Wacheck S, Stolle A, Fredriksson-Ahomaa M. Epidemiological data on pathogenic *Yersinia enterocolitica* in southern Germany during 2000-2006. Foodborne Pathog Dis. 2008 Jun;5(3):273-280.

171. Bhaduri S, Wesley IV, Bush EJ. Prevalence of pathogenic *Yersinia enterocolitica* strains in pigs in the United States. Appl Environ Microbiol. 2005;71(11):7117-7121.

172. Kechagia N, Nicolaou C, Ioannidou V, Kourti E, Ioannidis A, Legakis NJ, et al. Detection of chromosomal and plasmid — encoded virulence determinants in *Yersinia enterocolitica* and other *Yersinia* spp. isolated from food animals in Greece. Int J Food Microbiol. 2007;118(3):326-331.

173. White DG, Zhao S, Simjee S, Meng J, Walker RD, McDermott PF. Prevalence of antimicrobial-resistant bacteria in retail foods. In: Beier RC, Pillai SD, Phillips TD, editors. Preharvest and postharvest food safety. Ames, Iowa: Blackwell Publishing; 2004. p. 239-54.

174. Health Canada. Frequently Asked Technical Questions on "Policy on *Listeria monocytogenes* in Ready-To-Eat Foods" [webpage on the Internet]. Ottawa, Ontario: Government of Canada; [updated 2008-04-16; cited July 15, 2009]. Available at: http://www.hcsc.gc.ca/fn-an/legislation/pol/listeria_faq-eng.php.

175. Center for Food Safety and Applied Nutrition. Guidance for Industry Control of *Listeria monocytogenes* in Refrigerated or Frozen Ready-To-Eat Foods [webpage on the Internet]. United States Department of Health and Human Services, Food and Drug Administration; [updated 18 June 2009; cited July 7, 2009]. Available at: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodProcessingHACCP/ucm073110.htm.

176. Zhang Y, Yeh E, Hall G, Cripe J, Bhagwat AA, Meng J. Characterization of *Listeria monocytogenes* isolated from retail foods. Int J Food Microbiol. 2007;113(1):47-53.

177. Franco Abuin CM, Quinto Fernandez EJ, Fente Sampayo C, Rodriguez Otero JL, Dominguez Rodriguez L, Cepeda Saez A. Susceptibilities of *Listeria* species isolated from food to nine antimicrobial agents. Antimicrob Agents Chemother. 1994;38(7):1655-1657.

178. Abrahim A, Papa A, Soultos N, Ambrosiadis I, Antoniadis A. Antibiotic resistance of *Salmonella* spp. and *Listeria* spp. isolates from traditionally made fresh sausages in Greece. J Food Prot. 1998;61(10):1378-1380.

179. Jang SS, Choo E, Han K, Miyamoto T, Heu S, Ryu S. Antibiotic resistance and genetic diversity of *Listeria monocytogenes* isolated from chicken carcasses in Korea. J Microbiol Biotechnol. 2006;16(8):1276-1284.

180. Lyon SA, Berrang ME, Fedorka-Cray PJ, Fletcher DL, Meinersmann RJ. Antimicrobial resistance of *Listeria monocytogenes* isolated from a poultry further processing plant. Foodborne Pathog Dis. 2008;5(3):253-259.

181. Conter M, Paludi D, Zanardi E, Ghidini S, Vergara A, Ianieri A. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes.* Int J Food Microbiol. 2009;128(3):497-500.

182. Tsakris A, Papa A, Douboyas J, Antoniadis A. Neonatal meningitis due to multi-resistant *Listeria monocytogenes.* J Antimicrob Chemother. 1997;39:553-554.

183. Poyart-Salmeron C, Trieu-Cuot P, Carlier C, MacGowan A, McLauchlin J, Courvalin P. Genetic basis of tetracycline resistance in clinical isolates of *Listeria monocytogenes.* Antimicrob Agents Chemother. 1992;36(2):463-466.

184. The Panel on Biological Hazards. Assessment of the public health significance of meticillin resistant *Staphylococcus aureus* (MRSA) in animals and foods. 993. Parma, Italy: European Food Safety Authority (EFSA); 2009. [cited August 4, 2009]. Available from: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902408708.htm.

185. Committee for Medicinal Products for Veterinary Use (CMVP). Reflection paper on MRSA in food producing and companion animals in the European Union: Epidemiology and control options for human and animal health. EMEA/CVMP/SAGAM/68290/2009. London, England: European Medicines Agency; 2009. [cited August 4, 2009]. Available from: http://www.emea.europa.eu/pdfs/vet/sagam/6829009en.pdf.

186. Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister SK, Fosheim G, et al. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001-2002. J Infect Dis. 2006;193(2):169-171.

187. Hanselman B, Kruth S, Rousseau J, Weese JS. Methicillin-resistant Staphylococcus aureus colonization in school teachers in Ontario. Can J Med Microbiol Infect Dis. 2008;19(6):405-408.

188. Gorwitz RJ. Understanding the success of Methicillin-Resistant *Staphylococcus aureus* strains causing epidemic disease in the community. J Infect Dis. 2008;197(2):179-182.

189. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* Infections in the United States. JAMA. 2007;298(15):1763-1771.

190. Weese JS, Faires M, Rousseau J, Bersenas A, Mathews K. A cluster of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in a small animal intensive care unit. J Am Vet Med Assoc. 2007;231:1361-1364.

191. Weese JS. Antimicrobial resistance in companion animals. Anim Health Res Rev. 2008;9(S2):169.

192. Weese JS, Lefebvre S. Risk factors for methicillin-resistant *Staphylococcus aureus* colonization in horses admitted to a veterinary teaching hospital. Can Vet J. 2007;48:921-926.

193. Voss A, Loeffen F, Bakker J, Klassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005;11(12):1965-1966.

194. Meemken D, Cuny C, Witte W, Eichler U, Staudt R, Blaha T. Occurrence of MRSA in pigs and in humans involved in pig production--preliminary results of a study in the northwest of Germany. Dtsch Tierarztl Wochenschr. 2008;115(4):132-139.

195. Smith TC, Male MJ, Harper AL, Moritz-Koroloev E, Kroeger JS, Diekema DJ, et al. Isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from swine in the Midwestern United States. Proceedings of International Conference on Emerging Infections; 16-19 March 2008: Atlanta, Georgia: Center for Disease Control and Prevention; 2008.

196. Duijkeren Ev, Ikawaty R, Broekhuizen-Stins MJ, Jansen MD, Spalburg EC, de Neeling AJ, et al. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. Vet Microbiol. 2008;126(4):383-389.

197. de Neeling AJ, van den Broek MJ, Spalburg EC, van Santen-Verheuvel MG, Dam-Deisz WD, Boshuizen HC, et al. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol. 2007;122(3-4):366-372.

198. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol. 2008;128(3/4):298-303.

199. Guardabassi L, Stegger M, Skov R. Retrospective detection of methicillin resistant and susceptible *Staphylococcus aureus* ST398 in Danish slaughter pigs. Vet Microbiol. 2007;122(3-4):384-386.

200. Van den Broek IV, van Cleef BA, Haenen A, Broens EM, van der Wolf PJ, van den Broek MJ, et al. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. Epidemiol Infect. 2008;24:1-9.

201. Kluytmans J, van Leeuwen W, Goessens W, Hollis R, Messer S, Herwaldt L, et al. Food-initiated outbreak of methicillin-resistant *Staphylococcus aureus* analyzed by pheno- and genotyping. J Clin Microbiol. 1995;33(5):1121-1128.

202. Kitai S, Shimizu A, Kawano J, Sato E, Nakano C, Uji T, et al. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. J Vet Med Sci. 2005;67(1):107-110.

203. Kwon NH, Park KT, Jung WK, Youn HY, Lee Y, Kim SH, et al. Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet Microbiol. 2006;117(2-4):304-312.

204. de Boer E, Zwartkruis A, Wit B, Huijsdens X, de Neeling H,Heuvelink A. Prevalence of methicillin-resistant *Staphylococcus aureus* in raw meats. Proceedings of Food Micro 2008; 1-4 September 2008: Aberdeen, Scotland: Food Standards Agency; 2008. p. R2.

205. van Loo IHM, Diederen BMW, Savelkoul PHM, Woudenberg JHC, Roosendaal, R. van Belkum, A. Methicillin-resistant *Staphylococcus aureus* in meat products, the Netherlands. Emerg Infect Dis. 2007;13(11):1753-1755.

206. Lee JH. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol. 2003;69(11):6489-6494.

207. Normanno G, Corrente M, La Salandra G, Dambrosio A, Quaglia NC, Parisi A, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. Int J Food Microbiol. 2007;117(2):219-222.

208. Weese JS, Reid-Smith R, l Rousseau J,Avery B. Methicillin-resistant *Staphylococcus aureus* in retail pork. Proceedings of European Conference of Clinical Microbiology and Infectious Diseases; 16-19 May, 2009: Helsinki, Finland: European Society of Clinical Microbiology and Infectious Disease; 2009.

209. Denis O, Suetens C, Hallin M, Ramboer I, Catry B, Gordts B, et al. High prevalence of "livestock-associated" methicillin-resistant *Staphylococcus aureus* ST398 in swine and pig farmers in Belgium. Proceedings of 18th European Congress of Clinical Microbiology and Infectious Diseases; 19-22 April 2008: Barcelona, Spain: European Society of Clinical Microbiology and Infectious Diseases; 2008.

210. Kehrenberg C, Cuny C, Strommenger B, Schwarz S, Witte W. Methicillin-resistant and -susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene *cfr.* Antimicrob Agents Chemother. 2009;53(2):779-781.

211. Heimesaat MM, Granzow K, Leidinger H, Liesenfeld O. Prevalence of *Clostridium difficile* toxins A and B and *Clostridium perfringens* enterotoxin A in stool samples of patients with antibiotic-associated diarrhea. Infection. 2005;33:340-344.

212. Fekety R, Shah AB. Diagnosis and treatment of *Clostridium difficile* colitis. JAMA. 1993;269:71-75.

213. Bourgault AM, Yechouron A, Gaudreau C, Gilbert H, Lamothe F. Should all stool specimens be routinely tested for *Clostridium difficile?* Clin Microbiol Infect. 1999;5(219):223.

214. Dial S, Delaney JA, Barkun AN, Suissa S. Use of gastric acid-suppressive agents and the risk of community-acquired *Clostridium difficile*-associated disease. JAMA. 2005;294:2989-2995.

215. MacCannell D, Louie T, Gregson D. Molecular analysis of *Clostridium difficile* PCR ribotype 027 isolates from Eastern and Western Canada. J Clin Microbiol. (44):2147-2152.

216. Anonymous. Severe *Clostridium difficile*-associated disease in populations previously at low risk – four states, 2005. MMWR. 2005;54:1201-1205.

217. Kuijper EJ, van Dissel JT. Spectrum of *Clostridium difficile* infections outside health care facilities. Can Med Assoc J. 2008;179:747-748.

218. Rodriguez-Palacios A, Stämpfli H, Duffield T, et al. *Clostridium difficile* PCR ribotypes in calves, Canada. Emerg Infect Dis. 2006;12:1730-1736.

