



*Prevalence and antibiotic resistance of
Sveriges lantbruksuniversitet*
Fakulteten för Veterinärmedicin och husdjursvetenskap
Institutionen för biomedicin och veterinär folkhälsa

Prevalence and antibiotic resistance of *Salmonella enterica* in poultry and raw meat in the Can Tho province, Vietnam

Oskar Nilsson

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Oskar Nilsson

Handledare: Sofia Boqvist, Institutionen för biomedicin och veterinär folkhälsa

Examinator: Märit Pringle, Institutionen för biomedicin och veterinär folkhälsa

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ABSTRACT

The aim of this study was to get an overview of prevalence and resistance patterns of *Salmonella enterica* in chicken as well as antibiotic use and knowledge of antibiotic resistance among farmers in the Can Tho region, Vietnam. Material was sampled on local farms and markets during six weeks in October to November of 2008. Twelve markets were selected and each market was sampled once for a total of 96 samples of neck skin of chicken. In addition 20 farms were selected and rectal swabs were collected on a total of 96 chicken. Analyses of market samples were based on the principles of the Nordic Committee on Food Analysis but adapted to local practises. The sampling from farms was based on procedures used in humans and studies of other bacteria in chicken, thus being more uncertain in its outcome. A total of 11 samples, nine from farms and two from markets, were positive or uncertain on polyvalent antiserum testing and hence sent to Sweden for final confirmation and serotyping. Surprisingly only one out of the eleven isolates was shown to belong to the *Salmonella enterica* species, namely *S. Enteritidis*. The reason to the large number of negative samples has to be contributed to the inexperience of myself as well as to the use of modified methods in a foreign laboratory environment. The positive sample was tested for antibiotic resistance showing resistance to four different antibiotics; ampicillin, streptomycin, sulfamethoxazol and tetracyclin. Farmer awareness was studied through a questionnaire and showed low knowledge of antibiotic resistance development and moreover that antibiotics are used as a prophylactic and dependent on availability rather than in regard to its effect on specific disease causing agents.

SAMMANFATTNING (ABSTRACT IN SWEDISH)

Målet med studien var att undersöka prevalens och antibiotikaresistensmönster för *Salmonella enterica* i kyckling i Can Tho regionen i södra Vietnam. Utöver detta syftade studien till att undersöka kunskap och medvetenhet hos fjäderfäproducenterna i regionen avseende antibiotikaanvändning och resistensutveckling. Provtagningen utfördes på lokala gårdar och marknader under sex veckor i oktober och november 2008. Tolv marknader valdes ut och varje marknad provtogs en gång. Sammanlagt togs 96 prover av nackskinn från kyckling. Utöver detta valdes 20 gårdar ut, från vilka totalt 96 kloakprover från levande kyckling erhöles. Marknadsprover analyserades enligt NMKL-metoden, men modifierades efter lokala förutsättningar. Gårdsproverna följde ingen etablerad metod utan utgick från hur analysmetoder på prover från människa går till samt erfarenheter från SVA på liknande provtagning vid undersökning av andra bakterier. Således var resultatet av den senare analysen mer osäkert. Totalt 11 prover, nio gårdsprover och två marknadsprover, var positiva eller tveksamma vid agglutination med polyvalent antiserum och skickades till Sverige för slutgiltig konfirmering och serotypning. Förvånande nog tillhörde endast ett av elva skickade bakterieisolat *Salmonella enterica*, serotypat som *S. Enteritidis*. Orsaken till det stora antalet osäkra och vidare negativa proverna måste härledas till personlig brist på erfarenhet av bakteriologiska studier samt användandet av modifierade och relativt oprövade metoder i en främmande laboratoriemiljö. Det positiva provet testades för antibiotikaresistens och visade sig vara en multiresistent stam med höga MIC-värden mot följande antibiotika; ampicillin, streptomycin, sulfamethoxazol och tetracyclin. Producentmedvetenhet undersöktes med ett skriftligt frågeformulär och visade på en mycket liten kunskap om antibiotikaresistensutveckling samt att antibiotika används profylaktiskt och efter tillgång och kostnad snarare än med avseende på aktuell sjukdomsagens och passande antibiotikabehandling.

