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Epidemiological study on antimicrobial use and non-compliance with withdrawal times in broiler farms in Vietnam

par

Thi Viet Hang Nguyen

Département de pathologie et microbiologie

Faculté de médecine vétérinaire

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Résumé

L’utilisation d’antimicrobiens chez les animaux de consommation est une source de préoccupation importante pour la santé publique à travers le monde en raison de ses impacts potentiels sur l’émergence de micro-organismes résistants aux antimicrobiens et sur la présence de résidus antimicrobiens néfastes dans la viande. Cependant, dans les pays en développement, peu de données sont disponibles sur les pratiques d’utilisation des antimicrobiens à la ferme. Par conséquent, une étude épidémiologique transversale a été menée de juin à août 2011 dans des élevages de poulets de chair situés dans le sud du Vietnam, ayant pour objectifs de décrire la prévalence d’utilisation des antimicrobiens ajoutés à l’eau de boisson ou aux aliments à la ferme, et de tester les associations entre les caractéristiques des fermes et la non-conformité avec les périodes de retrait recommandés sur l’étiquette des produits. Un échantillon d’accommodement de 70 fermes a été sélectionné. Les propriétaires des fermes ont été interrogés en personne afin de compléter un questionnaire sur les caractéristiques des fermes et les pratiques d’utilisation d’antimicrobiens. Au cours des 6 mois précédant les entrevues, il a été rapporté que la colistine, la tylosine, l’ampicilline, l’enrofloxacine, la doxycycline, l’amoxicilline, la diavéridine et la sulfadimidine ont été utilisés au moins une fois dans les fermes échantillonnées, avec une fréquence descendante (de 75.7% à 30.0%). D’après deux scénarios de risque basés sur la comparaison de la période de retrait recommandée sur l’étiquette du produit et celle pratiquée à la ferme, de 14.3% à 44.3% des propriétaires de ferme interrogés n’ont pas respecté la période de retrait recommandée sur l’étiquette au moins une fois au cours des 6 derniers mois, et ce pour au moins un antimicrobien. Les facteurs de risque associés ($p<0.05$) avec une non-conformité avec la période de retrait recommandée sur l’étiquette pour au moins un des deux scénarios sont les suivants : élever des oiseaux qui n’appartiennent pas tous à des races d’origine asiatique, vacciner contre la bronchite infectieuse, avoir utilisé plus de 6 différents antimicrobiens à la ferme au cours des 6 derniers mois, et utiliser un mélange d’aliments fait maison et commerciaux. Nos résultats soulignent l’importance d’utiliser les antimicrobiens de façon judicieuse et en respectant les temps de retrait officiels, afin de protéger le consommateur contre les risques pour la santé causés par une exposition à des niveaux nocifs de résidus antimicrobiens.

Mots-clés : antimicrobien, résidu, facteurs de risque, poulet, période de retrait, Vietnam.

Abstract

Antimicrobial use in food-animal husbandry is an important public health concern worldwide due to its potential impact on the emergence of drug-resistant microbes and its harmful residues in meat. However, in developing countries, few data are available on farm drug use practices. Therefore a cross-sectional epidemiological study was conducted on broiler chicken farms in Southern Vietnam from June 2011 to August 2011 with the aim of both describing prevalence of antimicrobials added to feed or water at the farm level and ascertaining any associations between farm characteristics and non-compliance of antimicrobial withdrawal times on labels. A convenient sample of 70 broiler farms was surveyed via personal interviews with farm owners using a questionnaire pertaining to farm characteristics and drug use practices. Over the 6-month period prior to the interviews, colistin, tylosin, ampicillin, enrofloxacin, doxycyclin, amoxicillin, diaveridin, and sulfadimidin were used at least once in descending frequency (from 75.7% to 30.0%) by the farms surveyed. Following two risk scenarios based on the comparison of recommended label withdrawal times with actual withdrawal times practiced during this period, between 14.3% and 44.3% of farmers did not comply with on-label withdrawal times for at least one antimicrobial. Risk factors associated ($p<0.05$) with non-compliance with on-label withdrawal times in at least one risk scenario were: raising birds other than Asian-indigenous bird breeds only, vaccinating against infectious bronchitis, using more than 6 different antimicrobials on a farm during the last 6 months prior to the interview, and mixing home-made and commercial feed. Our results underline the importance of using antimicrobials judiciously and respecting official withdrawal times in order to protect the consumer from the health risks caused by exposure to harmful levels of antimicrobial residues.

Keywords: antimicrobial, residue, risk factors, broiler farm, withdrawal time, Vietnam.

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List of abbreviations

WTO: World Trade Organisation

GDP: Gross Domestic Product

AGPs: Antimicrobial Growth Promoters

OIE: World Organization for Animal Health

MRL: Maximum Residue Level

US: United States

EU: European Union

MIC: Minimum Inhibitory Concentration

MBC: Minimum Bactericidal Concentration

M: Mycoplasma

S: Salmonella

C: Campylobacter

To my dear family, mama Thu, papa Huong,
younger brother Duong and sister Ha for
their endless love and encouragement!

"Men are born to succeed, not fail."
- Thoreau

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1. Introduction

The globalization is bringing up numerous opportunities for international trade of food products. Yet, food safety standards and regulations can vary between countries, regions and continents. Thus, the need for harmonization on food safety standards is pressing. To do this, Vietnam has actively joined in the international food-safety organizations such as: Codex Alimentarius, World Organization for Animal Health, International Plant Protection Convention, as well as signed the Agreement on the Application of Sanitary and Phytosanitary Measures, the Agreement on Technical Barriers to Trade as a WTO member.

Moreover, Vietnam has been in efforts to establish the legal systems on food safety administration with Law on Food Safety (No. 55/2010/QH12) dated 17 June 2010 as the highest legal corridor, and then, under-law regulatory documents such as: Government Decrees guiding the implementation of the Law on Food Safety, Circulars guiding the detailed implementation of Decrees on Food Safety, National Technical Regulations and Standards. The National Regulations and Standards have been being established with taking international regulations and standards in consideration so that Vietnamese food products can reach not only the domestic market, but also export markets.

Additionally, to improve food safety management competence for Vietnamese governmental organisation, the international cooperation on food safety between developed and developing countries is an indispensable trend. The Food and Agricultural Products Quality Development and Control Project (FAPQDCP) funded by the Canadian International Development Agency (CIDA) has been implementing in Vietnam to improve the quality, safety and marketability of agro-food products by strengthening the production-processing-quality control system in food chain. As a small component of this FAPQDCP project, my field study is able to support Vietnamese governmental authorities on food safety to have insight to an angle of current situation on food safety, to evaluate the degree of the ratified legal document implementation in reality, then to make interventions by more workable policies to be reachable to international food safety standards. All of this finally is to promote human- and eco-health.

2. Literature Review

2.1. Poultry production in Vietnam

2.1.1. Roles of poultry production in Vietnam

Vietnam is a maritime country in Southeast Asia characterized by an S-shaped land area of about 330,000 km^2. It has over 3200 km of coastline, a subtropical monsoon climate, and a population of around 90 million (2011 census) (GSO, 2011).

To combat a series of crises that arose during the postwar years after 1975, the Vietnamese government has been enacting policies to transform the country from a central planned economy to a market-oriented economy through the economic and institutional innovative reforms called “Doi Moi” officially initiated in 1986 (Burgos, Hanh, Roland-Holst, & Burgos, 2007; FAO, 2006; Largo, 2002). Since then, economic growth and living standards have been improving and they have been moving toward global integration, becoming an official member of the World Trade Organization (WTO) in 2007. As a result, Vietnam has become one of the world’s fastest growing economies with an annual growth rate of gross domestic product (GDP) around 6% in 2011, despite the challenging global economy (Gafin, 2011).

Playing a remarkable role in the national economy, agriculture occupied over 20% of the total GDP in 2010 (GSO, 2010). Not only has Vietnam made great strides in improving national food security, but poverty has been cut in more than half, from 37.4% in 1998 to 14.5% in 2008, thanks to agricultural development (WorldBank, 2012). The exportation of key agricultural goods such as coffee, tea, rubber, crude oil, pepper, and fishery products makes up a large portion of total exports. In fact, Vietnam is the world's largest Robusta coffee, cashew nut and pepper exporter, and the second largest rice exporter (Burgos, Hinrichs, Otte, Pfeiffer, & Roland-Holst, 2008). This enabled Vietnam to enhance GDP per capita from below 200USD/capita in 1986 to around 1200USD/capita in 2010 (Vietnam's Department of Livestock, 2010), propelling Vietnam out of an underdeveloped status and into a middle income standing in 2008 (Dantri, 2011).

Relative to demographic distribution, around 69.8% of the total population in 2010 was settled in rural areas where livelihoods mainly depend on agriculture (GSO, 2011). One of the major sources of income for rural dwellers derives from livestock, which in 2010 made up 24% of the total agricultural economic value, second behind cultivation at 74%, with the remaining 2% in agriculture services (GSO, 2010).

Just behind pigs, the second most important livestock species is poultry production, a traditional occupation as old as the long-standing tradition of rice cultivation in Vietnam (Epprecht, 2005). As the archaeological evidence has shown, traditional farming operations have been in existence for as long as 3000-3500 years (Van Duc Nguyen & Tran, 2008). Poultry is raised in nearly all regions of the country (Figure 1) with chickens and ducks as the dominant species. The region with the densest population of poultry is the Red River Delta, which made up 26% of the total national poultry population in 2010 (GSO, 2010), contributing to around 19% of the total economic value of livestock in 2010 (GSO, 2010).

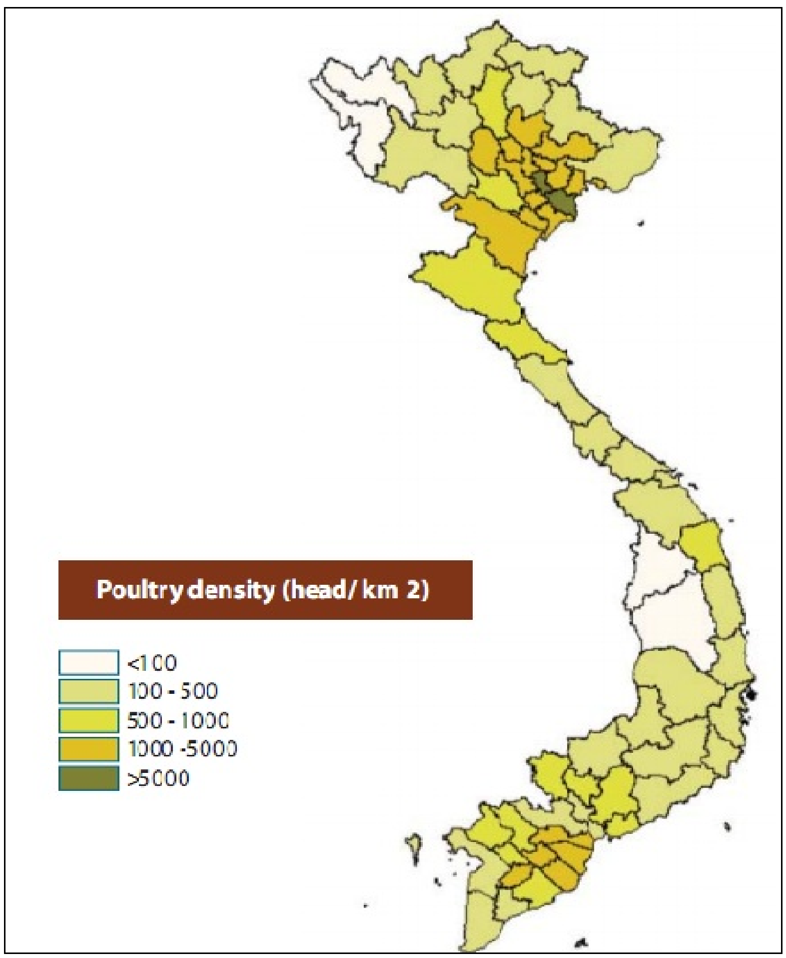

Figure 1. Poultry density in Vietnam in 2006. With permission from Stéphanie Desvaux.

In the past 10 years, the number of poultry heads has been increasing as a general trend to meet growing demands for poultry products, which is strongly associated with the rise of per capita income (Figure 2) (GSO, 2010). In particular, the per capita meat consumption of Vietnam was up to 5.9 kg/person/year for broilers in 2011 (FAPRI, 2010), in comparison to about 2.5 kg/person/year in 2001-2003 (Burgos et al., 2008). However, in the 2010 United States (US) and World Agricultural Outlook by the US Food and Agricultural Policy Research Institute, the domestic meat production of Vietnam will not be able to satisfy the rapidly growing demand for broiler meat consumption within the country by 2019 (FAPRI, 2010; U.S. Department of Agriculture, 2010).

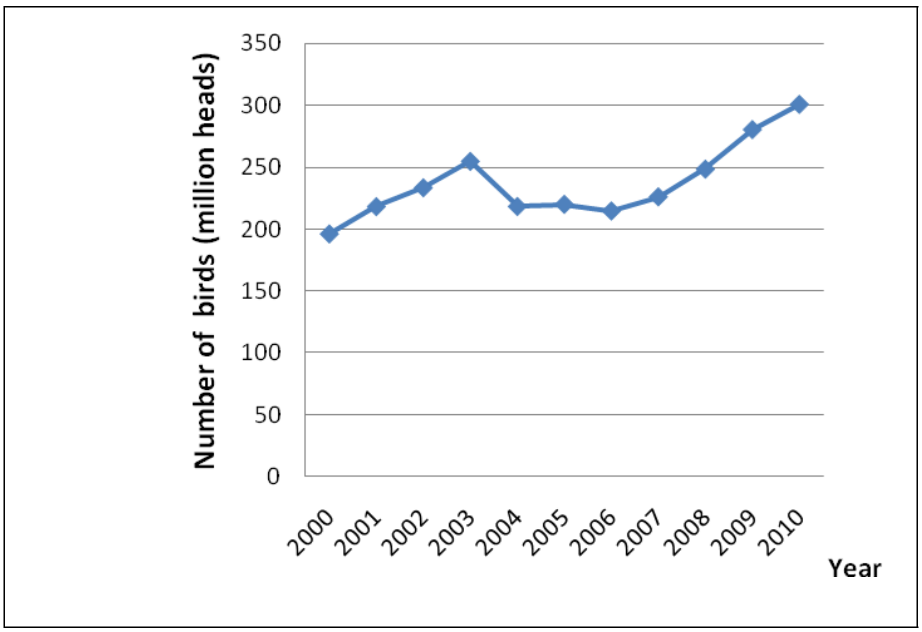

Figure 2. Number of birds in Vietnam from 2000 to 2010 (GSO, 2010)

From a poultry and livelihood perspective, there were 8.3 million poultry-holding households, which was 64% of all rural households at the time of the 2001 census. Poultry was most densely held in the Northeast with 85% of rural households and in the Northwest with 84% of rural households. Further, there is a difference in poultry-keeping operations between the north and the south of Vietnam. In the north, the operations are characterized by low-input investments and smaller numbers of birds kept by mostly rural households mainly for personal consumption, while in the south, raising chickens is practiced by fewer households but with larger livestock investments for commercial purposes. Poultry-derived income is used to cover various household expenditures such as other food stuffs for home consumption, school, home improvement, housewares, medications, bird replacements, and other asset and non-asset investments.

From a Vietnamese socio-cultural perspective, healthy birds and eggs can be given to neighbours, to sick people or to pregnant women as gifts. It is an honoured tradition for people to bring replacement birds to a beloved person who has lost some to disease (Van Duc Nguyen & Tran, 2008). Furthermore, long ago, chicken breeds from special areas were offered to the King, such as the Mia birds in Mia village, Dong Tao birds in Dong Tao village and Ho birds in Dong Ho village. If the King was satisfied with the quality of the indigenous birds, he would give gold to these villages so that they felt an honour about maintaining their indigenous bird breeds, thereby preserving important genetic resources and avoiding crossbreeding (Van Duc Nguyen & Tran, 2008). In addition, some special birds are fed and trained by experienced farmers only for cock fighting in local traditional fairs. Fighter cocks, called "Choi" chickens, will earn prizes and boasting rights for farmers if they win in competitions (Van Duc Nguyen & Tran, 2008). These poultry-related activities bring farmers together and build community, ensuring the continuation of a vibrant cultural tradition around raising poultry in Vietnam.

2.1.2. Poultry production systems

According to the proposed classification of poultry production, poultry production in Vietnam can be classified into three systems: 1) traditional, extensive backyard/household poultry productions with less than 50 birds/cycle; 2) semi-intensive, small to medium scale, market-oriented, commercial poultry productions with 51-2000 birds/cycle; 3) and intensive, large scale, industrial poultry productions with more than 2000 birds/cycle (Burgos et al., 2007; Burgos et al., 2008; T. H. H. Pham, Burgos, & Roland-Host, 2007). Characteristics of each system will be described below:

2.1.2.1. Traditional extensive backyard chicken production

This operation is the most popular by far, characterized by small flock size <50 birds free-ranging in backyards, gardens, courtyards, orchards and on neighboring land during the day (Burgos et al., 2007) and resting in a corner of the garden or in cattle or pig stables at night (Van Duc Nguyen & Tran, 2008).

In this operation, local breeds are more often raised such as Ri, Mia, Dong Tao and Ho birds in the North and Tau Vang in the South (Burgos et al., 2007; Van Duc Nguyen & Tran, 2008). In spite of lower productivity than imported breeds, they are more favored by consumers nationwide, particularly for traditional festivals, family gifts, marriages and for religious offerings because of their characteristic taste (Burgos et al., 2007; T. H. H. Pham et al., 2007).

Main sources of feed for chickens are derived from their own free-scavenging in gardens, fields and on roadsides including: crop remains, worms, insects or locusts. In the morning, birds are also supplemented with limited amounts of home-made grains: paddy rice, maize, cassava, potato or kitchen waste. The amount of feed given to birds depends mainly on the availability of agro-by-products at home and is not based on the birds' real demand for feed consumption. In the afternoon, farmers sometimes feed them some more (Burgos et al., 2007; Van Duc Nguyen & Tran, 2008).

Owners of these operations are predominantly smallholder farmers with bird-husbandry knowledge handed down from one generation to another (Van Duc Nguyen & Tran, 2008). Since bird husbandry is considered as a non-market-oriented sideline activity, primarily for home consumption (Burgos et al., 2007), householders may not know exactly how many birds they are raising. They just recognize changes in flock size after birds die or are sick (Van Duc Nguyen & Tran, 2008). Furthermore, together with poor nutrition, bird health care services are also very limited. In particular, only 30 to 40% of birds are vaccinated because multiple generations in the same farm make it difficult to vaccinate all of them at the same time and vaccination is costly for smallholder farmers (Van Duc Nguyen & Tran, 2008). These risk factors, along with free movement, cause birds to be more susceptible to infections (Delquign, Edan, Nguyen, Pham, & Gautier, 2004; Desvaux, Vu, Phan, & Pham, 2008), pushing up mortality as high as 40 to 50% (Burgos et al., 2007; GSO, 2004). Chicks are replaced mainly from hatched eggs from their own chickens, but sometimes chick replacements are bought from relatives, neighbors, local markets and/or traders (Burgos et al., 2007; Van Duc Nguyen & Tran, 2008).

In short, this traditional operation is characterized by free scavenging birds, low infrastructure investment, and limited care (Desvaux et al., 2008). This causes low productivity and high mortality, but chickens raised under these conditions is the most popular nationwide because the taste is favored in Vietnam (Burgos et al., 2007; T. H. H. Pham et al., 2007). Poultry products are primarily used for home consumption in self-sufficient farms and a small number of birds are sold at market or to neighbors when needed. This system is practiced in 85% of total households in Vietnam and contributes to more than 65% of the poultry products in the country (Van Duc Nguyen & Tran, 2008).

2.1.2.2. Semi-intensive, small to medium scale, market-oriented, commercial chicken production

Since 1990, some farm households switched from the traditional system to larger scale operations that are more commercially-oriented and combine traditional practices with more advanced technology (Van Duc Nguyen & Tran, 2008). Poultry are both kept in enclosures and/or are free to range in backyards, orchards and gardens (Burgos et al., 2007; Desvaux et al., 2008).

Breeds raised in this system are either indigenous or a mixture of indigenous and non-indigenous ones (Burgos et al., 2007). Some colored imported breeds are raised such as: Tam Hoang, Luong Phuong (China); Kabir (Israel); and Sasso, ISA Colour (France) (Desvaux et al., 2008; Van Duc Nguyen & Tran, 2008). This system has production cycles of around 70 to 90 days for broiler chickens (Burgos et al., 2007; T. H. H. Pham et al., 2007).

Besides naturally available feed resources such as worms, insects, pests, vegetables and grass during scavenging, birds are also fed with broken grains, home-food debris, feedstuffs such as brewery, soya waste and ensiled shrimp waste and/or commercial feeds (Burgos et al., 2007; T. K. D. Nguyen, 2005). Day-old-chicks are bought from national or private breeding centers for bird reproduction (Van Duc Nguyen & Tran, 2008).

Farmers involved in this operation mainly consist of former government employees, current local officers or wealthy farmers who have permanent income, some farming skills, and knowledge of marketing in particular (Burgos et al., 2007). They are also trained on how to feed their flock and employ veterinary services offered by the National Institute of Animal Husbandry and agricultural universities (Van Duc Nguyen & Tran, 2008). As a result, disease prevention and treatment measures are better practiced (Burgos et al., 2007), leading to lower mortality percentages of around 5 to 10% (Van Duc Nguyen & Tran, 2008). Output products are sold to various buyers such as brokers, wholesalers and consumers (Burgos et al., 2007).

In brief, this operation can be characterized by larger flock sizes, more investment in infrastructure, more technical improvements, and being more market-oriented, however bio-security is still at a medium level (Burgos et al., 2007; Desvaux et al., 2008; Van Duc Nguyen & Tran, 2008). In 2006, this system was in operation in about 15 to 20% of farm households and produced around 28% of Vietnam's chicken (Burgos et al., 2007; T. H. H. Pham et al., 2007).

2.1.2.3. Intensive, large scale, industrial chicken production

Modelled after modern industrial poultry systems found in OECD countries, these operations involve keeping birds indoors with semi-automatic or automatic well-equipped systems for internal feeding, nipple-enabled watering, centralized controls for humidity, ventilation, lighting, curtain movements and waste management and even remote monitoring and computerized controls (Burgos et al., 2007).

Many kinds of imported breeds with high-productivity are raised such as: AA (France), Ross 208, 308 and 508 (United Kingdom), Avian (Thailand), Lohmann (Germany), Cobb and Hubbard (United States).They have a short husbandry period of about 42-45 days (Burgos et al., 2007) so farmers can produce 4-5 batches per year (Van Duc Nguyen & Tran, 2008).

This operation with white commercial birds was promoted by multi-national agro-food conglomerates who provide some advanced technology, veterinary services and marketing assistance to large domestic farms as primary recipients through contracts (Burgos et al., 2007). With such optimal conditions, mortality often remains low around 5 to 7% in Vietnam (Van Duc Nguyen & Tran, 2008). Output products are marketed through three channels: brokers, foreign or domestic abattoirs, and marketing co-operatives. Foreign-owned abattoirs make up 45 to 50% of marketing (Burgos et al., 2007).

In sum, these operations are characterized by indoor automatic operating systems with centralized controls and optimal veterinary services (Burgos et al., 2007; Van Duc Nguyen & Tran, 2008).

2.2. Antimicrobial overview

2.2.1. Definition of an antimicrobial

Infectious diseases have always been lurking in the environment, threatening animal and human survival, driving mortality and morbidity up (Guardabassi et al., 2008). The discovery of the first antibiotic, penicillin, by Alexander Fleming in 1928, marked the advent of a revolutionary way of treating infections with antimicrobial drugs, saving numerous lives (Giguère et al., 2006).

According to the Nobel laureate S. A. Waksman, the term “antibiotic” refers to natural compounds originated from microbes, principally from bacteria and fungi, which are able to inhibit the growth of other microorganisms or kill other microorganisms in low concentrations (Guardabassi et al., 2008; Percival, Knottenbelt, & Cochrane, 2011). With industrialization, natural antibiotics have been modified to offer semi-synthetic (amoxicillin, doxycycline) and synthetic derivatives (sulfonamides, fluoroquinolones) (Giguère et al., 2006; Guardabassi et al., 2008; Percival et al., 2011), which often differ from their parent compounds in antimicrobial activities or pharmacological properties (Percival et al., 2011).

To encompass all synthetically, semi-synthetically, and naturally produced compounds, the term “antimicrobial” was preferred because of its broader definition over “antibiotic.” Antimicrobials able to kill other microbes or inhibit the growth of other microbes (such as bacteria, fungi, and protozoa) are called “microbiocidal” and “microbiostatic” respectively (Giguère et al., 2006; Guardabassi et al., 2008; Percival et al., 2011). Therefore, it is correct to say that an antibiotic is an antimicrobial agent, but the converse may not be true (Giguère et al., 2006). Nevertheless, in many publications, “antimicrobial” is often used as a synonym for “antibiotic” (Giguère et al., 2006). In the context of our study, the terminology “antimicrobial” will be used.

