Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Biomedical Sciences
and Veterinary Public Health

Prevalence and antibiotic resistance of *Campylobacter* spp. in poultry and raw meat in the Can Tho Province, Vietnam

Petter Schwan

Uppsala

2010

Degree project within the Veterinary Medicine Programme

ISSN 1652-8697
Degree project 2010:49

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Sveriges lantbruksuniversitet

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Petter Schwan

Supervisor: Helena Höök, Department of Biomedical Sciences and Veterinary Public Health
Assistant supervisor: Karl Ståhl, National Veterinary Institute (SVA)

Examiner: Märit Pringle, Department of Biomedical Sciences and Veterinary Public Health

Examensarbete inom veterinärprogrammet, Uppsala 2010
Fakulteten för Veterinärmedicin och husdjursvetenskap
Institutionen för biomedicin och veterinär folkhälsovetenskap
Kurskod: EX0234, Nivå X, 30hp

Online publication of this work: http://epsilon.slu.se
ISSN 1652-8697
Examensarbete 2010:49

Content

ABSTRACT

The aims of the study were to investigate the prevalence and antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* in chicken and chicken products, and to investigate farmer awareness of antibiotic resistance development. The study was conducted in the Can Tho province in Vietnam during six weeks in October to November 2008. Ninety-six samples from raw chickens were collected from twelve market places and analysed for the presence of *Campylobacter*. A total of 96 cloacal swabs from 20 farms were obtained and analysed for the presence of *Campylobacter*. Farmers were asked to answer eight questions concerning the housing of the chickens, modes of handling sick animals as well as use and knowledge of antibiotics and resistance development to antibiotics.

None of the market samples were positive for *Campylobacter* spp. most likely due to faulty handling of samples, wrong sample selection and contaminant bacterial overgrowth. Seventy-six percent of the individual farm samples were positive for *Campylobacter* spp. (73/96), and 95% (19/20) of the farms had at least one positive sample. From the 73 positive samples, 28 isolates were chosen for further analysis, species identification and antibiotic resistance testing. Twenty-one percent of the isolates (6/28) were *C. coli* and 79% (22/28) were *C. jejuni*. Resistance was found to all examined antibiotics: erythromycin 7%, ciprofloxacin 71%, tetracycline 71%, streptomycin 21%, gentamicin 7% and nalidixic acid 71%. *Campylobacter coli* had an overall higher level of resistance than *C. jejuni.*

Antibiotics were used in most farms and often in a profylactic manner. Most farmers chose substance depending on availability and cost and/or what the local salesperson recommended rather than consulting a veterinarian. Knowledge of resistance to antibiotics was generally low.

SAMMANFATTNING

Målet med studien var att undersöka prevalens för och antibiotikaresistens hos *Campylobacter jejuni* och *Campylobacter coli* från kyckling och kycklingprodukter samt att undersöka bönders medvetenhet om antibiotikaresistensutveckling. Studien genomfördes i Can Tho-provinsen i södra Vietnam under en sex-veckorsperiod i oktober och november 2008. Nittiosex prover från rå kyckling inhämtades från tolv olika marknadsplatser och undersöktes med avseende på *Campylobacter*-förekomst. Nittiosex kloakprover inhämtades från 20 gårdar och undersöktes för *Campylobacter*-förekomst. Bönderna utfrågades angående inhysningssystem för kycklingarna, handhavande av sjuka djur samt kunskap om och användning av antibiotika och resistensutveckling hos bakterier.

Samtliga marknadsprover var negativa för *Campylobacter* spp. Orsaken till detta var troligen felaktig hantering av prover, felaktigt val av prover och överväxt av kontaminant bakterieflora. Sjuttiosex procent (73/96) av kycklingarna på gårdarna hade positiv växt av *Campylobacter* spp. och 95 % (19/20) av gårdarna hade minst en individ med positiv växt. Av de 73 positiva proverna valdes 28 isolat ut för analys av antibiotikaresistensmönster samt artbestämning. Tjugoen procent av isolaten (6/28) visade sig vara *C. coli* och 79 % (22/28) var *C. jejuni.* Antibiotikaresistens hittades mot alla testade antibiotika: erytromycin 7 %, ciprofloxacin 71 %, tetracyklin 71 %, streptomycin 21 %, gentamicin 7 % och nalidixinsyra 71 %. *C. coli* var i större utsträckning resistent mot antibiotika än *C. jejuni.*

På de flesta av gårdarna användes antibiotika och ofta i ett profylaktiskt syfte. De flesta bönder valde typ av antibiotika utifrån tillgänglighet och kostnad samt beroende på vad den lokala försäljaren rekommenderade snarare än efter konsultering av veterinär. Medvetenheten om antibiotikaresistens var generellt låg.

Background

Enteric disease is a common and important illness in Vietnam as in many developing countries (Isenbarger et al. 2002). *Campylobacter* is one of the most important bacterial pathogens and is regarded as the major bacterial cause of human gastroenteritis worldwide (Allos 2001). Poultry and poultry products are considered the primary source of infection (Coker et al. 2002).

