UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Antimicrobial drug resistance at the human-animal interface in Vietnam

Nguyen, V.T.

[Link to publication](#)

Citation for published version (APA):
Nguyen, V. T. (2017). Antimicrobial drug resistance at the human-animal interface in Vietnam

CHAPTER 2
ANTIMICROBIAL USAGE IN CHICKEN PRODUCTION IN THE MEKONG DELTA OF VIETNAM

CHAPTER 2

Chapter 2: Antimicrobial usage in chicken production in the Mekong delta of Vietnam

Juan J Carrique-Mas[1], Nguyen V. Trung[1,2], Ngo T. Hoa[1], Ho Huynh Mai[3], Tuyen H. Thanh[1], James I. Campbell[1], Jaap A. Wagenaar[4], Anita Hardon[5], Thai Quoc Hieu[3] and Constance Schultsz[1,6]

[1] Nuffield Department of Medicine, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

[2] Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, The Netherlands

[3] Sub-Department of Animal Health Ly Thuong Kiet, Tien Giang, Vietnam

[4] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

[5] Center for Social Science and Global Health, University of Amsterdam, The Netherlands

[6] Department of Global Health - Amsterdam Institute for Global Health and Development, University of Amsterdam, The Netherlands

CHAPTER 2

Abstract

Antimicrobials are used extensively in chicken production in Vietnam, but to date no quantitative data are available. A 2012-2013 survey of 208 chicken farms in Tien Giang province, stratified by size (10-200 chickens; >200-2,000) was carried out to describe and quantify use of antibacterial antimicrobials (usage per week per chicken, and usage per 1,000 chickens produced) in the Mekong delta, and to investigate factors associated with usage. Twenty-eight types of antimicrobial belonging to 10 classes were reported. Sixty three per cent of all commercial formulations contained at least two antimicrobials. On 84% occasions antimicrobials were administered with a prophylactic purpose. The overall adjusted quantities of antimicrobials used/week/chicken and per 1,000 chickens produced (g) were 26.36mg (SE ±3.54) and 690.4g (SE ±203.6), respectively. Polypeptides, tetracyclines, penicillins and aminoglycosides were the antimicrobials used by most farms (18.6% farms, 17.5%, 11.3% and 10.1% farms, respectively), whereas penicillins, lincosamides, quinolones, and sulphonamides/trimethoprim, were quantitatively the most used compounds (8.27mg, 5.2mg, 3.16mg and 2.78mg per week per chicken, respectively). Factors statistically associated with higher levels of usage (per week per chicken) were: meat farms (OR=1.40) and farms run by a male farmer (OR=2.0). All-in-all out farming systems (correlated with medium farms) were associated with reduced levels of antimicrobial usage (OR=0.68). Usage levels to produced meat chickens were considerably higher than those reported in European countries. This should trigger the implementation of surveillance programmes to monitor sales of antimicrobials that should contribute to the rational administration of antimicrobials in order to preserve the efficacy of existing antimicrobials in Vietnam.

Keywords: Antimicrobial use, antimicrobial resistance, antibiotics, poultry, Vietnam

CHAPTER 2

Introduction

Antimicrobial resistance (AMR) is currently one of the most serious threats to global health, resulting in a decreasing repertoire of antimicrobials available to treat serious infections [1]. Almost all classes of antibiotics available to humans have also been used in animal production [1, 2], and AMR has been increasingly identified in animal pathogens [3-5]. Over recent years there has been mounting evidence that the use of antimicrobials in agriculture is a major factor driving AMR globally [6]. Antimicrobials are extensively used in animal farming with the aim of treating and prevent animal diseases, as well as improving growth performance [7]. Antimicrobial usage on farms selects for AMR bacteria and other genetic determinants that may spread to humans either through direct contact, consumption of meat or indirectly through environmental pathways [6, 8].

In Vietnam, high levels of resistance against a number of antimicrobials have been reported in food-borne pathogens such as non-typhoid *Salmonella* serovars and *Campylobacter* spp. in poultry, livestock and meat [9-14]. Compared with isolated from pigs and fish, *E. coli* from Vietnamese chickens have higher levels of AMR [15]. In Vietnam antimicrobials are available to farmers over the counter without prescription. Some reports have suggested high levels of usage of a range of antimicrobials in pig and poultry farming, although the quantities used are unknown [16, 17].