2.2.2. Antimicrobial classification

Numerous antimicrobials have been developed and are classified based on certain similar features (Giguère et al., 2006):

(1) Class of target microorganisms: if antimicrobials can actively fight bacteria, they are grouped into a class of antibacterial agents. Likewise, if antimicrobials can be very active against fungi or protozoa, they can be grouped into a class of antifungal or antiprotozoal agents, respectively. Furthermore, if an antibacterial agent is active against only bacteria, it is considered to be a narrow-spectrum antibacterial, but if it can inhibit bacteria, *mycoplasma (M), rickettsia*, and *chlamydia*, it is considered to be a broad-spectrum antibacterial agent (Giguère et al., 2006). More specifically, *mycoplasmas* are respiratory bacteria completely lacking cell walls and bounded by only a plasma membrane (Altman, 1997; Charlton & American Association of Avian Pathologists., 2006; Co., 2010; Saif & Barnes, 2008). *Chlamydia* are obligate intracellular bacterial parasites (Altman, 1997; Co., 2010) that cause systemic, respiratory, enteric infections in birds (Charlton & American Association of Avian Pathologists., 2006; Co., 2010). *Rickettsias* are also obligate intracellular bacterial parasites (Hackstadt, 1996; Ritchie, Harrison, Harrison, & Zantop, 1996; Weiss, 1982; Winkler, 1991), but are differentiated from *Chlamydia* by the absence of a developmental cycle and the ability for ATP-energy-rich compound synthesis (Ritchie et al., 1996). *Aegyptianella pullorum* is a strain of *Rickettsias* that can cause anemia and hepatitis for birds (Ritchie et al., 1996). For example, a class of antibacterial agents can consist of sub-classes such as beta-lactams (ampicillin, amoxicillin) or sulfonamides (sulfaquinoxaline, sulfadimidine), where beta-lactams are narrower-spectrum because they can inhibit only bacteria, and sulfonamides are broader-spectrum because they can inhibit bacteria, *mycoplasma, chlamydia* and protozoa (Dwight C. Hirsh, 2004; Giguère et al., 2006).

(2) Antibacterial activity: if an antibacterial agent can be active against only Gram-positive or Gram-negative bacteria or against primarily Gram-positive bacteria but still against some Gram-negatives, it is considered narrow-spectrum. In contrast, if an antibacterial agent can inhibit both Gram-positive and Gram-negative bacteria, it is considered broad-spectrum. For example, ampicillin and amoxicillin are narrow-spectrum while enrofloxacin and norfloxacin are broad-spectrum (Dwight C. Hirsh, 2004; Giguère et al., 2006).

(3) Bacteriostatic and bactericidal activity: an antibacterial agent can inhibit the growth of a specific bacterial pathogen at one concentration which is considered the minimum

inhibitory concentration (MIC), but it can also kill the same specific bacterial pathogen at a higher concentration which is considered the minimum bactericidal concentration (MBC) (Giguère et al., 2006; Percival et al., 2011; Scholar & Pratt, 2000). When there is a large dilution difference between MIC and MBC, the antibacterial agent is considered as bacteriostatic to a given pathogen. On the contrary, when there is a negligible dilution difference between MIC and MBC, the antibacterial agent is considered as bactericidal to a given pathogen (Giguère et al., 2006). However, the distinction is not absolute because it depends on the drug, concentration, type, quantity and growth rate of the microorganism (Carter & J.Wise, 2004; Dwight C. Hirsh, 2004; Giguère et al., 2006). For example, aminoglycosides and beta-lactams are often bactericidal, while tetracyclines are often bacteriostatic (Giguère et al., 2006). Another example of different activity is penicillin, which is more bactericidal against organisms in a rapidly growing state, but is much less bactericidal against organisms in a stationary state (Carter & J.Wise, 2004).

(4) Based on pharmacodynamic properties: antimicrobials can be classified into either concentration-dependent or time-dependent agents (Dwight C. Hirsh, 2004; Giguère et al., 2006). An antimicrobial agent is considered time-dependent if the rate of killing a target pathogen primarily depends on the length of time that the drug concentration in the serum of a given pathogen exceeds the MIC, but does not depend on the increase of drug concentration. In other words, if increasing the drug concentration is several-fold above the MIC, it does not significantly increase the microbial-killing rate. Thus, such antimicrobials will be of optimal efficacy if they are administered frequently within a sufficient dosing interval. Conversely, an antimicrobial agent is considered concentration-dependent if the rate of killing a target pathogen increases when the drug concentration in the serum of a given pathogen increases above the MIC. Thus, such antimicrobials will be optimally efficacious if they are administered in an acceptably high dose within a sufficient dosing interval. Some agents are both concentration- or time-dependent. For example, beta-lactams, macrolides, tetracyclines, and trimethoprim-sulfonamide combinations are time-dependent whereas aminoglycosides, fluoroquinolones are concentration-dependent (Giguère et al., 2006).

Additionally, in May 2007, at its 75th General Session, the International Committee of the World Organization for Animal Health (OIE) unanimously adopted a comprehensive list of veterinary antimicrobials. In my current research study, antimicrobials will be grouped into each class based on the list of veterinary antimicrobials established by OIE.

2.2.3. Antimicrobial use in poultry

Antimicrobial compounds are administered in poultry for three major reasons: growth promotion, disease prevention and treatment (A.R., 2008; Cromwell, 2005; Dang, 2010; Guardabassi et al., 2008; Singer & Hofacre, 2006).

First, with regard to antimicrobial growth promoters (AGPs), as early as the late 1940s, it was discovered that by-products fermented with tetracyclines enabled chickens to grow more rapidly (Guardabassi et al., 2008; Phillips et al., 2004). Over time, with the intensification of poultry production (Srivastava, 2010), other antimicrobial compounds were approved to add into animal feed as feed additives at low concentrations – called sub-therapeutic concentrations – for long durations to increase feed conversion and to enhance the performance of poultry (A.R., 2008; Barton, 2000; CDC, 2005; Cromwell, 2005; Dang, 2010; Dibner & Richards, 2005; Giguère et al., 2006; Guardabassi et al., 2008; Singer & Hofacre, 2006; Srivastava, 2010). Although the working mechanisms of AGPs are not completely clear, it is explainable on the basis of metabolism, nutrient absorption, and antibacterial activity against opportunistic microbes in the intestine (Srivastava, 2010). Namely, AGPs may reduce the thickness of cell walls to enhance nutrient digestibility or diminish nutrient competition between the host bird's normal intestinal flora and the host bird, and concurrently, may limit the colonization of harmful enteropathogens causing subclinical infections (Cromwell, 2005; Dibner & Richards, 2005; Hume, 2011; Huyghebaert, Ducatelle, & Van Immerseel, 2011; Srivastava, 2010). Making a decision to use AGPs for weight gain in poultry is primarily dependent upon economic factors (Giguère et al., 2006; Singer & Hofacre, 2006). According to the US's Code of Federal Regulations, title 21, antimicrobials approved as growth promotants in broiler chickens are: bacitracin, bambermycin, chlortetracycline, lincomycin, oxytetracycline, penicillin, tylosin and virginiamycin (Giguère et al., 2006; Singer & Hofacre, 2006) while all antimicrobial growth promoters were banned to use for poulty in EU since January 2006 (EU, 2005).

Second, with respect to antimicrobial use for prophylaxis, antimicrobial agents are administered at sub-therapeutic concentrations to birds as a preventative when pathogens are known to be present in the surroundings and when birds may be more susceptible to pathogenic infections (CDC, 2005; Council, 1999). Along with ubiquitous bacterial infections by *mycoplasma* in poultry, often prevented with tylosin or oxytetracycline (Council, 1999), antimicrobials are frequently used to prevent coccidiosis. Coccidiosis is caused by the protozoan genus *Eimeria,* and is widespread in poultry production (Saif & Barnes, 2008). Moreover, coccidiosis play a significant role in predisposing the occurrence of necrotic enteritis caused by *Clostridium perfringens* increasing mortality (Al-Sheikhly, 1980). Thus, prevention and control of coccidiosis with antiprotozoal agents and *Clostridium perfringens* - induced necrotic enteritis in broilers are imperative, especially in industrial scale industries (Council, 1999). The vast majority of coccidiostats are regulated by feed legislation as feed additives (Guardabassi et al., 2008). Sulfonamides are primarily used as coccidiostats for prophylactic and therapeutic use in poultry (Council, 1999; Giguère et al., 2006; Guardabassi et al., 2008; Sirdar, 2010) via feed additives and drinking water (Giguère et al., 2006; Guardabassi et al., 2008). However, sulfonamides may induce toxic effects such as bone marrow suppression, thrombocytopenia and depression of the lymphoid and immune function of birds, thus in the 1980s, a new group of coccidiostats called ionophores were added (Giguère et al., 2006). Ionophores can be used against coccidiosis and bacterial infections, for example, *Clostridium* infections (Giguère et al., 2006; Guardabassi et al., 2008), but ionophores are used almost exclusively for coccidiosis control in animals (Council, 1999; Giguère et al., 2006; Guardabassi et al., 2008). Moreover, due to their potent cardiovascular effects, ionophores are not used in human medicine (Bioagrimix, 2012; Guardabassi et al., 2008). The set of coccidiostats approved for broiler chickens by the US are: sulfonamides (sulfachloropyrazine, sulfamethazine, sulfadimethoxine, sulfamycin, sulfanitran, sulfaquinoxaline); ionophones (lasalocid, maduramycin, monensin, narasin, salinomycin) and others (amprolium, arsanilate, buquinolate, clopindol, dequinate, nequinate, nicarbazin, robenidine, zoalene) (Council, 1999). To prevent *Clostridium perfringens* - induced necrotic enteritis in broilers, it is important to prevent coccidiosis, as well as to use some feed additives such as virginiamycin, bacitracin and lincomycin (Merck Manual, 2012).

Third, with regard to the use of antimicrobials for therapy, antimicrobials are administered to birds when a disease has been identified in a flock (CDC, 2005; Singer &

Hofacre, 2006). Generally, therapy is done in order to target the pathogens after diagnosis and are governed in accordance with on-label or extra-label instructions but under prescription of veterinary professionals (Council, 1999). Antimicrobials used for disease treatment can rapidly produce therapeutic concentrations at a given site of infection within a sufficient length of time, reducing and eliminating the replication of target pathogens based on the host's specific and non-specific defense mechanisms, resulting in the termination of infections (Giguère et al., 2006). Therapeutic antimicrobials can be administered in various ways such as individual injection, direct oral administration, or via drinking water or feed to the whole flock (Guardabassi et al., 2008). In chickens, the drinking water route is the most popular because sick birds can stop eating but continue drinking (Giguère et al., 2006; Singer & Hofacre, 2006). On the basis of common diseases of poultry, a set of major antimicrobials used in birds are demonstrated as follows (Guardabassi et al., 2008):

(1) For dysbacteriosis mainly caused by *Clostridium* spp., necrotic enteritis mainly caused by *Clostridium perfringens*, and other clostridial infections, antimicrobials of choice include: benzylpenicillin, aminopenicillins (amoxicillin, ampicillin), and tylosin.

(2) For colibacillosis caused by *Escherichia coli* and salmonellosis caused by *Salmonella* spp, antimicrobials of choice include: potentiated sulfonamides, aminopenicillins, tetracyclines, colistin, spectinomycin, aminoglycosides and enrofloxacin.

(3) For mycoplasmosis caused by *M. gallisepticum*, *M. synoviae*, and *M. meleagridis*, antimicrobials of choice include: tiamulin, tetracyclines, lincomycin, macrolides and enrofloxacin.

(4) For *Ornithobacterium rhinotracheale* infections, antimicrobials of choice include: tiamulin, aminopenicillins, and tetracyclines.

(5) For *Staphylococcus* and *Streptococcus* infections, antimicrobials of choice include: benzylpenicillin, potentiated sulfonamides, aminopenicillins, tetracyclines, and macrolides.

(6) For fowl cholera, antimicrobials of choice include: potentiated sulfonamides, tetracyclines, spectinomycin and enrofloxacin.

(7) For *Pasteurella multocida* infections, antimicrobials of choice include: aminopenicillins.

(8) For infectious coryza caused by *Haemophilus paragallinarum*, antimicrobials of choice include: sulfonamides, potentiated sulfonamides, streptomycin, tetracyclines, lincomycin, spectinomycin, macrolides, and enrofloxacin.

2.2.4. Risks of antimicrobial use in food animals

Use of antimicrobial agents in food animals is associated with public health risks, including unwanted antimicrobial residues in meat, microbial resistance to antimicrobials and environmental problems (Lowenthal et al., 2000), which will be described below:

2.2.4.1. Antimicrobial residues in food

According to the Codex standard, the total residue of an antimicrobial compound in food encompasses the parent antimicrobial substance in addition to all the metabolites and impurities which remain in the food offered to the consumer. Using a radio-labelled drug, the amount of the total residue is expressed as the parent drug with the unit of mg/kg in food (Codex, 1993).

To control the amount of residual antimicrobials in food in order to ensure food safety for the consumer, the term "maximum residue limit" has been introduced. It is the maximum concentration of antimicrobial residues legally permitted or recognised as acceptable in or on food offered to the consumer (expressed in mg/kg on a fresh weight basis) (Codex, 1993).

One of the leading causes of the presence of antimicrobial residues in food is misuse of authorised veterinary drugs in animal production for any purpose regarding disease prevention, disease treatment or growth promotion (Botsoglou & Fletouris, 2001; Dang, 2010). This is explainable on the basis that antimicrobial residue is associated with the antimicrobial pharmacokinetic properties (Lees & Toutain, 2012). As opposed to the antimicrobial pharmaco-dynamic properties, which show how the antimicrobial agent behaves in the body, antimicrobial pharmacokinetic properties exhibit how the body behaves toward the antimicrobial agent in the body (Benet, 1984). In particular, antimicrobial pharmacokinetic properties consist of various processes: first, up-take via dissolution and absorption; second, distribution to the site of action; and third, elimination via metabolism (bio-transformation) and excretion (Dorrestein, van Gogh, & Rinzema, 1984). The excretion process is mainly via the liver and the kidneys (Berkow, Beers, &

Fletcher, 1997; Dorrestein et al., 1984; Goudah, 2009). It is needed to note that (1) the rate of absorption and distribution to tissues can vary from an antimicrobial to another because there are differences of physicochemical properties between antimicrobials; and (2) the antimicrobial metabolism and excretion process occur in parallel with the distribution to access the site of action (Merck Manual, 2012). Residues are more likely to be present in meat if antimicrobials are administered in an improper manner inconsistent with label instructions such as: larger doses, longer dosing duration, inappropriate route of administration, and application for non-approved species (Botsoglou & Fletouris, 2001; Giguère et al., 2006; Miller, 1997). Administering excessive doses for longer than recommended durations can slow down the rate of metabolism and elimination from the animal's body (Botsoglou & Fletouris, 2001; Chang et al., 2010). Relative to the routes of administration, a study on tissue residue level of moxifloxacin by route in broiler chickens was conducted with intramuscular and oral administration routes with the same dose of 5mg/kg body weight, once daily for 5 consecutive days. Results showed that 144 hours after the last dose, although residue of moxifloxacin was still detected in both the liver and kidneys in both routes, the amounts of moxifloxacin residue were higher in both the liver and kidneys with oral administration (Goudah, 2009).

As a potential factor for antibiotic residues, animal species and breed affect pharmacokinetics (Miller, 1997). This was confirmed by some studies where authors found a difference in the rate of amoxicillin elimination between pigs and poultry (Krasucka & Kowalski, 2010), and a difference in tissue residue concentration of florphenicol between Leghorn and Taiwan native chickens (Chang et al., 2010). In particular, after 60 hours from the last dose, florphenicol residue was still detected in kidneys of Taiwan native chickens, but was undetectable in kidneys of Leghorn chickens (Chang et al., 2010).

In particular, non-compliance with withdrawal times recommended on labels is considered a potential risk factor associated with antimicrobial residues (Botsoglou & Fletouris, 2001; Dang, 2010; Giguère et al., 2006; Miller, 1997). Withdrawal time is defined as the duration of time between the last administration of an antimicrobial agent for a given route of administration and the slaughter date, in order to ensure that residues fall below the maximum residue limits for food safety for consumers (Codex, 1993). Notably, medicated feeds at the end phase of the production cycle are associated with residue violations in marketed poultry meat (Giguère et al., 2006) because the withdrawal times of antimicrobial feed additives added to feeds are not properly observed. Besides, disease status may impact the pharmacokinetics of drugs, potentiating residues (Miller, 1997). Renal and liver diseases may diminish the biotransformation of drugs, lowering the elimination of drugs from the body, whereas presence of infection and/or inflammation may accumulate drugs at infection sites, leading to the prolongation of drug elimination (Botsoglou & Fletouris, 2001; Miller, 1997). Likewise, age is also the potential risk factor for residues because they are associated with kidney and liver functions, thus affecting drug metabolism and clearance (Botsoglou & Fletouris, 2001).

Additionally, another potential cause of antibiotic residues in meat is unintentional antibiotic contamination from coming into contact with antibiotic-contaminated surroundings (Dang, 2010). In particular, drinking water that is used for animal husbandry on farms and processing streams at slaughterhouses can be contaminated with antibiotics, creating the opportunity for acquiring accidental residues (Botsoglou & Fletouris, 2001; Kemper, 2008; National Academy of Argicultural Sciences, 2010). One more possible cause of antibiotic residues is that antibiotics are often intentionally and directly added to food to inhibit the growth of microbes for food preservation (Dang, 2010; Deatherage, 1957; Dols, 1968).

The presence of antimicrobial residues in foods brings public attention to their detrimental consequences, including toxicology, microbiology, immunology, pharmacology-related health risks and others (Botsoglou & Fletouris, 2001). In fact, the composition of intestinal bioflora needs to be relatively stable to metabolize endogenous compounds such as estrogens, vitamins, cholesterol and bile acids, as well as to inhibit the colonization of pathogens in the host (Botsoglou & Fletouris, 2001). When the indigenous gastro-intestinal micro-flora is disturbed by drug residues, metabolism could be altered (Giguère et al., 2006) and the flora's colonization resistance could be compromised, facilitating the invasion and colonization of enteric pathogens, leading to abnormally soft stools, diarrhea, colitis or septic conditions, especially in immune compromised individuals (Codex, 2009; Goldstein, 2011).

More noticeably, antimicrobial residues can cause allergic reactions when people are exposed to antimicrobial agents that result in antigen-antibody interactions (Codex, 2009; Giguère et al., 2006). Allergenicity can manifest in many ways, from life-threatening anaphylactic reactions to lesser reactions such as rashes, especially in immunological supersensitive persons. Among allergens, penicillin is the most frequently cited as triggering allergic reactions (Botsoglou & Fletouris, 2001). For example, there was a case in the early 90s in the United Kingdom where someone's allergic symptoms, which included facial and peripheral oedema with widespread urticaria, was due to the consumption of chicken meat with penicillin residue (Teh & Rigg, 1992). Allergic reactions to other drugs such as streptomycin, tetracyclines, sulphonamides, aminoglycosides, and β-lactams have been documented with sensitized individuals (Botsoglou & Fletouris, 2001; Giguère et al., 2006).

Adversely threatening human health, chloramphenicol, a broad-spectrum antibiotic, has been implicated as a causative agent in many cases of fatal aplastic anemia because chloramphenicol-suppressed bone marrow cannot produce a sufficient number of new cells to supplement blood cells (Ambekar et al., 2000; Botsoglou & Fletouris, 2001; Chylinski & Zegarski, 1971; Hartzen & Frimodt-Moller, 1986; Lokhande, Juvekar, & Kulkarni, 2007; Taka, Baras, & Chaudhry Bet, 2012). It has also been noted that adverse effects caused by chloramphenicol are non-dose-related and could potentially be induced at extremely low concentrations in food (Botsoglou & Fletouris, 2001; Turton, Andrews, Havard, & Williams, 2002). In fact, it was documented that a 73-year-old woman died after receiving only 82 mg chloramphenicol as an ophthalmic antibiotic. A small dose like this may be found in foods destined for human consumption, potentiating adverse effects on human health (Botsoglou & Fletouris, 2001). Until recently, it was controversial whether chloramphenicol-associated bone marrow was a toxigenic or allergenic effect (Botsoglou & Fletouris, 2001). Due to adverse risks to public health, chloramphenicol has been prohibited for use in animal production (Botsoglou & Fletouris, 2001; Polzer, Hackenberg, Stachel, & Gowik, 2006; Taka et al., 2012; Wesongah et al., 2007; Zeleny, Emteborg, & Schimmel, 2010).

As regards to effects caused by antibiotic residue, residues could induce pharmacological effects on consumers. In reality, the prolonged intake of tetracyclines from any source has serious effects on growing children's teeth and bones (Billings, Berkowitz, & Watson, 2004; Botsoglou & Fletouris, 2001; Sloan & Scheinfeld, 2008; Volovitz et al., 2007).

From the standpoint of other potential risks caused by antimicrobial residues, their presence in food may cause chronic toxicity such as carcinogenicity (A.R., 2008; Botsoglou & Fletouris, 2001; Codex, 2009). For instance, nitrofurans (furazolidone, furaltadone, nitrofurantoine, nitrofurazone, nifursol), which are antibacterials effectively used in pig and poultry productions, were classified as genotoxic carcinogens (Botsoglou & Fletouris, 2001). In fear of the carcinogenic effects by parent nitrofurans or their metabolites on human health, nitrofurans were banned for use in livestock production in many countries including the European Union (EU) in 1993 (Barbosa et al., 2011; Barbosa, Freitas, Mourao, Noronha da Silveira, & Ramos, 2012; McCracken, Van Rhijn, & Kennedy, 2005; Verdon, Couedor, & Sanders, 2007).

Finally, antimicrobial residues in foods can encourage the emergence of bacteria resistant to antimicrobial agents, especially those also used for humans. These antimicrobial-resistant bacteria can be transmissible to humans through airborne or food-borne pathways, causing diseases resistant to antibiotic treatment and therefore increasing morbidity and mortality (Council, 1999; Giguère et al., 2006; Guardabassi et al., 2008; Singer & Hofacre, 2006).

2.2.4.2. Microbial resistance to antimicrobials

Despite being an indispensable tool against infectious diseases, antimicrobial treatments for animals or humans can fail because some bacteria can survive under the selective pressure created by some antimicrobial exposure. Because of the ability to neutralize or evade the efficacy of a normally active concentration of an antimicrobial, one bacterium can remain alive and multiply, quickly replacing all the bacteria killed off and causing treatment failures (CDC, 2005; Giguère et al., 2006; Guardabassi et al., 2008; Singer & Hofacre, 2006). This microbial resistance to antimicrobial agents is a natural and unavoidable consequence of antimicrobial use (Guardabassi et al., 2008). Each species follows the Darwinian principle of "survival of the fittest" and adapts to the conditions in which they are living (Guardabassi et al., 2008; Vaarten, 2012). In other words, microbes can undergo an evolutionary process under the selective pressure induced by co-existence with antimicrobial agents (Shea, 2003; Vaarten, 2012).

In reality, there exist three fundamental phenotypes for bacteria: susceptibility, intrinsic resistance, or acquired resistance (Giguère et al., 2006; Soares et al., 2012). Intrinsic resistance is natural, resulting from a specific bacterial group's inherent structural or biochemical characteristics. For instance, the cell walls of Gram-negative bacteria are so small that macrolides cannot penetrate and reach their cytoplasmic target, making them naturally resistant to these agents (Giguère et al., 2006). Acquired resistance results from the genetic change of a normally susceptible microbe (Giguère et al., 2006) by vertically transferring housekeeping-genes through random mutation and clonal spread or by horizontally acquiring foreign genes from other microbes (Beovic, 2006; Frere et al., 1991; Guardabassi et al., 2008; Mazel & Davies, 1999).

Acquired resistance displays its biochemical mechanisms through the following five major ways: (1) hindering antimicrobials from reaching the intracellular action site by fostering efflux or impeding influx through the cell membrane with various kinds of pumps within the same organism; (2) lowering the permeability of the outer cell membrane by modifying the composition of the cell membrane, reducing uptake of antimicrobials; (3) inactivating antimicrobials by enzymes produced by the bacteria; (4) disabling an antimicrobial agent by changing the existing action site in which an antimicrobial agent can interfere such as: cell wall synthesis, cell membrane synthesis, bacterial protein and folic acid synthesis, or nucleic acid metabolism; and (5) developing by-pass mechanisms by duplicating the target action site (Acar & Moulin, 2012; Dever & Dermody, 1991; Mazel & Davies, 1999; Savjani, Gajjar, & Savjani, 2009; Sefton, 2002; Tenover, 2006; Wright, 2011).

Resistance genes are encoded on chromosomal DNA or more commonly on extra chromosomal DNA (plasmids). Acquisition of resistance genes is undertaken through clonal or horizontal gene transmission (CDC, 2005; Giguère et al., 2006; Shea, 2003). Horizontal transmission of resistance determinants is more commonly associated with the development of resistance (Walsh & Fanning, 2008). Mobile gene elements harbouring resistance genes such as plasmids, transposons and integrons are horizontally transferred between species via three main methods: (1) transformation: through the growth medium, a short fragment of free exogenous DNA moves from the transformable donor to the recipient's cell cytoplasm to integrate into the recipient's genome; (2) transduction: bacterial DNA is inserted into the recipient bacterial through a bacteria-infecting virus vector, namely a bacteriophage. The transduction is often limited to closely related bacteria; and (3) conjugation: bacterial DNA is directly transferred from the donor to the recipient through a bridge established at the time of physical cell-to-cell interaction. After genes are exchanged, they separate and the transconjugant recipient continues acting as a donor of resistance genes to other microbes. Among these modes, conjugation is the most significant because integrative and conjugative elements are long fragments of DNA located on mobile gene elements like plasmids, transposons and integrons and conjugation can occur on a broader range of hosts, including distantly related bacteria (Acar & Moulin, 2012; Apata, 2009; Chee-Sanford et al., 2009; Dröge, Pühler, & Selbitschka, 1999; Guardabassi et al., 2008).