219. al Saif N, Brazier JS. The distribution of *Clostridium difficile* in the environment of South Wales. J Med Microbiol. 1996;45:133-137.

220. Arroyo LG, Kruth SA, Willey BM, Staempfli HR, Low DE, Weese JS. PCR ribotyping of *Clostridium difficile* isolates originating from human and animal sources. Journal of Medical Microbiology. 2005;54:163-166.

221. Lefebvre S, Arroyo L, Weese J. Epidemic *Clostridium difficile* strain in hospital visitation dog. Emerg Infect Dis. 2006;12:1036-1037.

222. Rodriguez-Palacios A, Staempfli HR, Duffield T, Weese JS. *Clostridium difficile* in retail ground meat, Canada. Emerg Infect Dis. 2007;13(3):185-187.

223. Weese J, Armstrong J. Outbreak of *Clostridium difficile*-associated disease in a small animal veterinary teaching hospital. J Vet Intern Med. 2003;17:813-816.

224. Songer J, Uzal F. Clostridial enteric infections in pigs. J Vet Diagn Invest. 2005;17:528-536.

225. Songer JG, Trinh HT, Thompson AD, Killgore G, McDonald LC,Limbago BM. Isolation of *Clostridium difficile* from retail meats. Proceedings of 2nd International *Clostridium difficile* Symposium; 6-9 June 2007: Maribor, Slovenia: Marie Curie Actions; 2007.

226. Weese JS, Staempfli HR, Prescott JF, Kruth SA, Greenwood SJ, Weese HE. The roles of *Clostridium difficile* and enterotoxigenic *Clostridium perfringens* in diarrhea in dogs. J Vet Intern Med. 2001;15:374-378.

227. Weese JS, Staempfli HR, Prescott JF. A prospective study of the roles of *Clostridium difficile* and enterotoxigenic *Clostridium perfringens* in equine diarrhoea. Equine Vet J. 2001;33(403):409.

228. Jhung MA, Thompson A,Killgore G. An emerging *Clostridium difficile* toxinotype in humans and food animals. Proceedings of 2nd International *Clostridium difficile* Symposium; 6-9 June 2007: Maribor, Slovenia: Marie Curie Actions; 2007.

229. Limbago B, Long CM,Thompson A. Isolation and characterization of *Clostridium difficile* responsible for community-associated disease. Proceedings of 2nd International *Clostridium difficile* Symposium; 6-9 June 2007: Maribor, Slovenia: Marie Curie Actions; 2007.

230. Warny M, Pepin J, Fang A. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet. 2005;366(1079):1084.

231. McDonald LC, Killgore GE, Thompson A. An epidemic, toxin gene-variant strain of *Clostridium difficile.* N Engl J Med. 2005;353(2433):2441.

232. Martin H, Willey B, Low DE, Staempfli HR, McGeer A, Boerlin P, et al. Characterization of *Clostridium difficile* strains isolated from patients in Ontario, Canada, from 2004 to 2006. J Clin Microbiol. 2008;46(9):2999-3004.

233. Mulvey M, Miller M,Gravel D. Molecular epidemiology of Clostridium difficile in Canadian hospitals, 2004-2005. Proceedings of 2nd International *Clostridium difficile* Symposium; 6-9 June 2007: Maribor, Slovenia: Marie Curie Actions; 2007.

234. Buttrini M, Spigaglia P,Somenzi P. Epidemiology of *Clostridium difficile* strains with binary toxin genes among clinical isolates in an Italian hospital. Proceedings of 2nd International *Clostridium difficile* Symposium; 6-9 June 2007: Maribor, Slovenia: Marie Curie Actions; 2007.

235. Zimmerman O, Zemljic M, Janezic S, Gross U,Rupnik M. Prevalence of variant strains in hospital-associated *C. difficile* from Germany. Proceedings of 2nd International *Clostridium difficile* Symposium; 6-9 June 2007: Maribor, Slovenia: Marie Curie Actions; 2007.

236. Jhung MA, Thompson AD, Killgore GE. Toxinotype V *Clostridium difficile* in humans and food animals. Emerg Infect Dis. 2008;14:1039-1045.

237. Keel MK, Brazier JS, Post KW, Weese JS, Songer JG. Prevalence of PCR ribotypes among *Clostridium difficile* isolates from pigs, calves and other species. J Clin Microbiol. 2007;45:1963-1964.

238. Debast SB, van Leengoed LA, Goorhuis A, Harmanus C, Kuijper EJ, Bergwerff AA. *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. Environ Microbiol. 2009;11:505-511.

239. Zidaric V, Zemlijic M, Janezic S, Kocuvan A, Rupnik M. High diversity of *Clostridium difficile* genotypes isolated from a single poultry farm producing replacement laying hens. Anaerobes. 2008;14:325-327.

240. Simango C, Mwakurudza S. *Clostridium difficile* in broiler chickens sold at market places in Zimbabwe and their antimicrobial susceptibility. Int J Food Microbiol. 2008;124(3):268-270.

241. Metcalf D, Reid-Smtih R, Avery B, Weese JS. Prevalence of *Clostridium difficile* in retail pork. Can Vet J. ;in press.

242. Weese JS, Avery BP, Rousseau J, Reid-Smith RJ. Detection and enumeration of *Clostridium difficile* spores in retail beef and pork. Appl Environ Microbiol. 2009;75:5009-5011.

243. Bakri MM, Brown DJ, Butcher JP, Sutherland AD. *Clostridium difficile* in ready-to-eat salads, Scotland. Emerg Infect Dis. 2009;15(5):817-818.

244. Weese JS, Finley R, Reid-Smith R, Janeko N,Rousseau J. *Clostridium difficile* in dogs and the home environment: Prevalence and risk factors. Proceedings of European Conference of Clinical Microbiology and Infectious Diseases; 16-19 May, 2009: Helskinki, Finland: European Society of Clinical Microbiology and Infectious Diseases; 2009.

245. Thompson S. Update on CVM activities in antimicrobial resistance FDA. Veterinarian. 2000;15:4-5.

246. Bailar JC, Travers K. Review of assessments of the human health risk associated with the use of antimicrobial agents in agriculture. Clin Infect Dis. 2002;34 S3:S135-143.

247. O'Connor AM, Poppe C, McEwen S. Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. Can J Vet Res. 2002;66(-):145-150.

248. Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health. Uses of antimicrobials in food animals in Canada: Impact on resistance and human health. Ottawa, Ontario: Health Canada; 2002. Available from: http://www.hcsc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html.

249. McDermott PF, Zhao S, Wagner DD, Simjee S, Walker RD, White DG. The food safety perspective of antibiotic resistance. Anim Biotechnol. 2002;13:71-84.

250. Linton AH. Antibiotic resistance: The present situation reviewed. Vet Rec. 1977;100(17):354-360.

251. Blake DP, Humphry RW, Scott KP, Hillman K, Fenlon DR, Low JC. Influence of tetracycline exposure on tetracycline resistance and the carriage of tetracycline resistance genes within commensal *Escherichia coli* populations. J Appl Microbiol. 2003;94(6):1087-1097.

252. McGowan J,Jr, Gerding DN. Does antibiotic restriction prevent resistance? New Horiz. 1996;4(3):370-376.

253. Gaynes R, Monnet D. The contribution of antibiotic use on the frequency of antibiotic resistance in hospitals. Ciba Found Symp. 1997;207:47.

254. Aarestrup FM. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. Int J Antimicrob Agents. 1999;12(4):279-285.

255. Langlois BE, Cromwell GL, Stahly TS, Dawson KA, Hays VW. Antibiotic resistance of fecal coliforms after long-term withdrawal of therapeutic and subtherapeutic antibiotic use in a swine herd. Appl Environ Microbiol. 1983;46(6):1433-1434.

256. Smith HW. Antibiotic resistant bacteria ad associated problems in farm animals before and after the Swann report. In: Woodbine, editor. Antibiotic and antibiosis in agriculture. Woburn, MA: Buttersworths; 1977. p. 344-57.

257. Linton AH. Antibiotics, animal and man-an appraisal of a contentious subject. In: Woodbine, editor. Antibiotics and antibiosis in agriculture. Wodburn, MA: Buttersworths; 1977. p. 315-43.

258. Langlois B, Dawson K, Leak I, Aaron D. Antimicrobial resistance of fecal coliforms from pigs in a herd not exposed to antimicrobial agents for 126 months.* Vet Microbiol. 1988;18:147-153.

259. Castanon JIR. History of the use of antibiotic as growth promoters in European poultry feeds. Poult Sci. 2007;86:2466-2471.

260. Jones FT, Ricket SC. Observations on the history of the development of antimicrobials and their use in poultry feeds. Poult Sci. 2003;82(4):613-617.

261. Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds. The effects on human health of subtherapeutic use of antimicrobials in animal feeds. Washington, D.C.: National Academy of Sciences; 1980.

262. Tollefson L, Flynn W. Impact of antimicrobial resistance on regulatory policies in veterinary medicine: Status report. AAPS Pharm Sci. 2002;4(4):37.

263. Compendium of Veterinary Products [webpage on the Internet]. Hensall, Ontario, Canada: North American Compendiums; [updated July 31, 2009; cited August 4, 2009]. Available at: http://phac-aspc.naccvp.com/?u=country&p=msds.

264. Canadian Food Inspection Agency. Compendium of medicating ingredients brochure. Ottawa, Ontario: Government of Canada; 2009. [cited June 10, 2009]. Available from: http://www.inspection.gc.ca/english/anima/feebet/mib/mibtoce.shtml.

265. Jones RN, Ballow CH, Biedenbach DJ, Deinhart JA, Schentag JJ. Antimicrobial activity of quinupristin-dalfopristin (RP 59500, Synercid) tested against over 28,000 recent clinical isolates from 200 medical centers in the United States and Canada. Diagn Microbiol Infect Dis. 1998;31(3):437-451.

266. National Research Council (NRC), Institute of Medicine. The Use of Drugs in Food Animals: Benefits and Risks [webpage on the Internet]. Washington, D.C.: National Academy Press; cited July 15, 2009]. Available from: http://www.nap.edu/books/0309054346/html/18.html.

267. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. Clin Infect Dis. 2002;34(S3):S93-106.

268. Prescott JF. Antimicrobial use in food and companion animals. Anim Health Res Rev. 2008;9(S2):127.

269. Second joint FAO/ OIE/ WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: Management options. WHO/CDS/CPE/ZFK/2004.8. Geneva, Switzerland: World Health Organization; 2004. [cited July 27, 2009]. Available from: ftp://ftp.fao.org/es/esn/food/antimicro_2report.pdf.

270. World Health Organization. Global principles for the containment of antimicrobial resistance in animals intended for food. Geneva Switzerland: WHO; 2000. [cited August 4, 2009]. Available from: http://www.who.int/foodborne_disease/resistance/en/.

271. Helmuth R, Hensel A. Management recommendations of the federal institute for risk assessment (BfR) after the international BfR symposium "Towards a risk analysis of antibiotic resistance." Int J Med Microbiol. 2006;296S2:15-17.

272. United States Department of Health and Human Services. #152 guidance for industry, evaluating the safety of antimicrobial new animal drugs with regard to their microbiological effects on bacteria of human health concern. 2003. [cited July 2, 2009]. Available from: http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052519.pdf.