INTRODUCTION

Aims

The study, aimed at getting an overview of prevalence and resistance patterns of *Salmonella enterica* in chicken meat and live chicken as well as antibiotic use and awareness among farmers in the Can Tho province in the Mekong Delta, South Vietnam. By getting a gathered picture of a small specific region the study provided the opportunity for future studies in other regions with high comparability. Narrowing the study to a smaller region and thus a smaller population also enabled a high a level of confidence considering the number of samples we were limited to by time and resources.

Thus the study included the following:

- 1) A study of prevalence of *Salmonella enterica* on farms and at meat-markets.
- 2) An investigation of antibiotic resistance on isolates confirmed as *Salmonella enterica* .
- 3) An investigation on farmer-awareness of disease control and use of antibiotics through a small-scale questionnaire study.

Background

The study was performed in Vietnam and Sweden with supervisors from CTU (University of Can Tho), Can Tho, Vietnam and SLU (Swedish University of Agricultural Sciences) and SVA (National Veterinary Institute), Uppsala, Sweden. Funding was in part achieved through Sida (Swedish International Development Cooperation Agency) and the project was performed as a minor field study, MFS.

Poultry meat, mainly from chickens and ducks, is one of the main meat protein sources in Vietnam, poultry being available even amongst the poorer part of the population who cannot afford to keep pigs, cattle etc. Especially important is poultry protein for those living inland where fish and seafood, otherwise readily available protein sources are limited (Te & Duong 2000). Poultry can be found in almost every Vietnamese village where they are often kept free ranged among other animals and people. This means that the risk of contamination to food and water from *Salmonella* and other pathogen infected poultry is high (Kelly-Hope et al 2007).

Data concerning chicken production from the region in Vietnam studied, Can Tho City region in the Mekong delta South Vietnam, was not obtainable on location. Through interviews with local veterinarians and through work with the study some facts were made clearer for us. Chicken production in the study region is mostly family run, not industrialized, meaning there is no flocks in the tens of thousands and flocks are not kept in large housing systems. Most chicken farmers have their flocks outdoors and even the larger ones; having approximately 2000 heads, have all chicken in a single large enclosure without roofing or high fences. Many farmers have other poultry, mostly ducks, in the same enclosure as their chickens. Some also have pig stables and fish ponds within or very close by to the enclosure. As the Mekong Delta is full of streams, rivers and dikes, almost all farms have some connection to water thus so have the chickens/animals on the farms. The closeness between many different animals, both domesticated and wild, and humans as well as the short distance to temperate water, without

cleaning or buffering stages, is a concern both in disease transmission and antibiotic resistance development (Isenberger et al. 2002).

Figure 1. Small-scale chicken farm in the Mekong Delta



Diarrheic disease is a common and important illness in Vietnam (Kelly-Hope et al. 2008) as in many South East Asian countries (Padungtod et al. 2008). Among the most important species of bacterial enteric pathogens one finds *Salmonella enterica* (Isenbarger et al. 2002), which is also the most frequently reported cause of bacterial food borne illness worldwide (Foley & Lynne 2008). Poultry and poultry products are considered one of the primary sources of infection (Adams & Moss 2000).

Besides the concern to public health the economic consequences of salmonellosis are dire. In the US alone the cost of *Salmonella* infections are estimated in the vicinity of three to four billion USD annually (WHO 2008). No study has been made to estimate the cost for salmonellosis and its prevention in Vietnam but one can assume based on figures from other countries, as the one above, that the cost is substantial.

The prevalence of *Salmonella enterica* in raw chicken meat and other food stuffs in Vietnam has been investigated in a number of studies, results ranging between 10% and 60% (Anh D. H. T. & Thanh Y. P. 2006) (Van et al., 2007). The reason to the differences in prevalence can only be speculated upon, but reasons could include seasonal variability, regional difference, chosen samples and so forth.

There are a number of studies on other material besides raw chicken meat, notably studies performed on material from carcasses obtained from slaughterhouses or farms, such as caecal contents (Tran et al. 2004) and meat or organs (Tran et al.

2006). Studies on samples from live chicken through rectal swabs or collection of environmental samples in chicken farms are scarce but those performed (Tran et al. 2006) have shown a relatively low prevalence.

The use of antibiotics in animals in many countries follows in large the pattern for their use in humans (WHO 1998). Broad-spectrum antibiotics are used in excess because of the “over-the-counter” pharmacists operating in Vietnam and other similar countries (Duong et al. 1997).