Resistance genes can be grouped on self-transmissible mobile genetic elements and can cluster together in a single package. Thus, horizontal gene transference facilitates the recipient microbe's ability to resist unrelated multi-antimicrobials. A bacterium that is able to resist more than one antimicrobial is considered multi-resistant (CDC, 2005; Guardabassi et al., 2008; Shea, 2003). Multi-resistance may result from intrinsic resistance to multiple antimicrobial agents, or acquired cross- or co-resistance (Werckenthin et al., 2005). Cross-resistance is microbial resistance to two or more structurally-related-antimicrobials belonging to the same antimicrobial class with the condition that a single resistance gene or a mutation is present in a bacterium (Guardabassi et al., 2008; Werckenthin et al., 2005). Unlike cross-resistance, co-resistance is the microbial resistance to two or more antimicrobials belonging to different antimicrobial classes with the condition that distinct genes or mutations are present in the same bacterium (Canton & Ruiz-Garbajosa, 2011; Guardabassi et al., 2008; Marshall & Levy, 2011).

As is well-documented, the use of antimicrobial compounds in food animals is associated with the emergence of resistant microbes and genes (Aarestrup, 1999, 2004, 2005; Aidara-Kane, 2012; Sundsfjord & Sunde, 2008; Tollefson & Karp, 2004; Tollefson & Miller, 2000). The drug-resistant microbes and genes can be transmitted to humans in a variety of pathways encompassing: (1) direct transference by direct contact between animal carriers and farmers, farm workers, veterinarians and meat producers; (2) food-borne transference by exposure to contaminated foods; (3) environmental transference by contacting contaminated environments such as soil, water and air (Aidara-Kane, 2012; Guardabassi et al., 2008; Marshall & Levy, 2011).

Resistant pathogens may have consequences on human health. Non-pathogenic microbes, such as commensal and environmental bacteria, can serve as vast reservoirs of resistance genes received from pathogenic and non-pathogenic microbes alike, and can become donors of resistance genes for human pathogens in turn (Aidara-Kane, 2012; Guardabassi et al., 2008). They place a burden on public health, causing "infections which would not otherwise have occurred if the pathogens were not resistant." (Angulo, Nargund, et al., 2004; Guardabassi et al., 2008; Wassenaar, 2005). In particular, while an individual is taking an antimicrobial agent for treating the disease caused by a specific pathogen, foreign pathogens (e.g. a *Salmonella* strain secondary to the initial infection) can penetrate that individual's body via *Salmonella*-contaminated foodstuffs. The *Salmonella* strain, which had once been susceptible to the same therapeutic antimicrobial agent that the individual is taking, is encouraged to colonize under the selective pressure induced by the current antimicrobial use - also because the intestinal micro-flora is disturbed by the antimicrobial - resulting in clinical salmonellosis. In other words, the infection caused by the *Salmonella* strain would not have occurred if the *Salmonella* strain had not been resistant to the antimicrobial agent being used to treat the initial infection (Angulo, Nargund, et al., 2004; Guardabassi et al., 2008; Tollefson & Karp, 2004). The treatment may then fail or be prolonged or more costly, especially for populations at high-risk (Angulo, Nargund, et al., 2004; Collignon, 2012). These negative effects can be augmented when the same antimicrobials being used in food animals are prescribed for humans (Angulo, Baker, et al., 2004). It has been documented that resistant pathogens are selected during antimicrobial use in animals and transferred to humans (Guardabassi et al., 2008), in particular the zoonotic pathogens *Salmonella*, *Campylobacter* and the so-called indicator microorganisms such as *Enterococci* and *E.coli* (Angulo, Nargund, et al., 2004; Beovic, 2006). Antimicrobial consumption in both human beings and animals is associated with the emergence of microbial resistance to antimicrobials (Beovic, 2006), although antimicrobial use in humans is believed to be a bigger contributing factor than their use in animals

(Compassion in World Farming, 2011). The relative importance of antimicrobial use in animals in terms of contributing to the emergence of antimicrobial resistance in human pathogens, is still being debated (Beovic, 2006; Guardabassi et al., 2008; McDermott et al., 2002).

2.2.4.3. Environmental effects

The presence of antimicrobial agents and resistance bacteria in the environment originating from human and veterinary medicine has the potential to cause problems within both the environment and public health in general. The bio-chemical agents can enter the environment through the application of animal manure and slurry on agricultural fields as fertilizers (Kemper, 2008). Antimicrobial agents enter the environment via non-metabolized/unabsorbed antimicrobials and their metabolites in the urine and feces of animals (National Academy of Argicultural Sciences, 2010). These contaminants can spread in the air when their medium dries or travel down to water tables, thus being present not only on land and in surface water but in ground water (Kemper, 2008).

The presence of antimicrobials in soil potentiates the imbalance of indigenous soil microbial communities by changing their composition and diversity, leading to a reduced rate of organic matter decomposition in soil (Ding & He, 2010; Martinez, 2008; National Academy of Argicultural Sciences, 2010). Moreover, the presence of antimicrobial-related agents in terrestrial and aquatic environments and even in sources of drinking water can compromise water quality for humans and food safety of produce (Kemper, 2008; National Academy of Argicultural Sciences, 2010). That these residuals can be air-borne on dust also represents potential allergen risks to human beings (Kummerer, 2008).

More notably, antimicrobial-related agents can persist in the environment for long duration depending on the bio-physicochemical properties of antimicrobials, temperature and other edaphic factors (Kumar, 2005). These agents can encourage the emergence of resistance bacteria when bacteria and antimicrobial agents coexist (Martinez, 2008; Shea, 2003; Zhang, Zhang, & Fang, 2009). Thus, together with resistance bacteria originated from animal husbandry, non-pathogens including commensal and environmental bacteria can serve as a potential reservoir of mobile resistance gene elements for pathogens through horizontal gene transfer, leading to the potential consequences of multi-resistance for both animals and humans (Canton, 2009; Kemper, 2008).

2.2.5. Recommendations for limiting antimicrobial residues and resistance

2.2.5.1. Antimicrobial use principles

The goals of antimicrobial use are to anticipate infectious pathogens or to eliminate them from the host for disease prevention or treatment. In an effort to tackle the problems of antimicrobial residues in food and microbial resistance by reducing the use of antimicrobials while maximizing their medical efficacy, some fundamental principles for prophylactic and therapeutic use in veterinary practices should be followed:

(1) Disease prevention is better than a cure: it has been noted that antimicrobial use for prophylaxis should never be practiced as a substitute for poor preventive veterinary measures (Guardabassi et al., 2008). Therefore, preventive measures should be improved to enhance host immunity and prevent contagious pathogens from entering the flocks. In order to reduce the risk of infections, management and bio-security measures should be exercised that encompass: reducing live-bird transport time to minimize stress; separating new chicks from old birds; using an all in-all out system; restricting animal and human visitors; sanitizing feeders, watering troughs, bird houses and local surroundings; ensuring good manure waste management; isolating sick birds; preventing stress by reducing flock density; avoiding abnormal changes of ambient conditions such as temperature, light, humidity; and ensuring the good health of farm workers. At the same time, host immunity should be improved through high-quality feeds, good drinking water, nutrients, vitamins and minerals, and good vaccination practices (Guardabassi et al., 2008; Knechtges, 2012). Prophylactic use of antimicrobials should be employed at recommended levels (Guardabassi et al., 2008).

(2) Identification of pathogens at a given site of infection: when any pathogen is introduced to the host at an infection site, ideally, both clinical and laboratory diagnosis should be conducted to identify which pathogens are the causal infectious microorganisms (Altman, 1997; Giguère et al., 2006; Guardabassi et al., 2008; Ritchie et al., 1996). When

an outbreak spreads with high mortality, empirical therapy based on clinical diagnosis may be initiated, however it has been noted that empirical treatment should be avoided whenever possible (Guardabassi et al., 2008).

(3) Antimicrobial susceptibility of causal infectious pathogens: to predict which antimicrobials can be efficaciously administered to the diseased host, veterinary practitioners should base treatment plans on previous profiles of the antimicrobial susceptibility of corresponding pathogens. However, when the antimicrobial susceptibility of causal infectious pathogens cannot be predicted, antimicrobial susceptibility of pathogens should be tested at laboratories to identify which antimicrobials would be efficacious against pathogens (Altman, 1997; Giguère et al., 2006; Guardabassi et al., 2008; Ritchie et al., 1996).

(4) Selection of appropriate antimicrobials: with accurate diagnosis, veterinary professionals must ascertain on a case by case basis when no alternatives to antimicrobials can be given and when therapeutic use is necessary. Antimicrobials should be selected for administration on the basis of susceptibility, ability to reach an adequate concentration at a given infection site, toxicity to the host, risk for the spread of resistance, and cost of treatment (Altman, 1997; Giguère et al., 2006; Guardabassi et al., 2008; Ritchie et al., 1996). Thus, an antimicrobial agent is deemed ideal if it can eliminate the susceptible pathogen by reaching an effective concentration at a given infection site, and if it causes no toxicity and minimal stress to the host, minimizes resistance of the pathogen, and is cost-effective (Giguère et al., 2006).

(5) Antimicrobial treatment in compliance with label instructions: antimicrobial agents should be administered at recommended doses and at sufficient dosing intervals, treatment durations, and via approved administration routes as instructed on labels to eliminate infectious pathogens. An exceptional off-label use should only be undertaken when prescribed by veterinary professionals (Guardabassi et al., 2008). It has also been noted that withdrawal times must be observed to assure that meat is clear of antimicrobial residues (Giguère et al., 2006; Guardabassi et al., 2008).

2.2.5.2. Alternatives for antimicrobial growth promoters

Although AGPs make a tremendous contribution to enhancing the growth of birds and also to preventing diseases in intensive poultry productions, the use of growth promoters is considered an important potential risk factor in the emergence of antimicrobial resistance of enteric microbes in animal intestines, which can become communicable to humans through air-borne or food-borne pathways (Cromwell, 2005; Singer & Hofacre, 2006). It was recommended that therapeutic antimicrobials destined for humans should not be approved for animal growth promotion in the Swann report to the British Parliament in 1969 (Cromwell, 2005; Dibner & Richards, 2005; Guardabassi et al., 2008). This recommendation became the foundation for many countries for their consideration on either to abandon or limit the use of growth promoters to minimize the spread of antimicrobial resistance. In January 2006, the EU released a total ban on the use of all remaining in-feed antibiotics (avilamycin, flavophospholipol) as growth promoters in animal feed for poultry. This came after a ban on avoparcin in 1997 and a ban on virginiamycin, bacitracin, spiramycin and tylosin in 1999 (EU, 2005; Guardabassi et al., 2008). Although the US and other developing countries have not introduced the same restrictions as the EU, the use of growth promoters worldwide has followed a downward trend (Guardabassi et al., 2008). This is why non-antimicrobial additives are considered necessary as replacements for AGPs. Ideally, non-antimicrobial alternatives should have the same beneficial characteristics as AGPs (Huyghebaert et al., 2011). However, from a practical view, it is required that any alternatives have the same main attribute of AGPs, namely the ability to enhance the growth of animals (Huyghebaert et al., 2011). Such alternatives encompass:

(1) Probiotics are non-pathogenic living microorganisms that are either single or multiple microbial species. They are beneficial to the host by strengthening the intestinal micro-flora to be antagonistic against potential enteropathogens when taken in adequate amounts by the host (Ahmad, 2006; Fuller, 1989; Huyghebaert et al., 2011; Knechtges, 2012; Perié, Zikié, & Lukié, 2009). These favorable microorganisms can be derived from certain species of bacteria, fungi and yeasts, and be classified into 2 categories: colonizing (*Lactobacillus* spp., *Enterococcus* spp., and *Streptococcus* spp.) and free flowing, non-colonizing species (*Bacillus* spp. and *Saccharomyces cerevisiae*) (Huyghebaert et al., 2011; Patterson & Burkholder, 2003; Perié et al., 2009). Some mechanisms of action have been

reported: probiotics can inhibit the development of enteropathogens by producing bacteria-killing proteins called bacteriocins and by creating a restrictive environment due to a lowering of the pH though the production of organic acids; and probiotics can increase competition for nutrients and receptor sites in the intestinal mucous membranes, weakening the ability of enteropathogens to take hold (Ahmad, 2006; G.Piva & F.Rossi, 1998; Hume, 2011; Huyghebaert et al., 2011; Perié et al., 2009).

(2) Prebiotics are organic nutrients - not viable micro-organisms like probiotics - that can be added to feeds to promote the growth of certain beneficial bacteria antagonistic to the development of potential enteropathogens. It has been confirmed that prebiotics are not digested by the host (Hajati & Rezaei, 2010; Hume, 2011; Huyghebaert et al., 2011; Knechtges, 2012; Perié et al., 2009). The main components of prebiotics are oligosaccharides and any of hexose monosaccharides (such as: glucose, fructose, galactose, and mannose) (Hume, 2011; Huyghebaert et al., 2011; Perié et al., 2009).

(3) Synbiotics are mixtures of probiotics, prebiotics and other promoting agents to create the combined effect of minimizing potential enteropathogens and ultimately promoting weight gain for poultry (Perié et al., 2009).

(4) Enzymes are supplemented into feed to catalyse the metabolism of poor nutritive or indigestible feedstuffs and increase feed conversion for poultry (Perié et al., 2009). These days, almost all broiler feed contains enzymes such as xylanases and beta-glucanases to break down non-starch polysaccharides in feedstuffs which are soluble at a low rate (Huyghebaert et al., 2011).

(5) Acidifiers are organic acids supplemented into feed to create a restrictive environment against potential enteropathogens by reducing pH. They include butyric acid, formic acid, fumaric acid, etc. (Huyghebaert et al., 2011; Perié et al., 2009).

(6) Antioxidants are agents to prevent the formation of free radicals, which are very malicious to cells and cause the disruption of cell integrity. They include vitamin E, selenium, carotinoids, etc. (Perié et al., 2009).

(7) And finally, phytobiotics (phytotherapeutics, phytogenic additives) are medical plants, herbs and spices, plant extracts, and essential oils (Hume, 2011; Huyghebaert et al., 2011; Perié et al., 2009) with specific bio-active components and antibacterial properties to

reduce the development of pathogens by promoting the production of digestive secretions to improve feed conversion, exerting antioxidant qualities, and potentially improving host immunity (Hume, 2011; Huyghebaert et al., 2011; Perié et al., 2009). For example, the *Aloe* species (*Aloe* vera and *Aloe* spicata) is used to take care of chickens in Zimbabwe (Mwale, Bhebhe, Chimonyo, & Halimani, 2005).

Overall, the list of non-antimicrobial alternatives has the potential to grow, and these alternatives are promising in the context of improving poultry's performance to benefit the poultry producer and ensure food safety for the consumer.

2.3. Antimicrobial use in poultry in Vietnam

2.3.1. Vietnamese regulations on antimicrobial use in poultry

Together with national regulations on poultry husbandry practices such as: (1) Good Animal Husbandry Practices for Poultry in Vietnam (VIETGAHP) (Decision No. 1504/QĐ-BNN-KHCN dated 15 May 2008) and (2) compulsory vaccination of cattle and poultry (Decision No. 63/2005/QĐ-BNN dated 13 October 2005), the Ministry of Agriculture and Rural Development also promulgated the national regulations on antimicrobial use in food-animal production consisting of:

(1) Animal feeding stuffs - Maximum levels of antibiotics, drugs, microorganisms and heavy metals in completed feeds for chickens shown in QCVN 01 - 10: 2009/BNNPTNT in Circular No. 81/2009/TT-BNNPTNT dated 25 December 2009. Some details are showed in Table I.

(2) List of food safety criteria and maximum level corresponding to each criterion applied to animal-originated foods either imported or domestically produced within the ministry's authority in Circular No. 29/2010/TT-BNNPTNT dated 6 May 2010. Accordingly, maximum residue limits were regulated for antimicrobial residues in poultry products.

(3) List of drugs, chemicals and antimicrobials prohibited or restricted for use in agriculture in Circular No. 15/2009/TT-BNN dated 17 March 2009. Afterwards, some details in this circular were amended by Circular No. 29/2009/TT-BNNPTNT dated 6 June

2009 and 20/2010/TT-BNNPTNT dated 2 April 2010. The two lists relevant to the extent of this study are: (1) List of drugs, chemicals, and antibiotics banned for veterinary use in all species (excluding aquaculture) (Table II); and (2) List of drugs, chemicals and antibiotics limited for veterinary use in all species (excluding aquaculture) (Table III).

Table I. Maximum level of antibiotics and other veterinary drugs in completed feeds for broiler chickens in Vietnam

Antimicrobials and veterinary drugs	**Approved maximum dose (mg/kg)**	**Withdrawal time of medicated feed before slaughter (day)**
Amprolium	250	0
Axit Arsanilic	90	0
BMD (Bacitracin Methylene-Disalicylate)	50	0
Bacitracin Zinc	50	0
Chlortetracyline	50	0
Clopidol	250	5
Decoquinate	30	0
Lasalocid sodium	113	3
Lincomycin	4	0
Monensin	110	0
Narasin/Nicarbazin	72	5
Nitarsone	187	0
Oxytetracyline	50	0
Roxarsone	50	0
Salinomycin	60	0
Sulfadimethoxine + Ormetoprim 5:3	113	0
Tylosin phosphate	50	0
Virginiamycin	5	0
Zoalene	113.5	0

Table II. List of drugs, chemicals, and antibiotics banned for veterinary use in all species excluding aquaculture in Vietnam

No.	Chemicals, Antibiotics
1	Chloramphenicol (Chloromycetin, Chlornitromycin, Laevomycin, Chlorocid, Leukomycin)
2	Furazolidon and Nitrofuran derivatives (Nitrofuran, Furacillin, Nitrofurazon, Furacin, Nitrofurantoin, Furoxon, Orafuran, Furadonin, Furadantin, Furaltadon, Payzone, Furazolin, Nitrofurmethon, Nitrofuridin, Nitrovin)
3	Dimetridazole (Emtryl)
4	Metronidazole (Trichomonacid, Flagyl, Klion, Avimetronid)
5	Dipterex (Metriphonat,Trichlorfon, Neguvon, Chlorophos, DTHP); DDVP (Dichlorvos; Dichlorovos)
6	Eprofloxacin
7	Ciprofloxacin
8	Ofloxacin
9	Carbadox
10	Olaquidox
11	Bacitracin Zn*
12	Green Malachite (Malachite)
13	Gentian Violet (Crystal violet)

* Bacitracin Zn is banned to treat diseases but still approved to be used as a feed additive in completed feeds for chickens in Vietnam.

Table III. List of drugs, chemicals, and antibiotics limited for veterinary use in all species excluding aquaculture in Vietnam

No.	Chemicals, Antibiotics
1	Improvac (no. of registration: PFU-85 by Pfizer Australia Pty Limited)
2	Spiramycin
3	Avoparcin
4	Virginiamycin
5	Meticlorpidol
6	Meticlorpidol/Methylbenzoquate
7	Amprolium (powder)
8	Amprolium/ethopate
9	Nicarbazin
10	Flavophospholipol
11	Salinomycin
12	Avilamycin
13	Monensin

2.3.2. Antimicrobial use, residues and resistance in poultry in Vietnam

To make it easier to present information on antimicrobial use, residues and resistance in poultry in Vietnam, the following is a list of previous studies organized by region from the most recent to the least.

First of all, a cross-sectional study on antimicrobial use in chicken productions in Northern Vietnam using a questionnaire was conducted with a total of 210 chicken production entities with three different production systems consisting of household farms, semi-industrial farms and industrial farms in three provinces of Hai Duong, Thai Binh and Ha Noi between July 2009 and March 2010. The results showed that antimicrobial compounds were used not only for disease treatment and prevention, but also for growth promotion as feed additives. For disease treatment and prevention, selection of antimicrobial agents was mainly based on the farmer's personal experience. A group of the common antimicrobials for preventive and curative purposes in chickens involved: gentamicin (8.1%), amoxicillin (8.1%), ampicillin (21.0%), enrofloxacin (9.0%), tylosin (16.7%), sulfachloropyrazine (15.2%), sulfaguanidine (8.1%), doxycycline (7.1%), oxytetracycline (6.7%), tetracycline (8.6%), colistin (31.4%), diaveridin (6.2%), and trimethoprim (15.2%). Moreover, chloramphenicol, a prohibited antimicrobial, was found in the survey (2.4%). In addition, for growth promotion and coccidiosis prevention, a set of antimicrobials used as feed additives encompassed: chlortetracycline (13.8%), tetracycline (2.4%), maduramicin (1.4%), monensin (2.9%), salinomycin (18.1%), bambermycin (1.9%), bacitracin methylene-disalicylate (1.9%), colistin (2.9%) and diclazuril (4.3%) (Vu et al., 2010).

Recently, also in Northern Vietnam, another study on prevalence of antimicrobial residues in chicken meat was conducted as a Master's degree in Veterinary Science. A total of 75 chicken meat samples (15 samples/province) were randomly collected from markets and slaughterhouses in five provinces of Ha Noi, Hai Duong, Thai Binh, Nam Dinh and Ninh Binh in 2010. First, all of the 75 chicken samples were screened with an Enzyme-Linked ImmunoSorbent Assay (ELISA) test, then quantitatively analysed with High Performance Liquid Chromatography (HPLC) for three different antimicrobials: Tylosin, Enrofloxacin, and Streptomycin. The results were that tylosin was present in 60/75 carcass meat samples (80.0%) and detected to be prevalent in all of the five provinces, but only 2/75 samples (2.7%) were above a national legal Maximum Residue Level (MRL). Enrofloxacin was detected at much lower levels with 3/75 samples (4.0%) positive from only two provinces, Ha Noi and Thai Binh, and no samples were above MRL. Streptomycin was positively tested in 31/75 samples (41.0%) and detected to be prevalent in all the five provinces, but no samples were above MRL (Dang, 2010).

Furthermore, regarding antimicrobials used as feed additives, a recent study on prevalence of in-feed antibiotics in chicken feed was conducted in Northern Vietnam by Luong (2010). A total of 60 feed samples (20 samples/province) were collected from feeding troughs on farms in three provinces of Thai Binh, Vinh Phuc, and Hung Yen. All the feed samples were quantitatively tested for five different antibiotics: oxytetracycline and chlortetracycline were tested with HPLC and enrofloxacin, sulfamethazine, and tylosin with ELISA. Results indicated that there were three in-feed antibiotics, oxytetracycline, sulfamethazine, and tylosin found to be prevalent in Thai Binh in comparison with chlortetracycline, sulfamethazine, and tylosin that were detected in Hung Yen. Vinh Phuc was revealed to be using the same three antibiotics used in Hung Yen in addition to oxytetracycline. Enrofloxacin was not detected in any samples (Luong, 2010).

In regard to the antimicrobial use in Southern Vietnam, some previous studies on antibiotic use in broiler chickens in Ho Chi Minh city were conducted about 10 years ago (2000-2001). To summarize these five studies, the antibiotics commonly used in broiler chicken production in Ho Chi Minh city involved: colistin, enrofloxacin, diaveridin, sulfadimidine, trimethoprim, norfloxacin, oxytetracycline, gentamicin, oxolinic acid, sulfadiazine, flumequine, ampicillin, erythromycin, and tylosin (P. S. Huynh, 2000; T. N. D. Huynh, 2000; V. A. Nguyen, 2001; V. D. Nguyen, 2001; Vo, 2001). In a different province, a previous study in 2001 concluded that the five most common antibiotics in chicken broiler production in Binh Duong province included enrofloxacin, norfloxacin, diaveridin, trimethoprim, and colistin (Q. D. Pham, 2001).

Noticeably, another interesting study on the quality of veterinary drug products being marketed in Vietnam was conducted in 2008. A total of 280 drug samples including vitamins and minerals, antibiotics and other veterinary drugs were randomly collected from either manufacturers or veterinary pharmacy stores (139 samples in Northern Vietnam and 141 samples in Southern Vietnam), in which there were 178 samples for antibiotics only. These samples were quantitatively tested with HPLC. The findings were that 14.6% of antibiotic products were somewhat different than the described label, with either a lower or higher concentration of active compounds, a complete lack of active elements, or the presence of unlabelled active elements (Van Diep Nguyen, 2008).

From an antimicrobial resistance perspective, there were 2 previous studies on prevalence of antibiotic resistance of the two bacterial types, *Salmonella* (*S*) *enterica* and *Campylobacter* (*C*) spp. respectively, in poultry and raw meat (Nilsson, 2009; Schwan, 2010). Both of the studies were launched in Can Tho province, Southern Vietnam during six weeks in October to November in 2008 with the same size of samples taken on local farms and markets. In each study, there were a total of 96 samples of raw chickens collected from 12 markets and each market was sampled once. Additionally, there were a total of 96 rectal swabs collected from 20 local chicken farms. According to the results of the first study by Nilsson (2009), only one market sample was positive with a *Salmonella enterica* species, namely *S.* Enteritidis, and no farm samples were positive for *Salmonella* spp. The positive sample from the market was then tested for antibiotic resistance and resistance to four different antibiotics was detected, involving: ampicillin, streptomycin, sulfamethoxazole and tetracycline (Nilsson, 2009). According to the results of another study by Schwan (2010), none of the market samples were positive for *Campylobacter* spp., while 76% (73/96) of the individual farm samples were positively tested and 95% (19/20) of the farms had at least one positive sample for *Campylobacter* spp. They next selected 28 isolates from the 73 positive samples to identify the *Campylobacter* species and to test for antibiotic resistance. They found that 21% of the isolates (6/28) were *C. coli* and 79% (22/28) were *C. jejuni*, but *C. coli* had a higher resistance to all of the 6 examined antimicrobials than *C. jejuni* did. Overall, resistance of both *C. coli* and *C. jejuni* to antibiotics was demonstrated as follows: erythromycin 7%, ciprofloxacin 71%, tetracycline 71%, streptomycin 21%, gentamicin 7% and nalidixic acid 71%. (Schwan, 2010).