The use of antibiotics in animals in developing countries follows in large the pattern for use in humans (Allos 2001). Broad-spectrum antibiotics are used in excess because of the over-the-counter-pharmacists operating in these countries (Duong et al. 1997). The high level of antibiotic resistance in many food-borne pathogens in developing countries is a major reason to the negative effect on public health these microbes have, as last line antibiotics are expensive and already widely used (Allos 2001; WHO 2003). 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 (WHO 2003). Knowledge of the resistance patterns among pathogens is a prerequisite for effective medical treatment of humans as well as in farm animals (Allos 2001). Further, knowledge of the farmers reasons to treat their animals and which types of antibiotics they choose can facilitate prevention of antibiotic resistance development. If farmers were to choose antibiotics with increased knowledge of resistance development it could limit the use of broad-spectrum antibiotics as well as limit the use of antibiotics in general. The correct use of narrow-spectrum antibiotics reduces risk of resistance development and such antibiotics are often cheaper than broad-spectrum antibiotics (Duong et al. 1997; Allos 2001).

Campylobacter the bacterium

The first bacteria in the genus later classified as *Campylobacter* were isolated from aborted ovine foetuses in the beginning of the 20th century by McFadyean and Stockman (McFadyean & Stockman 1919). The first bacteria belonging to the group were first isolated and described as a human pathogen in 1938 in the context of a milk-borne diarrhoeal outbreak (Levy 1946). Later King, working with isolates from human blood found two different groups on the basis of their optimum growth temperature (King 1962). One corresponded to the bacteria found to cause abortion, with an optimum growth temperature of 37 ^{o}C, and the second, thermophilic group was associated with human cases of diarrhoea and grew best at 42 ^{o}C (King 1962). In the 1970s it was established that *Campylobacter jejuni* subsp. *jejuni.*, hereafter *C. jejuni*, and *Campylobacter coli* were responsible for a large percentage of diarrhoeal cases in many countries (Vandamme et al. 2005). This paper will hereafter focus on the most common *Campylobacter* spp. causing illness in human, i.e. *C. jejuni* and to a lesser extent *C. coli.*

In later years the *Campylobacter* genus has grown significantly and now consists of 21 species whereof five are further divided into two subspecies each (Euzéby 2009).

Campylobacters are small and slender gram negative spiral shaped rods with a typical corkscrew or darting motility due to uni- or bipolar flagella (Vandamme et al. 2005). The growth optimum is at an oxygen concentration of 3–15% and the optimum growth temperature varies from 15 to 30 °C (Vandamme et al. 2005). For *C. jejuni* and *C. coli* the incubation temperature for best selectivity lies between 42 and 43 °C, mainly due to the fact that the temperature eliminates inhibitory growth of other intestinal organisms (Vandamme et al. 2005). *Campylobacter coli* and *C. jejuni* are both oxidase and catalase positive (Vandamme et al. 2005). *Campylobacter jejuni* is susceptible to a variety of environmental conditions including low pH, drying, high oxygen conditions and does not grow below 30 °C (Montville & Matthews 2005). Despite this, campylobacters have been shown to survive for five weeks in urine held at 4 °C and in contaminated poultry the number of bacteria is reduced, but not to zero, when frozen to –20 °C (Montville & Matthews 2005). The bacteria do not survive in food products brought to adequate cooking temperatures.

Campylobacter in humans

Infection with enteropathogenic campylobacters in a naive individual causes an acute enterocolitis with symptoms like diarrhoea varying from profuse watery to bloody dysenteric stools, nausea, abdominal pain, fever and headache (Adams & Moss 2000). The incubation period varies from 1 to 11 days. Infection with *C. jejuni* and *C. coli* are clinically indistinguishable (Adams & Moss 2000). The infection is normally self-limiting over a period of 5–8 days, but may persist longer and require medical treatment (Brooks et al. 2004). The infective dose has not been set, but ingestion of less than 10^4 organisms have been shown sufficient to produce infection (Brooks et al. 2004). The organism multiplies in the small intestine, invades the epithelium and causes inflammation that results in diarrhoea and appearance of inflammatory cells and red blood cells in the stool (Brooks et al. 2004). Sometimes the bloodstream is invaded.

The pathogenicity factors of enteropathogenic campylobacters are relatively unknown, but as with other enteropathogens virulence factors like motility, chemotaxis, adherence, invasion and toxin production have been recognized (Vandamme et al. 2005). The virulence of *C. jejuni* is at least in some strains to a part caused by a heat-labile enterotoxin that share some common properties with that of *V. cholerae* and *E. coli* (Jay et al. 2005). Another virulence factor is a cytotoxin that together with the enterotoxin has shown to cause fluid accumulation in the jejunal loops of rats when experimentally infected. Also, the invasive abilities of the organism itself appears to be strongly linked to its toxicity (Jay et al. 2005).

Patients that have gone through a *C. jejuni* infection sometimes suffer from reactive arthritis, and in exceptional cases Guillain-Barré syndrome, GBS (Montville & Matthews 2005). GBS is a severe acute immune-mediated paralytic

disorder affecting the peripheral nervous system. The pathogenesis of campylobacter-induced GBS is not clear but it is estimated that one third of patients with GBS developed the disease 1–3 weeks after a *C. jejuni* enteritis (Jay et al. 2005).