273. Health Canada. Guidance for industry preparation of veterinary new drug submissions. 2003. [cited August 1, 2009]. Available from: http://www.hc-sc.gc.ca/dhpmps/vet/legislation/guide-ld/vdd_nds_guide-eng.php#9224.

274. GovTrack.us. Track Congress: A civic project to track Congress [webpage on the Internet]. [updated July 21, 2009; cited July 30, 2009]. Available at: http://www.govtrack.us.

275. Aaerstrup FM, Jenser LB. Use of antimicrobials in food animal production. In: Simjee S, editor. Foodborne diseases. Totowa, New Jersey: Humana Press; 2007. p. 405-17.

276. Jensen VF, Jacobsen E, Bager F. Veterinary antimicrobial-usage statistics based on standardized measures of dosage. Prev Vet Med. 2004;64:201-215.

277. Dunlop RH, McEwen SA, Meek AH, Friendship RA, Clarke RC, Black WD. Antimicrobial drug use and related management practices among Ontario swine producers. Can Vet J. 1998;39(2):87-96.

278. Singer RS, Reid-Smith R, Sischo WM. Stakeholder position paper: Epidemiological perspectives on antibiotic use in animals. Prev Vet Med. 2006;73:153-161.

279. Institute of Medicine. Human health risks with the subtherapeutic use of penicillin or tetracyclines in animal feeds. Washington, DC; 1989.

280. Mellon M, Benbrook C, Benbrook KL. Hogging it! Estimates of antimicrobial abuse in livestock. Cambridge: UCS Publications; 2001.

281. Viola C, DeVincent SJ. Overview of issues pertaining to the manufacture, distribution, and use of antimicrobials in animals and other information relevant to animal antimicrobial use data collection in the United States. Prev Vet Med. 2006;73(2-3):111-131.

282. Dunlop RH, McEwen SA, Meek AH, Black WD, Clarke RC, Friendship RM. Individual and group antimicrobial usage rates on 34 farrow-to-finish swine farms in Ontario, Canada. Prev Vet Med. 1998;34:247-264.

283. Akwar HT, Poppe C, Wilson J, Reid-Smith RJ, Dyck M, Waddington J, et al. Associations of antimicrobial uses with antimicrobial resistance of fecal *Escherichia coli* from pigs on 47 farrowto-finish farms in Ontario and British Columbia. Can J Vet Res. 2008;72:202-210.

284. Canadian pork council: Canadian quality assurance producer materials. 2007. [cited March 25, 2009]. Available from: http://www.cqa-aqc.ca/home_e.cfm.

285. Health Canada. Policy on extra-label drug use (ELDU) in food producing animals. 2008. [cited March 25, 2009]. Available from: http://www.hc-sc.gc.ca/dhp-mps/vet/labeletiquet/pol_eldu-umdde-eng.php.

286. Grignon-Boutet R, Ireland M, Adewoye L, Mehrotra M, Russell S, Alexander I. Health Canada's policy on extra-label drug use in food-producing animals in Canada. Can Vet J. 2008;49(7):689-693.

287. Bush EJ, Biehl LG. Use of antibiotics and feed additives in weaned market pigs by U.S. pork producers. Proceedings of American Association of Swine Veterinarians Kansas City, MO; 2002. p. 329-31.

288. Dewey CE, Cox BD, Straw BE, Bush EJ, Hurd HS. Use of antimicrobials in swine feeds in the United States. Journal of Swine Health and Production. 1999;7(1):19-25.

289. Cromwell GL. Why and how antibiotics are used in swine production. Anim Biotechnol. 2002;13(1):7-27.

290. Ouckema R, Phillipe C. *Salmonella* isolations: Historical OHSFP trends. Proceedings of Salmonellosis, Antimicrobial Use and Antimicrobial Resistance Symposium; May 6: Guleph, Ontario; 2009.

291. McDonald's global policy on antibiotic use in food animals. : McDonald's Corporation; 2003. [cited July 28, 2009]. Available from: http://www.crmcdonalds.com/publish/etc/medialib/mcdonalds_media_library/report/downloads/antibiotics_policy.Par.0001.File.tmp/antibiotics_policy.pdf.

292. Singer RS, Hofacre CL. Potential impacts of antibiotic use in poultry production. Avian Dis. 2006;50(2):161-172.

293. Rothman KJ, Greenland S. Modern epidemiology. Second ed. Philadelphia, PA: Lippincott-Raven Publishers; 1998.

294. Akwar HT, Poppe C, Wilson J, Reid-Smith RJ, Dyck M, Waddington J, et al. Prevalence and patterns of antimicrobial resistance of fecal *Escherichia coli* among pigs on 47 farrow-to185 finish farms with different in-feed medication policies in Ontario and British Columbia. Can J Vet Res. 2008;72(2S):195-201.

295. Akwar TH, Poppe C, Wilson J, Reid-Smith RJ, Dyck M, Waddington J, et al. Risk factors for antimicrobial resistance among fecal *Escherichia coli* from residents on forty-three swine farms. Microb Drug Resist. 2007;13(1):69-76.

296. Boerlin P, Wissing A, Aarestrup FM, Frey J, Nicolet J. Antimicrobial growth promoter ban and resistance to macrolides and vancomycin in enterococci from pigs. J Clin Microbiol. 2001;39(11):4193-4195.

297. Cavaco LM, Abatih E, Aarestrup FM, Guardabassi L. Selection and persistence of CTX-Mproducing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrob Agents Chemother. 2008;52(10):3612-3616.

298. Harada K, Asai T, Ozawa M, Kojima A, Takahashi T. Farm-level impact of therapeutic antimicrobial use on antimicrobial-resistant populations of *Escherichia coli* isolates from pigs. Microb Drug Resist. 2008;14(3):239-244.

299. Rosengren L, Waldner C, Reid-Smith R, Dowling P, Harding J. Associations between feed & water antimicrobial use in farrow-to-finish swine herds and antimicrobial resistance of fecal *Escherichia coli* from grow-finish pigs. Microb Drug Resist. 2007;13(4):261-269.

300. Thakur S, Tadesse DA, Morrow M, Gebreyes WA. Occurrence of multidrug resistant *Salmonella* in antimicrobial-free (ABF) swine production systems. Vet Microbiol. 2007;125(3-4):362-367.

301. Langlois BE, Dawson KA. Antimicrobial resistance of gram-negative enteric bacteria from pigs in a nonantimicrobial-exposed herd before and after transportation. J Food Prot. 1999;62(-):797-799.

302. Miranda JM, Vazquez BI, Fente CA, Barros-Velazquez J, Cepeda A, Franco CM. Evolution of resistance in poultry intestinal *Escherichia coli* during three commonly used antimicrobial therapeutic treatments in poultry. Poult Sci. 2008;87(8):1643-1648.

303. Gebreyes WA, Thakur S, Morrow WE. Comparison of prevalence, antimicrobial resistance, and occurrence of multidrug-resistant *Salmonella* in antimicrobial-free and conventional pig production. J Food Prot. 2006;69(4):743-748.

304. Emborg HD, Andersen JS, Seyfarth AM, Andersen SR, Boel J, Wegener HC. Relations between the occurrence of resistance to antimicrobial growth promoters among *Enterococcus faecium* isolated from broilers and broiler meat. Int J Food Microbiol. 2003;84(3):273-284.

305. da Costa PM, Bica A, Vaz-Pires P, Bernardo F. Effects of antimicrobial treatment on selection of resistant *Escherichia coli* in broiler fecal flora. Microb Drug Resist. 2008 Dec;14(4):299-306.

306. Butaye P, Devriese LA, Haesebrouck F. Effect of avilamycin fed to chickens on *E. faecium* counts and on the selection of avilamycin-resistant *E. faecium* populations. Microb Drug Resist. 2005;11(2):170-177.

307. Garcia-Migura L, Liebana E, Jensen LB, Barnes S, Pleydell E. A longitudinal study to assess the persistence of vancomycin-resistant *Enterococcus faecium* (VREF) on an intensive broiler farm in the United Kingdom. FEMS Microbiol Lett. 2007;275(2):319-325.

308. Borgen K, Sorum M, Wasteson Y, Kruse H. VanA-type vancomycin-resistant enterococci (VRE) remain prevalent in poultry carcasses 3 years after avoparcin was banned. Int J Food Microbiol. 2001;64(1-2):89-94.

309. Pleydell EJ, Brown PE, Woodward MJ, Davies RH, French NP. Sources of variation in the ampicillin-resistant *Escherichia coli* concentration in the feces of organic broiler chickens. Appl Environ Microbiol. 2007;73(1):203-210.

310. Price LB, Johnson E, Vailes R, Silbergeld E. Fluoroquinolone-resistant *Campylobacter* isolates from conventional and antibiotic-free chicken products. Environ Health Perspect. 2005;113(5):557-560.

311. Soonthornchaikul N, Garelick H, Jones H, Jacobs J, Ball D, Choudhury M. Resistance to three antimicrobial agents of *Campylobacter* isolated from organically- and intensively-reared chickens purchased from retail outlets. Int J Antimicrob Agents. 2006;27(2):125-130.

312. Berrang ME, Ladely SR, Meinersmann RJ, Fedorka-Cray PJ. Subtherapeutic tylosin phosphate in broiler feed affects *Campylobacter* on carcasses during processing. Poult Sci. 2007;86(6):1229-1233.

313. Griggs DJ, Johnson MM, Frost JA, Humphrey T, Jorgensen F, Piddock LJV. Incidence and mechanisms of ciprofloxacin resistance in *Campylobacter* spp. isolated from commercial poultry flocks in the United Kingdom before, during, and after fluoroquinolone treatment. Antimicrob Agents Chemother. 2005;49(2):699-707.

314. McDermott PF, Bodeis SM, English LL, White DG, Walker RD, Zhao S, et al. Ciprofloxacin resistance in *Campylobacter* jejuni evolves rapidly in chickens treated with fluoroquinolones. J Infect Dis. 2002;185:837-840.

315. Dutil L, CIPARS. CIPARS historical results and trends: Salmonellosis, antibiotic use and antimicrobial resistance. Proceedings of Salmonellosis, antimicrobial use ad antimicrobial resistance symposium; May 6, 2009: Guelph, Ontario: Public Health Agency of Canada; 2009.

316. Turnidge J. Antibiotic use in animals - prejudices, perceptions and realty. J Antimicrob Chemother. 2004;53:26-27.

317. Cox LA,Jr, Popken DA. Quantifying human health risks from virginiamycin used in chickens. Risk Anal. 2004;24(1):271-288.

318. Phillips I, Casewell M, Cox T, de Groot B, Friis C, Jones R, et al. Does the use of antibiotics in food animals pose a risk to human health? A reply to critics. J Antimicrob Chemother. 2004;54(1):276-278.

319. Kelly L, Smith DL, Snary EL, Johnson JA, Harris AD, Wooldridge M, et al. Animal growth promoters: To ban or not to ban? Int J Antimicrob Agents. 2004;24(3):205-212.

320. Hurd HS, Doores S, Hayes A, Maurer J, Silley P, Singer RS, et al. Public health consequences of macrolide use in food animals: A deterministic risk assessment. J Food Protect. 2004;67(5):980-992.