Obviously, the preceding studies illustrate antimicrobial misuse and residues as well as antimicrobial resistance, which were found in broiler chickens in Vietnam at varying rates. However, up to now, scant attention has been paid to risk factors associated with these problems in Vietnam. Thus, this study was conducted in Southern Vietnam with the following objectives: 1) to describe broiler chicken farm characteristics; 2) to describe veterinary drug use in broiler farms; 3) to evaluate the association between farm characteristics and producers' non-compliance with on-label recommended withdrawal times of antimicrobials to find associated risk factors. Based on the study results, some interventions will be possibly made to minimize antimicrobial misuse to protect public health.

3. Materials, methods and results

Scientific paper[1]: Prevalence of antimicrobial use and risk factors associated with non-compliance of label withdrawal times in broiler chicken farms in Vietnam

Thi Viet Hang Nguyen[1], Denise Bélanger[2], Martine Boulianne[2], Patricia Turgeon[3], Julie Arsenault[2*]

[1] *Vietnam Food Administration, Ministry of Health, 135 Nui Truc street, Ba Dinh, Ha Noi, Vietnam*

[2] *Faculty of Veterinary Medicine, Université de Montréal, 3200 Sicotte, St-Hyacinthe, Quebec, Canada*

[3] *Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Saint-Hyacinthe, Quebec, Canada*

[*] Corresponding author

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Abstract

Antimicrobial use in food-animal husbandry is an important public health concern worldwide due to its potential impact on the emergence of drug-resistant microbes and its harmful residues in meat. However, in developing countries, few data are available on farm drug use practices. Therefore a cross-sectional epidemiological study was conducted on broiler chicken farms in Southern Vietnam from June 2011 to August 2011 with the aim of both describing prevalence of antimicrobials added to feed or water at the farm level and ascertaining any associations between farm characteristics and non-compliance of antimicrobial withdrawal times on labels. A convenient sample of 70 broiler farms was surveyed via personal interviews with farm owners using a questionnaire pertaining to farm characteristics and drug use practices. Over the 6-month period prior to the interviews, colistin, tylosin, ampicillin, enrofloxacin, doxycyclin, amoxicillin, diaveridin, and sulfadimidin were used at least once in descending frequency (from 75.7% to 30.0%) by the farms surveyed. Following two risk scenarios based on the comparison of recommended label withdrawal times with actual withdrawal times practiced during this period, between 14.3% and 44.3% of farmers did not comply with on-label withdrawal times for at least one antimicrobial. Risk factors associated ($p<0.05$) with non-compliance with on-label withdrawal times in at least one risk scenario were: raising birds other than Asian-indigenous bird breeds only, vaccinating against infectious bronchitis, using more than 6 different antimicrobials on a farm during the last 6 months prior to the interview, and mixing home-made and commercial feed. Our results underline the importance of using antimicrobials judiciously and respecting official withdrawal times in order to protect the consumer from the health risks caused by exposure to harmful levels of antimicrobial residues.

Keywords: antimicrobial, residue, risk factors, broiler farm, withdrawal time, Vietnam.

3.1. Introduction

In order to improve animal health and productivity, antimicrobials are commonly used in the poultry industry for disease treatment, prevention and growth promotion *(1, 22, 27, 52)*. However, antimicrobial use in food-animal husbandry has been progressively perceived as a potential risk to public health owing to the increasing trend of microbial resistance to antimicrobials worldwide *(37, 39, 46, 56)*. In fact, antimicrobial use can exert a selective pressure on micro-organisms and promote the survival of resistant microbes *(13, 25, 27, 52)*. Resistant zoonotic pathogens can then be transmitted to humans through a variety of routes that involve either direct contact with animal carriers or indirect contact via food-borne or air-borne transmission *(3, 27, 34, 55)*. Moreover, resistance genes harbored on mobile genetic elements can be horizontally transmitted together as a single package to different microbial species, including pathogens and non-pathogens *(2, 8, 14, 27)*. As a consequence, multi-resistance is potentially disseminated broadly in animal and non-animal reservoirs, posing a potential health threat to both animals and humans by increasing morbidity and mortality due to treatment failures *(6, 11, 13, 27, 36, 43, 44, 51, 52)*. The degree to which antimicrobial use in animals contributes to the emergence of antimicrobial resistance in human pathogens is still being debated *(9, 37, 56)*. However, precautions should be taken in using veterinary drugs *(7, 17, 21, 34, 40, 49)* since a large number of antimicrobials used in food animals are the same as those used for human medicine *(5, 35)* and antimicrobials are necessary tools for sustaining animal health. In addition to antimicrobial resistance, there is also increased concern about the potential impact of antimicrobial use in animal husbandry on ecosystem health following the dispersal of antimicrobials or their metabolites in the soil and water after manure application *(31)*.

Furthermore, antimicrobial misuse in poultry can result in hazardous drug residues in meat offered to the consumer *(1, 11, 25, 38)*. The presence of residues is largely associated with off-label use of drugs or farmers' non-compliance with on-label recommended withdrawal times prior to slaughtering *(11, 22, 25, 38)*. Drug residues in foods may have potential adverse impacts on the consumer in a number of ways, including chronic toxicological effects like carcinogenicity and mutagenicity (e.g. furazolidone) *(1, 10, 11, 18, 24, 32)* and allergic reactions in sensitive individuals (ex. penicillin) *(1, 11, 12, 18, 19, 25)*. Lastly, antimicrobial residues may favor development of resistance among bacteria present in meat or in the digestive tract of humans following ingestion *(1, 20, 25, 27, 38, 39, 52)*.

In Vietnam, the amount of chicken meat consumed per capita is quite considerable, second behind pork *(23)*. Research studies about 10 years ago identified a set of antimicrobials commonly used in broiler chicken production in Southern Vietnam that involved colistin, diaveridin, trimethoprim, enrofloxacin, sulfadimidin, oxytetracyclin, ampicillin, and tylosin *(29, 30, 41, 42, 57)*. More recently, tylosin and enrofloxacin residues were detected in 60 and 3 of 75 chicken meat samples, respectively, and 2 samples were above the national maximum residue limits for tylosin *(22)*. Additionally, a study conducted in Vietnam reported that among 28 *Campylobacter* isolates from poultry meat, between 7% and 71% of isolates were resistant to erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin and nalidixic acid *(50)*. In Vietnam, antimicrobials are easily accessible from drug sellers, without veterinarian prescription. Thus, although farmers have access to local veterinarians, they can use antimicrobials mainly based on their personal experience or a drug-seller's consultation after describing disease symptoms. Moreover, laboratory diagnosis to identify pathogens are still not used on a regular basis for routine diagnosis *(58)*. This situation could encourage misuse of antimicrobials in chicken flocks. This study was conducted in Southern Vietnam with the following objectives: 1) to describe veterinary drug use in broiler farms; 2) to evaluate the association between farm characteristics and producers' non-compliance with on-label recommended withdrawal times.

3.2. Materials and methods

3.2.1. Study design:

A cross-sectional study was conducted in broiler chicken farms in Dong Nai and Long An provinces in Southern Vietnam (Figure 1) from June 2011 to August 2011. The study was limited to broiler farms having more than 1000 birds per cycle of production. For farms with birds owned by a company and with poultry management practices provided by the latter (e.g. type of poultry house, source for chicks, drug use), only one farm per company was selected for the study.

3.2.2. Broiler farm sampling:

The sampling unit was the broiler chicken farm. A convenience sampling method was used for selection, with a target sample size of 35 farms per province. This number was based on the maximal number of farms that could be visited during the sampling period allowed for the study. To select these broiler farms, formal requests originating from provincial Agricultural and Rural Development Service authorities were directed to local authorities. Officials from district Animal Health Stations or their collaborators selected broiler farms meeting the criteria for inclusion from the list of chicken farms under their responsibility and then contacted the broiler-farm owners (called farmers in the study) to invite them to enroll in the study.

3.2.3. Questionnaire:

A questionnaire consisting of 45 questions was developed, pertaining to general farm characteristics, vaccination status, farmers' education, animal health management, drinking and feeding methods, and drug use for all therapeutic and sub-therapeutic purposes within the last 6 months prior to the interview. For drug use, the farmer was asked to report the name of the drug (active compounds or commercial name of the product), the age of birds at time of administration, duration of administration, routes of administration, and diseases treated. Only drugs administered by the farmers to birds in feed or water were considered (i.e. feed additives added by feed mills were not considered). For each different drug, farmers were asked to report only the most recent use over the last 6 months. During the interviews, farmers were asked to show drug containers or product labels to help the researcher retrieve the information on active ingredients used. When commercial names were given by farmers but product labels were not available at the farms, active ingredients were retrieved from catalogues of veterinary drug stores in both Dong Nai and Long An provinces. When farmers remembered just the one or two most important active ingredients of the drug product, but did not remember the commercial product name, no more information about other ingredients included in the same drug product was gathered.

The questionnaire's format and content was revised by the research group and one collaborator at the provincial Agricultural and Rural Development Service in Vietnam prior to administration. Farm owners responded to the questionnaire during interviews of about one hour. All interviews were conducted in person by the first author and were followed by a visit of each farm.

3.2.4. Statistical analyses:

Descriptive statistics were used to present farm characteristics. The proportion of farms having used each drug at least once over the last 6 months prior to the interview was estimated with 95% confidence limits based on the exact binomial distribution.

Each farm was classified according to the compliance (yes, no) of on-label recommended withdrawal times for each antimicrobial administered by digestive route based on: 1) On-farm withdrawal time: estimated as the difference between the age of birds at slaughter and the time of the last drug administration. When a range of values for the age of birds at slaughter was provided, minimal and maximal on-farm withdrawal times were estimated; 2) On-label withdrawal time: collected from the labels of drugs marketed in Vietnam containing the same active antimicrobial and indicated for chicken use by digestive route. Because a range of values for the on-label withdrawal times were recommended depending on drug formulation or dosage, minimal and maximal on-label withdrawal times were noted. Non-compliance with on-label withdrawal times for each antimicrobial was considered in two scenarios (low-risk, high-risk) based on the comparison between on-farm withdrawal times and on-label withdrawal times: 1) Low-risk: a farm was considered as non-compliant with on-label withdrawal time if the maximal on-farm withdrawal time was smaller than the minimal on-label withdrawal time for at least one antimicrobial administration, and compliance with on-label withdrawal times was otherwise recorded; 2) High-risk: a farm was considered as non-compliant with on-label withdrawal time if the minimal on-farm withdrawal time was smaller than a maximal on-label withdrawal time for at least one administration, and compliance with on-label withdrawal times was otherwise recorded. Farms not reporting use of an antimicrobial were considered as compliant with on-label withdrawal times for this antimicrobial. The proportion of farms not complying with on-label withdrawal times according to the two scenarios was then estimated for each antimicrobial, and for all the antimicrobials combined, with 95% confidence limits based on the exact binomial distribution.

For evaluating associations between farm characteristics and producers' non-compliance with on-label withdrawal times, two logistic regression models were built with the following dichotomous outcome variables: 1) compliance with on-label withdrawal times in a high-risk scenario (yes/no) for at least one antimicrobial; 2) compliance with on-label withdrawal times in a low-risk scenario (yes/no) for at least one antimicrobial. Variables from the questionnaire were selected for inclusion in the model if the variable was expected to have a potential relationship with the outcome variable based on biological knowledge, had less than 4% of missing values, and could be categorized so that each category included at least 4% of the farms. Selected explanatory variables are presented in Table 1. As a first step, all explanatory variables were tested in a univariable logistic regression model. All variables with $P<0.25$ (likelihood-ratio χ^2 test) were then included in a multivariable logistic regression model. Next, a backward stepwise selection procedure was performed with P-value >0.05 (Wald chi-square χ^2 test) as a criteria for exclusion. However, if the removal of one variable was associated with a change of >30% in the natural logarithm of odds ratio estimates of statistically significant ($P\leq0.05$) variables included in the model, this variable was kept in the model as a potential confounder. As a final step, the fit of the model was assessed with the Hosmer-and-Lemeshow goodness-of-fit test. All analyses were conducted in SAS software version 9.3.

3.3. Results

A total of 70 broiler chicken farms located in 5 districts of Dong Nai province (Trang Bom, Vinh Cuu, Long Thanh, Thong Nhat, and Xuan Loc) and in 1 city and 2 districts of Long An province (Tan An city, Chau Thanh, and Tan Tru) were selected. Farm characteristics are described in Table I for categorical variables and in Table II for continuous variables. Most of the farms (91%) were private and the remaining farms had birds owned by companies, including Dolico, Thanh Binh, Binh Minh, CP, Japfa and Emivet. Most farms raised their flocks in open shelters (94%). The majority of farms (56%) raised 1000 to 5000 birds/cycle. Asian-indigenous bird breeds with colored feathers were raised in 65% of farms and included non-Vietnamese indigenous breeds, Tam Hoang and Luong Phuong (China), raised mainly in Dong Nai, as well as Vietnamese indigenous ones, Tau Vang and "Gà nòi lai," bred primarily in Long An. Non-Asian bird breeds from American or Western countries, characterized by white feathers, were AA (USA), Cobb (USA), Lohmann Meat (Germany), and Avian (USA, but imported from Thailand). The overall mean bird age at slaughtering was 72.0 days, but a large variation of age at slaughtering was observed by bird breed and gender. Indeed, both female and male non-Asian birds were slaughtered at a mean age of 45 days (cycle range of 42-48 days), while female Asian birds ranged from 52 to 150 days with a mean of 91 days and male Asian birds ranged from 52 to 125 days with a mean of 79 days. The presence of one or more other animal species, including dogs (n=47 farms), cats (n=6 farms), fish (n=11 farms), ducks (n=7 farms), and/or pigs (n=7 farms) was noted in most of the farms.

Regarding bird health management, all farmers practiced all-in/all-out, sanitizing shelters prior to chick introduction. Water from drilled wells was the main drinking water source for birds (96% of farms). A commercial feed source was more common, representing 93% of farms, than home-made and commercial mixed feed. All farmers vaccinated all flocks against Newcastle Disease and Infectious Bursal Disease (IBD). Vaccination against Infectious Bronchitis (IB), Marek's Disease, Fowl Pox, Avian Influenza, Fowl Cholera was less frequent, with percentage of farms ranging from 20% to 63%, depending on the vaccine.

In relation to farmer education, 46% of farmers had high school education levels (after 12 schooling years) based on the Vietnamese education system, compared to 39% with lower education levels and 16% with higher education levels. About 19% of farmers reported that they were aware of the national regulation entitled Good Animal Husbandry Practices for Poultry in Vietnam (VIETGAHP) at Decision No. 1504/QĐ-BNN-KHCN dated May 15th, 2008 issued by the Ministry of Agriculture and Rural Development.

3.3.1. Description of drug use:

A total of 37 different drugs including 34 antimicrobials and 3 others (analgine, bromhexine and dexamethasone) were reported as having been added to feed or water by the farmer at least once during the last 6 months preceding the interview (see Table 3). The antimicrobials used in the largest number of farms were colistin, tylosin, ampicillin, enrofloxacin, doxycyclin, and amoxicillin in proportions ranging from 76% down to 44%, respectively. Only one farm reported the use of an antimicrobial prohibited for chickens in Vietnam, i.e. chloramphenicol. Except for one farm, all drug administrations were given at the same time to all chickens on the farm within the same production cycle, whether birds were located in a single shelter or in multiple. According to the questionnaire, drugs were selected primarily based on farmer's personal experience, and the dosages used were mainly based on product labels (see Table I). Respiratory problems were the most common reason for drug use. Regarding specific practices for drug use, the dosing duration per course of treatment was 3.6 days on average (ranging from 1.7 to 7 days), and there was a mean of 2.0 (ranging from 1 to 8 times) courses per broiler production cycle for either a single drug or a mixture of multi-drugs (data available upon request). Drug dilution in drinking water was the most frequent route of administration, followed by drugs mixed with feeds on the farm.

3.3.2. Estimation of non-compliance with on-label withdrawal time:

Non-compliance with on-label withdrawal times for various antimicrobials administered in feed or water for broilers is presented in Table III. In the high-risk scenario, non-compliance with on-label withdrawal times involved 20 antimicrobials. Non-compliance with on-label withdrawal times was observed for at least one antimicrobial and for at least one administration over the 6-month period for a total of 44.3% (32.4-56.7%) of farms. For each specific antimicrobial, the percentage of farms that did not comply with on-label withdrawal times ranged from 1.4% to 25.7%.

In the low-risk scenario, non-compliance with on-label withdrawal times involved 13 antimicrobials. It was estimated that 14.3% (7.1-24.7%) of farms did not comply with on-label withdrawal times for at least one antimicrobial and for at least one administration over the 6-month period. For each specific antimicrobial, the percentage of farms non-compliant with on-label withdrawal times ranged from 1.4% to 4.3%.

3.3.3. Risk factors for non-compliance with recommended withdrawal times

3.3.3.1. Logistic regression model for the high-risk scenario

Out of the potential risk factors assessed in the univariable analyses (see Table I), 12 were selected for inclusion in the multivariable analysis (all P≤0.25). These 12 are: province (Dong Nai, Long An), bird breeds (Asian-indigenous only, others), bird density by m^2 (<9, ≥ 9), percentage of annual mortality (<6, ≥6), vaccination against Infectious Bronchitis (yes, no), vaccination against Fowl Pox (yes, no), vaccination against Fowl Cholera (yes, no), drug seller as a basis for drug selection (yes, no), veterinarian as a basis for drug selection (yes, no), drug seller as a basis for drug administration duration (yes, no), veterinarian as a basis for drug dosage (yes, no), and number of antimicrobials used per farm over the last 6 months <6, ≥6). Following data exploration, vaccination against Fowl Pox was not considered for inclusion as it was highly correlated with the "bird breed" variable (i.e vaccinated farms all had Asian-indigenous birds). Likewise, the variables "drug seller as a basis for drug selection" and "drug seller as a basis for drug administration duration" were highly correlated and conveyed the same idea, so only the one most statistically significantly associated with outcome was selected (i.e. drug seller as a basis for drug selection). Similarly, the variable "veterinarian as a basis for drug dosage" was not considered due to high correlation with the variable "veterinarian as a basis for drug selection." The final multivariable logistic regression model is presented in Table IV. The odds of producers' non-compliance with on-label recommended withdrawal times were statistically significantly higher in farms raising birds other than Asian-indigenous bird breeds, and farms that vaccinated against Infectious Bronchitis.

3.3.3.2. Logistic regression model for the low-risk scenario

Out of the potential risk factors at farm level assessed in the univariable analyses (see Table I), 7 were selected for inclusion in the multivariable analysis (all $P \leq 0.25$). These were: bird breeds (Asian-indigenous only, others), feed sources (only commercial feed, combination of commercial and home-made feed), farmers' education (>high school, high school, <high school), vaccination against Fowl Pox (yes, no), number of antimicrobials used per farm over the last 6 months (<6, ≥6), province (Dong Nai/Long An), and percentage of annual mortality (<6, ≥6). However, as described for the high-risk scenario, vaccination against Fowl Pox was not considered for inclusion in the model.

The final multivariable logistic regression model is shown in Table 4. The odds of producers' non-compliance with on-label recommended withdrawal times were statistically significantly higher in farms raising birds other than Asian-indigenous bird breeds, using a combination of commercial and homemade feeds, and having used at least 6 different antimicrobials per farm over the last 6 months.

3.4. Discussion

3.4.1. Description of drug use

We found that colistin, tylosin, ampicillin, enrofloxacin, doxycycline and amoxicillin were the antimicrobials reported in the largest number of selected broiler chicken farms. At least one of these antimicrobials was also reported to be commonly used in the previous studies conducted 10 years ago in broiler chicken production in other Vietnamese provinces such as in Ho Chi Minh city *(29, 30, 41, 42, 57)* and in Binh Duong province *(45)*. It is noteworthy that Ho Chi Minh City, the most important economic center, is geographically surrounded by Dong Nai, Long An and Binh Duong in Southern Vietnam. Most large-scale veterinary drug manufactories, importing companies, sales agents and distributors are primarily located in Ho Chi Minh, Dong Nai, Binh Duong, and Can Tho and supply veterinary drugs for the whole Southern Vietnam region, which could explain the similarity among drug usage between Southern provinces. Another study recently conducted in Hai Duong, Thai Binh and Ha Noi in Northern Vietnam also reported a set of antimicrobials commonly used in broiler chicken production including gentamicin, amoxicillin, ampicillin, enrofloxacin, norfloxacin, tylosin, doxycyclin, oxytetracyclin, colistin, diaveridin, trimethoprim, and sulfachlorpyridazin, which were also all reported to have been used by surveyed farmers in our study *(58)*. None of the farms included in our study reported the use of penicillin, which was previously reported in Northern Vietnam for preventive and curative purposes, although infrequently (3/210 of chicken farms) *(58)*. Penicillin is one of the most allergenic antimicrobials and when a sufficient amount of penicillin residual in food is consumed, there could be an allergic risk for sensitive individuals *(11, 26, 54)*. Although we might have failed to detect penicillin use due to sample size limitation, the absence of use of penicillin in our study is in agreement with previous studies conducted in Southern Vietnam, suggesting a negligible risk of allergy by penicillin when humans consume chicken meat produced in this region *(29, 30, 41, 42, 57)*.

The use of chloramphenicol, a banned antimicrobial in chicken production, was reported in one farm. This drug has the potential to cause bone marrow suppression in exposed humans *(1, 4, 11, 16, 28, 33, 47, 53)*. Ten years ago, the use of chloramphenicol was also reported in broiler chicken production in Southern Vietnam *(29, 30, 41, 42, 57)*. More recently, in 2010, chloramphenicol was used in chicken production for preventive and curative purposes with the proportion of 5/210 farms (2.4%) in Northern provinces *(58)*, which is consistent with our results.

It should be noted that all the drugs surveyed in this study did not involve drugs added to commercial feed by feed mills, which includes growth promoters or drugs for disease prevention. Thus, the overall use of antimicrobials administered to birds is probably underestimated. Also, the data collected in our study did not allow for estimation of the frequency of drug use at the flock or bird level, as we cumulated data on drug use over a 6-month period, which included 1 to 3 production cycles depending on the farm. Prevalence of antimicrobial use at the flock level probably ranges from 1 to 3 times lower than estimates (if the drug was used in only one flock) or estimates are similar (if the drug was given to all flocks).

From an antimicrobial practice perspective, antimicrobials were administered to chickens over a 3-4 day period on average, in conformity with the labels. The most popular route of drug administration was dilution with drinking water, which is also consistent with label instructions because sick birds may stop ingesting feed but will keep consuming water *(52)*.

3.4.2. Estimation of non-compliance with on-label withdrawal times

Non-compliance with on-label withdrawal times can lead to the presence of harmful antimicrobial residues in meat. Our study estimated the percentages of farms not complying with on-label withdrawal times for each antimicrobial and provides an estimate of the percentages of farms more at risk of having harmful antimicrobial residues in chicken meat offered to consumers. Interestingly, in a previous study conducted in Vietnam, residues above the national maximum limit were detected in chicken meat (2/75 samples) for tylosin *(22)*, an antimicrobial for which farmers often reported non-compliance with withdrawal time in the high-risk scenario of our study.

In both risk scenarios, producers' non-compliance with on-label withdrawal times was higher in farms raising birds other than Asian-indigenous breeds only. Asian-indigenous birds are likely better adapted to the Vietnamese climate than non-Asian-indigenous birds. Moreover, although these commercial breeds of white feathered chickens not indigenous to Asia have been genetically selected for rapid growth, it has been reported that this has resulted in a decreased resistance to disease *(15, 48)*. Thus, Asian-indigenous birds could be less susceptible to local diseases and as a result, might need shorter and less frequent antimicrobial administrations. It should be noted that compared to farms with non-Asian-indigenous breeds, all farms raising only Asian-indigenous birds were using vaccinations against Fowl Pox. This could also explain in part a lower risk of non-compliance due to less use of antimicrobials with these flocks, either due to a lesser extent of secondary infections or because they have better management practices overall.

In the high-risk scenario, the use of vaccination against infectious bronchitis virus was associated with higher probability of non-compliance with on-label withdrawal times.

Vaccination could be a proxy variable for the general health condition of chickens raised on the farms if the decision to vaccinate was motivated by the need to improve health status in farms facing high mortality or morbidity rates.

In the low-risk scenario, producers' non-compliance with on-label recommended withdrawal times was higher in farms using a combination of commercial and home-made feed than commercial feed only. Farmers using home-made feeds could be more likely to report the addition of antimicrobials as growth promoters to their feed, with potential non-compliance with withdrawal times, than farmers using commercial feeds only. In fact, it is possible that commercial feeds already include growth promoters added at the feed mills, which was not covered by our study. In addition, the use of 6 or more different antimicrobials was associated with a higher risk of non-compliance with recommended withdrawal times. This is attributed to a probable higher use of antimicrobials in general, with a potential higher risk of not complying with withdrawal times at least once.

3.4.3. Study limitations

A convenience sampling method was used for selecting farms from the list of chicken farms administrated by the animal health authorities. All selected farm owners agreed to participate in the study and were located in various geographical areas of the targeted provinces, favoring the representativeness of our sample. However, it is possible that selected farms had closer relationships with authorities, and we cannot exclude that they may have presented some differences in management practices compared to other farms. It should be noted that according to the Vietnamese law, local authorities are responsible for administrating all chicken farms within their localities, and thus contact with every farm is expected.