The aims of this study were: (1) to describe and quantify levels of antimicrobial usage, both in terms of usage per unit time as well as per chicken produced, in chicken farms in the Mekong delta; and (2) to identify factors associated with usage. Results from this study will serve to increase awareness and identify required efforts to reduce usage of antimicrobials in animal production in the region.

CHAPTER 2

Materials and methods

Survey design and data collection

Data on antimicrobial usage on chicken farms were obtained from a survey carried out on 208 chicken farms sampled from three districts containing 40% poultry population in Tien Giang province, Mekong delta, Vietnam. The survey was carried out between March 2012 and April 2013. For logistic reasons, sampling was stratified by size (10-200, 'household farms'; 201-2,000 chickens, 'small to-medium farms') and district (My Tho, Cho Gao and Chau Thanh) (total 6 strata), with ~34 farms sampled per stratum. Within each stratum, farms were randomly selected from the census by staff from Tien Giang sub-Department of Animal Health. The sample size per stratum (34) was determined based on requirements for determining the prevalence in each district of *E. coli* resistance against a number of antimicrobials. Questionnaires with both open and closed questions were used to obtain data on antimicrobial usage. Farm owners were asked about details on administration of any bacterial antimicrobial formulations over a period of time, including: (1) Method of administration (water, feed, injection, spray); (2) Type of use (prophylactic/therapeutic/both); (3) Timing of application: (a) continuously; (b) on arrival; (c) in response to disease; (c) periodic (i.e. change of season, changing feed, before selling). Quantitative data on each formulation administered over a set period of time were gathered, including the commercial name of the product, presentation and number of containers used. From these data the total amount of active antimicrobial compound was calculated. Questionnaires enquired about usage 'from restocking until the visit date' for small-to-medium farms. A fixed period of observation (90 days) was determined for household farms since household farms did not practice all-in-all-out production. In addition farmers were asked about the source of advice for using the antimicrobial (veterinary pharmacist; district veterinarian; chief animal health worker; drug company sales person; friend/neighbour; and 'other').

Calculation of antimicrobial usage

Two outcomes were of interest: (a) usage per chicken per time unit (or 'intensity' of usage); and (b) usage related to production output (usage per 1,000 chickens produced).

Usage per week per chicken (Uwc *milligrams*) was calculated by dividing in each farm the amount of each antimicrobial used (Ur *milligrams*) by the length of the reporting period for that

farm (*t weeks*), and then by the number of chickens present in the farm (*N chickens*) on the visit date. The 'amount of each antimicrobial used to produce 1,000 chickens' (in grams) (*U1000c grams*) is dependent on the length of production cycle in each farm. Therefore chicken output and antimicrobial usage were estimated in each study farm over one year.

Estimated annual antimicrobial usage (*Uy grams*) was calculated for each antimicrobial:

*U*y *grams*= *U*r *milligrams* * 0.001 *grams/milligram* * (52 *weeks*-[0.1*52 *weeks*]) /*t weeks*

From the above formula, *U*1000c *grams* was derived:

*U*1000c *grams* = *U*y *grams* * 1000 *chickens* /(*C* * *Nchickens*).

C (number of cycles of production per year) was obtained from:

C = 1/(*a* years + [0.1 * *a* years]), where *a* is the expected age of depopulation of chickens. These calculations assume a fixed downtime of 10% (i.e. time when the farm is not productive and therefore neither chickens are produced nor antimicrobials are used). Estimates of usage (farm prevalence of usage by farm and quantitative estimates) were calculated after adjusting for the stratified study design by assigning a stratum-specific sampling weight to each observation unit (farm). Standard errors were corrected to take into account potential similarities of usage between farms in each stratum [18].

Risk factor analyses

Risk factor analyses for usage were carried out by fitting proportional odds model (ordinal logistic model) [19] for the two outcomes describing total antimicrobial usage (i.e. usage in relation to time, usage in relation to production), after adjusting for the stratified survey design. Data on usage were categorised into three levels: no use; low level usage; high level usage. Low and high level usage categories were determined from dividing the farms that had consumed antimicrobials into two categories, based on the median quantity used. The following explanatory variables were investigated: (1) farmer's gender; (2) farmer's age (years) (log); (3) farmer's highest educational attainment (four levels: 'no formal education', 'primary school', 'secondary school' and 'higher'); (4) farmer's experience in chicken farming (years) (log); (5) number of chickens (log); (6) type of production (three levels: meat; layer; dual purpose); (7)