321. Singer RS, Cox LA, Dickson JS, Hurd HS, Phillips I, Miller GY. Modeling the relationship between food animal health and human foodborne illness. Prev Vet Med. 2007;79:186-203.

322. Hammerum AM, Heuer OE, Lester CH, Agerso Y, Seyfarth AM, Emborg H-, et al. Comment on: Withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. Int J Antimicrob Agents. 2007;30(5):466-468.

323. Cox J, L.a, Ricci PF. Causal regulations vs. political will: Why human zoonotic infections increase despite precautionary bans on animal antibiotics. Environ Int. 2008;34(4):459-475.

324. Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother. 2003;52(2):159-161.

325. Dawe J. The relationship between poultry health and food safety. Poult Inform Prof. 2004;77:1-6.

326. Russell SM. The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors, and populations of *Campylobacter* spp. and *Escherichia coli.* Poult Sci. 2003;82:1326-1331.

327. Phillips I. Withdrawl of growth-promoting antibiotics in Europe and its effects in relation to human health. Int J Antimicrob Agents. 2007;30(2):101-107.

328. Patrick ME, Christiansen LE, Wain<o> M, Ethelberg S, Madsen H, Wegener HC. Effects of climate on incidence of *Campylobacter* spp. in humans and prevalence in broiler flocks in Denmark. Appl Environ Microbiol. 2004;70(12):7474-7480.

329. Eurosurveillance Weekly. Campylobacteriosis in Norway 2001: incidence is still rising [webpage on the Internet]. Eurosurveillance Weekly 6 (24)2002; cited July 15, 2009]. Available at: http://www.eurosurveillance.org/Public/Articles/Archives.aspx.

330. European food safety authority reports on zoonotic diseases in the EU. Vet Rec. 2006;158(1):2.

331. Samuel M, Vugia D, Shallow S, Marcus R, Segler S, McGivern T, et al. Epidemiology of sporadic *Campylobacter* infection in the United States and declining trend in incidence, FoodNet 1996-1999. Clin Infect Dis. 2004;38:S165-S174.

332. Wingstrand A, Neimann J, Engberg J, Nielsen EM, Gerner-Smidt P, Wegener HC, et al. Fresh chicken as main risk factor for *Campylobacteriosis,* Denmark. Emerg Infect Dis. 2006;12(2):280-285.

333. Stern NJ, Robach MC. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. J Food Prot. 2003;66(9):1557-1563.

334. Veterinary Laboratories Agency. Surveillance report avian. Quarterly report [webpage on the Internet]. United Kingdom: DEFRA; [updated 2004; cited July 15, 2009]. Available at: http://www/defra.gov.uk.

335. Dahiya JP, Wilkie DC, van Kessel A, Drew MD. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim Feed Sci Technol. 2006;129(1):60-88.

336. Mywire. World-leading pork exporter Denmark sees sharp increase in pig mortality [webpage on the Internet]. Mywire; cited July, 15, 2009]. Available at: http://www.mywire.com/a/AFP/Worldleading-pork-exporter-Denmarksees/991278?&pbl=163&srchId=ws2.sj1.keepmedia.int3mtLfCIBAAA=&pos=1.

337. Ministry of Agriculture. Antimicrobial feed additives. SOU 1997:132. Stockholm, Sweden: Government Offices of Sweden; 1997. [cited August 4, 2009]. Available from: http://www.regeringen.se/sb/d/574/a/54899.

338. Veterinary Medicines Directorate. Sales of antimicrobial products authorized for use as veterinary medicines, antiprotozoals, antifungals, growth promoters and coccidiostats in the UK in 2006. Surey, United Kingdom 2007. [cited July 10, 2009]. Avialable from: http://www.vmd.gov.uk/publications/antibiotic/salesanti06.pdf.

339. Wegener HC. Antibiotics in animal feed and their role in resistance development. Curr Opin Microbiol. 2003;6(5):439-445.

340. Emborg HD, Ersboll AK, Wegener HC. The effect of discontinued use of antimicrobial growth promoters on the productivity in the Danish broiler production. Prev Vet Med. 2000;50:53-70.

341. Jacobsen L, Jensen HG, Lawson LG. Sector – and economy-wide effects of terminating the use of antimicrobial growth promoters in Denmark. Acta Agriculturae Scandinavia, Section C – Economy. 2006;3(1):1-11.

342. Callesen J. Working Papers for the WHO international review panel's evaluation: Effects of termination of AGP -use on pig welfare and productivity [webpage on the Internet]. Foulum, Denmark: World Health Organization (WH0); [updated 2002; cited August 8, 2009]. Available at: http://www.who.int/salmsurv/links/en/FoulumWorkingPapers.pdf.

343. Department of Health and Human Services. Final decision of the commissioner withdrawal of approval of the new animal drug application for enrofloxacin in poultry. 2000N-1571. United States Food and Drug Administration2005. [cited Jule 1, 209]. Available from: http://www.fda.gov/oc/antimicrobial/baytril.pdf.

344. Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM):. NOOH for poultry fluoroquinolones-background information [webpage on the Internet]. [updated December 7, 2000; cited July 15, 2009]. Available at: http://www/fda/gov/AnimalVeterinary/NewsEvents/CVMUpdates/default.htm.

345. Iversen A, Kuhn I, Rahman M, Franklin A, Burman LG, Olsson-Liljequist B, et al. Evidence for transmission between humans and the environment of a nosocomial strain of *Enterococcus faecium.* Environ Microbiol. 2004;6(1):55-59.

346. Pugh DM. The EU precautionary bans of animal feed additive antibiotics. Toxico Lett. 2002;128:35-44.

347. Aarestrup FM, Kruse H, Tast E, Hammerum AM, Jensen LB. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. Microb Drug Resist. 2000;6(1):63-70.

348. Grosso Md, Caprioli A, Chinzari P, Fontana MC, Pezzotti G, Manfrin A, et al. Detection and characterization of vancomycin-resistant enterococci in farm animals and raw meat products in Italy. Microb Drug Resist. 2000;6(4):313.

349. Robredo B, Singh KV, Baquero F, Murray BE, Torres C. Vancomycin-resistant enterococci isolated from animals and food. Int J Food Microbiol. 2000;54(3):197-204.

350. World Health Organisation. About WHO [webpage on the Internet]. [updated 2009; cited July/25]. Available at: http://www.who.int/asbout/en/.

351. FAO. About Food and Agriculture Organization of the United Nations [webpage on the Internet]. [updated 2009; cited July/25]. Available at: http://www.fao.org.cyber.usask.ca/about/about-fao/en/.

352. FAO/WHO. Understanding the Codex Alimentarius. ISBN 92-5-104248-9. Rome, Italy: FAO Corporate Document Repository; 1999. [cited August 4, 2009]. Available from: http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/w9114e/w9114e00.htm.

353. CODEX Alimentarius. Code of practice on good animal feeding. CAC/RCP 54-2004.: FAO / WHO Food Standards; 2004. [cited July 15, 2009]. Available from: http://www.Codexalimentarius.net/search/advancedsearch.do?=&cat=15&doctext=&key=&qlang=&titletext=&type=4#.

354. Office International des Episooties. About Us [webpage on the Internet]. [updated 2009; cited July/20]. Available at: http://www.oie.int/eng/OIE/en_about.htm?e1d1.

355. Office International des Episooties. Terrestrial Animal Health Code [webpage on the Internet]. [updated 2009; cited July/20]. Available at: http://www.oie.int/eng/normes/en_mcode.htm?e1d10.

356. World Trade Organisation. Sanitary and Phytosanitary Measures: Introduction; Understanding the WTO agreement on Sanitary and Phytosanitary Measures [webpage on the Internet]. [updated 1998 May; cited July/25]. Available at: http://www.wto.org/english/tratop_e/sps_e/spsund_e.htm.

357. World Trade Organization. What is the WTO? [webpage on the Internet]. Geneva, Switzerland: cited July 25, 2009]. Available at: http://www.wto.org/english/thewto_e/whatis_e/whatis_e.htm.

358. WHO. The medical impact of the use of antimicrobials in food animals. report of a WHO meeting. Berlin, Germany, 13-17 October. WHO/EMC/ZOO/97.4. Berlin, Germany: World Health Organization; 1997. [cited August 1, 2009]. Available from: http://www.who.int/emc.

359. FAO/OIE/WHO. Joint first FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: Scientific assessment. Geneva, Switzerland: World Health Organization; 2003. [cited July 27, 2009]. Available from: http://www.who.int/foodsafety/publications/micro/en/amr.pdf.

360. Franklin A, Acar J, Anthony F, Gupta R, Nicholls T, Tamura Y, et al. Antimicrobial resistance: Harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. Rev sci tech Off int Epiz; Rev Sci Tech. 2001;20(3):859-870.

361. White DG, Acar J, Anthony F, Franklin A, Gupta R, Nicholls T, et al. Antimicrobial resistance: Standardisation and harmonisation of laboratory methodologies for the detection and quantification of antimicrobial resistance. Rev – Off Int Epizoot. 2001;20(3):849-858.

362. FAO/OIE/WHO. Joint FAO/WHO/OIE expert meeting on critically important antimicrobials. Proceedings of Report of the FAO/WHO/OIE Expert Meeting; 2007 Nov 26-30: Rome, Italy; 2007.

363. Veterinary Drugs Directorate. Categorization of antimicrobial drugs based on importance in human medicine (table 23). Ottawa, Ontario: Health Canada; 2006. [cited August, 11, 2009]. Available from: http://www.hc-sc.gc.ca/dhp-mps/consultation/vet/consultations/amr_ram_hummed_e.html.

364. Health Canada, Veterinary Drugs Directorate. Guidance for industry preparation of veterinary new drug submissions. Version 1.1. Ottawa, Ontario2007. [cited August 1, 2009]. Available from: http://www.hc-sc.gc.ca/dhp-mps/vet/legislation/guide-ld/vdd_nds_guideeng.php#9224.

365. Swann MM. Report of the Joint Committee on the use of antibiotics in animal husbandry and veterinary medicine. London: Her Majesty's Stationary Office; 1969.

366. Wierup M. The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microb Drug Resist. 2001;7(2):183-190.

367. van den Bogaard, A. E., Bruinsam N, Stobberingh EE. The effect of banning avoparcin on VRE carriage in the Netherlands. J Antimicrob Chemother. 2000;46:145-153.

368. Kruse H, Johansen BK, Rorvik LM, Schaller G. The use of avoparcin as a growth promoter and the occurrence of vancomycin-resistant *Enterococcus* species in Norwegian poultry and swine production. Microb Drug Resist. 1999;5(2):135-139.

369. Bager F, Madsen M, Christensen J, Aarestrup FM. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. Prev Vet Med. 1997;31(1-2):95-112.

370. Dowling P. The Canadian gFARAD: Extralabel antimicrobial use in poultry. Proceedings of Salmonellosis, Antimicrobial Use and Antimicrobial Resistance Symposium; 6-May-2009: Guelph, Ontario: Public Health Agency of Canada; 2009.

371. Scholar EM, Pratt WB editors. The antimicrobial drugs. 2nd ed. New York: Oxford University Press; 2000.

372. Wright GD. A new target for antibiotic development. Science. 2007;315:1373-1374.

373. FDA proposes new industry draft guidance for evaluating the safety of antimicrobial new animal drugs. FDA Veterinarian Newsletter. 2002;XVI(V).