A questionnaire was used to collect the data. This questionnaire was not formally validated prior to use. However, at least for the variables directly observable at the farm (e.g. type of shelter, breed of birds, etc.), concordance of the responses was favored by the on-site administration of the questionnaire. For these variables, no difference was noted between farmer responses and direct observations. Drug use was based on the 6-month period prior to the interview. As most farmers did not have drug records, answers were based on memory and thus subject to recall bias. Information bias on drug use could be potentially higher when farmers only remembered the name of the most important active ingredients of the drug product, but did not remember the commercial product name, so that all the active ingredients of the drug product could not be retrieved from drug labels. It is also possible that farmers aware of Vietnamese regulations about drug use were reluctant to provide information about prohibited drug use, resulting in a potential underestimation of such drug use. Moreover, as data for each antimicrobial was collected only for the most recent use on each farm, it is possible that non-compliance with withdrawal time was underestimated if the use was different between flocks. However, as only the most recent data on use for a specific antimicrobial was collected, the information is probably more exact due to a lower recall bias. Finally, regarding the statistical models, variables such as company owned or not, were not selected for inclusion in the final model, but statistical power could have been an issue. Many different variables were tested on the other side, increasing the likelihood that some of them were statistically significant only by chance. Indeed, the risk factors found in our study should be considered as potentially important variables in predicting non-compliance with withdrawal time, therefore warranting further study.

3.5. Conclusions

The antimicrobials used in the largest number of chicken farms in Vietnam involve: colistin, tylosin, ampicillin, enrofloxacin, amoxicillin, and doxycycline. Among the risk factors associated with the non-compliance with on-label withdrawal times, bird breed was the most consistent. Antimicrobial use with non-compliance with on-label recommended withdrawal times posed a potential risk for the presence of harmful residues of antimicrobials in chicken meat offered to the consumer. To safeguard the consumer from health risks caused by exposure to antimicrobial residues, efforts should be directed toward the reduction of antimicrobial use by promoting biosecurity measures and good management practices to enhance chicken flock health, especially given the fact that only

19% of the farmers were familiar with the regulation entitled Good Animal Husbandry Practices for Poultry in Vietnam. Further, the implementation of a surveillance and control program on antimicrobial residues in chicken meat in Vietnam is recommended.

Bibliography

1. A.R., N. 2008. Antibiotic Residues - A global health hazard. *Veterinary World*. 1(12):375-377.
2. Acar, J. F., and G. Moulin. 2012. Antimicrobial resistance: a complex issue. *Revue scientifique et technique (International Office of Epizootics)*. 31:23-31.
3. Aidara-Kane, A. 2012. Containment of antimicrobial resistance due to use of antimicrobial agents in animals intended for food: WHO perspective. *Revue scientifique et technique (International Office of Epizootics)*. 31:277-87.
4. Ambekar, C. S., B. Cheung, J. Lee, L. C. Chan, R. Liang, and C. R. Kumana. 2000. Metabolism of chloramphenicol succinate in human bone marrow. *European journal of clinical pharmacology*. 56:405-9.
5. Angulo, F. J., N. L. Baker, S. J. Olsen, A. Anderson, and T. J. Barrett. 2004. Antimicrobial use in agriculture: controlling the transfer of antimicrobial resistance to humans. *Seminars in pediatric infectious diseases*. 15:78-85.
6. Angulo, F. J., V. N. Nargund, and T. C. Chiller. 2004. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health*. 51:374-9.
7. Anthony, F., J. Acar, A. Franklin, R. Gupta, T. Nicholls, Y. Tamura, S. Thompson, E. J. Threlfall, D. Vose, M. van Vuuren, and D. G. White. 2001. Antimicrobial resistance: responsible and prudent use of antimicrobial agents in veterinary medicine. *Revue scientifique et technique (International Office of Epizootics)*. 20:829-39.
8. Apata, D. F. 2009. Antibiotic resistance in poultry. *International Journal of Poultry Science*. 8:404-408.
9. Beovic, B. 2006. The issue of antimicrobial resistance in human medicine. *International journal of food microbiology*. 112:280-7.
10. Berendsen, B., L. Stolker, J. de Jong, M. Nielen, E. Tserendorj, R. Sodnomdarjaa, A. Cannavan, and C. Elliott. 2010. Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. *Analytical and bioanalytical chemistry*. 397:1955-63.
11. Botsoglou, N. A., and D. J. Fletouris. 2001. Drug residues in foods : pharmacology, food safety, and analysis. Marcel Dekker, New York.
12. Caimmi, S., D. Caimmi, E. Lombardi, G. Crisafulli, F. Franceschini, G. Ricci, and G. L. Marseglia. 2011. Antibiotic allergy. *International journal of immunopathology and pharmacology*. 24:S47-53.

13. CDC. Date, 2005, National antimicrobial resistance monitoring system - Frequently asked questions about antibiotic resistance. Available at: http://www.cdc.gov/narms/faq_pages/7.htm. Accessed 4 February, 2012.
14. Chee-Sanford, J. C., R. I. Mackie, S. Koike, I. G. Krapac, Y. F. Lin, A. C. Yannarell, S. Maxwell, and R. I. Aminov. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *Journal of environmental quality*. 38:1086-108.
15. Cheema, M. A., M. A. Qureshi, and G. B. Havenstein. 2003. A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. *Poultry science*. 82:1519-29.
16. Chylinski, J., and W. Zegarski. 1971. Case of toxic bone marrow depression following treatment with chloramphenicol. *Polski tygodnik lekarski*. 26:801-2.
17. Codex. 2005. Code of Practice to minimize and contain antimicrobial resistance. p. 15. *In*.
18. Codex. 2009. Guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals. p. 38. *In*.
19. Comte, D., S. Petitpierre, F. Spertini, and P. A. Bart. 2012. Allergy to beta-lactam antibiotics. *Revue médicale suisse*. 8:836, 838-42.
20. Council, N. R. 1999. The use of drugs in food animals : benefits and risks. National Academy Press, Washington, D.C.
21. Cox, L. A., Jr., and D. A. Popken. 2006. Quantifying potential human health impacts of animal antibiotic use: enrofloxacin and macrolides in chickens. *Risk analysis*. 26:135-46.
22. Dang, B. N. 2010. Prevalence of antibiotic residues in pork and chicken meat in some slaughterhouses and markets in the Northern region in Vietnam. p. 82. *In*, Faculty of Veterinary Medicine vol. Master thesis. Hanoi University of Agriculture, Vietnam.
23. Epprecht, M. 2005. Geographic dimensions of livestock holdings in Vietnam. Spatial relationships among poverty, infrastructure and the environment. p. 51. *In*, A living from livestock. Pro-poor livestock policy initiative.
24. Farber, T. M., and G. B. Guest. 1984. A strategy for the assessment of the carcinogenic potency of veterinary drug residues: recommendations of the FDA. *Food additives and contaminants*. 1:213-9.
25. Giguère, S., J. F. Prescott, J. D. Baggot, R. D. Walker, and P. M.Dowling. 2006. Antimicrobial therapy in veterinary medicine. Blackwell.
26. Gracey, J., D. S.Collins, and R. Huey. 1999. Meat Hygiene. Harcourt Brace and Company.
27. Guardabassi, L., L. B. Jensen, and H. Kruse. 2008. Guide to antimicrobial use in animals. Blackwell Pub., Oxford, UK ; Ames, Iowa.
28. Hartzen, S. H., and N. Frimodt-Moller. 1986. Toxic bone marrow depression after local use of chloramphenicol eye agents. *Ugeskrift for laege*. 148:2225-7.

29. Huynh, P. S. 2000. Survey on antibiotic use in Thu Duc and No.9 Districts in Ho Chi Minh city. p. 58. *In*, vol. Veterinary Doctor at Undergraduate Level. Agricultural University in Ho Chi Minh city, Vietnam.

30. Huynh, T. N. D. 2000. Survey on antibiotic use in industrial broiler chicken production in Hoc Mon distric in Ho Chi Minh city. p. 58. *In*, Veterinary medicine and husbandry faculty, vol. Veterinary Doctor at Undergraduate Level. Agricultural University in Ho Chi Minh city, Vietnam.

31. Kemper, N. 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological Indicators*. 8:1-13.

32. Liu, Y., L. Huang, Y. Wang, B. Yang, A. Ishan, K. Fang, D. Peng, Z. Liu, M. Dai, and Z. Yuan. 2010. Tissue depletion and concentration correlations between edible tissues and biological fluids of 3-amino-2-oxazolidinone in pigs fed with a furazolidone-medicated feed. *Journal of agricultural and food chemistry*. 58:6774-9.

33. Lokhande, J., A. S. Juvekar, and K. P. Kulkarni. 2007. Chloramphenicol: screening and review to evaluate its potential beneficial effects in leukaemia. *Journal of the Indian Medical Association*. 105:224, 226-8.

34. Marshall, B. M., and S. B. Levy. 2011. Food animals and antimicrobials: impacts on human health. *Clinical microbiology reviews*. 24:718-33.

35. Mather, A. E., L. Matthews, D. J. Mellor, R. Reeve, M. J. Denwood, P. Boerlin, R. J. Reid-Smith, D. J. Brown, J. E. Coia, L. M. Browning, D. T. Haydon, and S. W. Reid. 2012. An ecological approach to assessing the epidemiology of antimicrobial resistance in animal and human populations. *Proceedings. Biological sciences / The Royal Society*. 279:1630-9.

36. Mathew, A. G., R. Cissell, and S. Liamthong. 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne pathogens and disease*. 4:115-33.

37. McDermott, P. F., S. Zhao, D. D. Wagner, S. Simjee, R. D. Walker, and D. G. White. 2002. The food safety perspective of antibiotic resistance. *Animal biotechnology*. 13:71-84.

38. Miller, J. B. K. a. R. 1997. Problems associated with drug residues in beef from feeds and therapy. *Revue scientifique et technique (International Office of Epizootics)*. 16(2):694-708.

39. Molbak, K. 2004. Spread of resistant bacteria and resistance genes from animals to humans-the public health consequences. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health*. 51:364-9.

40. Morley, P. S., M. D. Apley, T. E. Besser, D. P. Burney, P. J. Fedorka-Cray, M. G. Papich, J. L. Traub-Dargatz, and J. S. Weese. 2005. Antimicrobial drug use in veterinary medicine. *Journal of veterinary internal medicine*. 19:617-29.

41. Nguyen, V. A. 2001. Survey on antibiotic use in industrial boiler chicken production in district no.12 in Ho Chi Minh city. p. 64. *In*, Animal Husbandry and Veterinary Medicine Faculty, vol. Veterinary Doctor at Undergraduate level. Agricultural University in Ho Chi Minh city, Vietnam.

42. Nguyen, V. D. 2001. Survey on antibiotic use in industrial broiler chicken production in Cu Chi district in Ho Chi Minh city. p. 54. *In*, Animal Husbandry and Veterinary Medicine Faculty, vol. Veterinary Doctor at Undergraduate level. Agricultural University in Ho Chi Minh city, Vietnam.

43. Obeng, A. S., H. Rickard, M. Sexton, Y. Pang, H. Peng, and M. Barton. 2012. Antimicrobial susceptibilities and resistance genes in Campylobacter strains isolated from poultry and pigs in Australia. *Journal of applied microbiology*. 113:294-307.

44. Oliver, S. P., S. E. Murinda, and B. M. Jayarao. 2011. Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. *Foodborne pathogens and disease*. 8:337-55.

45. Pham, Q. D. 2001. Survey on antibiotic use in boiler chicken produciton in Ben Cat and Phu Giao districts in Binh Duong province. p. 41. *In*, vol. Undergraduate thesis. Agricultural University in Ho Chi Minh city, Vietnam.

46. Phillips, I., M. Casewell, T. Cox, B. De Groot, C. Friis, R. Jones, C. Nightingale, R. Preston, and J. Waddell. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of antimicrobial chemotherapy*. 53:28-52.

47. Plumb, D. C. 2011. Plumb's veterinary drug handbook. PharmaVet, Stockholm, Wis.; Ames, Iowa.

48. Qureshi, M. A., and G. B. Havenstein. 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed "typical" 1957 and 1991 broiler diets. *Poultry science*. 73:1805-12.

49. Rodriguez, L. D., M. A. Romo, M. C. Calonge, and S. T. Pena. 2010. Prudent use of antimicrobial agents and proposals for improvement in veterinary medicine. *Enfermedades infecciosas y microbiología clínica*. 28 Suppl 4:40-4.

50. Schwan, P. 2010. Prevalence and antibiotic resistance of Campylobacter spp. in poultry and raw meat in the Can Tho province, Vietnam. p. 21. *In* Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine and Animal Science, Department of Biomedical Sciences and Veterinary Public Health.

51. Shea, K. M. 2003. Antibiotic resistance: what is the impact of agricultural uses of antibiotics on children's health. *Pediatrics*. 112 (Supp.1):253-258.

52. Singer, R. S., and C. L. Hofacre. 2006. Potential impacts of antibiotic use in poultry production. *Avian diseases*. 50:161-72.

53. Taka, T., M. C. Baras, and Z. F. Chaudhry Bet. 2012. Validation of a rapid and sensitive routine method for determination of chloramphenicol in honey by LC-MS/MS. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment*. 29:596-601.

54. Teh, W. L., and A. S. Rigg. 1992. Possible penicillin allergy after eating chicken. *Lancet*. 339:620.

55. Teuber, M. 2001. Veterinary use and antibiotic resistance. *Current opinion in microbiology*. 4:493-9.

56. Van den Bogaard, A. E., and E. E. Stobberingh. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. *Drugs*. 58:589-607.

57. Vo, T. T. A. 2001. Antibiotic use and residue in chicken meat on industrial boiler chicken farms in Ho Chi Minh city. p. 88. *In*, Animal Husbandry and Veterinary Medicine Faculty, vol. Master thesis. Agricultural University in Ho Chi Minh city, Vietnam.

58. Vu, D. T., P. K. Dang, and M. L. Scippo. 2010. Antibiotic Utilization in Pig and Chicken Production in Vietnam-Case study in Red River Delta. *International Conference on Hygiene and Importation Management of Livestock Products*:93-101.

Table I. Description of farm characteristics with percentages of farms not complying with on-label withdrawal times for selected variables (n=70 broiler chicken farms in two provinces of South Vietnam, 2011)

Variables	Number of farms	Percentage of farms non-compliant with on-label withdrawal times	
		High-risk scenario	Low-risk scenario
I. General farm characteristics			
Province[a,b]			
Dong Nai	34	55.9	8.8
Long An	36	33.3	19.4
Bird ownership			
Farmers	64	43.8	14.1
Companies	6	50.0	16.7
Shelter type			
Open shelters only	66	43.9	15.2
Closed shelters +/-open shelters	4	50.0	0.0
Multiple ages in the same shelter			
No	68		
Yes	2		
Farms by flock size (birds/cycle)			
1.000-5.000	39	35.9	12.8
>5.000-20.000	20	55.0	15.0
>20.000-50.000	11	54.6	18.2
Bird types raised on the farm			
Broilers only	67		
Broilers and layers/ breeders	3		
Bird breeds[a,b]			
Asian-indigenous only	45	26.7	4.4
Others[c]	25	76.0	32.0
Chick origin			
Day-old chicks from the hatchery	69		
Own-stock eggs laid on farm	1		

Variables	Number of farms	Percentage of farms non-compliant with on-label withdrawal times	
		High-risk scenario	Low-risk scenario
Other animals on the same farm			
Yes	55	56.4	18.2
No	15	0.0	0.0
Birds separated from other animals by fence			
Yes, in open shelters	52		
Yes, in closed shelters	4		
No, freely moving	0		
Missing values	14		
Bird density[a]			
<9 birds/m2	34	32.4	14.7
≥9 bird/m2	36	55.6	13.9
Annual mortality[a,b]			
<6%	40	57.5	20.0
≥6%	30	26.7	6.7
II. Chicken health management			
New day-old chicks raised in separate shelters			
No	2		
Yes	68		
Sick birds separated from the others (different pens, within the same or different poultry house)			
No	3	33.3	0.0
Yes	67	44.8	14.9
Management of dead-birds			
For human consumption			
Yes	0		
No	70		
Burying on farm			
Yes	22		
No	48		
Thrown into rivers/lakes/ponds...			
Yes	0		

Variables	Number of farms	Percentage of farms non-compliant with on-label withdrawal times	
		High-risk scenario	Low-risk scenario
No	70		
Feed for other animals (fish, crocodiles, python)			
Yes	46		
No	24		
Burning on farm			
Yes	28		
No	42		
Sanitary practices			
All in – all out			
Yes	70		
No	0		
Sanitizing[d] shelters before introduction of new day-old chicks			
Yes	70		
No	0		
Sanitizing[d] shelters during farming			
Yes	70		
No	0		
Keep sanitized[d] shelters empty before introduction of new day-old chicks			
Yes, and in:			
03-10 days	19		
11-20 days	16		
21-30 days	25		
31-40 days	1		
41-90 days	9		
No	0		
Drinking water sources			
Water from drilled wells	67		
Water from dug wells	3		
Feed sources[b]			
Only commercial feed	65	43.1	10.8

Variables	Number of farms	Percentage of farms non-compliant with on-label withdrawal times	
		High-risk scenario	Low-risk scenario
Combination of homemade and commercial feed	5	60.0	60.0
III. Vaccination			
Vaccination			
Yes	70		
No	0		
Diseases for vaccination			
Newcastle Disease			
Yes	70		
No	0		
Infectious Bronchitis [a]			
Yes	44	61.4	15.9
No	26	15.4	11.5
Marek's Disease			
Yes	22	0.0	0.0
No	48	64.6	20.8
Infectious Bursal Disease			
Yes	70		
No	0		
Fowl Pox[a,b]			
Yes	34	20.6	2.9
No	36	66.7	25.0
Avian Influenza			
Yes	37		
No	33		
Fowl Cholera[a]			
Yes	14	7.1	7.1
No	55	52.7	14.6
Missing value	1		
IV. Education and awareness			
Educational level[b]			

Variables	Number of farms	Percentage of farms non-compliant with on-label withdrawal times	
		High-risk scenario	Low-risk scenario
< High school	27	40.7	3.7
High school	32	43.8	21.9
>High school	11	54.6	18.2
Awareness of VIETGAHP[e]			
No	57	45.6	15.8
Yes	13	38.5	7.7
V. Drug use practices			
Drugs used for treatment, prevention or growth promotion			
Yes	70		
No	0		
Bases for drug selection[f]			
Personal experience			
Yes	59	44.1	13.6
No	11	45.5	18.2
Drug seller[a]			
Yes	22	22.7	13.6
No	48	54.2	14.6
Veterinarian[a]			
Yes	32	31.3	12.5
No	38	55.3	15.8
Product label			
Yes	3		
No	67		
Husbandry company			
Yes	11	45.5	9.1
No	59	44.1	15.3
Others			
Yes	5		
No	65		
Bases for duration of drug administration[f]			

Variables	Number of farms	Percentage of farms non-compliant with on-label withdrawal times	
		High-risk scenario	Low-risk scenario
Personal experience			
Yes	46	43.5	13.0
No	24	45.8	16.7
Drugseller[a]			
Yes	15	20.0	6.7
No	55	50.9	16.4
Veterinarian			
Yes	15	40.0	13.3
No	55	45.5	14.6
Product label			
Yes	23	34.8	13.0
No	47	48.9	14.9
Husbandry company			
Yes	7		
No	63		
Others			
Yes	4		
No	66		
Bases for drug dosage[f]			
Personal experience			
Yes	24	50.0	16.7
No	46	41.3	13.0
Drug seller			
Yes	8	37.5	25.0
No	62	45.2	12.9
Veterinarian[a]			
Yes	12	16.7	8.3
No	58	50.0	15.5
Product label			
Yes	49	42.9	16.3
No	21	47.6	9.5

Variables	Number of farms	Percentage of farms non-compliant with on-label withdrawal times	
		High-risk scenario	Low-risk scenario
Husbandry company			
Yes	8		
No	62		
Others			
Yes	1		
No	69		
Number of antimicrobials per farm over the last 6 months prior to interview[a,b]			
<6	32	53.1	6.3
>=6	38	36.8	21.1

[a] Variables selected for multi-variable analysis of the logistic regression model in a high-risk scenario with p-value<0.25.
[b] Variables selected for multi-variable analysis of the logistic regression model in a low-risk scenario with p-value<0.25.
[c] These 25 farms were raising non-Asian-indigenous white birds, although 3 among these farms were raising both types of chickens.
[d] Sanitizing included cleaning with only water, cleaning with water and soaking, cleaning with water and disinfectant agents, and/or spray with disinfectant agents.
[e] VIETGAHP: Good Animal Husbandry Practices for Poultry in Vietnam regulated at Decision No. 1504/QĐ-BNN-KHCN dated May 15th, 2008 issued by Ministry of Agriculture and Rural Development.
[f] Multiple choices were possible.

Table II. Descriptive statistics of farm characteristics in 70 broiler chicken farms in South Vietnam, 2011 for continuous variables

Variables	Minimum	Mean	Standard Deviation	Maximum
Number of layers (or floors)	1.0	1.0	0.1	2.0
Bird density (birds/m^2)	1.0	8.3	3.1	20.0
Bird age at slaughtering (days)	42.0	72.0	27.0	125.0
Flock size (birds per cycle)	1000.0	9553.6	10889.2	50000.0
Annual mortality (%)	1.5	6.3	2.7	12.5

Table III. Percentage with 95% confidence limits (95% CL) of farms having reported the use of various drugs over a 6-month period and not complying with on-label withdrawal based on two risk scenarios (n=70 broiler chicken farms in two provinces of South Vietnam, 2011)

Classes (drugs)	Percentage of farms using drugs (95% CL)	Withdrawal times on-label[d] (days)		Percent of farms not complying with on-label withdrawal times (95% CL)	
		Min	Max	High-risk scenario	Low-risk scenario
Aminoglycosides					
Gentamicin	10.0 (4.1-19.5)	7	21	2.9 (0.3-9.9)	0.0 (0.0-5.1)
Apramycin	7.1 (2.4-15.9)	21	21	4.3 (0.9-12.0)	4.3 (0.9-12.0)
Neomycin	7.1 (2.4-15.9)	14	30	4.3 (0.9-12.0)	1.4 (0.0-7.7)
Spectinomycin	1.4 (0.0-7.7)	3	15	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Streptomycin	1.4 (0.0-7.7)	21	21	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Beta-lactams					
Ampicillin	61.4 (49.0-72.8)	12	21	12.9 (6.1-23.0)	1.4 (0.0-7.7)
Amoxicillin	44.3 (32.4-56.7)	7	15	7.1 (2.4-15.9)	0.0 (0.0-5.1)
Cephalosporins					
Cephalexin	1.4 (0.0-7.7)	7	7	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Diaminopyrimidines					
Diaveridin	30.0 (19.6-42.1)	7	7	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Trimethoprim	11.4 (5.1-21.3)	5	12	5.7 (1.6-14.0)	2.9 (0.3-9.9)
Pyrimethamine	8.6 (3.2-17.7)	7	7	1.4 (0.0-7.7)	1.4 (0.0-7.7)
Lincosamides					
Lincomycin	1.4 (0.0-7.7)	3	15	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Macrolides					
Tylosin	70.0 (57.9-80.4)	7	21	22.9 (13.7-34.4)	0.0 (0.0-5.1)

Classes (drugs)	Percentage of farms using drugs (95% CL)	Withdrawal times on-label[d] (days)		Percent of farms not complying with on-label withdrawal times (95% CL)	
		Min	Max	High-risk scenario	Low-risk scenario
Tilmicosin	11.4 (5.1-21.3)	12	12	2.9 (0.3-9.9)	2.9 (0.3-9.9)
Erythromycin	8.6 (3.2-17.7)	2	15	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Josamycin	7.1 (2.4-15.9)	5	7	1.4 (0.0-7.7)	1.4 (0.0-7.7)
Phenicols					
Florphenicol	10.0 (4.1-19.5)	7	28	8.6 (3.2-17.7)	0.0 (0.0-5.1)
Chloramphenicol[a]	1.4 (0.0-7.7)				
Thiamphenicol	1.4 (0.0-7.7)	21	21	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Polypeptides					
Colistin	75.7 (64.0-85.2)	6	21	25.7 (16.0-37.6)	2.9 (0.3-9.9)
Quinolones					
Enrofloxacin	50.0 (37.8-62.2)	11	15	4.3 (0.9-12.0)	1.4 (0.0-7.7)
Norfloxacin	10.0 (4.1-19.5)	5	7	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Oxolinic acid	8.6 (3.2-17.7)	7	7	1.4 (0.0-7.7)	1.4 (0.0-7.7)
Flumequin	1.4 (0.0-7.7)	5	7	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Sulfonamides					
Sulfadimidin	30.0 (19.6-42.1)	12	12	1.4 (0.0-7.7)	1.4 (0.0-7.7)
Sulfaquinoxalin	14.3 (7.1-24.7)	7	10	2.9 (0.3-9.9)	1.4 (0.0-7.7)
Sulfachlorpyridazin	7.1 (2.4-15.9)	7	21	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Sulfadiazine	2.9 (0.3-9.9)	5	7	1.4 (0.0-7.7)	1.4 (0.0-7.7)
Tetracyclines					
Doxycyclin	44.3 (32.4-56.7)	7	21	8.6 (3.2-17.7)	0.0 (0.0-5.1)
Oxytetracyclin	10.0 (4.1-19.5)	7	30	2.9 (0.3-9.9)	0.0 (0.0-5.1)
Tetracyclin	8.6 (3.2-17.7)	4	4	0.0 (0.0-5.1)	0.0 (0.0-5.1)

Classes (drugs)	Percentage of farms using drugs (95% CL)	Withdrawal times on-label[d] (days) Min	Max	Percent of farms not complying with on-label withdrawal times (95% CL) High-risk scenario	Low-risk scenario
Chlortetracyclin	1.4 (0.0-7.7)	1	6	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Other anti-coccidials					
Amprolium	2.9 (0.3-9.9)	3	3	0.0 (0.0-5.1)	0.0 (0.0-5.1)
Toltrazuril	20.0 (11.4-31.3)	4	12	1.4 (0.0-7.7)	0.0 (0.0-5.1)
Others[b]					
Analgine[c]	1.4 (0.0-7.7)				
Bromhexine	2.9 (0.3-9.9)				
Dexamethason	1.4 (0.0-7.7)				

[a]Antibiotic prohibited in Vietnam according to Circular No. 15/2009/TT-BNN dated 17[th] March 2009.