The high level of antibiotic resistance in many food-borne pathogens in both developing and industrialized countries is a major reason for the negative effect on public health these microbes have, as new or last line antibiotics are getting rare and are very expensive (WHO 1998). This means that a common infection, otherwise easily treatable even for people with limited economic resources, can require a high level of costly medical care thus making treatment unavailable for a large number of people in both industrialized and developing countries (STRAMA 2004). Knowing the resistance patterns among pathogens is a prerequisite for effective medical treatment of humans as well as in farm-animals (Foley & Lynne 2008). Further, knowing the reasons for farmers to treat their animals and which types of antibiotics chosen can facilitate prevention of antibiotic resistance development. If farmers were to choose, or be guided by skilled veterinarians or pharmacists to usage of, correct antibiotics it could limit the use of broad-spectrum antibiotics as well as limiting the use of antibiotics in general (SVARM 2007). The correct use of narrow-spectrum antibiotics reduces risk of resistance development and is often cheaper than broad-spectrum ones (WHO 1998).

MATERIALS AND METHODS

Study region and study population

For the study a number of farms and markets in the Can Tho region were chosen. These were selected by local supervisors based on accessibility and logistic reasons primarily, though aiming to study farms of different sizes, type of production and economic conditions was the original intent.

The number of samples required estimating prevalence in a large population with a 95% level of confidence and an absolute precision of 10% is 96 samples (Thrusfield 2000). Therefore twelve markets and twenty farms were chosen and 4-18 samples were taken on each market for a total of 96 samples, and 4-5 samples were taken on each farm for a total of 96 samples.

Sample quantity is based on the assumption that all farms and all markets respectively can be considered to be a part of the same poultry-population. The reason for this is to limit the number of samples one must attain from each farm while keeping a high level of confidence.

Sampling

Samples were taken with sterile cotton swabs from the cloacae of live poultry and suspended in 5 ml of buffered peptone water (BPW) and kept cold during transport. Raw meat was sampled on local markets in separate sterile plastic zip-lock bags and kept cold during the short transport back to the university laboratory.

For meat samples neck skin of chicken was chosen as this has proven to be suitable material for finding *Salmonella enterica* in chicken in Sweden (National Veterinary Institute of Sweden 1994).

Laboratory analyses

All except antibiotic resistance testing and final serotyping was performed on location with assistance from the coordinator from CTU.

Agars, broths and other reagents were prepared continuously and ingredients bought through the veterinary department of CTU. Materials brought from Sweden were of logistical reasons limited, thus only the pre-made blue (Bromocresol-purple lactose) agar and polyvalent H and O antigen were transported to Vietnam. Materials from Sweden was kept cold throughout the entire transport and controls of the temperature by hand were made during stop-overs.

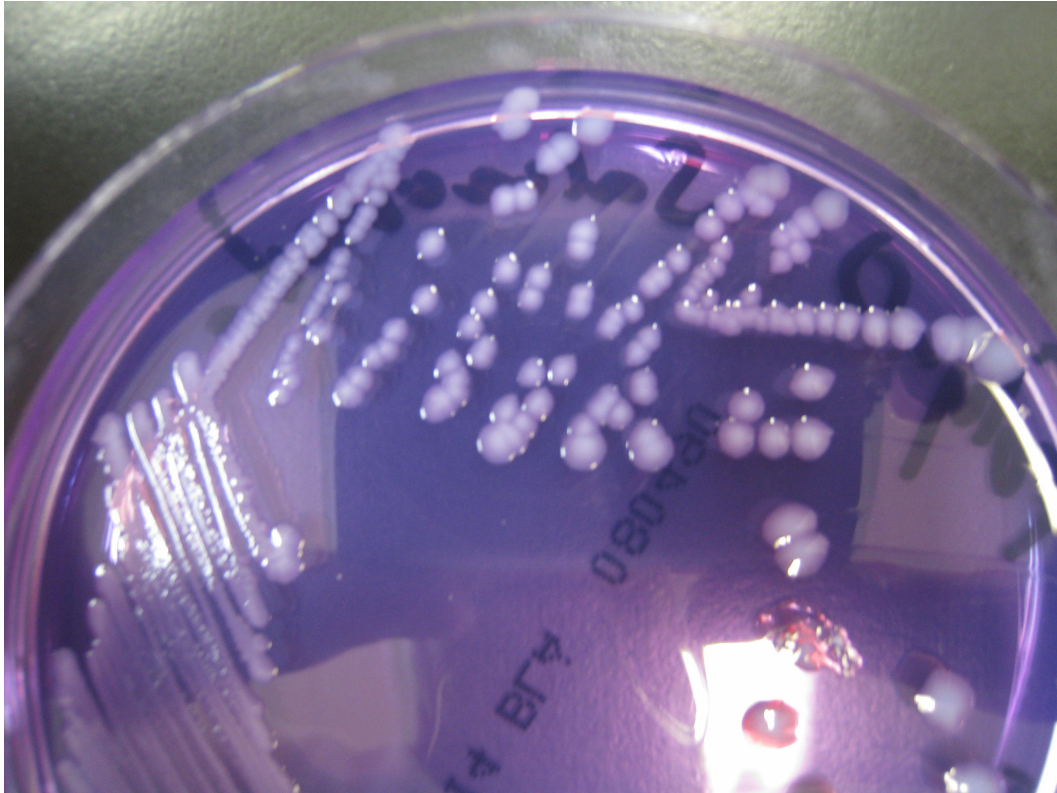
The basis of the analyses was the methodology of the Nordic Committee on Food Analysis (NMKL 1999) but adapted to local factors as described below.