Campylobacter in a global perspective

Infection with *Campylobacter* spp. is considered as one of the most common bacterial causes of human gastroenteritis worldwide (Allos 2001). In Europe alone more than 200 000 confirmed cases were reported to the European Food Safety Authority in 2007 (EFSA 2009). The main vehicle of infection is considered to be poultry and poultry products. Outbreaks of campylobacter-caused enteritis are rare. The largest recorded outbreak in the U.S. was traced to a water supply and about 2000 individuals were infected (Jay et al. 2005). Other outbreaks have occurred due to consumption of unpasteurized milk, in which *Campylobacter* spp. can often be isolated (Jay et al. 2005).

In developing countries campylobacteriosis is considered hyperendemic, and therefore most children have gone through a campylobacter enteritis at young age and often developed antibodies to the bacterium (Coker et al. 2002; Montville & Matthews 2005). The major sources of human infection in these countries are environmental contamination and food (Coker et al. 2002). A study has shown that *Campylobacter* strains isolated from faeces from humans and chickens were related phenotypically and genotypically. This indicates that chickens are important carriers of campylobateriosis in developing countries (Coker et al. 2002).

Campylobacter in poultry

Very few studies have been published on *Campylobacter* prevalence in faeces of live poultry in Asia. The ones performed have all found campylobacter in relatively substantial quantities. In one study from Taiwan on campylobacter prevalence in ducks *Campylobacter* spp. were isolated from 92% of the duck farms and from 43.5% of the individual samples (Tsai & Hsiang 2005). A significantly lower level of positive samples were isolated from ducklings under two weeks of age than from older age groups. Most of the isolates, 94.8%, were *C. jejuni* and the rest were *C. coli* (Tsai & Hsiang 2005). Another study on campylobacter prevalence in broiler flocks in southern Japan showed a 32.1% prevalence on flock level (Chuma et al. 2001). In a study from northern Thailand campylobacters could be found in 64% of chicken individuals at farm level (Padungtod & Kaneene 2005). The majority of the samples, 52%, were shown to be *C. coli.*

Poultry, mainly chicken and ducks, is one of the main meat protein sources in Vietnam. Poultry is especially important amongst the poorer part of the population who cannot afford to keep pigs or cattle. Chicken and ducks can be found in almost every Vietnamese village where they often are kept free ranged among

other animals and people. This means that the risk of contamination to food and water from campylobacter infected poultry is high (Coker et al. 2002).

Campylobacter in Vietnam

Studies on campylobacteriosis from Vietnam have shown that the bacterium is a major cause of diarrhoea, especially in children under one year of age (Isenbarger et al. 2001). In older children campylobacter can be found both in the faeces of children with diarrhoea and in children without clinical symptoms (Isenbarger et al. 2001). Presence of campylobacter in faeces whithout clinical symptoms is rare in the western countries.

Studies from Hanoi in 2003–2005 show that approximately 30% of raw chicken in school and hospital canteens and at retail markets contain campylobacter (Huong et al. 2006; Dao & Yen 2006). Except these, few published studies have been made on campylobacter in poultry in the country.

Aims

The aims with the study were to get an assessment of the prevalence of *Campylobacter* spp. in chicken meat from local markets and in chicken faeces at farm level in the Can Tho Province in the Mekong Delta of Southern Vietnam, to investigate the resistance patterns of a selection of isolates found and to get an overview of farmers use of antibiotics and awareness of disease control. The study was performed as a minor field study (MFS) with financial support from Sida and in close co-operation with professors and students at the Can Tho University, the Swedish National Veterinary Institute (SVA) and the Swedish University of Agricultural Sciences (SLU).

MATERIALS AND METHODS

Study area and study population

This study was performed in the Can Tho province in southern Vietnam. The province is a rich farmland, irregated by the Hao river and the Mekong delta (Sawadee.com). The area of the province is 2965 km^2 with a population of approximately 1,8 million people. The region is rich in rice fields and small scale family-run farms. Rice is the major export product of the province (Sawadee.com).

No information of the animal population of the region could be obtained at location. Through interviews with local veterinarians we understood that poultry in the region are mostly kept at small scale farms with number of heads ranging from just a few up to a few thousand. The animals in the small farms are mostly kept outdoors while some of the larger farms have simple fencing and roofing. Some farms combine poultry production with fish farming, using the droppings from the birds to feed the fish. The proximity to water in the Mekong delta makes

the farms part of a complex ecosystem with little possibility of segregation and epidemiological barriers. Other animals than poultry and fish is scarce in the region although smaller pig and cattle stocks were seen.

For epidemiological and statistical reasons, the poultry in the region Can Tho was considered as one epidemiological unit. All samples from farms and markets were considered as a part of this large population of poultry. The farms were chosen by our local coordinator on basis of accessibility and willingness to participate. Moreover, the choice of farms were aimed at farms of different sizes and production types. To achieve a confidence level of 90% and an absolute precision of 10% on prevalence, 96 samples from faeces and meat, respectively, had to be obtained (Thrusfield 2000). Therefore 20 farms were visited and 4–5 samples were taken from each farm. The markets visited, all in the nearby region of Can Tho city, were of different sizes. Twelve markets were visited and 2–18 samples were obtained from each market.