[b]Drugs other than antimicrobials were not evaluated for compliance with withdrawal times.

[c]Analgine was manufactured for human medicine, not veterinary use, but was administered to birds by one farmer.

Table IV. Multivariable logistic regressions modeling the probability of non-compliance with on-label withdrawal times for at least one antimicrobial administered in food or water according to two risk scenarios (n=70 broiler farms, in two provinces of South Vietnam, 2011).

Predictor	Number of farms	Odds ratio		
		Estimate	95% CL	p-value
High-risk scenario[a]				
Bird breeds				
Asian-indigenous only	45	Reference		
Others[b]	25	5.4	1.6-18.4	<0.01
Vaccination against Infectious Bronchitis				
No	26	Reference		
Yes	44	7.9	1.9-32.6	<0.01
Low-risk scenario[a]				
Bird breeds				
Asian-indigenous only	45	Reference		
Others[b]	25	42.3	3.0-599.0	<0.01
Feed sources				
Commercial feed only	65	Reference		
Mixture of commercial and home-made feed	5	51.2	3.4-779.5	<0.01
Number of antimicrobials per farm over the last 6 months prior to the interview				
<6	32	Reference		
≥6	38	16.1	1.2-211.8	0.03

[a]Hosmer and Lemeshow Goodness-of-Fit Test: p= 0.31 (high-risk) and p = 0.73 (low-risk)
[b]The 25 farms were raising non-Asian-indigenous white birds, although 3 among these farms were raising both types of chickens.

Figure 1. Location of the two provinces surveyed (Dong Nai, Long An) in the study on antimicrobial use in South Vietnam, 2011

4. General discussion

4.1. Discussion of main results

It is of interest to compare drug use in broiler farms in Vietnam with usage in other countries. A research study conducted on 101 broiler farms in Thailand in 2007 showed that the most commonly used antimicrobials consisted of enrofloxacin, amoxicillin, doxycycline, colistine and roxithromycin (Na lampang, Chongsuvivatwong, & Kitikoon, 2007). The first four antimicrobial agents were also reported as the most commonly used in Vietnam and evidence of roxithromycin use was not found in Vietnam by the current study. Another research study conducted in 32 randomly selected broiler farms in Belgium between the years of 2007-2008 found that amoxicillin and tylosin were the most commonly used (Persoons et al., 2012), which are the same findings of the current study in Vietnam. In addition, the combination of trimethoprim-sulfonamide was found to be one of the most common multi-drug mixtures used against coccidiosis in Belgium (Persoons et al., 2012) and in the current study.

Regarding drug administration practices, drugs were administered either as a single drug or in a multi-drug combination with an average of 3.6 days (ranging from 1.7 to 7 days) for dosing duration per course of administration, and there was a mean of 2.0 (ranging from 1 to 8 times) administration courses per broiler production cycle for either a single drug or a mixture of multi-drugs (see Appendix 3). As reported, one farm raising non-Asian indigenous birds used tilmicosin 8 times (3 days/administration) per production cycle. Thus, 24 days of antimicrobial use in a 45-day production cycle might be either overuse or overrepresented because of recall bias. In addition, two farms reported the use of gentamicin or amoxicillin for the treatment or prevention of coccidiosis. Coccidiosis is caused by a protozoan genus of *Eimeria*, and is a disease of major importance in the chicken industry, causing mortality, reduced weight gain and increasing the susceptibility to other diseases (Co., 2010; Yegani & Korver, 2008). However, gentamicin and amoxicillin are not reported to be effective against protozoa (Giguère et al., 2006), which might represent a drug misuse. On the other hand, it might also be mistakenly reported by farmers when being interviewed because of lack of knowledge of diseases and veterinary medicine. In other words, they might have actually used the 2 antibiotics for different target diseases, and not for coccidiosis. This might be because farmers administered antimicrobials mainly based on their personal experience or drug-sellers' consultation after only a description of disease symptoms (Vu et al., 2010), a phenomena reported in the current study. After listening to farmer descriptions of disease symptoms, drug-sellers could give farmers drug products based on their drug-selling experience and instructions shown on product labels. Consequently, although farmers used appropriate drugs following drug-seller instructions, farmers might not exactly remember the target disease at the time of being interviewed and could have inaccurately answered the question about targeted disease. Furthermore, some producers remembered only the active ingredients used, but not the commercial name of the product, and we could not retrieve the whole mixture of all the active ingredients in one product, leading to misinformation. In other words, these single ingredients might have been used in combination with other ingredients to treat or prevent two kinds of concurrent digestive diseases caused by both bacteria and protozoa, rather than solely one digestive protozoal disease like coccidiosis.

It is worth noting that for colistin and tylosin, around 25% of farms did not comply with withdrawal times based on our estimates from the high-risk scenario, representing a drug misuse. Interestingly, in a previous study conducted in Vietnam, residues above the national maximum were detected in chicken meat (2/75 samples) for tylosin (Dang, 2010), which is consistent with the results from our study.

Some farm characteristics were associated with the probability of producers' non-compliance with on-label withdrawal times. In both risk scenarios, producers' non-compliance with on-label withdrawal times was higher in farms raising birds other than Asian-indigenous breeds. Bird breed was the only risk factor common in both risk scenarios. This association was expected since bird breeds are highly correlated with resistance to infectious diseases and possibly flock management. In fact, all farms raising only Asian-indigenous birds were using vaccination against Fowl Pox. This could explain in part a lower risk of non-compliance due to a lesser use of antimicrobials in these flocks, either due to a smaller instance of secondary infections or because they have better management practices overall. However, a vaccination variable (Infectious Bronchitis) was selected in the high risk scenario only, and could be interpreted more as a proxy for more recurrent health problems overall and more misuse of antimicrobials.

In the low-risk scenario, producers' non-compliance with on-label recommended withdrawal times was higher in farms using a combination of commercial and home-made feed than commercial feed only. In fact, farmers using home-made feeds could be more likely to report or be aware of the addition of antimicrobials as growth promoters to their feed, with potential non-compliance with withdrawal times, than farmers only using commercial feeds. This might also be because farmers added antimicrobials to home-made feed for growth promotion or disease prevention. Moreover, the mixture of commercial and home-made feeds may cause some health risks for birds if home-made feed is already contaminated. Indeed, home-made feeds noted in the survey involve corn, other grains, human food leftovers from home, and slaughter by-products. Leftovers from human food and slaughter by-products can potentially be contaminated with micro-organisms such as *E.coli* and *Salmonella* because slaughter by-products are not normally preserved in hygienic conditions on farms in Vietnam and thus micro-organisms present in slaughter by-products might rapidly develop, especially in the high humidity in Vietnam. Consequently, birds are more vulnerable to being infected by pathogenical opportunistic microbes. As a consequence, antimicrobials may be employed as a therapy for infections, potentiating antimicrobial residues, especially treatments at the end of rearing due to poor health conditions. Furthermore, the use of 6 or more different antimicrobials was associated with a higher risk of non-compliance with recommended withdrawal times. This is attributed to a probable higher use of antimicrobials in general, with a potential higher risk of not complying with withdrawal times at least once.

Risk factor differences between both low and high-risk scenarios might be explained by the fact that the producer's non-compliance with on-label recommended withdrawal times varied between the two scenarios, which were estimated on the basis of differences between on-farm withdrawal times and on-label withdrawal times. In fact, some farms were non-compliant with on-label recommended withdrawal times in the high-risk scenario but were compliant with on-label recommended withdrawal times in the low-risk scenario. The producer's non-compliance with on-label recommended withdrawal times varied between 14.3% in the low-risk scenario and 44.3% in the high-risk scenario. Consequently, farm characteristics selected as explanatory variables for logistic regression models were potentially different between both scenarios depending on the level of compliance with on-label recommended withdrawal times in each scenario. In other words, the distribution of explanatory variables were probably different between farms for which we have greater evidence of non-compliance with label withdrawal times compared to those that were closer to compliance.

4.2. Study strengths and limitations

To the best of our knowledge, this research study is the first to date that evaluated the association between farm characteristics and non-compliance with on-label mandatory withdrawal times in broiler productions in Vietnam or in other countries. Potential risk factors were tested for two scenarios defined as low-risk and high-risk scenarios based on non-compliance with on-label withdrawal times. Establishing what the biggest potential risk factors are can help interventions for preventing harmful antimicrobial residues likely present in broiler meat. The collection of antimicrobials suspected as residues in this study could also serve as a foundation for a follow-up surveillance and control program on antimicrobial residues in chicken meat. In particular, this should be prioritized to control harmful residues of the most commonly used antimicrobials that were suspected in chicken meat from the highest percentage of farms non-compliant with label withdrawal times, which were: ampicillin, amoxicillin, colistin, tylosin, enrofloxacin, and doxycyclin. The surveillance and control program should first target farms raising non-Asian birds in Dong Nai and Long An. Further, results from a surveillance and control program like this can support a future risk analysis study of antimicrobial residues in chicken meat in Dong Nai and Long An.

Concerning the selection of the farms, a convenience sampling method was used from the list of chicken farms administrated by the animal health authorities. They directly contacted broiler-farm owners on their list to invite them to enroll in the research study. As sampled, the 70 selected broiler farms met the criterion of having more than 1000 birds within the selected regions of the study: the 5 districts of Dong Nai (Trang Bom, Vinh Cuu, Long Thanh, Thong Nhat, and Xuan Loc) and the 1 city and 2 districts of Long An (Tan An city, Chau Thanh, and Tan Tru). Thus, the source population from which the 70 broiler farms were drawn might be defined as the broiler farms listed on the overall list of all the broiler farms with more than 1000 birds within the selected regions. According to direct phone calls between local officials and farmers, all 70 broiler farm owners agreed to participate in the study after the first contact. This high participation rate favors the representativeness of our sample. However, the authorities might have had a better connection with all these broiler-farm owners, and therefore, not all broiler-farm owners might have had the same probability of being recruited into the study. In other words, we cannot exclude the possibility that the enrolled farms presented some differences in management practices compared to other farms. Despite this fact, we had no indication of significant differences between sampled and non-sampled farms during the field work. Moreover, the selected farms were located in various geographical areas of the targeted provinces, favoring the representativeness of our sample. It should also be noted that according to national law, the local authorities are responsible to administrate every chicken farm within their regions. Thus, contact with every farm was expected.

The target population might be defined as the broiler farms with more than 1000 birds across all the cities and districts in both of Dong Nai and Long An (beyond the 8 regions specified above). To increase the representativeness of the sample, a formal random sampling procedure could have been carried out from the source population which was shown in the overall list of all the farms having more than 1000 birds across all of Dong

Nai and Long An. In this case, the source population would also be the target population, and thus, internal and external inferences would be more valid.

Regarding questionnaire design, there was a wording problem with question 41 (see Appendix 2) regarding sanitary practices which were also considered as potential risk factors associated with antimicrobial use. In particular, the definition of "cleaning" and "disinfection" were not differentiated and classified into: cleaning with only water, cleaning with water and soaking, cleaning with water and disinfectant agents, and/or spray with disinfectant agents. When being translated to Vietnamese, the two terms cleaning and disinfection were similar to the term "sanitation," which encompasses all of the above practices. As a result, all sanitation practices were applied to all the farms (see Table I), thus could not be included in the logistic regression analyses.

Additional risk factors associated with bird health and therefore with antimicrobial use could have been considered for inclusion in the questionnaire, such as: use of competitive exclusion products (called antimicrobial alternatives), and use of nutrients (vitamins). This could be considered a gap in the questionnaire design. Given that farmers expressed impatience with the duration of the one hour questionnaire, an ideal decision would have been to include these questions but limit the questions about antimicrobial administrations to the most recent flock sent to slaughter prior to the interview instead of the last 6-month period where 1-3 flocks had been produced.

That drug use was often reported for only the most recent use over the last 6 months prior to the interview might lead to underestimation of non-compliance with label withdrawal times. In particular, if the same drug was used in more than one flock in a range of 1-3 flocks within the last 6 months, that drug was reported for only the most recent use and thus, non-compliance with label withdrawal times might be underestimated if the same drug was used between the flocks raised over the last 6 months but with shorter withdrawal times actually practiced on farm.

Moreover, most farmers did not have drug records when being interviewed, so it was subject to recall bias. Information bias on drug use could be potentially higher when farmers only remembered the name of the most important active ingredients of the drug product, but did not remember the commercial product name, so that all the active ingredients of the drug product could not be retrieved from drug label. Thus, this recall bias might lead to overrepresentation of single drugs and underrepresentation of multi-drug mixtures as mentioned in Appendix 3. Moreover, information bias might have happened with the information about kinds of "diseases" listed in Appendix 3 because the diseases were identified primarily by the farmer's clinical (not laboratory) diagnosis. Thus, some farmers might not have been able to differentiate between digestive coccidiosis by protozoa and other digestive diseases by other pathogens. Thus, they might have misreported the two kinds of digestive diseases.

After most interviews, the researcher visited places on the farms where packaging/labels might be found or stored, such as drug cabinets, garbage areas, and feed storage rooms. All the labels that were tracked down during farm visits were the same as the information provided by farmers during interviews. However, a systematic procedure of locating labels on farms was not undertaken for all the visited farms, and therefore, it is difficult to assess how much misinformation might exist in our data. However, many measures were taken to exploit as many drugs as possible and also, to reduce recall bias : (1) during the interviews, the farmers were asked to show the drug products so that the researcher could compare their responses with the drug product labels and retrieve the active ingredients of the drug product; (2) the commercial names of the drug products were given by farm owners if the labels of the drug products were not available on farms and the active ingredients of the drug product were retrieved by using drug labels from catalogues collected from veterinary drug stores in both Dong Nai and Long An provinces and by accessing websites of veterinary drug production companies; (3) to remind farmers of the antimicrobials they may have used, they were presented with a list of the antimicrobial agents most commonly used in chicken production in Northern Vietnam as surveyed in a recent study (Vu et al., 2010): amoxicillin, ampicillin, gentamicin, enrofloxacin, tylosin, doxycylin, oxytetracyclin, tetracycline, colistin, and sulfa-trimethoprim; (4) the places on farms where labels might be found were visited together by both the researcher and the farmer to identify as many labels as possible.

The risk factors found in our study should be considered as potentially important variables in predicting non-compliance with withdrawal times and warrant further study in any initiative focusing on intervention. The validity of the association between the potential risk factors and non-compliance with withdrawal times on labels in the logistic regression models is supported by the following:

(1) The explanatory variables included for analyses: although this questionnaire was not formally validated prior to use, at least for the variables directly observable at the farm (ex. type of shelter, breed of birds, etc.), validity of the responses was favored by the on-site administration of the questionnaire. For these variables, no difference was noted between farmer responses and direct observations, increasing the likelihood that explanatory variables were valid. However, explanatory variables such as the number of antimicrobials per farm over the last 6 months prior to the interview could not be validated because there were no retrospective six-month drug records available on the farms.

(2) The outcome variable (compliance with label withdrawal time): To avoid inaccuracy about drug use, compliance with label withdrawal times was defined according to a range of days. On-farm withdrawal times of each specific antimicrobial were estimated to be in the range from the minimal to the maximal based on the variation of the age of birds at slaughter and the last day of antimicrobial use to increase the likelihood that true on-farm withdrawal times were somewhere within the estimated range. Farmers' non-compliance with on-label withdrawal times was considered within the two low- and high-risk scenarios based on the comparison between the range of the minimal-to-maximal on-farm withdrawal times and the range of the minimal-to-maximal on-label withdrawal times for each antimicrobial to make sure that the true percentage of farms non-compliant with on-label withdrawal times was somewhere among the range of estimates between the two scenarios for each antimicrobial, then again for all antimicrobials. However, we cannot exclude that misclassification of farmer's compliance with on-label withdrawal times might exist due to recall bias of drug use over the last 6 months.

However, when inferences about the association between farm characteristics and non-compliance with on-label withdrawal times from the sample data to both of the source and target populations, the association may be also subject to a type I error (α = 5%), which is the probability of rejecting the null hypothesis that there is no real association between farm characteristics and non-compliance with on-label withdrawal times. In our study, many different variables were tested for the statistical models, increasing the likelihood that some of them were statistically significant only by chance. Regarding the statistical models, variables such as "company owned or not" were not selected for inclusion in the final model, but statistical power could have been an issue. Finally, the statistically significant risk factors found here may not have a real effect if removed from the population. In fact, the observed associations could be confounded by other factors and not be causal relationships. No previous studies, in Vietnam or elsewhere, on the evaluation of association between farm characteristics and non-compliance with label withdrawal times in broiler productions were found with which to compare. Thus, to have science-based evidence that the association was not found by chance and was likely causal, other follow-up studies should be done in order to validate the significant risk factors found in this study.

4.3. Interventions and recommendations:

In order to prevent antimicrobial residues in chicken carcass meat offered to the consumer, it is necessary to base risk analysis for the whole production process from farm to table in Vietnam. Particularly, the following fundamental principles should be adhered to:

(1) Disease prevention: With the approach "prevention is better than a cure," it has been noted that antimicrobial use for prophylaxis should never be practiced as a substitute for poor preventive veterinary measures (Guardabassi et al., 2008). Therefore, preventive measures should be improved to prevent contagious pathogens from entering the flocks and to enhance host immunity.

In order to reduce the risk of infections, bio-security measures should be exercised following Good Animal Husbandry Practices for Poultry in Vietnam (VIETGAHP) at

Decision No. 1504/QĐ-BNN-KHCN dated 15 May 2008 because the survey result demonstrated that only 13/70 farms were aware of this regulation. From our database and through direct observations on farm, we identified some indications of bio-security measures that should be improved: ensure good manure waste management to prevent coccidiosis; isolate sick birds into a separate place away from healthy birds to prevent pathogen transmission to healthy birds; disinfect the places where dead birds were buried or burned to prevent the re-emergence of surviving pathogens; separate birds from other animals completely; disinfect equipment and boots, hands, gloves, etc. of workers and visitors; and ensure the good health of farm workers through periodic health examinations.

At the same time, host immunity should be improved through high-quality feeds, good drinking water, nutrients, vitamins and minerals, and good vaccination practices (Guardabassi et al., 2008; Knechtges, 2012):

(a) The quality of drinking water needs to be tested to satisfy the Vietnamese technical regulation of drinking water quality promulgated at QCVN 01:2009/BYT by Circular No. 04/2009/TT- BYT dated 17 June 2009 before using.

(b) The quality of commercial and home-made feed mixture needs to be quantitatively tested to verify whether it can meet the Vietnamese technical regulation on the maximum levels of antibiotics, drugs, microorganisms and heavy metals in completed feeds for chickens promulgated at QCVN 01 - 10: 2009/BNNPTNT by Circular No. 81/2009/TT-BNNPTNT dated 25 December 2009, since the kind of feed was found to be a potential risk factor associated with antimicrobial residues suspected in the study. Also, the withdrawal time of antimicrobial feed additives in chicken feed must be observed.

(c) The efficacy of the vaccination against Infectious Bronchitis needs to be evaluated in Dong Nai and Long An because it was found to be a potential risk factor associated with antimicrobial residues suspected in chicken meat based on non-compliance with on-label mandatory withdrawal times.

Additionally, alternatives for antimicrobial growth promoters, such as probiotics, prebiotics, synbiotics, enzymes, acidifiers, antioxidants, and phytobiotics, are suggested for promoting growth and preventing diseases for animals (Ahmad, 2006; Fuller, 1989; Hajati

& Rezaei, 2010; Hume, 2011; Huyghebaert et al., 2011; Knechtges, 2012; Perié et al., 2009).

(2) Disease treatment: when no alternatives to antimicrobials can be given and when antimicrobial therapy is necessary to eliminate infectious target pathogens from the host, some principles should be followed:

(a) It is necessary to identify causal target pathogens at a given site of infection through clinical and laboratory diagnosis (Altman, 1997; Giguère et al., 2006; Guardabassi et al., 2008; Ritchie et al., 1996). To do this, veterinary laboratories in Vietnam need to be well equipped with chemicals, testing equipment, human resources, etc. to accurately diagnose target pathogens.

(b) It is necessary to select the antimicrobials to which causal infectious pathogens are susceptible for administration to the diseased host (Altman, 1997; Giguère et al., 2006; Guardabassi et al., 2008; Ritchie et al., 1996). An antimicrobial agent is deemed ideal if it can eliminate the susceptible pathogen by reaching an effective concentration at a given infection site, cause no toxicity and minimal stress to the host, minimize resistance of the pathogen, and be as cost-effective as possible (Giguère et al., 2006). To do this, veterinary laboratories in Vietnam need to be well equipped with chemicals, testing equipment, human resources, etc. to test antimicrobial susceptibility of isolated pathogens and to prescribe the appropriate antimicrobial agents for the target pathogen after accurate diagnosis

(c) It is necessary to comply with the instructions on licensed antimicrobial treatments and to use reasonable doses in sufficient dosing intervals during treatment durations via approved administration routes as instructed on labels to eliminate infectious pathogens. An exceptional off-label use should only be undertaken when prescribed by veterinary professionals (Guardabassi et al., 2008). It has also been noted that withdrawal times must be observed to assure that meat is clear of antimicrobial residues (Giguère et al., 2006; Guardabassi et al., 2008). To do this, farmers need to be trained about how to use antimicrobials to efficaciously treat diseases and to prevent antimicrobial residues and resistance.

(3) Surveillance and control programs on antimicrobial residues in chicken meat in Vietnam should be conducted by authorities in cooperation with relevant institutes or agencies to ensure that authorised antimicrobials are absent or at acceptable levels in chicken meat in conformity with Maximum Residue Levels lawfully laid down before handing to the consumer, satisfying Circular No. 29/2010/TT-BNNPTNT dated 6 May 2010.

(4) The state administration on veterinary antimicrobial use in Vietnam needs to be reinforced to stop farmers from using forbidden drugs and to limit use of restricted drugs regulated at Circular No. 15/2009/TT-BNN dated 17 March 2009. (Details in this circular were amended by Circular No. 29 /2009/TT-BNNPTNT dated 6 June 2009 and 20/2010/TT-BNNPTNT dated 2 April 2010). At the same time, authorities need to raise awareness that veterinary antimicrobials are also important human medicines.

(5) Research programs on alternatives for antimicrobials should be launched by institutes and universities, with the focus on alternatives sourced from herbal or organic natural products available in Vietnam.

4.4. Future studies

In view of the results from our study, the following research avenues are proposed:

(1) Quantitatively analysing antimicrobial residues (estimated to be likely present in chicken meat in Vietnam based on non-compliance with on-label withdrawal times found in this study) in both Asian and non-Asian indigenous flocks, since bird breed was found to be a potential risk factor in the current study. A quantitative assessment should be made about an association between antimicrobial residue levels (below MRL/above MRL) and bird breed (non-Asian and Asian indigenous) by Chi-square test to be sure that an intervention based on bird breed in Vietnam is based on scientific evidence and that the relationship between antimicrobial residue levels and bird breed is statistically significant with $p <0.05$.

(2) Evaluating the prevalence of microbial resistance of bacteria that are common in chickens and that cause enteric bacterial diseases in humans: *E.coli*, *Clostridium perfringens, Salmonella and Campylobacter*. Testing should be for resistance to the

commonly used antimicrobials found in this study: colistin, tylosin, ampicillin, enrofloxacin, amoxicillin, and doxycycline. And identification of risk factors associated with prevalence based on multivariable logistic regression models should be performed.

(3) Evaluating prevalence of microbial resistance of common protozoan-type parasites (*Eimeria tenella, Eimeria necatrix*) which cause coccidiosis in chickens. Testing should be for resistance to the commonly used anti-protozoals found in this study: amprolium, diaveridin, sulfaquinoxalin, sulfadimidin, sulfadiazine, sulfachlorpyridazin, trimethoprim, and pyrimethamine. Identification of risk factors associated with prevalence based on multivariable logistic regression models should also be performed.

(4) If implemented, results from a future surveillance and control program conducted in Dong Nai and Long An could be used for a future risk analysis study of antimicrobial residues in chicken meat in Dong Nai and Long An.

5. General conclusions

This study provides insight into the prevalence of drug use in broiler chicken farms in Vietnam and also gives indications as to which harmful antimicrobial residues are possibly present in chicken meat based on non-compliance with on-label mandatory withdrawal times. Furthermore, to the best of our knowledge, this research study is the first to assess the association between farm characteristics and non-compliance with on-label mandatory withdrawal times by broiler productions in Vietnam and has identified bird breed as a potential risk factor associated with non-compliance with on-label withdrawal times. To safeguard the consumer from health risks caused by exposure to harmful antimicrobial residues present in chicken meat, it is suggested that risk management measures should be well implemented from farm to table to reduce antimicrobial use and misuse. Future studies should be conducted on the quantification of antimicrobial residues in chicken meat in Vietnam and on the association between antimicrobial residue limits and bird breed.

Bibliography

A.R., N. (2008). Antibiotic Residues - A global health hazard. *Veterinary World, 1(12)*, 375-377.

Aarestrup, F. M. (1999). Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. [Review]. *International journal of antimicrobial agents, 12*(4), 279-285.

Aarestrup, F. M. (2004). Monitoring of antimicrobial resistance among food animals: principles and limitations. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health, 51*(8-9), 380-388.

Aarestrup, F. M. (2005). Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. [Review]. *Basic and clinical pharmacology and toxicology, 96*(4), 271-281.

Acar, J. F., & Moulin, G. (2012). Antimicrobial resistance: a complex issue. *Revue scientifique et technique (International Office of Epizootics), 31*(1), 23-31.

Ahmad, I. (2006). Effect of probiotics on broilers performace. *International Journal of Poultry Science, 5*(6), 593-597.