Market samples

Sampled raw meat-material was cultivated as follows:

- 1) Pre-enrichment of 25 g of sample in 225 ml of buffered peptone water (Merck®), 24 hours incubation in 37°C.
- 2) Selective enrichment of 1 ml of BPW-solution (Merck®) to 5 ml of Rappaport-Vasiljadis medium (Oxoid®) 20-24 hours in 37°C incubator.
- 3) Plating on XLD (Merck®) and Brilliant-Green-Agar (Merck®), 24 hours incubation in 37°C incubator.
- 4) Confirmation test was performed after obtaining pure cultures macroscopically similar to *Salmonella enterica* on XLD and BG to blue agar (Produced by SVA), after 24 hours incubation in 37°C incubator.

Figure 2. *Salmonella enterica*-like cultures on blue agar (Confirmed as *S. Enteritidis* in later testing)



Farm samples

Sampled fecal swabs were cultivated as follows:

- 1) Transport of swab in transport tube containing approximately 5 ml of buffered peptone water (Merck®), vortexing before transfer to selective medium.
- 2) Selective enrichment of 0.1 ml of vortexed fecal/BPW-solution to 5 ml of Rappaport-Vasilijadis medium (Oxoid®), 44-48 hours in 37°C incubator.
- 3) Plating on XLD (Merck®) and Brilliant-Green-Agar (Merck®), 24 hours incubation in 37°C incubator.
- 4) Confirmation test was performed after obtaining pure cultures macroscopically similar to *Salmonella enterica* on XLD and BG to blue agar (SVA), after 24 hours incubation in 37°C incubator.

All cultures, both from farms and markets, that were pure and without lactase production, thus not coloring the medium yellow, was then chosen for further testing with Polyvalent H and O antisera (Oxoid®) agglutination. Those samples with a clear agglutination, as well as those with an unclear agglutination or unclear false positive control in sodium chloride, was transferred to semi solid nutrient stock agar and kept cold in refrigerator. Three separate cultures were chosen for confirmation from each separate blue agar. Confirmation and final serotyping was performed in Sweden as described below.

Further analyses

Samples sent to Sweden for antibiotic susceptibility tests and final serotyping were transferred, by staff from the department of Biomedical Sciences and Veterinary public Health at Swedish University of Agricultural Sciences (SLU), to BHI broth with 17% glycerol for storage in -70° C. Samples were stored for approximately five weeks.

After thawing, samples were re-cultured on bovine blood agar and incubated in 37°C for 24 hours and then transferred to blue agar and incubated in 37°C for 24 hours.

Serotyping was performed in accordance to methods used at, and under supervision of staff from the salmonella laboratory of National Veterinary Institute of Sweden (SVA), with O-antisera testing from blue agar and H-antisera testing from semi solid swarm agar. Samples were also transferred to a fermentation tube series and incubated 24 hours in 37°C.