Data collection

To get a general appreciation of the size and type of prodution on each farm, as well as to evaluate the farmer awareness of the adverse effects of using antibiotics and in which extent antibiotics were used, a questionnaire with eight questions was created and translated by our local coordinator (Appendix 1). While the sampling was done, the farmer was taken aside by our local coordinator and asked to answer the questions. The farms were then stratified by size into three groups: small (1–50 heads), medium (50–200 heads) and large (200 heads and above).

Sample collection

During two weeks, from October 27 to November 5, 96 chicken samples from local markets were collected during the first opening hours of the morning, from approximately 8 am to 11 am. During this time the samples were kept cold by cooling clamps and an insulated cooling box. Approximately 25 samples were collected at each occasion from different vendors at the market. Two samples were chosen from each salesperson and kept separate in clean zip-lock bags. The samples were mainly raw chicken necks, but in six cases, when no necks were available, samples of raw chicken breast or thigh were taken.

The 96 faecal samples were collected from 20 different farms in the nearby region of Can Tho city. Two days of sampling were needed (November 12 and November 19). The sampling took place from 8 am to 12 pm. The samples were kept cool by cooling clamps and an insulated container. Sterile milk tubes were filled with 4–5 ml of Bolton broth as transport medium. On the farms four or five chickens were arbitrarily selected from the herd and swabbed in the cloacae with sterile cotton swabs to obtain approximately 1 g of faeces. When no faecal material could be obtained from one chicken another one was chosen for sampling. In some cases when the correlation between a chicken and fresh droppings was evident, faecal material was collected from the ground. To minimize the risk of contamination new latex gloves were used for every individual tested.

Laboratory analyses

Market samples

For this study, a modified form of the ISO 10272-1 method was used (ISO/CEN 2009). The modifications were made mainly due to difficulty in transporting equipment without spoiling it. From each market sample 25 g of neck skin was collected and put into a stomacher bag with 225 ml of buffered peptone water (Merck®) of room temperature. The sample was then thoroughly mixed by hand for approximately 3 minutes after which 5 ml of the fluid was transferred into stomacher bags containing 45 ml Bolton preenrichment broth (ratio 1:9, Oxoid®). The bags were then put into an airtight container together with a campygen catalyst bag (Oxoid®) creating a microaerobic environment. The containers were then incubated for 3–4 hours at 37 ºC and thereafter at 41.5 ºC for 40–48 hours. After this time, one loop (10 µl) of the culture was transferred to campylobacter selective mCCD agar (modified Charcoal Cefoperazone Deoxycholate agar, Oxoid®) and incubated microaerobically for another 40–48 hours.

For identification of campylobacters, suspect colonies were examined in microscope for typical morphology and motility.

Farm samples

At the laboratory the samples were vortexed and one loop of medium was transferred to mCCD agar which was then incubated microaerobically for 3–4 hours at 37 ºC and then at 41.5 ºC for another 40–48 hours.

The identification of campylobacter from the agars was conducted as with the market samples. One or two samples from each farm, in total 28 samples, were selected for isolation on sheep blood agar and later for antibiotic resistance testing. Since no equipment for resistance testing was brought to Vietnam, the isolates were transferred to sterile cotton swabs, placed in Amies agar gel with charcoal (Oxoid® TS0002) and sent by air to SVA, Uppsala, where they were re-cultured and frozen to −75 ºC in brain heart infusion medium (BHI, Oxoid® CM 225) with addition of 17% glycerol.

Figure 1: The author in the process of sampling faeces from the cloacae of a chicken on a small scale chicken farm in the Can Tho region, South Vietnam.

Species identification and antimicrobial resistance testing

At the laboratory of the Department of Biomedical Sciences and Veterinary Public Health at SLU in Uppsala, the 28 campylobacter isolates were checked for oxidase and catalase reaction, and were tested for hippurate hydrolysis to identify *C. jejuni* (Hwang & Ederer 1975). The isolates that were negative on hippurate hydrolysis were then tested with a *Campylobacter*-specific PCR (Denis et al. 1999). The antimicrobial susceptibility testing was carried out by experienced personnel at the Department of Antibiotics, SVA, Uppsala, Sweden. The department uses a MIC-based broth microdilution method specific for testing of *Campylobacter* spp., VetMIC^{TM} Camp., testing for susceptibility to erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin and nalidixic acid (SVARM 2004).

RESULTS

Market samples

From the market places, a total of 96 samples were collected and tested for presence of campylobacter. None of the 96 samples were positive for *Campylobacter* spp. All agar dishes showed a similar growth pattern with a mixed bacterial flora of two to four morphologically different colonies.

Farm samples

On farm level, 73 of the 96 (76%) animals tested were positive for campylobacter. Nineteen of the twenty farms tested had at least one positive sample out of the four or five animals tested, thus giving a 95% prevalence on farm level.