374. Vose D, Acar J, Anthony F, Gupta R, Nicholls T, Tamura Y, et al. Antimicrobial resistance: Risk analysis methodology for the potential impact on public health of antimicrobial resistant bacteria of animal origin. Rev – Off Int Epizoot. 2001;20(3):811-827.

375. Rubin PH. The FDA's antibiotic resistance. Regulation. 2004;27(4):34-37.

376. Nelson JM, Chiller TM, Powers JH, Angulo FJ. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones for use in poultry: A public health success story. Clin Infect Dis. 2007;44(7):977-980.

377. U.S. Food and Drug Administration. FDA Approved Animal Drug Products: Section 6.0: Voluntary Withdrawals [webpage on the Internet]. [updated 2008 Jan 15; cited July/25]. Available at: http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042864.pdf.

378. United States Department of Health and Human Services, Food and Drug Administration. 21 CFR part 530 [docket no. FDA-2008N-0326] new animal drugs; cephalosporin drugs; extralabel animal drug use; order of prohibition. Federal Register. 2008;73(129):38110-38113.

379. U.S. Food and Drug Administration. Green Book [webpage on the Internet]. [updated 2008 Jan 15; cited July/25]. Available at: http://www.accessdata.fda.gov/scripts/AnimalDrugsAtFDA/.

380. Webster P. The perils of poultry. Can Med Assoc J. 2009;181(12):21.

381. U.S. Food and Drug Administration. Animal and Veterinary: FDA Extends Cephalosporin Comment Period and Delays Final Rule [webpage on the Internet]. [updated 2008 Aug 15; cited July/24]. Available at: http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm047901.htm.

382. GovTrack.us. Track Congress: A civic project to track Congress [webpage on the Internet]. [updated July 21, 2009; cited July 30, 2009]. Available at: http://www.govtrack.us.

383. Health Products and Food Branch. Strategic Plan [webpage on the Internet]. Ottawa, Ontario: Health Canada; [updated 2006 Feb 6; cited July 25, 2009]. Available at: http://www.hcsc.gc.ca/ahc-asc/pubs/hpfb-dgpsa/vet/strategic_plan_apr2006_mar2009_final-eng.php#2c.

384. Health Canada and the Canadian Infectious Disease Society. Controlling antimicrobial resistance: An integrated action plan for Canadians. Can Commun Dis Rep. 1997;23(S7):1-32.

385. Health Canada. European Commission's Audit [webpage on the Internet]. [updated 2006-02-10; cited 07/09]. Available at: http://www.hc-sc.gc.ca/dhp-mps/vet/index-eng.php.

386. Health Canada. Advisory Committees [webpage on the Internet]. [updated 2008 Feb 19; cited July/20]. Available at: http://www.hc-sc.gc.ca/dhp-mps/consultation/vet/com/index-eng.php.

387. Canadian Committee on Antibiotic Resistance. Antimicrobial resistance: An update for the Canadian committee on antibiotic resistance. Can J Infect Dis Med Microbiol. 2005;16(5):309-311.

388. Health Canada. Ranking of the importance of antimicrobials against bacteria which affect human health through food commodities. 2006. [cited March 25, 2009]. Available from: http://www.fsc.go.jp/senmon/hisiryou/taiseikin_rank_english.pdf.

389. Health Canada. Proposed Response to the Final Report of the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health [webpage on the Internet]. [updated 2009 Jan 16; cited July/25]. Available at: http://www.hc-sc.gc.ca/dhpmps/pubs/vet/amr-ram_final_response-reponse_ac-cc_tc-tm-eng.php.

390. Canadian Veterinary Medical Association (CVMA). CVMA prudent use guidelines 2008 for beef cattle, dairy cattle, poultry and swine. Ottawa, ON: CVMA; 2009. [cited July 27, 2009]. Available from: http://Canadianveterinarians.net/Documents/Resources/Files/1211_11385_CVMA_pug_e_webFINALMay14'09.pdf.

391. Veterinary Drugs Directorate (VDD). Issue identification paper work in progress: Extra-label drug use in animals (ELDU) developing a common understanding. Ottawa, Ontario: Government of Canada; Health Products and Food Branch Health Canada; 2004. [cited June 1, 2009]. Available from: http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/pubs/elduumdde_issue-enjeux_final_10-12-2004_e.pdf.

392. Health Products and Food Branch. Policy for the importation of sale of active pharmaceutical ingredients for veterinary use. POL-0018. Ottawa, Ontario: Government of Canada; Health Canada; 2007. [cited April 15, 2009]. Available from: http://www.hc-sc.gc.ca/dhpmps/alt_formats/hpfb-dgpsa/pdf/compli-conform/pol_0018-eng.pdf.

393. Anonymous. Industry-led committee urges delay in closing loophole allowing import of unapproved antibiotics for animals. Can Med Assoc J. 2009 April 8, 2009;180(9):914-916.

394. Health Canada's task force on own-use importation (OUI) final report. Ottawa, Ontario: Government of Canada; Health Canada; 2008. [cited July 15, 2009]. Available from: http://www.wcabp.com/pdfs/Task%20force%20on%20own%20use.pdf.

395. Weese JS. Prudent use of antimicrobials. In: Giguère S, Prescott JF, Baggot JD, Walker RD, Dowling PM, editors. Antimicrobial therapy in veterinary medicine. Fourth ed. Ames, Iowa: Blackwell Publishing; 2006. p. 437-46.

396. DeVincent SJ, Viola C. Deliberations of an advisory committee regarding priorities, sources, and methods for collecting animal antimicrobial use data in the United States. Prev Vet Med. 2006;73:133-151.

397. Heuser W. Ethical considerations for medication use by swine veterinarians. Proceedings of Western Canadian Association of Swine Practitioners; October 14-15, 2005: Saskatoon, SK: WCASP; 2005. p. 1-7.

398. Baines D. A doggone shame: Vets have effectively cornered the retail market for pet drugs. Canadian Business. 2009;82(11):24-25.

399. Harremoes P, Gee P, MacGarvin M, Stirling A, Keys J, Wyne B. Late lessons from early warnings: The precautionary principle 1896-2000. Environment Issue Report No22. Copenhagen: European Environment Agency; 2001.

400. Goldstein B, Carruth RS. The precautionary principle and/or risk assessment in world trade organisation decisions: A possible role for risk perception. Risk Anal. 2004;24(2).

401. Nicholls T, Acar J, Anthony F, Franklin A, Gupta R, Tamura Y, et al. Antimicrobial resistance: Monitoring the quantities of antimicrobials used in animal husbandry. Rev Sci Tech Off int Epiz; Rev Sci Tech. 2001;20(3):841-847.

402. Buehler JW. Surveillance. In: Rothman KJ, Greenland S, editors. Modern epidemiology. Second ed. Philadelphia, PA: Lippincott-Raven Publishers; 1998. p. 435-58.

403. Salman MD editor. Animal disease surveillance and survey systems. Ames, Iowa: Blackwell Publishing; 2003.

404. Waldner CL, Kennedy RI, Rosengren LB, Pollock CM, Clark EG. A description of gross post-mortem and histologic examination findings from abortion losses and calf mortalities in western Canadian beef herds Can Vet J. 2009;In press.

405. Hald B, Skovgard H, Pedersen K, Bunkenborg H. Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. Poult Sci. 2008;87(7):1428-1434.

406. Stephan C, Parmely J, Dawson-Coates J, Fraser E, Conly J. Obstacles to developing a multinational report card on antimicrobial resistance for Canada: An evidence-based review. Microb Drug Resist. 2007;13(4).

407. NARMS. National Antimicrobial Resistance Monitoring System (NARMS): Enteric Bacteria [webpage on the Internet]. [updated July 10 2009; cited July/20]. Available at: http://www.cdc.gov/NARMS/.

408. DeVincent SJ, Viola C. Introduction to animal antimicrobial use data collection in the United States: Methodological options. Prev Vet Med. 2006;73(2-3):105-109.

409. National Animal Health Monitoring System (NAHMS) Program Unit. Animal Health Monitoring & Surveillance; NAHMS Swine Health Studies [webpage on the Internet]. United States Department of Agriculture (USDA); Animal and Plant Inspection Services; [updated February, 2009; cited 07/09]. Available at: http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/swine/index.htm.

410. Wray C, Gnanou J-. Antibiotic resistance monitoring in bacteria of animal origin: Analysis of national monitoring programs. Int J Antimicrob Agents. 2000;14:291-294.

411. Bager F. DANMAP: Monitoring antimicrobial resistance in Denmark. Int J Antimicrob Agents. 2000;14(4):271-274.

412. Stege H, Bager F, Jacobsen E, Thougaard A. VETSTAT-the Danish system for surveillance of the veterinary use of drugs for production animals. Prev Vet Med. 2003;57(3):105-115.

413. Asai T, Esaki H, Kojima A, Ishihara K, Tamura Y, Takahashi T. Antimicrobial resistance in *Salmonella* isolates from apparently healthy food-producing animal from 2000 to 2003: The first stage of Japanese veterinary antimicrobial resistance monitoring (JVARM). J Vet Med Sci. 2006;68(8):881-884.

414. Esaki H, Morioka A, Ishihara K, Kojima A, Shiroki S, Tamura Y, et al. Antimicrobial susceptibility of *Salmonella* isolated from cattle, swine and poultry (2001-2002): Report from the Japanese veterinary antimicrobial resistance monitoring program. J Antimicrob Chemother. 2004;53(2):266-270.

415. Tamura Y. National surveillance of antimicrobial resistance in food-producing animals in Japan. Proceedings of 5th International Symposium on Antimicrobial Agents and Resistance; 27-29 April, 2005: Seoul, Korea: Asian Network for Surveillance of Resistant Pathogens (ANSORP); 2005. p. 2-4.

416. Asai T, Ishihara K, Harada K, Kojima A, Tamura Y, Sato S, et al. Long-term prevalence of antimicrobial-resistant *Salmonella enterica* subspecies *enterica* serovar Infantis in the broiler chicken industry in Japan. Microbiol Immunol. 2007;51(1):111-115.

417. Harada K, Asai T, Kojima A, Sameshima T, Takahashi T. Contribution of multi-antimicrobial resistance to the population of antimicrobial resistant *Escherichia coli* isolated from apparently healthy pigs in Japan. Microb Immun. 2007;51(5):493-499.

418. Katsunuma Y, Hanazumi M, Fujisaki H, Minato H, Hashimoto Y, Yonemochi C. Associations between the use of antimicrobial agents for growth promotion and the occurrence of antimicrobial-resistant *Escherichia coli* and enterococci in the feces of livestock and livestock farmers in Japan. J Gen Appl Microbiol. 2007;53(5):273-279.

419. Zaidi MB, Calva JJ, Estrada-Garcia MT, Leon V, Vazquez G, Figueroa G, et al. Integrated food chain surveillance system for *Salmonella* spp. in Mexico. Emerg Infect Dis. 2008 Mar;14(3):429-435.

420. Nel H, Van Vuuren M, Swan GE. Towards the establishment and standardization of a veterinary antimicrobial resistance surveillance and monitoring programme in South Africa. Onderstepoort J Vet Res. 2004;71(3):239.