Those with fermentation atypical for *Salmonella enterica* were thereafter cultivated on API® 20 E according to the manufacturer's (Biomérieux®) specifications. Results were checked against the manufacturer's database to get the specified bacterial species. The reason for this was to see which species of bacteria, if any, that could have been mistaken for *Salmonella enterica*, when tested in Vietnam.

The antibiotic susceptibility was tested in VetMIC™ microdilution panels in the laboratory of, and with supervision from staff at the Department of Animal Health and Antimicrobial Strategies, SVA, Uppsala, Sweden. Methods used were those currently in use in the Swedish Veterinary Antimicrobial Resistance Monitoring program (CLSI 2004 and 2007) and a pre-evaluated *E. coli* was used as control culture. Sample cultures, taken from blue agar, and the control culture were pre-enriched in cation adjusted Mueller Hinton broth (CAMBH) in 37°C for 6 hours before diluted and transferred to the VetMIC™ panel and further incubated in 37°C for 16 hours before final reading.

Data collection

Apart from laboratory work the project also included a survey in the form of a questionnaire developed with, and translated by, an English speaking assistant from CTU. The assistant also performed the actual interviews on the farms as no farmers could speak English. All farms were included. The questionnaire contained eight questions and each question had three or four answer alternatives. The complete questionnaire can be found in appendix 1.

RESULTS

Prevalence and serotyping

After sampling of 12 markets and a total of 96 meat samples, two samples showed macroscopic likeness to *Salmonella enterica* on BGA and blue agar as well as being positive on polyvalent O- and H-antisera.

After sampling of 20 farms and a total of 96 rectal swabs, nine samples showed macroscopic likeness to *Salmonella enterica* on BGA and blue agar. Only seven of the nine had a positive result on polyvalent O- and H-antisera test. It was decided that all nine samples should be further investigated in Sweden.

In contrast to the results in Vietnam only one of the eleven samples belonged to the *Salmonella enterica* species, namely to the *S. Enteritidis* serovar, when tested in Sweden. If this would be in line with actual facts it would give a prevalence of *Salmonella enterica* in market samples of $1/48 \approx 2,1\%$, and in farm samples a prevalence of 0%.

The other ten samples where other fecal and environmental bacteria (table.1). Reasons to the positive antisera response in *Citrobacter* and *Morganella* has not been followed up.

Table 1. Results from serotyping in Sweden

Sample	Poly O	Poly H	Na Cl	Fermentation typical for <i>Salmonella</i> spp	Result from API® 20 E	Serovar according to Kauffman-White Tables
Markets						
M47	+	+	-	+	n/a	<i>Salmonella</i> Enteritidis
M48	+	+	-	-	<i>Morganella morganii</i>	n/a
Farms						
F12	-	-	n/a	-	<i>E. coli</i>	n/a
F38	(+)*	-	+	-	<i>Pseudomonas</i> spp**	n/a
F40	(+)*	(+)*	-	-	<i>E. coli</i>	n/a
F42	(+)*	(+)*	-	-	<i>E. coli</i>	n/a
F44	+	+	-	-	<i>Citrobacter farmeri</i>	n/a
F82	+	-	+	-	<i>Escherichia fergusonii</i>	n/a
F83	+	-	+	-	<i>Escherichia fergusonii</i>	n/a
F88	+	-	+	-	<i>E. coli</i>	n/a
F96	-	-	n/a	-	<i>Enterobacter cloacae</i>	n/a

*Weak and atypical agglutination. ** No specific sp. given.

Antibiotic resistance

The one *S. Enteritidis* found, was a multi-resistant isolate, here defined as resistant against more than three compounds, and showed high MIC (minimum inhibitory concentration) values compared to the control (figure 3). MIC level for resistance were those used in Sweden and the EU in 2007 (EFSA 2007).

Figure 3. Results from VetMIC™ antimicrobial susceptibility testing, showing resistance against amoxicillin, streptomycin, tetracycline and sulphamethoxazole.