Species identification and antimicrobial resistance testing

Of the 28 samples that were brought back to Sweden six were *C. coli* and the other 22 were *C. jejuni*, giving a proportion of 79% *C. jejuni* and 21% *C. coli.*

The results from the antimicrobial resistance testing are presented in Table 1, Table 2 and Diagram 1 below.

Table 1: Results from the VetMIC™ antimicrobial susceptibility testing of 22* C. jejuni *and 6* C. coli *isolates from Vietnamese chickens, 2008. Table shows Distribution (%) of MICs (mg/l)*.

Substance	R %	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
EM	7					63.0	14.8	3.7	11.1	3.7				3.7	
CI	71			21.4	7.1			3.6		10.7	57.1				
TE	71			25.0		3.6					10.7	60.7			
SR	21						64.3	14.3				7.1	10.7	3.6	
GM	7				10.7	78.6	3.6					7.1			
NA	71								17.9	7.1	3.6		3.6	67.9	

*The white fields denote the range of dilutions tested for each substance. Yellow fields are outside the tested range. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the the lowest concentration tested are given as the lowest tested concentration. Bold vertical lines indicate cut-off values for resistance (EFSA 2007). Numbers in red are above the cut-off values, thus resistant to the tested substance.

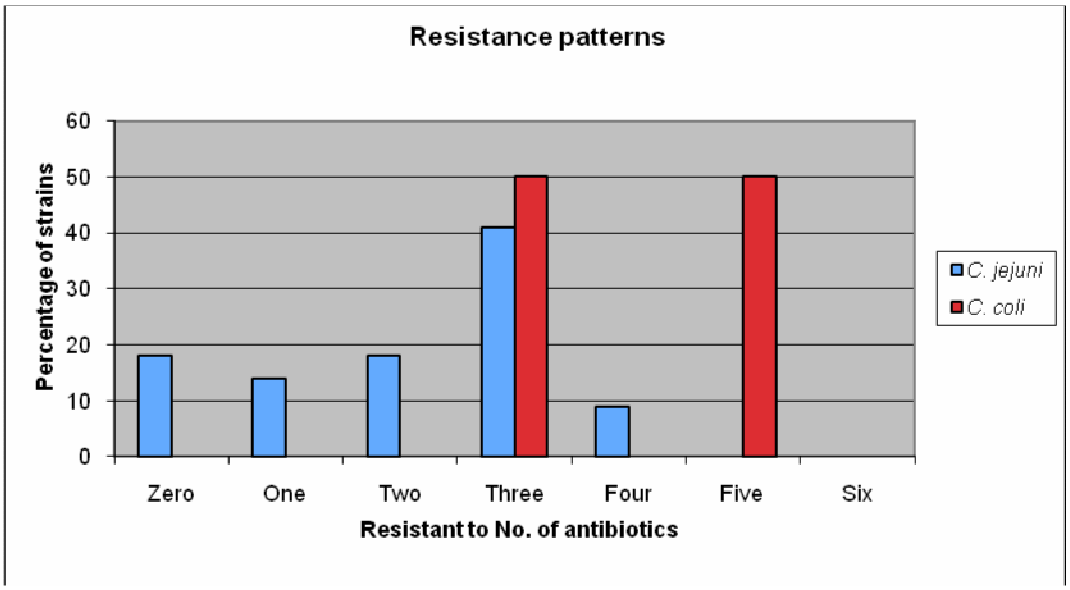
EM=Erythromycin, CI=Ciprofloxacin, TE=Tetracycline, SR=Streptomycin, GM=Gentamicin, NA=Nalidixic Acid.

R%= Percentage of isolates resistant to each antibiotic.

Table 2: Antimicrobial resistance in 6* C. coli *and 22* C. jejuni *isolates from Vietnamese chickens, 2008, showed as percentage of isolates resistant to a given antimicrobial substance.

	Antimicrobial					
	Erythro-mycin	Cipro-floxacin	Tetra-cycline	Strepto-mycin	Gentamicin	Nalidixic-acid
	% Resistance					
C. jejuni	0	64	68	14	9	64
C. coli	33	100	83	50	33	100
C. total	7	71	71	21	7	71

Diagram 1: Antimicrobial resistance among the tested isolates (n=28) showing the percentage of isolates from fully susceptible (zero resistance) to multiresistant (resistant to four or more antimicrobials).