density of chickens (chickens/sq. metre) in houses; (8) all-in-all-out production; (9) chickens lost to disease over the previous 90 days; (10) presence of species other than chickens (pigs, cattle/buffalo, or ducks) in the farm; and (11) district (Cho Gao, Chau Thanh, My Tho). Candidate variables were those significant in the univariable models for any of the two outcomes (p<0.05). Variables were ranked by their degree of significance and were included in the model starting with the most significant ones using a step-wise forward approach [20]. In the final multivariable model, variables were retained if their p-value was less than 0.05 for any of the two outcome variables. All interactions between all significant variables in the model were assessed. The suitability of each new variable included in the model was assessed using the AIC information criterion [21]. All interactions between final significant variables were tested. All statistical analyses were performed using R (packages 'epicalc', 'epiR', and 'survey') (http://www.r-project.org).

Results

Antimicrobial usage in study farms

A total of 123 farms (59.1%) reported administration of at least one antimicrobial formulation. A total of 168 administrations of an antimicrobial formulation were reported. A higher percentage of owners of medium farms reported administering antimicrobials in their farms, compared with owners of small farms (71.2% vs. 47.1%, respectively) (χ^2= 11.5, p<0.001). Owners of small-to-medium farms reported usage over a median of 140 days [56-224] (i.e. the median age of flocks visited), whereas owners of household farms reported usage over a fixed period of 90 days (Table 1). For 157/168 (95.7%) administrations the active ingredients (antimicrobial compounds) could be accurately described, either by direct observation of the container, or by farmer's recollection. A total of 28 different antimicrobial compounds belonging to 10 classes were identified (Table 2). A total of 100/157 administrations (63.7%) consisted of formulations containing at least two antimicrobial classes (Table 3). After adjusting for the sampling frame, polypeptides, tetracyclines, penicillins and aminoglycosides were the antimicrobials used by most farms.

CHAPTER 2

Table 1. Number of antimicrobial formulations used by chicken farmers, stratified by farm size (Tien Giang province, Vietnam).

	All farms (N=208)	Household farms			Small-to-medium farms		
		All (N=104)	Meat (N=79)	Mixed (N=25)	All (N=104)	Meat (N=40)	Eggs (N=63)
No. of antimicrobial formulations used	168	68	52	16	100	39	59
No. of farms that used antimicrobial formulations (%)	123 (59.1%)	49 (47.1%)	40 (50.6%)	9 (36.0%)	74 (71.2%)	30 (76.9%)	43 (68.2%)
No. of different antimicrobial formulations used per farm							
0	85	55	39	16	30	10	20
1	85	32	29	3	53	22	31
2	32	15	10	5	17	7	9
3	5	2	1	1	3	1	2
4	1		0	0	1		1
Median observation time per farm (days)[IQR]	90 [90-140]	90 [90-90]	90 [90-90]	90 [90-90]	140 [56-224]	49[28-70]	196 [157-280]

Table 2. Types of antimicrobials administered in 123 chicken farms, Tien Giang, Vietnam (2012-2013).

Class of antimicrobial	Name of antimicrobial	No. (%) formulations administered containing the antimicrobial (N=157) (%)	Percent farms using antimicrobial (N=208) (%)	Adjusted % farms using antimicrobial (95% CI)
Tetracyclines	Docycycline, oxytetracycline, tetracycline	57 (36.3%)	52 (25.0%)	17.5 (16.9-18.1)
Polypeptides	Colistin	48 (30.6%)	39 (18.8%)	18.6 (9.9-27.3)
Macrolides	Tylosin, tilmicosin, erythromycin, spiramycin	40 (25.5%)	40 (19.2%)	9.7 (8.2-11.3)
Penicillins	Ampicillin, amoxicillin	41 (26.1%)	33 (15.9%)	11.3 (10.4-12.1)
Quinolones	Flumequine, oxolinic acid, norfloxacin, enrofloxacin	22 (14.0%)	19 (9.1%)	6.0 (0.1-12.1)
Aminoglycosides	Spectinomycin, neomycine, gentamicin, apramycin, streptomycin	19 (12.1%)	19 (9.1%)	10.1 (8.0-12.1)
Phenicols	Florfenicol, thiamphenicol	14 (8.9%)	13 (6.3%)	0.90 (0-2.5)
Sulphonamides/ trimethoprim	Sulfamethoxazole, sulphadimidine, sulphadimetoxine, sulphadimerazine, trimethoprim	12 (7.6%)	12 (5.8%)	6.1 (4.7-7.5)
Lincosamides	Lincomycin	4 (2.5%)	4 (1.9%)	4.3 (1.9-6.8)
Pleuromutilins	Tiamulin	1 (0.6%)	1 (0.5%)	0.03 (0.01-0.05)