Vet MIC	Am	Ci	Ff	Nal	Gm	Sm	Tc	Ctx	Su	Tm	Cm	Km
	64 S>	1	32	256	32	256	64 S	8	1024 S>	32	256	16 R
	32	0.5	16 R	128	16	128 S	32	4	512	16	128	8
	16	0.25	8	64	8	64	16	2 R	256 R	8	64	4 X
	8	0.12	4 X S	32	4	32 R	8 R	1	128	4	32	2 S
	4 X, R	0.06 R	2	16 R	2 R	16	4	0.5	64	2 R	16 R	1
	2	0.03 X S	Ci 8*	8	1 X S	8 X	2	0.25	32 X	1 X	8	0.5
	1	0.016	4	4 S	0.5	4	1 X	0.12 X S	16	0.5 S	4 X S	tri-cit X S cont**
	0.5	0.008	2	2 X ≤	0.25	2	0.5	0.06	8	0.25	2	dist X S cont**

X, ≤ = Control MIC. S, > = S. Enteritidis MIC. R = Res-point according to EFSA 2007 (Note that ceftiofur was used in EFSA 2007 compared to cefotaxime in VetMIC 2008)

**Ci cont'd of aesthetic reasons. ** Growth control wells.*

*Am=Ampicillin, Ci=Ciprofloxacin, Ff=Florfenicol, Nal=Nalidixic acid,
Gm=Gentamicin, Sm=Streptomycin, Tc=Tetracycline, Ctx=Cefotaxime,
Su=Sulphamethoxazole, Tm=Trimetoprim, Cm=Chloramphenicol,
Km=Kanamycin*

Questionnaire

All 20 farmers answered the questionnaire. One farmer had only information about a few of the questions since he temporarily was tending the chickens for a family member. The complete questionnaire in English can be found in *appendix 1*.

Question 1, farm size. The smallest farm had 9 chickens and the largest had 1700. The mean farm size was 308 heads while the median was 100. The farms were divided into three size groups; 1-50 as small (S)(6 farms), 51-200 as medium (M)(7 farms) and 201 and above as large sized farms (L)(7 farms)

Question 2, production. All small farms (6) and most medium sized farms (4) produced chicken for family use. The rest of the medium farms (3) and some of the large (3) produced chicken for small scale buyers and local markets. Only some of the large farms (4) had production for large scale or international buyers.

Question 3, other animals. A few of the medium (2) and large farms (6) had ducks. Half of these (1 and 3 respectively) also had pigs. No farm had cattle.

Question 4, housing. Most of the farms kept their chickens free ranged without fencing or within simpler compounds (10 and 4 respectively). Out of the remaining six farms who kept their chickens in a separate area only one of the smaller and one of the large had separate enclosed buildings for their animals.

Question 5, use of antibiotics. Only the two smallest farms did not use antibiotics. Most (1 S, 3 M and 6 L) used it rarely and some (1 S, 3 M and 1 L farm) used it regularly. One did not answer.

Question 6, reason for use of antibiotics. Out of the seventeen that used antibiotics eight farms (1 S, 4 M and 3 L) used it to treat clinically sick animals and nine (3 S, 2 M and 4 L) used it as a prophylactic measure, especially against respiratory disorders during rain season.

Question 7, choice of antibiotics. Three farmers (1 S, 1 M and 1 L) used antibiotics depending on what they could find and afford. Eight (3 S, 3 M and 2 L) used what antibiotics that the sales person recommended. Six farmers used antibiotics depending on the actual illness and/or after consultation with a veterinarian. Out of the later, four were large farms and two were medium sized farms.

Question 8, knowledge of antibiotic resistance. Most of the farmers (11; 5 S, 3 M and 3 L) had never heard of antibiotic resistance. Some (5; 1 S, 1 M, and 3 L) had heard of it but didn't know how it affected their farm. Only three farms (2 M and 1 L) knew about it and took any measures to limit their use of antibiotics. One did not answer.

DISCUSSION

Prevalence

The prevalence of *Salmonella enterica* in this study must be considered too low to be a valid result; considered the higher prevalence found in other studies (Anh D. H. T. & Thanh Y. P. 2006) (Van et al., 2007). Because of this fact the discussion concerning prevalence will instead be primarily on the methods used and how they contributed to the prevalence found.