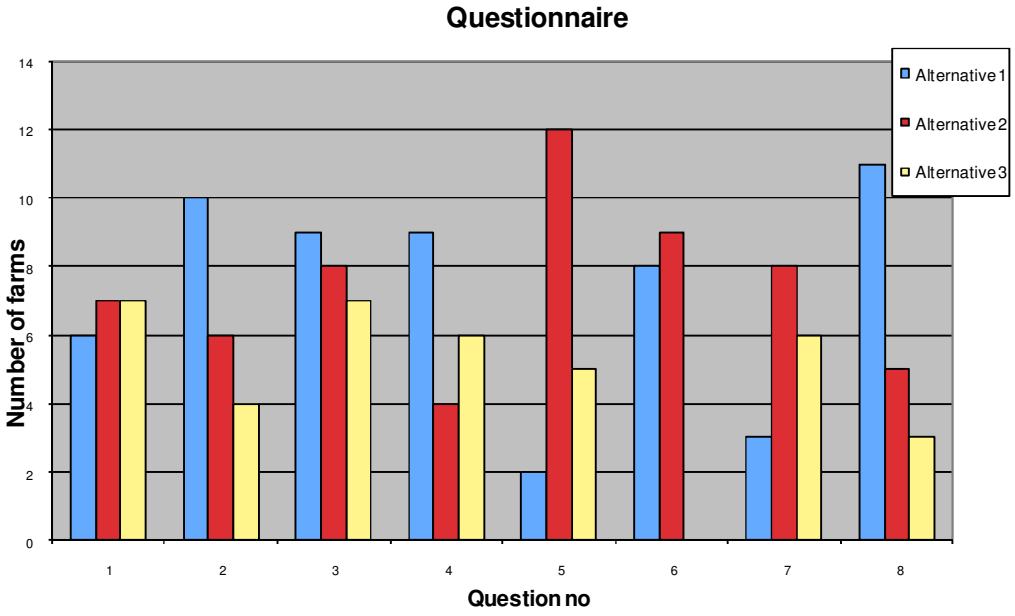


Questionnaire

The result of the questionnaire was interpreted and translated together with our local coordinator. The questionnaire in the English version before translation can be viewed in Appendix 1. All 20 farmers answered the questions although from

one farmer only the first three questions could be answered because the owner was not present at the time of visit. Results from the questionnaire can be viewed in Diagram 2 below.

Diagram 2: Results from the questions asked to twenty farmers in the Can Tho region in Vietnam autumn 2008. The questions and response alternatives are listed below the diagram.



1. How many poultry (chickens) does your farm approximately include?
Alt 1: Small (1-50) *Alt 2:* Medium (50-200) *Alt 3:* Large (200 or above)

2. For what purpose do you mainly farm your poultry?
Alt 1: Home consumption *Alt 2:* Selling at local markets *Alt 3:* Selling to large-scale buyers

3. Does your farm include other livestock animals as well?
Alt 1: No *Alt 2:* Yes, pigs *Alt 3:* Yes, ducks

4. Do you keep your poultry separate from other livestock (animals) in the farm/village?
Alt 1: No *Alt 2:* Yes, separate from other flocks *Alt 3:* Yes contained separate from other animals

5. Are antibiotics used on your farm?
Alt 1: No *Alt 2:* Yes, but rarely *Alt 3:* Yes, regularly

6. If yes in question 5, when do you use it?
Alt 1: To treat sick animals *Alt 2:* Prevent animals from getting sick *Alt 3:* As growth promoter

7. If yes in question 5, how do you choose which antibiotics to use?
Alt 1: Depending on price and availability *Alt 2:* What salesperson recommend *Alt 3:* Consulting veterinarian or depending on what illness is present

8. Are you aware of resistance of some bacteria to antibiotics?
Alt 1: No *Alt 2:* Heard of but not aware of effects *Alt 3:* Aware and use antibiotics restrictive

Results from the questionnaire are in some cases inconclusive although a tendency can be seen that the smaller the farm, the less the use of antibiotics. Of the farms using antibiotics about 50% used it to prevent animals from getting sick, and the other half used it to treat already sick animals. Close to 60% of the farmers were not aware that use of antibiotics could have adverse effects such as development of resistance in bacteria. No conclusions could be drawn from the questionnaire linking the size of the farm or the use of antibiotics to the resistance patterns of the bacteria. Also, in some farms one multiresistant isolate was found in the faeces from one individual, and another individual carried a fully susceptible isolate.

When asked what type of food additives that were used, one of the farmers on a medium scale farm presented a plastic bag with several types of commercially produced food additives mostly containing varying kinds of vitamines, but also gentamicin, tetracyclin, thiamphenicol and cortizone.

DISCUSSION

In this study none of the samples of chicken meat from the markets were positive for campylobacter. Surveillance studies in European countries of *Campylobacter* in broiler meat at retail have during the last years shown around 30% positive samples with a range from 4.3% to 67% in 2007 (EFSA 2009). An earlier study from Hanoi in 2005 (Huong et al. 2006) showed a 31% prevalence of campylobacter in raw chicken at retail markets. Another study in 2003–2004 on *C. jejuni* in raw poultry from canteens showed a 28% prevalence (Dao & Yen 2006). The three Vietnamese studies can not easily be compared since no information on the use of disinfectants on the carcasses was available. The sample selection, i.e. neck skin, in this study was based on studies from Sweden (Hansson 2007) where it has been shown more likely to isolate campylobacters from this part of the carcass than any other. The method is based on large scale abattoir techniques where water is used through the whole process and the carcasses are hanging upside down. Unfortunately no information of the poultry slaughter techniques of the region was available and neck skin is likely less polluted with bacteria if a dry slaughter method is used. The handling and cooling of the samples in our study was unfortunately not optimal, possibly resulting in overgrowth of contaminant bacterial flora.