CI=Confidence interval

The most common antimicrobial formulations combining multiple antimicrobial classes included penicillins and polypeptides (21% of all formulations) followed by macrolides plus tetracyclines (15.9%). A total of 28/157 (17.8%) of products reported included a combination of an antimicrobial considered to be bacteriostatic with another antimicrobial considered bactericidal (Table 3). Formulations including bacteriostatic/bactericidal combinations included: aminoglycosides combined with tetracyclines (i.e. gentamycin/doxcycyline, neomycin/tetracycline) or polypeptides combined either with tetracyclines (i.e. colistin/ oxytetracyclin), sulphonamides (colistin/sulphadimethoxine); or macrolides (colistin/tylosin)

Antimicrobial formulations were administered in water on 137 (81.5%) occasions, followed by both in feed and water (9.5%), and in feed only (4.2%). In 4.2% cases antimicrobial formulations were injected. In 141 (84%) cases farmers reported that the antimicrobial formulation was administered for prevention of disease (prophylaxis), and in 21 (12%) cases they were used exclusively for therapeutic reasons (i.e. to treat disease). On 6 (3.8%) occasions farmers reported using the antimicrobial formulation with a double purpose (both to prevent and treat). The most commonly reported timing of use was: on arrival (34.4%) followed by periodic (28.7%) and continuously (18.5%). The most common sources of advice with regards to antimicrobial formulations used were: the drug seller (56%), the district veterinarian (18%), a friend/neighbour (12%), a salesperson (12%), and 'other' (2%).

Household farms used 24.9 mg (SE ±7.91) of antimicrobial per chicken per week, compared with 5.21 mg (SE ±0. 91) used by small-to-medium farms (Kruskal-Wallis test; p=0.014). Likewise, household farms used greater amounts to produce 1,000 chickens, compared with small-to-medium farms (543.4g, SE±223.4 vs. 172.8g, SE±25.2) (Kruskal-Wallis test; p=0.360). After adjusting for the sampling frame, estimates of usage increased, since household farms in the district of Chao Gao reported by far the highest levels of usage, and household farms in this district had the greatest sampling weight (since they contained 46% of chickens of the study area according to the census) (Table 4). The adjusted levels of usage of antimicrobial per week per chicken and per 1,000 chickens (Uwc and U1000c) produced were, respectively 26.36 mg (SE ±3.54) and 690.4g (SE ±203.6). The model derived estimates of antimicrobial consumed per 1,000 meat and layer chickens produced were 470.4g (SE ±184.1) and 870.1g (SE ±263.9), respectively (model derived p=0.325).

Table 3. Combination of antimicrobials reported in 157 administrations of antimicrobial reported by 123 chicken farmers, Tien Giang province, Vietnam. The highlighted cells indicate a combination of a bacteriostatic and a bactericidal antimicrobial.

	Penicillin	Polypeptide	Macrolides	Tetracyclines	Quinolones	Phenicols	Aminoglycosides	Sulphonamides	Lincosamides	Pleuromutiins	Total	No. applications with each class of antimicrobial
Penicillin	6	33		1				1			41	41
Polipeptides		2	2	5	1		1	3			14	48
Macrolides			3	25*			5	4		1	38	40
Tetracyclines				13		6	7				26	57
Quinolones					20		1				21	22
Phenicols						8					8	14
Aminoglycosides							1	1	3		5	19
Sulphonamides								3			3	12
Lincosamides									1		1	4
Pleuromutilin											0	1
Total												258

*One administration of a macrolide/tetracycline product included a polypeptide antimicrobial

Quantitative estimates of antimicrobial usage

Penicillins, lincosamides, quinolones, and sulphonamides/trimethoprim, were the four most commonly antimicrobials consumed, with average Uwc values of 8.27mg, 5.20mg, 3.16mg and 2.78mg/week/chicken, and average U1000c values of 142.4g, 38.7g, 35.6g and 38.0g per 1,000 chickens produced (Figure 1).