Aidara-Kane, A. (2012). Containment of antimicrobial resistance due to use of antimicrobial agents in animals intended for food: WHO perspective. *Revue scientifique et technique (International Office of Epizootics), 31*(1), 277-287.

Altman, R. B. (1997). *Avian medicine and surgery*. Philadelphia: Saunders.

Ambekar, C. S., Cheung, B., Lee, J., Chan, L. C., Liang, R., & Kumana, C. R. (2000). Metabolism of chloramphenicol succinate in human bone marrow. *European journal of clinical pharmacology, 56*(5), 405-409.

Angulo, F. J., Baker, N. L., Olsen, S. J., Anderson, A., & Barrett, T. J. (2004). Antimicrobial use in agriculture: controlling the transfer of antimicrobial resistance to humans. [Review]. *Seminars in pediatric infectious diseases, 15*(2), 78-85.

Angulo, F. J., Nargund, V. N., & Chiller, T. C. (2004). Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. [Review]. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health, 51*(8-9), 374-379.

Anthony, F., Acar, J., Franklin, A., Gupta, R., Nicholls, T., Tamura, Y., Thompson, S., Threlfall, E. J., Vose, D., Van Vuuren, M., White, D. G. (2001). Antimicrobial resistance: responsible and prudent use of antimicrobial agents in veterinary medicine. *Revue scientifique et technique (International Office of Epizootics), 20*(3), 829-839.

Apata, D. F. (2009). Antibiotic resistance in poultry. *International Journal of Poultry Science, 8*(4), 404-408.

Barbosa, J., Freitas, A., Moura, S., Mourao, J. L., Noronha da Silveira, M. I., & Ramos, F. (2011). Detection, accumulation, distribution, and depletion of furaltadone and nifursol residues in poultry muscle, liver, and gizzard. *Journal of agricultural and food chemistry, 59*(22), 11927-11934.

Barbosa, J., Freitas, A., Mourao, J. L., Noronha da Silveira, M. I., & Ramos, F. (2012). Determination of furaltadone and nifursol residues in poultry eggs by liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of agricultural and food chemistry, 60*(17), 4227-4234.

Barton, M. D. (2000). Antibiotic use in animal feed and its impact on human health. *Nutrition Research Reviews, 13*(2), 279-299.

Benet, L. Z. (1984). Pharmacokinetic parameters: which are necessary to define a drug substance? *European journal of respiratory diseases. Supplement., 134*, 45-61.

Beovic, B. (2006). The issue of antimicrobial resistance in human medicine. [Review]. *International journal of food microbiology, 112*(3), 280-287.

Berendsen, B., Stolker, L., de Jong, J., Nielen, M., Tserendorj, E., Sodnomdarjaa, R., Cannavan, A., Elliott, C. (2010). Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. *Analytical and bioanalytical chemistry, 397*(5), 1955-1963.

Berkow, R., Beers, M. H., & Fletcher, A. J. (1997). *The Merck manual of medical information* (Home ed.). Whitehouse Station, N.J.: Merck Research Laboratories.

Billings, R. J., Berkowitz, R. J., & Watson, G. (2004). Teeth. [Review]. *Pediatrics, 113*(4 Suppl), 1120-1127.

Bioagrimix. (2012, 17 September 2012). Monensin and Salinomycin in Veterinary Medicine - Overview, from *http://www.bioagrimix.com/haccp/html/ionophores.html*

Botsoglou, N. A., & Fletouris, D. J. (2001). *Drug residues in foods : pharmacology, food safety, and analysis*. New York: Marcel Dekker.

Burgos, S., Hanh, P. T. H., Roland-Holst, D., & Burgos, S. A. (2007). Characterization of poultry production systems in Vietnam. *International Journal of Poultry Science, 6*(10), 709-712.

Burgos, S., Hinrichs, J., Otte, J., Pfeiffer, D., & Roland-Holst, D. (2008). Poultry, HPAI and livelihoods in Vietnam - A review. Rome.

Caimmi, S., Caimmi, D., Lombardi, E., Crisafulli, G., Franceschini, F., Ricci, G., & Marseglia, G. L. (2011). Antibiotic allergy. [Review]. *International journal of immunopathology and pharmacology, 24*(3 Suppl), S47-53.

Canton, R. (2009). Antibiotic resistance genes from the environment: a perspective through newly identified antibiotic resistance mechanisms in the clinical setting. [Review]. *Clinical microbiology and infection, 15 Suppl 1*, 20-25.

Canton, R., & Ruiz-Garbajosa, P. (2011). Co-resistance: an opportunity for the bacteria and resistance genes. [Review]. *Current opinion in pharmacology, 11*(5), 477-485.

Carter, G. R., & J.Wise, D. (2004). *Essentials of veterinary bacteriology and mycology* (6th ed.): Blackwell.

CDC. (2005, 1 June 2005). National antimicrobial resistance monitoring system - Frequently asked questions about antibiotic resistance Retrieved 4 February, 2012, from http://www.cdc.gov/narms/faq_pages/7.htm

Chang, S. K., Davis, J. L., Cheng, C. N., Shien, R. H., Hsieh, M. K., Koh, B. W., & Chou, C. C. (2010). Pharmacokinetics and tissue depletion of florfenicol in Leghorn and Taiwan Native chickens. *Journal of veterinary pharmacology and therapeutics, 33*(5), 471-479.

Charlton, B. R., & American Association of Avian Pathologists. (2006). *Avian disease manual* (6th ed.). Athens, Ga.: Order from American Association of Avian Pathologists.

Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., Maxwell, S., Aminov, R. I. (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. [Review]. *Journal of environmental quality, 38*(3), 1086-1108.

Chylinski, J., & Zegarski, W. (1971). Case of toxic bone marrow depression following treatment with chloramphenicol. *Polski tygodnik lekarski, 26*(21), 801-802.

Co., M. (2010). *The Merck Veterinary Manual* (10th ed.). Whitehouse Station, NJ: Merck & Co., Inc.

Codex. (1993). *Glossary of terms and definitions (residues of veterinary drugs in foods)*.

Codex. (2009). *Guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals*.

Codex Alimentarius Commission. (2005). Code of Practice to minimize and contain antimicrobial resistance.

Collignon, P. (2012). Clinical impact of antimicrobial resistance in humans. *Revue scientifique et technique (International Office of Epizootics), 31*(1), 211-220.

Compassion in World Farming. (2011). Antibiotics in animal farming-public health and animal welfare. Retrieved from http://www.ciwf.org.uk/includes/documents/cm_docs/2011/a/antibiotics_in_animal_farming.pdf
http://www.fao.org/fileadmin/user_upload/animalwelfare/antibiotics_in_animal_farming.pdf

Comte, D., Petitpierre, S., Spertini, F., & Bart, P. A. (2012). Allergy to beta-lactam antibiotics. *Revue médicale suisse, 8*(337), 836, 838-842.

Council, N. R. (1999). *The use of drugs in food animals : benefits and risks*. Washington, D.C.: National Academy Press.

Cox, L. A., Jr., & Popken, D. A. (2006). Quantifying potential human health impacts of animal antibiotic use: enrofloxacin and macrolides in chickens. *Risk analysis, 26*(1), 135-146.

Cromwell, G. L. (2005). Feed supplements: antibiotics *Encyclopedia of animal science*.

Dang, B. N. (2010). *Prevalence of antibiotic residues in pork and chicken meat in some slaughterhouses and markets in the Northern region in Vietnam.* Master thesis, Hanoi University of Agriculture, Vietnam.

Dantri (Producer). (2011, 26 January 2012). ADB: Vietnam has potential to avoid "middle-income trap". Retrieved from http://dantri.com.vn/c76/s76-478029/ADB-Viet-am-co-tiem-nang-de-thoat-bay-thu-nhap-trung-binh.htm

Deatherage, F. E. (1957). Use of antibiotics in the preservation of meats and other food products. *Am J Public Health Nations Health, 47*(5), 594-600.

Delquign, T., Edan, M., Nguyen, D. H., Pham, T. K., & Gautier, P. (2004). Evolution and impact of avian influenza epidemic and description of the avian production in Vietnam.

Desvaux, S., Vu, D. T., Phan, D. T., & Pham, T. T. H. (2008). A general review and a description on the poultry production in Vietnam: Agricultural Publishing House.

Dever, L. A., & Dermody, T. S. (1991). Mechanisms of bacterial resistance to antibiotics. [Review]. *Archives of internal medicine, 151*(5), 886-895.

Dibner, J. J., & Richards, J. D. (2005). Antibiotic growth promoters in agriculture: history and mode of action. [Review]. *Poultry science, 84*(4), 634-643.

Ding, C., & He, J. (2010). Effect of antibiotics in the environment on microbial populations. [Review]. *Applied microbiology and biotechnology, 87*(3), 925-941.

Dols, M. J. (1968). The use of antibiotics for food preservation. *Bibl Nutr Dieta, 10*, 77-80.

Dorrestein, G. M., van Gogh, H., & Rinzema, J. D. (1984). Pharmacokinetic aspects of penicillins, aminoglycosides and chloramphenicol in birds compared to mammals. A review. [Review]. *Veterinary quarterly, 6*(4), 216-224.

Dröge, M., Pühler, A., & Selbitschka, W. (1999). Horizontal gene transfer among bacteria in terrestrial and aquatic habitats as assessed by microcosm and field studies. [Review]. *Biology and fertility of soils, 29*, 221-245.

Dwight C. Hirsh, N. J. M., Richard L. Walker. (2004). Veterinary microbiology Retrieved from http://books.google.ca/books?id=FYdWYCUOFxsC&pg=PA26&dq=veterinary+Antibiotic+classification&hl=en&sa=X&ei=IGgrT5aUI8SOgwePsL3tDw&sqi=2&ved=0CHMQ6AEwCQ#v=snippet&q=Antibiotic%20classification&f=false

Epprecht, M. (2005). Geographic dimensions of livestock holdings in Vietnam. Spatial relationships among poverty, infrastructure and the environment *A living from livestock. Pro-poor livestock policy initiative*.

EU. (2005). Ban on antibiotics as growth promoters in animal feed enters into effect. (IP/05/1687). Retrieved 20 June 2012 http://europa.eu/rapid/pressReleasesAction.do?reference=IP/05/1687&format=HTML&aged=0&language=EN

FAO. (2006). Rapid growth of selected Asian economies. Lessons and implications for agriculture and food security. Republic of Korea, Thailand and Vietnam.

FAPRI. (2010). World Agricultural Outlook Retrieved from http://www.fapri.iastate.edu/outlook/2010/

Farber, T. M., & Guest, G. B. (1984). A strategy for the assessment of the carcinogenic potency of veterinary drug residues: recommendations of the FDA. *Food additives and contaminants, 1*(2), 213-219.

Frere, J. M., Joris, B., Granier, B., Matagne, A., Jacob, F., & Bourguignon-Bellefroid, C. (1991). Diversity of the mechanisms of resistance to beta-lactam antibiotics. [Review]. *Research in microbiology, 142*(6), 705-710.

Fuller, R. (1989). Probiotics in man and animals. [Review]. *Journal of applied bacteriology, 66*(5), 365-378.

G.Piva, & F.Rossi. (1998). Possible alternatives to the use of antibiotics as growth promotors. New additives. *Ciheam - Options Mediterraneennes*, 88-106.

Gafin (Producer). (2011, 26 January 2012). EIU forecasts Vietnam's expected GDP growth in 2011 down to 6%. Retrieved from http://gafin.vn/20110818033158463p0c33/eiu-ha-du-bao-tang-truong-gdp-viet-nam-nam-2011-xuong-6.htm

Giguère, S., Prescott, J. F., Baggot, J. D., Walker, R. D., & M.Dowling, P. (2006). *Antimicrobial therapy in veterinary medicine* (4rd ed.). Blackwell.

Goldstein, E. J. (2011). Beyond the target pathogen: ecological effects of the hospital formulary. [Review]. *Current opinion in infectious diseases, 24 Suppl 1*, S21-31.

Goudah, A. (2009). Pharmacokinetics and tissue residues of moxifloxacin in broiler chickens. [Comparative Study]. *British poultry science, 50*(2), 251-258.

GSO. (2004). Socio-economic impact of avian influenza (F. a. F. S. Agricultural, Trans.).

GSO. (2010). Stastics by domain. Retrieved 20 January 2012 http://www.gso.gov.vn/default.aspx?tabid=512&idmid=6

GSO. (2011). Statistical Yearbook of Viet Nam

Guardabassi, L., Jensen, L. B., & Kruse, H. (2008). *Guide to antimicrobial use in animals*. Oxford, UK ; Ames, Iowa: Blackwell Pub.

Hackstadt, T. (1996). The biology of rickettsiae. [Review]. *Infectious agents and disease, 5*(3), 127-143.

Hajati, H., & Rezaei, M. (2010). The application of prebiotics in poultry production. *International Journal of Poultry Science, 9*(3), 298-304.

Hartzen, S. H., & Frimodt-Moller, N. (1986). Toxic bone marrow depression after local use of chloramphenicol eye agents. *Ugeskrift for laege, 148*(35), 2225-2227.

Hume, M. E. (2011). Historic perspective: prebiotics, probiotics, and other alternatives to antibiotics. [Historical Article]. *Poultry science, 90*(11), 2663-2669.

Huyghebaert, G., Ducatelle, R., & Van Immerseel, F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. [Review]. *Veterinary journal, 187*(2), 182-188.

Huynh, P. S. (2000). *Survey on antibiotic use in Thu Duc and No.9 Districts in Ho Chi Minh city.* Veterinary Doctor at Undergraduate Level, Agricultural University in Ho Chi Minh city, Vietnam.

Huynh, T. N. D. (2000). *Survey on antibiotic use in industrial broiler chicken production in Hoc Mon distric in Ho Chi Minh city.* Veterinary Doctor at Undergraduate Level, Agricultural University in Ho Chi Minh city, Vietnam.

Kemper, N. (2008). Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological Indicators, 8*(1), 1-13.

Knechtges, P. L. (2012). *Food safety : theory and practice*. Sudbury, Mass.: Jones & Bartlett Learning.

Krasucka, D., & Kowalski, C. J. (2010). Pharmacokinetic parameters of amoxicillin in pigs and poultry. [Comparative Study]. *Acta poloniae pharmaceutica, 67*(6), 729-732.

Kummerer, K. (2008). *Pharmaceuticals in the environment : sources, fate, effects and risks* (3rd ed.). New York: Springer.

Largo, V. (2002). *Vietnam : current issues and historical background*. New York: Nova Science Publishers.

Lees, P., & Toutain, P. L. (2012). The role of pharmacokinetics in veterinary drug residues. *Drug testing and analysis, 4 Suppl 1*, 34-39.

Liu, Y., Huang, L., Wang, Y., Yang, B., Ishan, A., Fang, K., . . . Yuan, Z. (2010). Tissue depletion and concentration correlations between edible tissues and biological fluids of 3-amino-2-oxazolidinone in pigs fed with a furazolidone-medicated feed. *Journal of agricultural and food chemistry, 58*(11), 6774-6779.

Lokhande, J., Juvekar, A. S., & Kulkarni, K. P. (2007). Chloramphenicol: screening and review to evaluate its potential beneficial effects in leukaemia. *Journal of the Indian Medical Association, 105*(4), 224, 226-228.

Lowenthal, J. W., Lambrecht, B., van den Berg, T. P., Andrew, M. E., Strom, A. D., & Bean, A. G. (2000). Avian cytokines - the natural approach to therapeutics. [Review]. *Developmental and comparative immunology, 24*(2-3), 355-365.

Luong, T. T. (2010). *Evaluation on antibiotic use on pig and chicken farms in provinces of Northen Vietnam.* Hanoi University of Argiculture, Vietnam.

Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: impacts on human health. [Review]. *Clinical microbiology reviews, 24*(4), 718-733.

Martinez, J. L. (2008). Antibiotics and antibiotic resistance genes in natural environments. *Science, 321*(5887), 365-367.

Mather, A. E., Matthews, L., Mellor, D. J., Reeve, R., Denwood, M. J., Boerlin, P., . . . Reid, S. W. (2012). An ecological approach to assessing the epidemiology of antimicrobial resistance in animal and human populations. *Proceedings. Biological sciences / The Royal Society, 279*(1733), 1630-1639.

Mathew, A. G., Cissell, R., & Liamthong, S. (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. [Review]. *Foodborne pathogens and disease, 4*(2), 115-133.

Mazel, D., & Davies, J. (1999). Antibiotic resistance in microbes. [Review]. *Cellular and molecular life sciences : CMLS, 56*(9-10), 742-754.

McCracken, R. J., Van Rhijn, J. A., & Kennedy, D. G. (2005). The occurrence of nitrofuran metabolites in the tissues of chickens exposed to very low dietary concentrations of the nitrofurans. *Food additives and contaminants, 22*(6), 567-572.

McDermott, P. F., Zhao, S., Wagner, D. D., Simjee, S., Walker, R. D., & White, D. G. (2002). The food safety perspective of antibiotic resistance. [Review]. *Animal biotechnology, 13*(1), 71-84.

Merck Manual. (2012). Pharmacokinetics. http://www.merckmanuals.com/professional/clinical_pharmacology/pharmacokinetics/drug_absorption.html

Miller, J. B. K. a. R. (1997). Problems associated with drug residues in beef from feeds and therapy. *Revue scientifique et technique (International Office of Epizootics), 16(2)*, 694-708.

Molbak, K. (2004). Spread of resistant bacteria and resistance genes from animals to humans-the public health consequences. [Review]. *Journal of veterinary medicine. B, Infectious diseases and veterinary public health, 51*(8-9), 364-369.

Morley, P. S., Apley, M. D., Besser, T. E., Burney, D. P., Fedorka-Cray, P. J., Papich, M. G., Traub-Dargatz, J. L., Weese, J. S. (2005). Antimicrobial drug use in veterinary medicine. [Review]. *Journal of veterinary internal medicine, 19*(4), 617-629.

Mwale, M., Bhebhe, E., Chimonyo, M., & Halimani, T. E. (2005). Use of herbal plants in poultry health management in the Mushagashe small-scale commercial farming area in Zimbabwe. *International journal of applied research in veterinary medicine., 3*(2), 163-170.

Na lampang, K., Chongsuvivatwong, V., & Kitikoon, V. (2007). Pattern and determinant of antibiotics used on broiler farms in Songkhla province, southern Thailand. *Tropical animal health and production, 39*(5), 355-361.

National Academy of Argicultural Sciences. (2010). Antibiotics in manure and soil - A grave threat to human and animal health.

Nguyen, T. K. D. (2005). *Evaluation of agri-industrial by-products as protein source fo Duck production in the Mekong delta of Vietnam.* Doctoral Dissertation, Swedish University of Agricultural Sciences. Retrieved from http://pub.epsilon.slu.se/907/1/Thesis18-8-05.pdf

Nguyen, V. A. (2001). *Survey on antibiotic use in industrial boiler chicken production in district no.12 in Ho Chi Minh city.* Veterinary Doctor at Undergraduate level, Agricultural University in Ho Chi Minh city, Vietnam.

Nguyen, V. D. (2001). *Survey on antibiotic use in industrial broiler chicken production in Cu Chi district in Ho Chi Minh city.* Veterinary Doctor at Undergraduate level, Agricultural University in Ho Chi Minh city, Vietnam.

Nguyen, V. D. (2008). *Evaluation the quality of veterinary drugs being marketted in Vietnam.* Master thesis, Hanoi University of Agriculture, Vietnam.

Nguyen, V. D., & Tran, L. (2008). Poultry production systems in Vietnam (N. I. o. A. H. Animal Genetics and Breeding Department, Vietnam, Trans.).

Nilsson, O. (2009). Prevalence and antibiotic resistance of Salmonella enterica in poultry and raw meat in the Can Tho province, Vietnam (Vol. 33): Fakulteten för Veterinärmedicin och husdjursvetenskap, Institutionen för biomedicin och veterinär folkhälsa.

Obeng, A. S., Rickard, H., Sexton, M., Pang, Y., Peng, H., & Barton, M. (2012). Antimicrobial susceptibilities and resistance genes in Campylobacter strains isolated from poultry and pigs in Australia. *Journal of applied microbiology, 113*(2), 294-307.

Oliver, S. P., Murinda, S. E., & Jayarao, B. M. (2011). Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. [Review]. *Foodborne pathogens and disease, 8*(3), 337-355.

Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. [Review]. *Poultry science, 82*(4), 627-631.

Percival, S. L., Knottenbelt, D. C., & Cochrane, C. A. (2011). *Biofilms and veterinary medicine*. Heidelberg ; New York: Springer.

Perié, L., Zikié, D., & Lukié, M. (2009). Application of alternative growth promoters in broiler production. *Biotechnology in Animal Husbandry, 25*(5-6), 387-397.

Persoons, D., Dewulf, J., Smet, A., Herman, L., Heyndrickx, M., Martel, A., Catry, B., Butaye, P., Haesebrouck, F. (2012). Antimicrobial use in Belgian broiler production. *Preventive veterinary medicine, 105*(4), 320-325.

Pham, Q. D. (2001). *Survey on antibiotic use in boiler chicken produciton in Ben Cat and Phu Giao districts in Binh Duong province.* Undergraduate thesis, Agricultural University in Ho Chi Minh city, Vietnam.

Pham, T. H. H., Burgos, S., & Roland-Host, D. (2007). The poultry sector in Vietnam: prospects for smallholder producers in the aftermath of the HPAI crisis *Pro-poor livestock policy initiative. A living from livestock*.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Cabrera, L., Preston, R., Waddell, J. (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. [Review]. *Journal of antimicrobial chemotherapy, 53*(1), 28-52.

Polzer, J., Hackenberg, R., Stachel, C., & Gowik, P. (2006). Determination of chloramphenicol residues in crustaceans: preparation and evaluation of a proficiency test in Germany. [Evaluation Studies]. *Food additives and contaminants, 23*(11), 1132-1140.

Ritchie, B. W., Harrison, G. J., Harrison, L. R., & Zantop, D. W. (1996). *Avian medicine : principles and application* (Abridged ed.). Lake Worth, Fla.: Wingers Pub.

Rodriguez, L. D., Romo, M. A., Calonge, M. C., & Pena, S. T. (2010). Prudent use of antimicrobial agents and proposals for improvement in veterinary medicine. *Enfermedades infecciosas y microbiología clínica, 28 Suppl 4*, 40-44.

Saif, Y. M., & Barnes, H. J. (2008). *Diseases of poultry* (12th ed.). Ames, Iowa: Blackwell Pub. Professional.

Savjani, J. K., Gajjar, A. K., & Savjani, K. T. (2009). Mechanisms of resistance: useful tool to design antibacterial agents for drug - resistant bacteria. [Review]. *Mini Rev Med Chem, 9*(2), 194-205.

Scholar, E. M., & Pratt, W. B. (2000). *The antimicrobial drugs* (2nd ed.). New York: Oxford University Press.

Schwan, P. (2010). Prevalence and antibiotic resistance of Campylobacter spp. in poultry and raw meat in the Can Tho province, Vietnam: Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine and Animal Science, Department of Biomedical Sciences and Veterinary Public Health.

Sefton, A. M. (2002). Mechanisms of antimicrobial resistance: their clinical relevance in the new millennium. [Review]. *Drugs, 62*(4), 557-566.

Shea, K. M. (2003). Antibiotic resistance: what is the impact of agricultural uses of antibiotics on children's health. *Pediatrics, 112 (Supp.1)*, 253-258.

Singer, R. S., & Hofacre, C. L. (2006). Potential impacts of antibiotic use in poultry production. [Review]. *Avian diseases, 50*(2), 161-172.

Sirdar, M. M. (2010). *Antibiotic residues in commercial layer hens in Khartoum state, Sudan, 2007-2008.* Master thesis, University of Pretoria.

Sloan, B., & Scheinfeld, N. (2008). The use and safety of doxycycline hyclate and other second-generation tetracyclines. [Review]. *Expert opinion on drug safety, 7*(5), 571-577.

Soares, G. M., Figueiredo, L. C., Faveri, M., Cortelli, S. C., Duarte, P. M., & Feres, M. (2012). Mechanisms of action of systemic antibiotics used in periodontal treatment and mechanisms of bacterial resistance to these drugs. *Journal of applied oral science, 20*(3), 295-309.

Srivastava, M. K. (2010). Antibiotic growth-promoters in food animals *Pharmar Times*. Leeds, United Kingdom: Division of Microbiology, School of Biochemistry and Molecular Biology, University of Leeds.

Sundsfjord, A., & Sunde, M. (2008). Antimicrobial resistance after antibiotic use in animals-impact on human health. [Review]. *Tidsskr Nor Laegeforen, 128*(21), 2457-2461.

Taka, T., Baras, M. C., & Chaudhry Bet, Z. F. (2012). Validation of a rapid and sensitive routine method for determination of chloramphenicol in honey by LC-MS/MS. [Validation Studies]. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment, 29*(4), 596-601.

Teh, W. L., & Rigg, A. S. (1992). Possible penicillin allergy after eating chicken. *Lancet, 339*(8793), 620.

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. [Review]. *American journal of infection control, 34*(5 Suppl 1), S3-10; discussion S64-73.

Teuber, M. (2001). Veterinary use and antibiotic resistance. [Review]. *Current opinion in microbiology, 4*(5), 493-499.

Tollefson, L., & Karp, B. E. (2004). Human health impact from antimicrobial use in food animals. [Review]. *Médecine et maladies infectieuses, 34*(11), 514-521.

Tollefson, L., & Miller, M. A. (2000). Antibiotic use in food animals: controlling the human health impact. [Review]. *Journal of the Association of Official Agricultural Chemists, 83*(2), 245-254.

Turton, J. A., Andrews, C. M., Havard, A. C., & Williams, T. C. (2002). Studies on the haemotoxicity of chloramphenicol succinate in the Dunkin Hartley guinea pig. *International journal of experimental pathology, 83*(5), 225-238.