Our study demonstrated a 95% herd level prevalence of campylobacter. This result is in no sense extraordinary or surprising since many countries in the EU show close to similar prevalence in the broiler production (EFSA 2007). In the Scandinavian countries the number of campylobacter positive broiler flocks has gradually fallen due to the introduction of hygenic standards in the production chain (Humphrey et al. 2007). In the Swedish surveillance programme for campylobacter from 2007, campylobacters were isolated in 12.6% of the studied broiler flocks. The prevalence varies greatly over the year with a distinct peak during the summer months (Zoonoses in Sweden 2007). This pattern can be observed in many temperate countries but is not seen in subtropical climates such

as southern Vietnam where campylobacter is constantly present and seen as hyperendemic (Allos 2001).

The attempts to decrease the number of campylobacter-positive broiler flocks in the southern part of Europe has not been equally successful, possibly because of the differences in climate (Coker et al. 2002). Eradication of campylobacter in the poultry production in a climate like the one in Vietnam, and in a production system where the chickens mostly roam freely, in contact with wild birds, and drink river water seems very unlikely. The only mode of coping with the pathogen is therefore through food hygiene and keeping the animals away from the cooking area (Coker et al. 2002).

Earlier studies from the Swedish broiler production have shown that once introduced into a group, campylobacter has a tendency to spread rapidly and leading to close to 100% prevalence (Berndtson 1996). In the Swedish surveillance programme for campylobacter, however, also broiler flocks with low within-flock prevalence have been observed (Hansson 2007). Different explanations for this have been presented, such as recent introduction of the bacterium in the flock, contamination during transport, or a true low within-flock prevalence. Few parallels can be drawn between Swedish broiler production, with a strict all-in-all-out regime, and the small scale Vietnamese poultry farms in which our samples were taken. The sometimes low within-flock prevalence in our study can not be explained by either late introduction or by transport contamination since the groups tested were all long since established as a flock and no true sectioning was made. The cloacal samples were in some cases not free from acidous urine, which could possibly kill the relatively pH-sensitive campylobacters and lead to a prevalence of less than 100%. Other explanations might be the large area available for the chicken to roam, possibly resulting in a dilution of the faeces and less infection pressure. Yet another possibility is that some of the tested chickens in this study had already been infected by campylobacter, developed immunity and excreted the bacteria so that it was no longer present in the faeces at the time of sampling. No records were kept of the age of the chickens from which samples were obtained making it difficult to evaluate this hypothesis.

For the antimicrobial resistance testing the results varied greatly, but with an overall high level of resistance to many antimicrobials. In accordance to the situation in the rest of the world, *C. coli* isolates had higher levels of resistance than did *C. jejuni.* No earlier studies have been made in Vietnam on antimicrobial resistance in campylobacter from poultry. The studies that have been made in Vietnam are either on campylobacter prevalence from food products or campylobacter as a cause of enteritis in humans. The latter have also included antibiotic susceptibility testing. Studies in Sweden (SVARM 2004) show that the level of resistance of campylobacter found in broiler faeces correlated well to the level of resistance found in campylobacter in human faeces from patients with diarrhoea thought to have been infected within the country. If this holds true also for Vietnam the levels of resistance to nalidixic acid, ciprofloxacin and macrolides in campylobacter have increased markedly since 1996–1999 when the last published study was made (Isenbarger er et al. 2002). The levels of resistance in our study almost reach the levels of resistance seen in Thailand 1996–1999. For *C. jejuni* the proportion of resistant isolates have increased from 1% to 64% for

nalidixic acid, from 1% to 64% for ciprofloxacin and is stabile at 0% for erythromycin. For *C. coli* the proportion of resistant isolates have increased from 29% to 100% for nalidixic acid, from 31% to 100% for ciprofloxacin and from 0% to 33% for erythromycin (Isenbarger er et al. 2002). Cautious interpretation and comparison of the two studies are needed, since the bacterial isolates studied come from humans and chickens respectively, and from different geographical areas in Vietnam.

Comparison of the resistance to the situation in the EU where the EFSA on a yearly basis collects data from the member states and summarises it in a report can readily be done. Data from the member states show a varied level of resistance, with Spain and Italy having a high percentage of isolates from both *C. coli* and *C. jejuni* resistant to ciprofloxacin, streptomycin, tetracyclin and erythromycin, and the Nordic countries a markedly lower level of resistance (EFSA 2007).

The results on antibiotic resistance for campylobacter in our study support earlier studies that suggest fluoroquinolone antibiotics to have played out their role in treating more difficult cases of campylobacter-induced diarrhoea (Allos 2001). Instead erythromycin has been suggested as the drug of choice, which is also supported by the results of this study with a resistance of 7% compared to 71% for fluoroquinolones.

The microdilution plates used for testing of antibiotic resistance in campylobacter in Sweden do not contain chloramphenicol nor ampicillin. In the case of chloramphenicol this is because it is prohibited to use in food producing animals in the EU and in the case of ampicillin because of its lack of importance in campylobacter therapy today due to high levels of resistance (Allos 2001). It would be interesting to evaluate the resistance to chloramphenicol or related substances in campylobacters in Vietnam. The use of thiamphenicol as a feed additive in treating of sick flocks seemed to be a common feature in both large and small scale poultry farming in Vietnam. Other commercially available antibiotics observed and used as feed additives on farms were gentamicin and tetracyclin.