Risk factors for antimicrobial usage

Results indicated a significantly higher prevalence of usage per unit time (Uwc) for farms located in Cho Gao (OR=1.49) and Chau Thanh (OR=1.53) compared with My Tho (baseline, Table 5). Male farmers used more antimicrobials per unit time (OR=2.02). Meat farms used higher amounts of antimicrobial per unit time, compared with layer and dual purpose production

(OR=1.40). All-in-all out systems (highly correlated with small-to-medium farms) had reduced levels of usage per unit time compared with farms with continuous production (correlated with household farms) (OR=0.68). No interactions were significant.

Table 4. Sampling weights and sampling fraction and administration of antimicrobials by in poultry farms belonging to each survey stratum, Tien Giang, Vietnam.

| Stratum	No. farms sampled	No. chickens sampled	No. chickens (census)	Fraction sampled (%)	Sampling weight	Milligrams of active compound used per week per chicken (±SE)	Grams of active compound per 1,000 chickens produced
CG, hh	34	2,890	409,850	0.007	141.8	30.4 (±15.6)	901.2 (±622.8)
CG, sm	34	47,970	128,250	0.374	2.7	5.3 (±1.5)	167.5 (±63.9)
CT, hh	36	4,505	268,295	0.017	59.5	5.6 (±1.4)	327.8 (±122.4)
CT, sm	36	50,230	56,700	0.886	1.1	18.6 (±7.2)	193.1 (±57.3)
MT, hh	34	2,290	58,310	0.039	25.5	26.4 (±17.2)	413.8 (±256.4)
MT, sm	34	52,500	73,300	0.716	1.4	4.7 (±1.9)	156.6 (±63.7)
All	208	160,385	994,705	0.161		15.1 (±4.0)	358.1 (±113.5)

CG=Cho Chao; CT=Chau Thanh; MT=My Tho; hh=household farms; sm=small-to-medium farms

Fig. 1A: Antimicrobial usage per week per chicken (milligrams of active compound) (both unadjusted and adjusted by the survey design), 208 chicken farms, Tien Giang, Vietnam
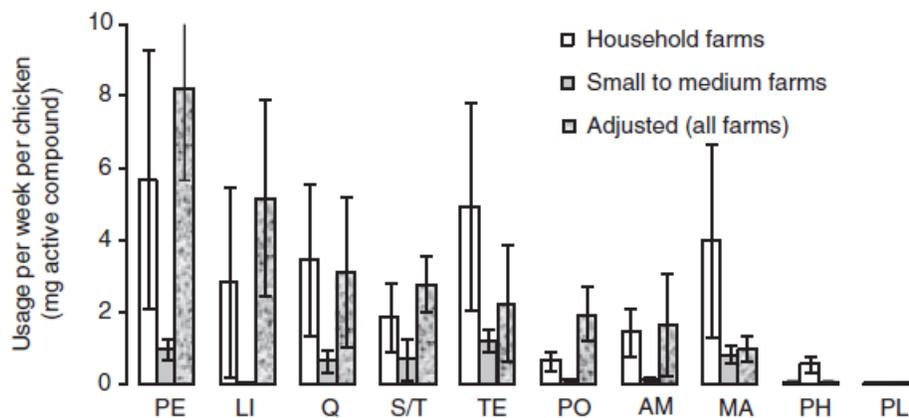
Fig. 1B: Antimicrobial usage per 1000 chickens produced (grams of active compound) (unadjusted and adjusted by survey design), 208 chicken farms, Tien Giang, Vietnam
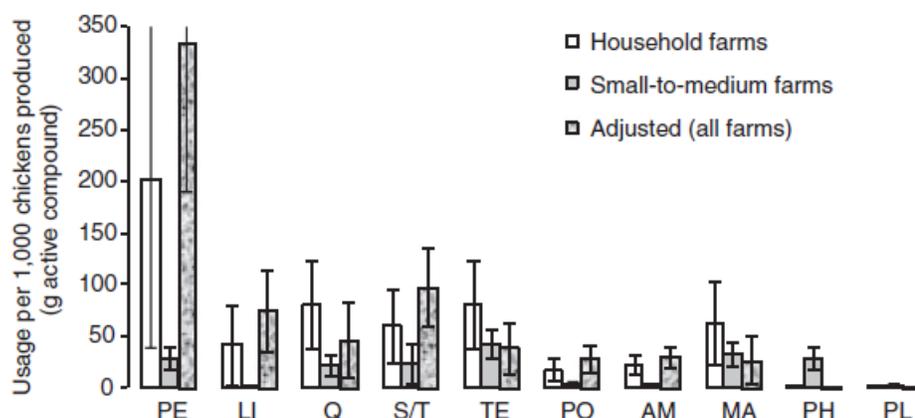
Key: PE, Penicillins; LI, Lincosamides; Q, Quinolones; S/T, Sulphonamides/trimethoprim; TE, Tetracyclines; PO, Polypeptides; AM, Aminoglycosides; MA, Macrolides; PH, Phenicols; PL, Pleuromutilins.