421. Joint Expert Advisory Committee on Antibiotic Resistance. The use of antibiotics in food-producing animals: Antibiotic-resistant bacteria in animals and humans. ISBN 1 86496 061 2. Canberra, Australia: Commonwealth Department of Health and Aged Care; 1999. [cited August 11, 2009]. Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/2A8435C711929352CA256F180057901E/$File/jetacar.pdf.

422. Hart WS, Heuzenroeder MW, Barton MD. Antimicrobial resistance in *Campylobacter* spp., *Escherichia coli* and enterococci associated with pigs in Australia. J Vet Med B. 2004;51:216-221.

423. Dias de Oliveira S, Siqueira Flores F, dos Santos LR, Brandelli A. Antimicrobial resistance in *Salmonella enteritidis* strains isolated from broiler carcasses, food, human and poultry-related samples. Int J Food Microbiol. 2005;97(3):297-305.

424. Dadi L, Asrat D. Prevalence and antimicrobial susceptibility profiles of thermo-tolerant *Campylobacter* strains in retail raw meat products in Ethiopia. Ethiop J Health Develop. 2008;22(2):195-200.

425. Minas A, Petridou E, Bourtzi-Chatzopoulou E, Krikelis V, Papaioannou A, Plageras P. Antibiotic resistance in intestinal commensal bacteria isolated from faecal samples from pigs and pig farm workers in Greece. Res J Biologic Sci. 2008;3(2):193-200.

426. Moniri R, Dastehgoli K. Antimicrobial resistance among *Escherichia coli* strains isolated from healthy and septicemic chickens. Pak J Biol Sci. 2007;10(17):2984-2987.

427. Lauderdale TW, Shiau YR, Wand HY, Lai JF, Huang IW, Chen PC, et al. Effect of banning vancomycin analogue avoparcin on vancomycin-resistant enterococci in chicken farms in Taiwan. Environ Microb. 2007;9(3):819-823.

428. Baekbo P. Disease control in Denmark. Proceedings of Canadian Swine Health Board Forum; 8-9 July, 2009: Saskatoon, Saskatchewan: Canadian Swine Health Board; 2009.

429. United States Food and Drug Administration. Framework Document. A proposed framework for evaluating and assuring the human safety of the microbial effects of antimicrobial new animal drugs intended for use in food-producing animals [webpage on the Internet]. [updated May 5, 2009; cited August 10, 2009]. Available at: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm126607.htm#statement.

430. United States Food and Drug Administration. Advisory Committees; Committees and Meeting Materials; Veterinary Medicine Advisory Committee; Questions – 1999 VMAC [webpage on the Internet]. [updated April 30, 2009; cited August 10, 2009]. Available at: http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm127752.htm.

431. Chapman HD. Use of anticoccidial drugs in broiler chickens in the USA: Analysis for the years 1995 to 1999. Poult Sci. 2001;80(5):572-580.

432. Communication from the commission on a community strategy against antimicrobial resistance. COM(2001) 333 final. Brussels: Commission of the European Communities; 2001. [cited June 28, 2009]. Available from: http://europa.eu/eurlex/en/com/cnc/2001/act333en01/com2001_0333en01-02.pdf.

433. FAAIR Scientific Advisory Panel. Policy recommendations. Clin Infect Dis. 2002;34(S3):S67-S77.

434. Government of Canada. Production of poultry, by province. CANSIM Tables 003-0018, 003-0019 & Catalogue no. 23-015-X. Ottawa, Ontario: Statistics Canada; 2009. [cited July 2, 2009]. Available from: http://www.statcan.gc.ca/pub/23-015-x/2009001/t001-eng.pdf.

435. Government of Canada. 2006 census of agriculture; farms classified by industry. Ottawa, Ontario: Statistics Canada; 2008. [cited July 17, 2009]. Available from: www.statscan.gc.ca.

436. Chicken Farmers of Canada. Market Information: Chicken Data Booklet [webpage on the Internet]. Ottawa, Ontario: [updated 2009; cited July 30, 2009]. Available at: www.chicken.ca.

437. Government of Canada. Pigs, by province, (quarterly). CANSIM Table 003-0004, Catalogue no 23-010-X. Ottawa, Ontario: Statistics Canada; 2009. [cited March 25, 2009]. Available from: http://www40.statcan.gc.ca/l01/cst01/prim51k-eng.htm.

438. United States Department of Agriculture, Economic Research Service. Briefing Rooms, Hog: Trade [webpage on the Internet]. USDA; [updated April 28, 2009; cited 07/09]. Available at: http://www.ers.usda.gov/Briefing/Hogs/Trade.htm.

439. Government of Canada. Food statistics. Catalogue no. 21-020-X. Ottawa, Ontario: Statistics Canada; 2008. [cited July 28, 2009]. Available from: http://www.statcan.gc.ca/bsolc/olccel/olc-cel?catno=21-020-X&lang=eng.

440. Ontario Ministry of Agriculture Food & Rural Affairs. Supply Management Systems Factsheet [webpage on the Internet]. Queens Printer for Ontario; [updated 2007; cited 07/09]. Available at: http://www.omafra.gov.on.ca/english/farmproducts/factsheets/supply.htm.

441. Agriculture and Agri-Food Canada. Profile of The Canadian Chicken Industry (2006) [webpage on the Internet]. Ottawa, Ontario: Government of Canada; [updated 2009-07-24; cited July 30, 2009]. Available at: http://www.agr.gc.ca/poultry-volaille/index_eng.htm.

442. Better Farming. Quota exemption for Ontario's small chicken farmers [webpage on the Internet]. Ontario: AgMedia Inc; [updated December 19, 2008; cited July 30, 2009]. Available at: http://www.betterfarming.com/online-news/quota-exemption-Ontario%E2%80%99s-smallchicken-farmers-1484.

443. PEW Commission on Industrial Farm Animal Production. Putting Meat on the Table: Industrial Farm Animal Production in America [webpage on the Internet]. Baltimore, MD: John Hopkins Bloomberg School of Public Health; [updated April 30, 2008; cited August 4, 2009]. Available at: http://www.ncifap.org/.

444. Canadian Pork Council. The Canadian Pork Industry at Work [webpage on the Internet]. Ottawa, Ontario: [updated 2009; cited July 30, 2009]. Available at: http://www.cpc-ccp.com/.

445. Hughes L, Hermans P, Morgan K. Risk factors for the use of prescription antibiotics on UK broiler farms. J Antimicrob Chemother. 2008;61:947-952.

446. Na lampang K, Chongsuvivatwong V, Kitikoon V. Pattern and determinant of antibiotics used on broiler farms in Songkhla province, Southern Thailand. Trop Anim Health Prod. 2007;5(355):361.

447. National Chicken Council. Animal Welfare Guidelines and Audit Checklist [webpage on the Internet]. [updated 2005; cited August 10, 2009]. Available at: http://www.nationalchickencouncil.com/aboutIndustry/detail.cfm?id=19.

448. Chicken Farmers of Canada. Animal care program draft implementation guide for farmers implementing safe, safer, safest. Ottawa, Ontario: CFC; 2007. . p. 1-16.

449. Agriculture Canada. Recommended code of practice for the care and handling of poultry from hatchery to processing plant. 1757/E.: Government of Canada; 1990. [cited August 3, 2009]. Available from: http://www.agr.ca/misb/aisd/poultry/pub1757e.pdf.

450. CODEX. Ad hoc Codex intergovernmental task force on antimicrobial resistance, joint FAO/WHO food standards program, proposed draft guidance to contain foodborne antimicrobial resistant microorganisms. CX/AMR 08/2/6.2008. [cited March 6, 2009]. Available from: ftp://ftp.fao.org/codex/ccamr2/am02_06e.pdf.

451. Mathews KH, Bernstein J, Buzby JC. International trade of meat / poultry products and food safety issues. AER-828. Washington, DC: United States Department of Agriculture, Economic Research Service, Agriculture Information Bulletins; 2004. [cited July 22, 2009]. Available from: http://www.ers.usda.gov/publications/aer828/aer828f.pdf.

452. Canadian Quality Assurance (CQA) Program. Communication to producers and validators RE: CQA® drug use policy. Ottawa Ontario: Canadian Pork Council; 2007. . p. 1-3.

453. Dowd SE, Thurston-Enriquez JA, Brashears M. Environmental reservoirs and transmission of foodborne pathogens. In: Beier RC, Pillai SD, Phillips TD, Ziprin RL, editors. Preharvest and postharvest food safety. Ames, Iowa: Blackwell Publishing; 2004. p. 161-71.

454. Keeton JT, Harris KB. The hazard analysis and critical control point system and importance of verification procedures. In: Beier RC, Pillai SD, Phillips TD, editors. Preharvest and postharvest food safety. Ames, Iowa: Blackwell Publishing; 2004. p. 257-70.

455. Bolder NM. Microbial challenges of poultry meat production. Worlds Poult Sci J. 2007;63(3):401-411.

456. American Meat Institute [webpage on the Internet]. Washington, DC: American Meat Institute; [updated July 20, 2009; cited July 30, 2009]. Available at: www.meatami.com.

457. Canadian Food Inspection Agency. Hazard Analysis Critical Control Points / Food Safety Enhancement Program [webpage on the Internet]. [updated 2007-06-02; cited August 9, 2009]. Available at: http://www.inspection.gc.ca/english/fssa/polstrat/haccp/haccpe.shtml.

458. Chicken Farmers of Canada. Safe, safer, safest on-farm food safety assurance program. Ottawa, Ontario: CFC; 2005. [cited August 4, 2009]. Available from: http://www.cfo.on.ca/_pdfs/SafeSaferSafest2005.pdf.

459. Potturi-Venkata LP, Backert S, Vieira SL, Oyarzabal OA. Evaluation of logistic processing to reduce cross-contamination of commercial broiler carcasses with *Campylobacter* spp. J Food Prot. 2007;70(11):2549-2554.

460. European Food Safety Authority--Working Group on Developing Harmonised Schemes for Monitoring Antimicrobial Resistance in Zoonotic Agents. Clin Microbiol Infect. 2008 Jun;14(6):522-533.

461. Shales DM, Gerding DN, John JF, Craig WA, Bornstein DL, Duncan RA, et al. Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the prevention of antimicrobial resistance: Guidelines for the prevention of antimicrobial resistance in hospitals. Infect Control Hosp Epidemiol. 1997;18:275-291.

462. Canadian Committee on Antibiotic Resistance. (CCAR) [webpage on the Internet]. Gabroila, British Columbia: [updated June 4, 2009; cited August 6, 2009]. Available at: www.ccarccra.com.

463. Aliabadi FS, Lees P. Antibiotic treatment for animals: Effect on bacterial population and dosage regimen optimization. Int J Antimicrob Agents. 2000;14:307-311.

464. Lees P, Aliabadi FS. Rational dosing of antimicrobial drugs: Animals versus humans. Int J Antimicrob Agents. 2002;19:269-284.

465. Kroll JJ, Roof MB, Hoffman LJ, Dickson JS, Harris HDL. Proliferative enteropathy: A global enteric disease of pigs caused by *Lawsonia intracellularis.* Anim Health Res Rev. 2005;6(02):173.