In interpreting the outcome of the questionnaire, one should take into consideration the effect that different interviewers can have on the answers. On the first day of sampling a female post graduate was accompanying us, asking the farmers the questions in the questionnaire. On the second day of sampling a male post graduate helped with the questionnaire and the outcome was very different even though the selection of farm size etc. was largely the same. The answers on the second day were more in accordance to what would be seen as "right answers", making the interpretation of the results very difficult. Despite this, a general response of a very high use of antibiotics, often in a profylactic manner, was seen. Notable is that none of the farms used antibiotics as a growth promoter. In choosing the drugs to treat the animals, more than 65% of the farmers chose the drug that they could afford for the moment, or the one that was recommended by the salesperson on the pharmacy. There was also a general unawareness of the development of antibiotic resistance, even in the larger farms and among the local veterinary practitioners accompanying us.

A concern for the future development of antibiotic resistance is the competitive situation on the pharmaceutical market (Duong et al. 1997). Today there are many small pharmacies, and sometimes even prescribing veterinarians or farm owners having their own pharmacy, all with a need for profit, thus with very little incentive in being restrictive with the use of antibiotics. Also, since most farmers turn to the pharmacies when having problems with their animals, the pharmacist have a very conciderable responsibility in knowing what drug or what measures to use in treating a certain disease. This is a problem that the WHO has acknowledged as a major problem for the future well-being of the people in Vietnam (WHO 2003). Suggestions have been made that the government of Vietnam should retake some of the former control of the pharmacies (WHO 2003). With this, and with education of the pharmacists in proper use of drugs in general, and antibiotics in particular, the people of the country could benefit from a better and more controlled medical counselling (Duong et al. 1997; WHO 2003).

There are today no legislations to control the use of antibiotics, and since there is very little knowledge of the adverse effects of using broad-spectrum antibiotics, and these are beneficial for pharmacies to sell, the only thing stopping a more widespread use is the economical limitations of the farmer. There is a need for better information to make the farmers aware of the situation. Another way to restrict the use is governmental pressure, legislations or monetary support to eco-farmers.

The project was in large successful in achieving the aims of the study. Only the results from the market samples must be seen as a failure in methodology and/or as a problem due to lack of knowledge of slaughter and cutting-up procedures in the country. The questionnaire did not cover some parts of the production such as the presence of small scale fish cultures in close proximity to, or even incorporated in, the chicken production. This information could have proved interesting in the interpretation of the prevalence and antibiotic resistance situation of the farm since the epidemiology and spread of campylobacters and antibiotic resistance is not only linked to poultry. The closeness to water is an effective way of spreading the bacteria and antibiotic resistance. Also the markedly different answers in the questionnaire from day one to day two of sampling makes it difficult to trust that the answers are not affected by the questioner.

Campylobacters are common inhabitants of the intestine of chicken in the studied area in the Can Tho region. Eradication of the bacteria must be seen as impossible and the only method of coping with the pathogen is through knowledge of their presence and how to avoid contamination of food products and water supplies. Also, the neutralization of bacteria through proper cooking is an important knowledge, one that is already well understood looking at the fact that the author was not once sick though indulging in the food culture of the country.

There is an obvious problem with the development of multiresistant bacteria in general, and with campylobacters and other human pathogens in particular. One can assume that self treatment with fluoroquinolones or tetracyclines are to a large extent ineffective in treating a severe infection by campylobacters deriving from chicken from the study region. Treatment with aminoglycosides or macrolides are more likely to be effective, although if the trend of a rising resistance even to

these substances continues it will prove a problem for the future. There is little awareness of development and presence of resistance in bacteria among farmers in the Can Tho region of Vietnam. The relatively well functioning political and governmental control systems and developing economy of the region and the country as whole enable creation of legislations and information campaigns that are crucial in battling the development of multiresistance in campylobacters and other bacteria.

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APPENDIX 1

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 (chickens) does your farm approximately include?

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

( ) Egg and meat production for my home consumption( ) Selling meat and egg to local markets

( ) Selling to large-scale buyers/distributors

3. Does your farm include other livestock animals as well? (Please choose one)

( ) No

( ) Yes, pigs

( ) Yes, ducks

( ) Yes, cattle

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

( ) No, they are able to move freely around the farm/village

( ) Yes, I keep my flock separate from the others in the farm/village

( ) Yes, I keep them in a separate area away from other animals

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

( ) No (continue to question 8)

( ) Yes, but rarely (continue to next question)

( ) Yes, regularly (continue to next question)

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

( ) To treat animals when they are sick

( ) I give it to prevent animals from getting sick

( ) I give it to animals as it makes them grow faster

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

( ) I use different antibiotics from time to time depending on what I can find or afford

( ) I use what the salesperson recommends

( ) I use antibiotics depending on what illness is present and/or after consultation with a veterinarian

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

( ) No

( ) I have heard of it but don't know how it affects my farm

( ) Yes, I am aware of the problem and try to restrict my use of certain antibiotics

Thank you very much for your participation!