Table 5. Results showing final multivariable proportional odds model (ordinal logistic model) investigating the outcomes: (i) antimicrobial usage per week per chicken (Uwc); and (ii) antimicrobial usage per 1000 chickens produced (U_{1000c}). Only variables remaining significant in either model are kept. 208 chicken farms, Tien Giang, Vietnam

	Usage per chicken per week (Uwc)			Usage per 1000 chickens produced (U_{1000c})		
	OR	95% CI	P-value	OR	95% CI	P-value
District (baseline=MyTho)	1.0			1.0	1.0	
Cho Gao	1.49	1.42–1.55	<0.001	1.01	0.50–2.01	0.998
Chau Thanh	1.53	1.47–1.59	<0.001	1.22	0.61–2.45	0.575
Male farmer(baseline=Female)	2.02	1.53–2.61	<0.001	2.18	1.12–3.98	0.019
Meat production (baseline layer and 'dual purpose')	1.40	1.01–1.89	0.040	0.65	0.43–1.38	0.374
All-in-all-out	0.68	0.56–0.81	<0.001	0.76	0.39–1.23	0.211

OR, Odds ratio; CI, Confidence interval.

Discussion

To our knowledge this is the first study quantifying antimicrobial usage in chicken farms in Vietnam. The key findings are: (1) An extensive range of antimicrobials compounds (28) belonging to ten antimicrobial classes were used, including macrolides, quinolones, and polypeptides; (2) A majority of antimicrobials (84%) were used to prevent, rather than to treat clinical diseases of chickens; (3) Higher levels of usage (per unit time) were associated with meat and household production systems.

CHAPTER 2

We estimated usage of antimicrobials for chicken production in the Mekong delta region from a detailed survey of 208 farms in Tien Giang province. Although we believe that chicken production systems are quite homogeneous across the Mekong delta, results must be interpreted with caution given the limited geographical scope of our sample (i.e. three districts) and the limited sample size. Even small recall errors on behalf of the farmers may have skewed the results in unforeseen directions. In particular the reported higher usage (in quantitative terms) in smaller farms may well reflect a recall bias of usage over an arbitrary period of 90 days. For medium farms recall biases are likely to be less important, since the questionnaire gathered information about 'any antimicrobials used since restocking', which is generally easier to remember. Results reported here are likely to underestimate total antimicrobial usage, since commercial feed commonly includes sub-therapeutic amounts of chlortetracycline and bacitracin, among other antimicrobials. Unfortunately data on feed consumption were not systematically collected.

Our results suggest that a total of 470.4mg of antimicrobials were used to produce one 'meat' chicken in the Mekong delta. These results contrast with data from other European countries (2009), where sales ranged from 14mg/chicken produced (Norway) to 165 mg (Netherlands), with an overall country average of 77.0 mg (SD=53.4) [2]. However it is important to highlight that the average production cycles of meat chickens are longer in the Mekong Delta (20.2 weeks SE±0.62 in our data set) compared with most developed countries (7-8 weeks). In addition, a considerable proportion (24%) of farms in our dataset were 'dual purpose' systems, which (per unit time) used less amount of antimicrobials compared with 'specialised' meat chicken farms.

Furthermore, after statistical adjustment quantitative estimates were much higher due to the higher weight of observations from household farms in the district of Cho Gao. Household farms (<200 chickens) represented 74% of the chicken census in our study population, a similar figure for the whole of Vietnam (79% of chicken production). The observed higher levels of usage among household farms may reflect either lack of technical ability to administer antimicrobials correctly, or a higher perception of risk of disease by household farm owners. This suggests that training of household farmers on the correct administration of antimicrobials would be an effective strategy aiming at reducing overall antimicrobial usage on poultry farms.