466. Van Immerseel F, Rood JI, Moore RJ, Titball RW. Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. Trends Microbiol. 2009;17(1):32-36.

467. Desrosiers R. Experiences with the use of Enterisol Ileitis in Canadian breeding animals. Proceedings of Boehringer Ingelheim Ileitis Symposium; 28 June 2004: Hamburg, Germany: International Pig Veterinary Society (IPVS); 2004.

468. McOrist S, Gebhart C, Pohlenz J, Voets H, Hardge T, Ohlinger V, et al. Ileitis Technical Manual 3.0 [webpage on the Internet]. Boehringer Ingelheim Animal Health GmbH; cited 07/09]. Available at: http://www.thepigsite.com/publications/2/ileitis.

469. Leger D, Gow S, Deckert A. CIPARS on-farm swine surveillance. Proceedings of Western Canadian Association of Swine Veterinarians; 3-4 October 2008: Saskatoon, SK: WCASP; 2008.

470. Elanco. Bacterial enteritis global impact assessment: New global findings on a common and costly disease. : Elanco Canada; 2005.

471. Ferket PR. Alternatives to antibiotics in poultry production: Responses, practical experience and recommendations. Proceedings of Alltech's 20th Annual Symposium Lexington, Kentucky: Alltech; 2004.

472. Anonymous. New strategy to reduce in-feed medication – passive immunity a viable strategy for Canada's Dr. Neil Ambrose. CocciForum 13: the Poultry Site; 2008. [cited August 4, 2009]. Available from: www.thepoultrysite.com.

473. Schrader JS. Product Development Netvax [webpage on the Internet]. cited April 22, 2008]. Available at: http://www.netvaxforpoultry.com/document_library/Schrader.pdf.

474. Aarestrup FM, Oliver Duran C, Burch DGS. Antimicrobial resistance in swine production. Anim Health Res Rev. 2008;9(S2):135.

475. Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN, et al. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim Health Res Rev. 2008;9(S2):217.

476. Canadian Poultry Consultants Ltd. [webpage on the Internet]. Abbotsford, British Columbia: Ritchie, S. J.; [updated 2008; cited 07/09]. Available at: www.canadianpoultry.ca.

477. Langhout P. New additives for broiler chickens. World Poultry. 2000;16:22-27.

478. Collett SR. Strategies for improving gut health in commercial broiler operations. Proceedings of Create Innovate Elevate Nutritional Biotechnology in the Feed and Food Industries, 21st International Symposium Lexington, Kentucky: Alltech; 2005. p. 17-30.

479. Anadon A, Martinez-Larranaga MR, Marinez MA. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. Regul Toxic Pharmacol. 2006;45(1):91-95.

480. Weese JS, Sharif S, Rodriguez A. Probiotics in veterinary medicine. In: Versalocic J, Wilson M, editors. Therapeutic microbiology. 1st ed. Washington, DC: ASM Press; 2008. p. 341-56.

481. Timmerman HM, Koning CJM, Mulder l, Rombouts FM, Beyen AC. Monostrain, multistrain and multispecies probiotics – A comparison of functionality and efficacy. Int J Food Microbiol. 2004;96(3):219-233.

482. Wagner RD, Cerniglia CE. Antimicrobial susceptibility patterns of competitive exclusion bacteria applied to newly hatched chickens. Int J Food Microbiol. 2005;102(3):349-353.

483. Veterinary Drugs Directorate. Qs and As – Veterinary Natural Health Products [webpage on the Internet]. Ottawa, Ontario: Health Canada; [updated 2006; cited June 2, 2009]. Available at: http://www.hc-sc.gc.ca/dhp-mps/vet/faq/qa_health_prod_sante_qr-eng.php.

484. Business Decisions Limited. Benchmarking the competitiveness of the Canadian animal health industry: Commissioned by the Canadian Animal Health Institute and the International Federation for Animal Health; 2007. . p. 1-102.

485. Kim LM, Gray JT, Bailey JS, Jones RD, Fedorka-Cray PJ. Effect of porcine-derived mucosal competitive exclusion culture on antimicrobial resistance in *Escherichia coli* from growing piglets. Foodborne Pathog Dis. 2005;2(4):317-329.

486. Patterson JA. Prebiotic feed additives: Rationale and use in pigs. In: Foxcroft GR, Ball RO, editors. Proceedings of Advances in Pork Production; Banff Pork Seminar: Banff, Alberta: University of Alberta, Department of Agricultural Food and Nutritional Sciences; 2005. p. 149-59.

487. Shane SM. Evaluating consumer-acceptable performance enhancers. World Poult. 2003;19(7):32-33.

488. Hooge DM. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993-2003. Int J Poult Sci. 2004;3(3):163-174.

489. Rosen GD. Holo-analysis of the efficacy of bio-mos(R) in broiler nutrition. Br Poult Sci. 2007;48(1):21-26.

490. Rosen GD. Optimizing the replacement of pronutrient antibiotics in poultry nutrition. Proceedings of Alltech's 20th Annual International Symposium; 2004: Lexington, Kentucky: Alltech; 2004. p. 94-101.

491. Grave K, Jensen VF, Odensvik K, Wierup M, Bangen M. Usage of veterinary therapeutic antimicrobials in Denmark, Norway and Sweden following termination of antimicrobial growth promoter use. Prev Vet Med. 2006;75(1).

492. Grave K, Kaldhusdal M, Harr L, Kruse H, Flatlandsmo K. What has happened in Norway after the avoparcin ban? Consumption of antimicrobials in poultry. Prev Vet Med. 2004;62:59-72.

493. Lovland A, Kaldhusdal M. Severely impaired production performance in broiler flocks with high incidence of *Clostridium perfringens*-associated hepatitis. Avian Patholog. 2001;30(1):73-81.

494. Kaldhusdal M, Lovland A. The economic impact of *Clostridium perfringens* is greater than anticipated. World Poultry. 2000;16:50-51.

495. Poole TL, Genovese KJ, Beier RC, Callaway TR, Bischoff KM. Antimicrobial resistance and the microflora of the gastrointestinal tract. In: Beier RC, Pillai SD, Phillips TD, editors. Preharvest and postharvest food safety. Ames, Iowa: Blackwell Publishing; 2004. p. 213-25.

496. Beltran R, Schatzmayr G,Klimitch A. Evaluation of a probiotic product on cecal colonization and organ invasion of *Salmonella enteritidis* in broilers. In: Scott TA, editor. Proceedings of 17th Australian Poultry Science Symposium; 7-9 Feb 2005: Sydney Australia: Poultry Research Foundation; 2005. p. 69-71.

497. Reid G, Friendship R. Alternatives to antibiotic use: Probiotics for the gut. Anim Biotechnol. 2002;13(1):97-112.

498. Jamalludeen N, Johnson RP, Friendship R, Kropinski AM, Lingohr EJ, Gyles CL. Isolation and characterization of nine bacteriophages that lyse O149 enterotoxigenic *Escherichia coli.* Vet Microbiol. 2007;124(1-2):47-57.

499. Ma YL, Lu CP. Isolation and identification of a bacteriophage capable of infecting *Streptococcus suis* type 2 strains. Vet Micro. 2008;132(3-4):340-347.

500. McLaughlin MR, Balaa MF, Sims J, King R. Isolation of *Salmonella* bacteriophages from swine effluent lagoons. J Environ Qual. 2006;35(2):522-528.

501. O'Flynn G, Coffey A, Fitzgerald GF, Ross RP. The newly isolated lytic bacteriophages st104a and st104b are highly virulent against *Salmonella enterica.* J Appl Microbiol. 2006;101(1):251-259.

502. Oliveira A, Sillankorva S, Quinta R, Henriques A, Sereno R, Azeredo J. Isolation and characterization of bacteriophages for avian pathogenic *E. coli* strains. J Appl Microbiol. 2009;6(1919):1927.

503. Jamalludeen N, Johnson RP, Shewen PE, Gyles CL. Evaluation of bacteriophages for prevention and treatment of diarrhea due to experimental enterotoxigenic *Escherichia coli* O149 infection of pigs. Vet Microbiol. 2009;136(1-2):135-141.

504. Jamalludeen N, She YM, Lingohr EJ, Griffiths M. Isolation and characterization of virulent bacteriophages against *Escherichia coli* serogroups O1, O2, and O78. Poult Sci. 2009;88(8):1694-1702.

505. Higgins JP, Andreatti Filho RL, Higgins SE, Wolfenden AD, Tellez G, Hargis BM. Evaluation of *Salmonella*-lytic properties of bacteriophages isolated from commercial broiler houses. Avian Dis. 2008;52(1):139-142.

506. Atterbury RJ, Van Bergen MAP, Ortiz F, Lovell MA, Harris JA, De Boer A, et al. Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. Appl Environ Microbiol. 2007;73(14):4543-4549.

507. Borie C, Albala I, Sánchez P, Sánchez ML, Ramirez S, Navarro C, et al. Bacteriophage treatment reduces *Salmonella* colonization of infected chickens. Avian Dis. 2008;52(1):64-67.

508. El-Shibiny A, Scott A, Timms A, Metawea Y, Connerton P, Connerton I. Application of a group II *Campylobacter* bacteriophage to reduce strains of *Campylobacter jejuni* and *Campylobacter coli* colonizing broiler chickens. J Food Prot. 2009;72(4):733-740.

509. Kwon HJ, Cho SH, Kim TE, Won YJ, Jeong J, Park SC, et al. Characterization of a T7-like lytic bacteriophage (phiSG-JL2) of *Salmonella enterica* serovar *gallinarum* biovar *gallinarum.* Appl Environ Microbiol. 2008;74(22):6970-6979.

510. Mateos GG, Gonzalez-Alvarado JM,Lazaro R. Facing the realities of poultry health and performance without antibiotics in Europe. Proceedings of Re-imaging the Feed Industry, Nutritional Biotechnology in the Feed and Food Industry Lexington, Kentucky: Alltech; 2004. p.69-79.

511. Tronoe N. Consequences of the termination of antimicrobial growth promoter use for broiler health and usage of antimicrobials for therapy and prophylaxis. WHO/CDS/CPE/ZFK/2003.1. Foulum, Denmark: World Health Organization; 2002. . Available from: http://www.dfvf.dk/Default.asp?ID=9203#74162.

512. Pritchard G, Dennis I, Waddilove J. Biosecurity: Reducing disease risks to pig breeding herds. In practice. 2005;27(5):230-237.

513. Tablante NL, Myint MS, Johnson YJ, Rhodes K, Colby M, Hohenhaus G. A survey of biosecurity practices as risk factors affecting broiler performance on the Delmarva peninsula. Avian Dis. 2002;46:730-734.

514. Amass SF, Clark LK. Biosecurity considerations for pork production units. J Swine Health Prod. 1999;7(5):217-228.

515. Sanei B, Innes P. Biosecurity recommendations for commercial poultry flocks in Ontario. 450/10. Ontario: Ontario Ministry of Agriculture Food & Rural Affairs; 2005. [cited August 1, 2009]. Available from: http://www.omafra.gov.on.ca/english/livestock/poultry/facts/05-077.htm.