Methods and laboratory environment. The use of modified methods is always a risk, especially when performed in a foreign laboratory environment. In hindsight one can always argue that one should have followed another protocol or used standardized methods. The choice of neck skin as sample material in markets in line with Swedish sampling methods was perhaps not the most suitable material in the study region. In Sweden slaughter of chickens is extensively automated and uses a lot of flushing water which make neck skin suitable sampling material as fecal material from machine damaged entrails runs with the water to the lowest point on the decapitated carcass hanging from its legs; namely the neck skin. In Vietnam many of the slaughter stages are done by hand and water isn't used in excess, moreover the heads aren't separated from the chicken in the slaughter house. Also much of the chickens

are slaughtered on the farms or by small manual butcher shops. However, the choice of methods was discussed both in Sweden and Vietnam with supervisors and the problems became apparent late in the analytic process in Vietnam. Some of the modifications had to be decided on location. Especially the rectal swab method had never been tried before the start of the project and was therefore difficult to assess when on location. Some of the changes made that might have had a big impact on the result was due to the lack of a 41,5°C incubator for RV incubation. The high temperature is an important stage in inhibiting some ambient bacteria from growing, which became apparent on the market samples with heavy overgrowth by *Proteus* spp., for example. Furthermore because of the difference in resources and laboratory standard all of a sudden there was a need to learn and carry out more, and pre-study seldom performed, steps within the set study time; making agars, autoclaving of used glass Petri dishes and so on. Because of the special circumstances concerning sampling and laboratory work; needing regional permits and also the language barrier, sampling without assistants would have proven very difficult. This together with only a limited time span for sampling and laboratory work made it almost impossible to alter sampling or analysis methods by and by.

Sampling and transports. Sampling was always carried out in the mornings and because of special circumstances markets were sampled twice a week and only in the mornings, and farms only once a week. This meant that many markets had to be sampled each time and even more farms. Because of the large number of markets/farms, an equally large amount of samples had to be kept cold on the way to the lab with the few cooling clamps we had available. The many samples then had to be prepared in the same day to keep schedule. All in all, the large number of samples and the inadequate cooling, pre and post sampling, could certainly have been a factor to overgrowth of ambient bacteria and the difficulty to find *Salmonella* or *Salmonella*-like cultures.

Experience and knowledge. Knowledge about the study area is essential before undertaking any scientific study and lack thereof can seriously hinder or alter your result. This could certainly be said about this study. Inadequate knowledge of the author about slaughter, chicken production and laboratory resources in Vietnam meant that many parts of the study had to be altered and those that couldn't be altered less suitable. A good way to avoid these mistakes would have been to make a trial run in Sweden before traveling to Vietnam; using the methods in a, as much as possible, similar environment as the one on location. Besides knowledge of the location, good knowledge of and skill in using the study methods are needed. Personal inexperience in bacteriology and culturing of *Salmonella* most certainly had an impact on the results.

Antibiotic resistance

The one *S. Enteritidis* isolate found in this study was multi-resistant and though it is only a single sample, other studies in Vietnam (Ogasawara et al. 2008) and elsewhere in South East Asia (Padungtod et al. 2008) have proven that resistance is wide-spread in *Salmonella* isolates. The need to battle this development is crucial but it will prove to be a costly and difficult battle (Foley and Lynne 2008).

Today there is little awareness about resistance development in South East Asia as well as in many other countries world wide, not only among farmers but also among veterinarians and governmental representatives. Larger measures against resistance developing are not prioritized in many countries even though it is listed by the UN as a major health issue (WHO 1998).

Some small steps are taken all the time though. For example a recent ban in Vietnam on using more than two different types of antibiotic compounds in one medical product (personal communication by local supervisor). This example proves that awareness is rising and, development is on its way. At the same time as it is concerning to know that a number of mixed antibiotics have been used in animal feed additives for a long time. This problem is certainly neither unique to Vietnam nor to any specific region of the world.

The Vietnamese government with its relatively sound economy and stable bureaucracy has a good chance of implementing restrictions and setting up control programs. A better control mechanism about drug prescription would be a good start and give the tools for further actions (Chuc & Tomson 1999). Today the knowledge of how much and for what purpose drugs in general and antibiotics in specific are lacking. This is true for both human and animal medicine (Duong et al. 1997) (Larsson et al. 2000).

Questionnaire

The purpose of the questionnaire was to get information on the farms, concerning size, other animals besides poultry, the level of use of antibiotics for poultry on sampled farms and why they are used, contributing to the understanding of the development of antibiotic resistance in the specific region of Vietnam. It also provided an opportunity to get a closer contact with the farmers in the chosen region and finally provided information helpful in interpreting how ambient factors influence prevalence and antibiotic resistance in specific farms/samples.