CHAPTER 2

Results from the study have highlighted important discrepancies between qualitative and quantitative estimates of usage. For example, polypeptides, tetracyclines, penicillins and aminoglycosides were the most commonly used antimicrobials in terms of reported usage by farms; however penicillins, lincolsamides, quinolones and sulphonamides/trimethoprim were used more in quantitative terms. Differences in the doses and concentration of active principles of the different antimicrobials used may explain these differences. There were also some differences in the quantitative assessment of antimicrobial usage, depending on the chosen estimate. For example, lincosamides ranked second to penicillins in terms of 'usage per unit time' (Uwc) (19.7% of total usage), but third in terms of usage per chicken produced (U1000c) (11.1% of total usage). The reason for these discrepancies lie in the variable levels of usage of antimicrobials in different production systems. Antimicrobials used with similar intensity (per unit time) in layer and meat flocks, will result in overall higher estimates of usage per 1,000 chickens, compared with antimicrobials used more commonly in meat flocks, since layer flocks have a longer lifespan. In particular lincosamides were administered to relatively few layer flocks (data not shown).

Most of the reported antimicrobial usage was 'prophylactic', that is in the absence of clinical disease, to prevent infection. This explains why the variable 'chickens lost to disease in the last three months' was not associated with higher usage in our risk analyses. Our results contrast with studies in chicken farms in Europe and Africa where usage was largely explained by history of disease in the flocks a response to disease [22, 23].

Quinolones and macrolides, both listed by the World Health Organization as antimicrobials 'critically important for human medicine' [24], represented 15.8% (per unit time) and 11.0% (per chicken produced) of overall antimicrobial usage. Neither the use of glycopeptides nor cephalosporins were reported in our study, although avoparcin (a glycopeptide) is sometimes used in feed, and ceftiofur and cefquinome (third/fourth generation cephalosporins) are currently licenced for animal production in Vietnam [25]. Polypeptides (colistin) were the second most commonly used antimicrobials, and represented 4-7% of all usage in quantitative terms in our study, compared with 1.6% reported from nine European countries [2]. This is a concern since colistin is a very valuable to treat serious nosocomial infections caused by multidrug-resistant

gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in humans [26].

The finding that female farmers used less antimicrobials merits further investigation, and suggests that cultural factors may also explain behaviour related to antimicrobial usage on farms. In our study females accounted for 35% of all farmers.

In Vietnam chicken production represents only a small fraction of total animal production, fish and pork being more common animal protein sources [27]. Usage of antimicrobials in Vietnamese aquaculture has been reported to be high compared with most other countries (700 g per tonne of production, compared to 1-200 g per tonne in three European countries, Canada and Chile) [28]. In order to provide an accurate estimate of the selective pressure for antimicrobial resistance in each species, it would be important to determine the comparative levels of usage in all relevant types of animal production, as well as in humans, as has been recommended internationally [29, 30]. Quantitative data on antimicrobial usage on chicken farms should ideally be complemented with surveillance of antimicrobial resistance of selected bacterial species both in main farmed species, food and humans. This should allow accurate monitoring of potential reductions in use and resistance in animal production as well as in humans.

References

1. Anonymous. *WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food. WHO, Geneva.* 2000; Available from: http://www.who.int/foodsafety/publications/containment-amr/en/.
2. Anonymous. *Trends in the sales of veterinary antimicrobial agents in nine European countries (2005-2009). European Medicines Agency.* 2011; Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2011/09/WC500112309.pdf.
3. Katsuda, K., et al., *Antimicrobial resistance and genetic characterization of fluoroquinolone-resistant Mannheimia haemolytica isolates from cattle with bovine pneumonia.* Vet Microbiol, 2009. **139**(1-2): p. 74-9.
4. Pitkala, A., et al., *Bovine mastitis in Finland 2001-prevalence, distribution of bacteria, and antimicrobial resistance.* J Dairy Sci, 2004. **87**(8): p. 2433-41.
5. Wissing, A., J. Nicolet, and P. Boerlin, *The current antimicrobial resistance situation in Swiss veterinary medicine.* Schweiz Arch Tierheilkd, 2001. **143**(10): p. 503-10.
6. Silbergeld, E.K., J. Graham, and L.B. Price, *Industrial food animal production, antimicrobial resistance, and human health.* Annu Rev Public Health, 2008. **29**: p. 151-69.
7. Pagel, S.W. and P. Gautier, *Use of antimicrobial agents in livestock.* Rev Sci Tech, 2012. **31**(1): p. 145-88.
8. Aarestrup, F.M. and H.C. Wegener, *The effects of antibiotic usage in food animals on the development of antimicrobial resistance of importance for humans in Campylobacter and Escherichia coli.* Microbes Infect, 1999. **1**(8): p. 639-44.
9. Carrique-Mas, J.J., et al., *An epidemiological investigation of Campylobacter in pig and poultry farms in the Mekong delta of Vietnam.* Epidemiol Infect, 2014. **142**(7): p. 1425-36.