U.S. Department of Agriculture. (2010). International Egg and Poultry Review (Vol. 15).

Vaarten, J. (2012). Clinical impact of antimicrobial resistance in animals. *Revue scientifique et technique (International Office of Epizootics) 31*(1), 221-229.

Van den Bogaard, A. E., & Stobberingh, E. E. (1999). Antibiotic usage in animals: impact on bacterial resistance and public health. [Review]. *Drugs, 58*(4), 589-607.

Verdon, E., Couedor, P., & Sanders, P. (2007). Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoine, nifursol) in poultry muscle tissue through the detection of their five major metabolites (AOZ, AMOZ, SEM, AHD, DNSAH) by liquid chromatography coupled to electrospray tandem mass spectrometry--in-house validation in line with Commission Decision 657/2002/EC. *Analytica chimica acta, 586*(1-2), 336-347.

Vietnam's Department of Livestock. (2010). Vietnam livestock in 2000-2010.

Vo, T. T. A. (2001). *Antibiotic use and residue in chicken meat on industrial boiler chicken farms in Ho Chi Minh city.* Master thesis, Agricultural University in Ho Chi Minh city, Vietnam.

Volovitz, B., Shkap, R., Amir, J., Calderon, S., Varsano, I., & Nussinovitch, M. (2007). Absence of tooth staining with doxycycline treatment in young children. [Randomized Controlled Trial]. *Clin Pediatr (Phila), 46*(2), 121-126.

Vu, D. T., Dang, P. K., & Scippo, M. L. (2010). *Antibiotic Utilization in Pig and Chicken Production in Vietnam-Case study in Red River Delta*. Paper presented at the International Conference on Hygiene and Importation Management of Livestock Products.

Walsh, C., & Fanning, S. (2008). Antimicrobial resistance in foodborne pathogens--a cause for concern? [Review]. *Current drug targets, 9*(9), 808-815.

Wassenaar, T. M. (2005). Use of antimicrobial agents in veterinary medicine and implications for human health. [Review]. *Critical reviews in microbiology, 31*(3), 155-169.

Weiss, E. (1982). The biology of rickettsiae. [Review]. *Annual review of microbiology, 36*, 345-370.

Werckenthin, C., Bottner, A., Hafez, H. M., Hartmann, K., Kaske, M., Kehrenberg, C., Kietzmann, M., Klarmann, D., Klein, G., Krabisch, P., Kuhn, T., Luhofer, G., Richter, A., Schulz, B., Schwarz, S., Sigge, C., Traeder, W., Waldmann, K. H., Wallmann, J. (2005). Cross-resistance between antimicrobial agents used in veterinary medicine: molecular background and practical consequences for susceptibility testing. [Review]. *Berl Munch Tierarztl Wochenschr, 118*(11-12), 471-480.

Wesongah, J. O., Murilla, G. A., Guantai, A. N., Elliot, C., Fodey, T., & Cannavan, A. (2007). A competitive enzyme-linked immunosorbent assay for determination of chloramphenicol. [Validation Studies]. *Journal of veterinary pharmacology and therapeutics, 30*(1), 68-73.

Winkler, H. H. (1991). Molecular biology of rickettsiae. [Review]. *European journal of epidemiology, 7*(3), 207-212.

WorldBank. (2012). Data by country Retrieved 26 January, 2012, from http://data.worldbank.org/country/vietnam

Wright, G. D. (2011). Molecular mechanisms of antibiotic resistance. *Chemical communications (Cambridge, England), 47*(14), 4055-4061.

Yegani, M., & Korver, D. R. (2008). Factors affecting intestinal health in poultry. [Review]. *Poultry science, 87*(10), 2052-2063.

Zeleny, R., Emteborg, H., & Schimmel, H. (2010). Assessment of commutability for candidate certified reference material ERM-BB130 "chloramphenicol in pork". *Analytical and bioanalytical chemistry, 398*(3), 1457-1465.

Zhang, X. X., Zhang, T., & Fang, H. H. (2009). Antibiotic resistance genes in water environment. [Review]. *Applied microbiology and biotechnology, 82*(3), 397-414.

Appendices

Appendix 1. Questionnaire on farm characteristics and risk factors associated with antimicrobial use in broiler chicken farms in Vietnam

Within the Canadian-Vietnamese government cooperation on food & agricultural product quality development and control in Vietnam, this questionnaire is being distributed only as a survey on farm characteristics and risk factors for antimicrobial use in chicken farms in Vietnam. The responder to this questionnaire should be the person in charge of taking direct care of chickens, and not necessarily the owner, referred to as the "farmer." To help ensure a bright future for the developing chicken industry in Vietnam, please spend your valuable time of around 60 minutes to complete this questionnaire. All the information gathered here, after being statistically analyzed, will be presented in scientific presentations and publications. However, individual data will be kept in total confidentiality.

QUESTIONNAIRE ON FARM CHARACTERISTICS & RISK FACTORS ASSOCIATED WITH ANTIMICROBIAL USE IN BROILER CHICKEN FARMS IN VIETNAM

SECTION 1: GENERAL INFORMATION Code of farm:

1. What is your name (farmer)? ....

2. What is the address of the farm? ....

....

3. What is your phone number? ....

....

Fax:...Email:..

4. Which type of farm applies to you? *(Please select one)*:

☐ A single private farm ....

☐ A family private farm: ....

☐ A husbandry company:..

☐ Others: ....

5. In total, how many farms donated to raising chickens in Vietnam do you have? *(Please provide more information)*:

....

SECTION 2:FARM CHARACTERISTICS

Note: The next questions (Q6-21) are only intended for the particular farm under study.

6. Which type(s) of design characterizes the shelters on your farm? *(Please select one or more if applicable)*:

☐ Open shelters (natural ventilation): →Number............

☐ Closed shelters (artificial ventilation system with temperature and humidity control): →Number.............

7. How many floors are there in a shelter on your farm? *(Please provide more information)*:

....

8. What is the average density of birds when birds are ready to be slaughtered (birds/m^2 of each floor)? *(Please provide more information)*:

....

9. What is the age of the birds when ready to be slaughtered (days)? *(Please provide more information)*:

....

10. Are there chickens of multiple ages in the same shelter? *(Please select one)*:

☐ Yes ☐ No

11. On average, how many pre-slaughter chickens in total per year (or day) die on your farm? *(Please provide more information)*:

....

12. How many chickens in total per year are raised in your farm (chickens alive until slaughter)? *(Please provide more information)*:

....

13. Which types of chickens are being raised on your farm? *(Please select one or more)*:

☐ Chicken-broiler ☐ Chicken-layer

☐ Chicken twofold (meat & egg) ☐ Chicken-Breeder

14. Which breeds of chickens are being raised on your farm? *(Please select one or more):*

☐ Gà tàu vàng ☐ Tam Hoàng 882 ☐ Lương Phượng ☐ Kabir

☐ Sasso (SA31) ☐ ISA.MPK ☐ ISA.Color ☐ Hubbard

☐ AA (Arbor Acress)☐ Avian ☐ Cobb

☐ Ross ☐ Others: ....

15. What is the origin of the chickens being raised on your farm? *(Please select one)*:

☐ Eggs from your farm ☐ Birds from the hatchery ☐ Both

16. Can sunshine directly enter bird houses ? *(Please select one)*:

☐ No ☐ Yes→ ....hours/day

17. Are there other animals, besides chickens, on the farm? *(Please select one)*:

☐ Yes → continue to next questions ☐ No → continue to question **20**

18. Which animals, besides chickens, are on the farm? *(Please select one or more)*:

☐ Dog ☐ Cat ☐ Duck ☐ Goose

☐ Pig ☐ Beef ☐ Buffalo ☐ Others:...........

19. Are chickens raised separately in fenced areas, away from other animals? *(Please select one)*:

☐ No, freely moving. ☐ Yes, in closed shelters. ☐Yes, in open shelters.

20. Is there a separate storage place for feed? *(Please select one)*:

☐ Yes ☐ No

21. Is there a separate storage place for drugs, vaccines? *(Please select one)*:

☐ Yes ☐ No

SECTION 3.1: DRUG USE FOR GENERAL INFORMATION

Note: The next questions (Q22-30) are only intended for chickens raised in the last 6 months.

22. Have you used any drugs for treatment, prevention or growth promotion for chickens in the last 6 months? *(Please select one)*:

☐ Yes → continue to next questions ☐ No → continue to question **31**

23. On which bases have your drug choices been considered? *(Please select one or more)*:

☐ Experience ☐ Drug seller ☐ Veterinarian ☐ Product label

☐ Husbandry company ☐ Others:...

24. On which bases has the duration of administration been considered? *(Please select one or more)*:

☐ Experience ☐ Drug seller ☐ Veterinarian ☐ Product label

☐ Husbandry company ☐ Others:...

25. On which bases has the dosage been considered? *(Please select one or more)*:

☐ Experience ☐ Drug seller ☐ Veterinarian ☐ Product label

☐ Husbandry company ☐ Others:...

26. Once you buy any drug, do you read information on the product label?

☐ No. → continue to question **30** ☐ Yes. → continue to the next question

27. Have you complied with the withdrawal time indicated on the product label? *(Please select one)*:

☐ Yes ☐ No idea

☐ No → Explain the reason: ....

28. Have you ever used expired drugs on your chickens? *(Please select one)*:

☐ No ☐ No idea

☐ Yes → Explain the reason: ....

29. Do you store the drugs as indicated on product labels? *(Please select one)*:

☐ Yes ☐ No idea

☐ No → Explain the reason: ....

30. Have you kept a record on drug use? *(Please select one)*:

☐ No ☐Yes, but not a full record ☐ Yes, with a full record.

SECTION 3.2: DRUG USE FOR DETAILED INFORMATION

31. Please provide some information on drugs used since last year **in Table 1**

Table 1. Drugs used since the last 6 months

Commercial Name	Reasons	% of birds treated overall	Age of birds at administration (days)	Duration of administration (days)	Administration Route	Age of birds at slaughtering (days)
	☐ Therapy ↳☐ Respiratory diseases. Month, % birds sick: .... ☐ Digestive diseases. Month, % birds sick: .... ☐ Skin and feather diseases. Month, % birds sick: .... ☐ Other diseases. Month, % birds sick:				☐ Feed ↳☐ Top dressed by farmer ☐ Mixed by farmer ☐ Others:... ☐ Water ↳ ☐ Automatic dispenser ☐ Directly put in a drinking bowl by farmer ☐ Others:... ☐ Injection ☐ Topical	
	☐ Prophylaxis ↳☐ Respiratory diseases. Month: .... ☐ Digestive diseases Month: .... ☐ Skin and feather diseases. Month: .... ☐ Other diseases. Month :				☐ Feed ↳ ☐ Top dressed by farmer ☐ Mixed by farmer ☐ Others:... ☐ Water ↳ ☐ Automatic dispenser ☐ Directly put in a drinking bowl by farmer ☐ Others:... ☐ Injection ☐ Topical	

	☐ Growth promoter				☐ Feed ☐ Top dressed by farmer ☐ Mixed by farmer ☐ Others:.. ☐ Water ☐ Automatic dispenser ☐ Directly put in a drinking bowl by farmer ☐ Others:..	
	☐Others:...				☐ Feed ☐ Top dressed by farmer ☐ Mixed by farmer ☐ Others:.. ☐ Water ☐ Automatic dispenser ☐ Directly put in a drinking bowl by farmer ☐ Others:.. ☐ Injection ☐ Topical	

SECTION 4: VACCINATION

32. Have your chickens been vaccinated in the last 6 months? *(Please select one):*

☐ Yes → continue to the next question ☐ No→ continue to question **34**

33. Which vaccines have been used for your chickens? (*Please give details)*:

Name of Vaccine	For which disease

SECTION 5: EDUCATION AND AWARENESS

34. What is your educational level? *(Please select one)*

☐ Never went to school ☐ Intermediate level (2 years)

☐ Primary school (1 - 5) ☐ College (3 years)

☐ Secondary school (6 - 9) ☐ University (≥ 4 years)

☐ High school (10 - 12) ☐ Post-graduate

35. Are you aware of Decision No. 1504/QĐ-BNN-KHCN dated 15 May 2008 on Good Animal Husbandry Practices for Poultry in Vietnam (VIETGAHP)? *(Please select one)*:

☐ Yes → continue to the next question ☐ No → continue to question **37**

36. How do you evaluate your own application of Decision No. 1504/QĐ-BNN-KHCN dated 15 May 2008 on Good Animal Husbandry Practices for Poultry in Vietnam (VIETGAHP)? *(Please select one)*:

☐ Excellent ☐ Very good ☐ Good ☐ Average ☐ Poor

☐ Other:...

37. Which elements are the most important to getting more financial benefit from your farm? *(Please select one or more)*:

☐ Reducing mortality rate ☐ Improving growth rate ☐ Improving marketing

☐ Others:...

SECTION 6: HEALTH MANAGEMENT

38. Are new birds raised in a separate shelter away from other remaining birds? *(Please select one)*:

☐ No ☐ Yes → for………days

39. Do you keep sick-birds separate from the others? *(Please select one)*:

☐ No ☐ Yes, but still in the same shelter ☐ Yes, and in the entirely independent shelter

40. How do you manage dead-birds? *(Please select one or more)*

☐ Still used for human consumption ☐ Feed for other animals raised on the farm

☐ Buried on your farm ☐ Burned on your farm

☐ Thrown into rivers/lakes/ponds... ☐ Other:...

41. Which sanitary practices have you undertaken on your farm? *(Please select one or more)*:

☐ All in/all out ☐ Cleaning shelters before introduction of new birds

☐ Cleaning shelters during farming

☐ Keeping disinfected shelters empty before introduction of new birds → if yes, for…………days

SECTION 7: DRINKING, FEED

42. Which water sources are used as the drinking water for chickens? *(Please select one or more)*:

☐ Public drilled water ☐ Private drilled water ☐ Public well

☐ Private well ☐ Spring/river/stream ☐ Lake/pond...

☐ Rain water

☐ Other: ....

43. Which types of feed are used for chickens? *(Please select one)*

☐ Only home-made feed ☐ Only commercial feed

☐ Combination of both feeds

☐ Other *(Please provide details)*:

....

SECTION 8: FUTURE DEVELOPMENT

44. What are the main purposes of rearing chickens? *(Please select one or more)*:

☐ For your home consumption

☐ For small-scale markets (within your province)

☐ For large-scale markets (beyond your province)

45. Do you know the pilot project on chicken quality development sponsored by CIDA? *(Please select one)*:

☐ Never heard (this is the first time).

☐ Heard of it, but this farm has not been selected or involved in this project yet.

☐ Heard of it and have been selected and involved in this project.

Date

Signature

THANK YOU FOR YOUR PARTICIPATION!!!

Appendix 2. Description of drug use practices in70 broiler chicken farms in two provinces of Vietnam, 2011

		Dosing duration per course of drug administration* (days)			Number of courses of drug administration per production cycle (times)			Routes[c] (% of total farms)		Diseases[d] (% of total farms)		
Drugs	% farms	Min	Mean	Max	Min	Mean	Max	F	W	R	D	C
Amoxicillin	27.1	2.0	3.4	5.0	1.0	1.7	3.0	0.0	27.1	8.6	24.3	1.4
Amoxicillin+Analgine[a] +Dexamethason+Bromhexine	1.4	2.0	2.0	2.0	1.0	1.0	1.0	0.0	1.4	1.4	1.4	0.0
Amoxicillin+Colistin	17.1	2.9	3.6	5.0	1.0	2.7	6.0	0.0	17.1	8.6	17.1	0.0
Amoxicillin+Tylosin	1.4	4.5	4.5	4.5	2.0	2.0	2.0	0.0	1.4	1.4	0.0	0.0
Ampicillin	10.0	3.0	3.9	4.5	1.0	1.9	2.0	0.0	10.0	1.4	10.0	0.0
Ampicillin+Colistin	51.4	2.0	3.6	5.5	1.0	2.2	5.0	0.0	51.4	24.3	50.0	0.0
Ampicillin+Colistin+Erythromycin	1.4	3.0	3.0	3.0	4.0	4.0	4.0	0.0	1.4	1.4	1.4	0.0
Amprolium	1.4	3.0	3.0	3.0	1.0	1.0	1.0	0.0	1.4	0.0	0.0	1.4
Amprolium+Sulfaquinoxalin	1.4	3.8	3.8	3.8	2.0	2.0	2.0	0.0	1.4	0.0	0.0	1.4
Apramycin	5.7	3.0	3.0	3.0	2.0	3.5	6.0	0.0	5.7	1.4	5.7	0.0
Apramycin+Tylosin	1.4	6.0	6.0	6.0	2.0	2.0	2.0	0.0	1.4	1.4	0.0	0.0
Bromhexine	1.4	4.0	4.0	4.0	1.0	1.0	1.0	0.0	1.4	1.4	0.0	0.0
Cephalexin	1.4	3.0	3.0	3.0	1.0	1.0	1.0	0.0	1.4	1.4	0.0	0.0
Chloramphenicol[b]	1.4	4.5	4.5	4.5	2.0	2.0	2.0	0.0	1.4	0.0	1.4	0.0
Chlortetracyclin+Erythromycin	1.4	2.5	2.5	2.5	2.0	2.0	2.0	0.0	1.4	1.4	0.0	0.0
Colistin	14.3	3.0	3.9	5.0	1.0	2.3	6.0	0.0	14.3	1.4	14.3	0.0

Drugs	% farms	Dosing duration per course of drug administration* (days)			Number of courses of drug administration per production cycle (times)			Routes[c] (% of total farms)		Diseases[d] (% of total farms)		
		Min	Mean	Max	Min	Mean	Max	F	W	R	D	C
Colistin+Doxycyclin	1.4	4.5	4.5	4.5	3.0	3.0	3.0	0.0	1.4	1.4	1.4	0.0
Colistin+Enrofloxacin	2.9	3.0	3.5	4.0	2.0	3.5	5.0	0.0	2.9	1.4	2.9	0.0
Colistin+Neomycin	2.9	3.0	3.3	3.5	2.0	2.0	2.0	0.0	2.9	2.9	2.9	0.0
Colistin+Oxytetracyclin	1.4	3.0	3.0	3.0	2.0	2.0	2.0	0.0	1.4	1.4	1.4	0.0
Colistin+Tilmicosin	2.9	3.0	3.5	4.0	3.0	3.5	4.0	0.0	2.9	2.9	2.9	0.0
Colistin+Trimethoprim	1.4	3.0	3.0	3.0	2.0	2.0	2.0	0.0	1.4	0.0	1.4	0.0
Colistin+Tylosin	2.9	3.0	3.8	4.5	3.0	3.0	3.0	0.0	2.9	2.9	1.4	0.0
Diaveridin+Sulfadimidin	28.6	2.0	3.2	5.5	1.0	2.6	6.0	1.4	27.1	0.0	0.0	28.6
Diaveridin+Sulfaquinoxalin	1.4	3.0	3.0	3.0	1.0	1.0	1.0	0.0	1.4	0.0	0.0	1.4
Doxycyclin	22.9	2.0	3.7	6.0	1.0	2.0	6.0	0.0	22.9	21.4	2.9	0.0
Doxycyclin+Florphenicol	2.9	2.5	3.3	4.0	1.0	1.5	2.0	0.0	2.9	1.4	2.9	0.0
Doxycyclin+Gentamicin	5.7	2.0	3.0	4.0	1.0	2.5	5.0	0.0	5.7	2.9	5.7	0.0
Doxycyclin+Josamycin	2.9	3.0	3.5	4.0	1.0	1.0	1.0	0.0	2.9	2.9	0.0	0.0
Doxycyclin+Thiamphenicol	1.4	4.5	4.5	4.5	2.0	2.0	2.0	0.0	1.4	1.4	0.0	0.0
Doxycyclin+Tylosin	10.0	3.0	3.6	5.0	1.0	1.4	2.0	0.0	10.0	10.0	5.7	0.0
Enrofloxacin	44.3	1.7	3.8	6.0	1.0	1.7	4.0	0.0	44.3	40.0	24.3	0.0
Enrofloxacin+Gentamicin	1.4	4.0	4.0	4.0	1.0	1.0	1.0	0.0	1.4	1.4	1.4	0.0

		Dosing duration per course of drug administration* (days)			Number of courses of drug administration per production cycle (times)			Routes[c] (% of total farms)		Diseases[d] (% of total farms)		
Drugs	% farms	Min	Mean	Max	Min	Mean	Max	F	W	R	D	C
Enrofloxacin+Tylosin	2.9	4.0	4.5	5.0	1.0	1.0	1.0	0.0	2.9	2.9	1.4	0.0
Erythromycin	5.7	3.0	3.3	4.0	2.0	3.5	6.0	0.0	5.7	5.7	0.0	0.0
Florphenicol	7.1	2.0	3.7	5.0	1.0	1.8	3.0	0.0	7.1	4.3	2.9	0.0
Flumequin	1.4	3.5	3.5	3.5	1.0	1.0	1.0	0.0	1.4	1.4	1.4	0.0
Gentamicin	1.4	3.0	3.0	3.0	1.0	1.0	1.0	0.0	1.4	0.0	0.0	1.4
Gentamicin+Sulfadimidin	1.4	2.0	2.0	2.0	4.0	4.0	4.0	0.0	1.4	0.0	1.4	0.0
Josamycin+Oxolinicacid +Trimethoprim	1.4	4.0	4.0	4.0	2.0	2.0	2.0	0.0	1.4	1.4	1.4	0.0
Josamycin+Trimethoprim	2.9	2.5	2.8	3.0	2.0	2.0	2.0	0.0	2.9	2.9	0.0	0.0
Lincomycin+Spectinomycin	1.4	4.0	4.0	4.0	1.0	1.0	1.0	1.4	0.0	1.4	0.0	0.0
Neomycin+Oxytetracyclin	4.3	3.0	3.3	4.0	1.0	2.0	3.0	0.0	4.3	2.9	4.3	0.0
Norfloxacin	10.0	3.0	3.7	5.0	1.0	1.3	2.0	0.0	10.0	8.6	5.7	0.0
Oxolinic acid	7.1	2.0	3.0	4.0	1.0	2.0	4.0	0.0	7.1	0.0	7.1	0.0
Oxytetracyclin	4.3	3.0	3.3	4.0	1.0	1.3	2.0	0.0	4.3	4.3	0.0	0.0
Pyrimethamine+Sulfaquinoxalin	8.6	3.0	3.7	4.0	1.0	2.2	3.0	0.0	8.6	0.0	0.0	8.6
Streptomycin+Tetracyclin	1.4	4.0	4.0	4.0	4.0	4.0	4.0	0.0	1.4	1.4	0.0	0.0
Sulfachlorpyridazin	5.7	3.0	3.9	5.0	1.0	1.3	2.0	0.0	5.7	2.9	5.7	0.0

		Dosing duration per course of drug administration* (days)			Number of courses of drug administration per production cycle (times)			Routes[c] (% of total farms)		Diseases[d] (% of total farms)		
Drugs	**% farms**	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**	**F**	**W**	**R**	**D**	**C**
Sulfachlorpyridazin+Trimethoprim	1.4	5.0	5.0	5.0	1.0	1.0	1.0	0.0	1.4	0.0	0.0	1.4
Sulfadiazine+Trimethoprim	2.9	4.0	4.0	4.0	1.0	1.0	1.0	0.0	2.9	1.4	1.4	2.9
Sulfaquinoxalin+Trimethoprim	1.4	3.0	3.0	3.0	4.0	4.0	4.0	0.0	1.4	0.0	0.0	1.4
Sulfaquinoxalin+Tylosin	2.9	2.0	2.5	3.0	1.0	2.0	3.0	0.0	2.9	0.0	1.4	1.4
Tetracyclin	7.1	3.0	3.8	4.5	1.0	2.4	5.0	0.0	7.1	1.4	5.7	0.0
Tilmicosin	10.0	2.5	3.6	7.0	1.0	2.7	8.0	2.9	7.1	10.0	0.0	0.0
Toltrazuril	20.0	2.0	3.6	6.0	1.0	2.0	5.0	0.0	20.0	0.0	0.0	20.0
Tylosin	60.0	2.5	3.8	6.0	1.0	1.8	4.0	0.0	60.0	60.0	4.3	0.0
Average	**7.89**	**3.08**	**3.56**	**4.19**	**1.56**	**2.04**	**2.96**	**0.10**	**7.79**	**4.61**	**3.98**	**1.25**
Min	**1.4**	**1.7**	**2.0**	**2.0**	**1.0**	**1.0**	**1.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Max	**60.0**	**6.0**	**6.0**	**7.0**	**4.0**	**4.0**	**8.0**	**2.9**	**60.0**	**60.0**	**50.0**	**28.6**

[a]Analgine which was manufactured for human medicine, not veterinary use, was also administered for birds by one farmer.

[b]Antibiotic prohibited in Vietnam according to Circular No. 15/2009/TT-BNN dated on 17 March 2009.

[c] Routes of drug administration:

- F (Feed): drugs were separately bought, then mixed with feed, but not in-feed drugs which were available in commercial feed as feed additives.
- W (Water): drugs were separately bought, then watered with drinking water by hand or by machine.

[d] Diseases:

		Dosing duration per course of drug administration* (days)			Number of courses of drug administration per production cycle (times)			Routes[c] (% of total farms)		Diseases[d] (% of total farms)		
Drugs	**% farms**	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**	**F**	**W**	**R**	**D**	**C**

- R: Respiratory diseases.
- D: Digestive diseases (Coccidiosis exclusively).
- C: Coccidiosis.
- No skin and feature diseases were responded to the questionnaire from the surveyed farmers.

* Dosing duration per course of drug administration was estimated based on the mean dosing duration on a farm because a range of values for the dosing duration was provided by farmers.