Unfortunately because of the lack of a complete prevalence study the results from the questionnaire couldn't be used exactly as intended, in regards to epidemiological analyses. Despite this, the information gained by such a simple questionnaire proved to be very interesting and provided much information about the study region and the farmers' situation. For example; the questions 5, 6, 7 and 8 concerning antibiotic use and resistance awareness show that there is difference in how and why antibiotics are used, as well as knowledge about antibiotic resistance in small scale farms compared to the medium or large scale farms. Note that because of the small total number this fact is not statistically proven. There does not seem to be an evident difference between medium or large scale farms. The reason to this can certainly be that the size categories are not entirely representative for actual differences in production type and available resources.

Of course some of the questions weren't adapted to local circumstances, for example should question 3 have included fish as well.

A larger study with more participants and questions more adapted to regional factors together with a successful prevalence study would have been a good contribution to the knowledge about which factors influence the spread of *Salmonella enterica* and the development of antibiotic resistance in Vietnam.

Conclusion

Salmonella is and will be a major disease causing agent in Vietnam and in the world long into the foreseeable future. As more and more people get access to antibiotics for the treatment of both human and animals the concern is great for a growing development of antibiotic resistance in *Salmonella* and other pathogens. Knowledge about prevalence and resistance patterns together with knowledge about extent and purpose of use of antimicrobial drugs are essential tools in the fight against these pathogens. Unfortunately, as is often the case with pilot studies using modified methods, the study result wasn't complete. Still a similar study modified to local factors with more valid results and carried out in a longer time span could give much needed information. The tendency in available studies to only focus on the bacterium as such and not look at the surrounding factors and attitudes is, in my personal opinion, limiting or delaying measures against disease spread as well as spread of antibiotic resistance. Of course there is a great need of large prevalence and antibiotic resistance studies but one must not forget to search beyond this and see the environment in which these factors develop. Further studies are needed both in Vietnam and elsewhere as no one country has the same factors influencing spread and disease outbreaks of *Salmonella* and the pathogen will prove a major public health concern for many years to come.

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APPENDICES

1. Questionnaire as dealt out to farmers

Note that each alternative are followed by a comment within bars; (*number of farms with that answer; small/medium/large*) eg. (Nn; X/Y/Z)

Questionnaire

Please choose the alternative most suited for your farm! Make a note in the area () in front of each chosen alternative. Please note as there are only three alternatives, not all answers will match well to every farm or production-type.

1. How many poultry (chicken or ducks) does your farm include?

2. For what purpose do you mainly farm your poultry? (Please choose one)

- () Egg and meat production for my home consumption (10; 6/4/0)
- () Selling meat and egg to local markets (6; 0/3/3)
- () Selling to large-scale buyers/distributors (4; 0/0/4)

3. Does your farm include other livestock animals besides chicken? (choose one or more)

- () No (9; 2/5/2)
- () Pigs (8; 1/2/5)
- () Ducks (7; 3/1/3)
- () Cattle (0)

4. Do you keep your poultry separate from other animals in the farm/village? (Please choose one)

- () No, they are able to move freely around the farm/village (9; 4/3/2)
- () Yes, I keep my flock separate from the others in the farm/village (4; 1/1/2)
- () Yes, I keep them in a separate area away from other animals (6; 1/2/3)

5. Are antibiotics used on your farm? (Please choose one)

- () No (continue to question 8) (2; 2/0/0)
- () Yes, but rarely (continue to next question) (12; 3/3/6)
- () Yes, regularly (continue to next question) (5; 1/3/1)

6. If yes in question 5, when do you use it? (Choose one or more)

- () To treat animals when they are sick (8; 1/4/3)
- () I give it to prevent animals from getting sick (9; 3/2/4)
- () I give it to animals as it makes them grow faster (0)

7. If yes in question 5, how do you choose which antibiotics to use? (Please choose one)

- () I use different antibiotics depending on what I can find or afford (3; 1/1/1)
- () I use what the salesperson recommends (8; 3/3/2)
- () I use antibiotics depending on what illness is present and/or after consultation with a veterinarian (6; 0/2/4)

8. Are you aware of resistance of some bacteria to antibiotics? (Please choose one)

- () No (11; 5/3/3)
- () I have heard of it but don't know how it affects my farm (5; 1/1/3)
- () Yes, I am aware of the problem and try to restrict my use of certain antibiotics (3; 0/2/1)

Thank you very much for your participation!