CHAPTER 2

10.   Garin, B., et al., *Prevalence, quantification and antimicrobial resistance of Campylobacter spp. on chicken neck-skins at points of slaughter in 5 major cities located on 4 continents.* Int J Food Microbiol, 2012. **157**(1): p. 102-7.

11.   Thai, T.H., et al., *Antimicrobial resistance of Salmonella serovars isolated from beef at retail markets in the north Vietnam.* J Vet Med Sci, 2012. **74**(9): p. 1163-9.

12.   Thai, T.H., et al., *Antibiotic resistance profiles of Salmonella serovars isolated from retail pork and chicken meat in North Vietnam.* Int J Food Microbiol, 2012. **156**(2): p. 147-51.

13.   Thai, T.H., et al., *Antimicrobial resistance in Salmonella serovars isolated from meat shops at the markets in North Vietnam.* Foodborne Pathog Dis, 2012. **9**(11): p. 986-91.

14.   Thai, T.H. and R. Yamaguchi, *Molecular characterization of antibiotic-resistant Salmonella isolates from retail meat from markets in Northern Vietnam.* J Food Prot, 2012. **75**(9): p. 1709-14.

15.   Van, T.T., et al., *Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes.* Int J Food Microbiol, 2008. **124**(3): p. 217-23.

16.   Anonymous. *Situation Analysis: Antibiotic use and resistance in Vietnam. In: E. a. P. Center for Disease Dynamics (ed.). Center for Disease Dynamics, Economics and Policy, Washington DC.* 2010; Available from: http://www.cddep.org/publications/situation_analysis_antibiotic_use_and_resistance_vietnam#sthash.YWpTVblU.dpbs.

17.   Dang, S.T., et al., *Impact of medicated feed on the development of antimicrobial resistance in bacteria at integrated pig-fish farms in Vietnam.* Appl Environ Microbiol, 2011. **77**(13): p. 4494-8.

18.   Dohoo, I., S. Martin, and H. Stryhn, *Veterinary Epidemiologic Research.* 1st edn ed. 2003, Charlottetown, Canada: AVC Inc.

19.   McCullagh, P., *Regression models for ordinal data.* J. R. Stat. Soc., 1980(42): p. 109-142.

20.   Hosmer, D. and S. Lemeshow, *Applied logistic regression.* 1st Edn ed. 1989: Jonh Wiley and Sons, New York.

21.   Thrusfield, M., *Veterinary Epidemiology.* 2007: Blackwell Publishing, Oxford.

22.   Hughes, L., P. Hermans, and K. Morgan, *Risk factors for the use of prescription antibiotics on UK broiler farms.* J Antimicrob Chemother, 2008. **61**(4): p. 947-52.

23.   Mitema, E.S., et al., *An assessment of antimicrobial consumption in food producing animals in Kenya.* J Vet Pharmacol Ther, 2001. **24**(6): p. 385-90.

24.   Anonymous. *Critically important antimicrobials for human medicine. 3rd revision (2011). World Health Organization, Geneva.* 2011; Available from: http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf.

25.   Anonymous. *List of antimicrobials authorized in agriculture. Ministry of Agriculture, Hanoi, Vietnam.* 2013; Available from: http://vanban.chinhphu.vn/portal/page/portal/chinhphu/hethongvanban?class_id=1&mode=detail&document_id=168233.

26.   Kadar, B., et al., *The renaissance of polymyxins.* Curr Med Chem, 2013. **20**(30): p. 3759-73.

27.   Anonymous. *Review of the livestock sector in the Mekong countries. FAO, Rome.* 2004; Available from: http://www.fao.org/ag/againfo/resources/es/publications/sector_reports/lsr_mekong.pdf.

28.   Smith, P., *Antimicrobial resistance in aquaculture.* Rev Sci Tech, 2008. **27**(1): p. 243-64.

29.   Anonymous. *Second Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance (AMR). Management options. Oslo, Norway.* 2004; Available from: https://www.oie.int/doc/ged/D895.PDF.

30.   Anonymous. *Critically important antimicrobials for human medicine: categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use : report of the second WHO Expert Meeting, Copenhagen.* 2007; Available from: http://apps.who.int/iris/bitstream/10665/43765/1/9789241595742_eng.pdf