516. Schuppers ME, Stephan R, Ledergerber U, Danuser J, Bissig-Choisat B, Stark KD, et al. Clinical herd health, farm management and antimicrobial resistance in *Campylobacter coli* on finishing pig farms in Switzerland. Prev Vet Med. 2005;69(3-4):189-202.

517. Petersen A, Christensen JP, Kuhnert P, Bisgaard M, Olsen JE. Vertical transmission of a fluoroquinolone-resistant *Escherichia coli* within an integrated broiler operation. Vet Microbiol. 2006;116(1-3):120-128.

518. Amass SF, Halbur PG, Byrne BA, Schneider JL, Koons CW, Cornick N, et al. Mechanical transmission of enterotoxigenic *Escherichia coli* to weaned pigs by people, and biosecurity procedures that prevented such transmission. J Swine Health Prod. 2003;11(2):61-68.

519. Allen VM, Weaver H, Ridley AM, Harris JA, Sharma M, Emery J, et al. Sources and spread of thermophilic *Campylobacter* spp. during partial depopulation of broiler chicken flocks. J Food Prot. 2008;71(2):264-270.

520. Campbell K. H1N1 influenza in Canada. Proceedings of Canadian Swine Health Forum; July 7-8, 2009: Saskatoon, SK: Canadian Swine Health Board; 2009.

521. Gibbens JC, Pascoe SJS, Evans SJ, Davies RH, Sayers AR. A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. Prev Vet Med. 2001;48(2):85-99.

522. Halvorson DA, Hueston WD. The development of an exposure risk index as a rational guide for biosecurity programs. Avian Dis. 2006;50:516-519.

523. Beach RH, Poulos C, Pattanayak K. Farm economics of bird flu. Can J Agric Econ. 2007;55:471-483.

524. Power C. The source and means of spread of the Avian Influenza Virus in the Lower Fraser Valley of British Columbia during an outbreak in the winter of 2004. Ottawa, Ontario: Canadian Food Inspection Agency; 2005. [cited August 4, 2009]. Available from: http://www.inspection.gc.ca.cyber.usask.ca/english/anima/heasan/disemala/avflu/2004rep/epie.shtml.

525. Hurd HS, Brudvig J, Dickson J, Mirceta J, Polovinski M, Matthews N, et al. Swine health impact on carcass contamination and human foodborne risk. Public Health Rep. 2008;123(3):343-351.

526. Cox LAJ. Potential human health benefits of antibiotics used in food animals: A case study of virginiamycin. Environ Int. 2005;31:549-563.

527. Engster HM, Marvil D, Stewart-Brown B. The effect of withdrawing growth promoting antimicrobials from broiler chickens, a long-term commercial industry study. J Appl Poult Res. 2002;11:431-436.

528. Canadian Food Inspection Agency. Meat hygiene manual of procedures. Ottawa, Ontario: Government of Canada; 2009. [cited July 20, 2009]. Available from: http://www.inspection.gc.ca/english/fssa/meavia/man/mane.shtml.

529. Johnson RP, Gyles CL, Huff WE, Ojha S, Huff GR, Rath NC, et al. Bacteriophages for prophylaxis and therapy in cattle, poultry and pigs. Anim Health Res Rev. 2008;9(S2):201.

530. Azanza PV. Hydrogen peroxide, peroxyacetic acid, octanoic acid, peroxyoctanoic acid, and 1-hydroxyethylidene-1, 1-diphosphonic acid (HEDP) as components of antimicrobial washing solution - chemical and technical assessment. 63rd Joint FAO/WHO Expert Committee for Food Additives.: FAO; 2004. [cited August 4, 2009]. Available from: ftp://ftp.fao.org/es/esn/jecfa/cta/CTA_63_Antimicrobials.pdf.

531. Joint FAO/WHO Expert Committee of Food Additives. Summary and conclusions. JECFA/63/SC. Geneva, Switzerland: FAO/WHO; 2004. [cited August 3, 2009]. Available from: ftp://ftp.fao.org/es/esn/jecfa/jecfa63_summary.pdf.

532. Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC). Treatment of poultry carcasses with chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids. EFSA q-2005-002.: The EFSA Journal; 2005. [cited August 4, 2009]. Available from: http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/afc_op_ej297_poultrytreatment_opinon_en-rev2,0.pdf.

533. Assessment of the possible effects of the four antimicrobial treatment substances on the emergence of antimicrobial resistance. Scientific opinion of the panel on biological hazards. Question No EFSA-Q-2007-203. Parma, Italy: European Food Safety Authority; 2008. [cited August 4, 2009]. Available from: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178697425124.htm.

534. D'Costa VM, McGrann KM, Hughes DW, Wright GD. Sampling the antibiotic resistome. Science. 2006;311:374-377.

535. Chauvin C, Madec F, Guillemont D, Sanders P. The crucial question of standardization when measuring drug consumption. Vet Res. 2001;32:533-543.

536. CODEX. Ad hoc Codex intergovernmental task force on antimicrobial resistance: Proposed draft guidance on creating risk profiles for antimicrobial resistant foodborne microorganisms for setting risk assessment and management priorities. CX/AMR 08/2/5.2008. [cited June 3, 2009]. Available from: www.codexalimentarius.net/download/report/708/am02_01e.pdf.

537. CODEX. Principles and guidelines for the conduct of microbial risk management (MRM). CAC/GL 63-2007.2007. [cited 2009/03/06]. Available from: www.codexalimentarius.net/download/standards/10741/cxg_063e.pdf.

538. CODEX. Working principles for risk analysis for food safety applications by governments. CAC/GL 62-2007.2007. [cited 2009/03/06]. Available from: ftp://ftp.fao.org/codex/Publications/Booklets/Risk/Risk_EN_FR_ES.pdf.

539. Kirby J. The corporate climate crusaders: Think big business is against new climate change laws? Well, think again. Maclean's. 2009;122(27).

Appendix 1: Search Strategy

The following search terms and combinations of key words were provided to the librarian. These were used, along with her expert knowledge regarding database capabilities and medical subject headings (MeSH), to conduct the systematic searches of CAB, EMBASE, and Medline, and the strategic searches of Scopus and Agricola. Searches were generally limited to the word being in the title, original title, abstract, name of substance word, subject heading word, or unique identifier. An English language restriction and publication date restrictions were used (First set; 1990 to present; Second set; 1999 to present).

MeSH terms, truncation, and Boolean operaters were used at the librarian's discretion. Additional consultation between the librarian and team members occurred to identify inclusion criteria with incomplete/insufficient citations identified, and ad hoc searches were conducted but not recorded.

The search results were downloaded into RefWorks ©. Duplicates were automatically removed thus the numbers of citations obtained prior to removing duplicates are not available.

Database	**Citations obtained**[a]
Embase/Medline[b]	1524
CAB	485
Scopus	114
Agricola	Not recorded

[a] The citations obtained refers to unique citations after duplicates were automatically removed.

[b] Embase and Medline were combined due to high degree of overlap.

Search Number	Search Terms
1.1	E* coli or Esherichia coli
1.2	Campylobact* OR C* coli OR C* jejuni
1.3	Salmonell* NOT Typhi
1.4	Enterococc* OR VRE OR (vancomycin NEAR Enterococc*)
1.5	Clostridi* AND (difficile OR perfringens)
1.6	Yersini*
1.7	MRSA OR (Staph* AND aureus AND methicillin)
1.8	Lact* AND bacteria
1.9	Listeri*
1	COMBINE TO MAKE SET #1
2.1	Poult* OR Chicken OR Broiler OR breeder OR Hatch* NOT (egg OR turkey OR layer)
2.2	Swine OR pig OR porcine
2.3	Food* OR Meat OR agri* OR muscle AND (Chicken OR Pork) NOT (beef)
2.4	2.3 AND (consumption OR handl*)
2.5	2.1 or 2.2 AND (fecal or feces or ceacal or cecal)
2	COMBINE TO MAKE SET #2
3.1	Anti* NEAR (bacterial OR biotic OR microb* OR drug)
3.2	3.1 NEAR (resist* OR suscept* OR sensitiv*)
3.3	3.1 AND Class NEAR (rank OR classification OR order OR importance)
3.4	ionophore OR "feed additive" OR "feed antibiotic" therapeutic or *drug or drug*
3	COMBINE TO MAKE SET #3
4.1	1 OR 2 OR 3 NEAR (control OR interven* OR prevent* OR transmi* OR monitor OR surveil*)
4.2	1 OR 2 OR 3 NEAR (policy OR protocol OR action OR program OR strategy OR position)
4.3	1 OR 2 OR 3 NEAR (guidelines OR "best practice" OR regulat* OR legislat* OR legal)
4.4	1 OR 2 OR 3 NEAR (good NEAR management OR production)
4.5	1 OR 2 OR 3 NEAR ("critical control point" OR "standard operating" OR HACCP)
4.6	1 OR 2 OR 3 NEAR (monitor* OR surveillance OR "public health" OR stewardship)

5.1	2 NEAR (Industry OR produce* OR farm* OR process* OR Abattoir OR Distribut* OR commodity)
5.2	(govern* OR regulat*) NEAR (health OR agri*)
5.3	5.2 AND (Canada OR United States OR US* OR Mexico)
5.4	5.2 AND (Europ* OR "South America" OR … specific inclusion criteria
5.5	(Vet* OR Practition*) AND (Association OR Organization)
5.6	2 AND pharmaceutical
6.1	2 and 5 (best if includes 1 or 4) AND nutri* OR feed
6.2	2 and 5 (best if includes 1 or 4) AND feed NEAR (probiotic OR prebiotic OR "essential oil" OR herd OR alternative)
6.3	2 and 5 (best if includes 1 or 4) AND organic OR (anti* NEAR free)
6.4	2 and 5 (best if includes 1 or 4) AND biosecurity OR (wash OR "down time" or boot OR fly OR shower)
6.5	2 and 5 (best if includes 1 or 4) NEAR (disease OR health)
6.6	2 and 5 (best if includes 1 or 4) AND hygiene OR disinfect* OR detergent OR sanitation OR wash OR dry
6.7	2 and 5 (best if includes 1 or 4) AND batch OR (all NEAR in)
6.8	2 and 5 (best if includes 1 or 4) AND dead OR cull OR compost OR mortality OR condemn* OR render
6.9	2 and 5 (best if includes 1 or 4) AND morbidity OR sick OR treatment
7.0	2 and 5 (best if includes 1 or 4) AND (medication OR vaccine OR drug) NEAR (storage OR handling OR use)
7.1	2 and 5 (best if includes 1 or 4) AND (air NEAR ventilation OR quality OR gas OR humidity OR flow OR ammonia)
7.2	2 and 5 (best if includes 1 or 4) AND (water NEAR pipes OR biofilm OR quality OR chlorin*)
7.3	2 and 5 (best if includes 1 or 4) AND ship* OR transport* OR handl* OR lairage
7.4	2 and 5 (best if includes 1 or 4) AND litter
7.5	2 and 5 (best if includes 1 or 4) AND brooding
7.6	2 and 5 (best if includes 1 or 4) AND bacteria AND (preservative OR *radiat* OR wash OR bacteriocin OR lactibiotic OR bacteriophage)
7.7	3 and 5 (best if includes 1 or 4) AND bacteria AND (chill OR steam OR OR packag*)

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