

CHAPTER II

LITERATURE REVIEW

2.1 General information of *Campylobacter* spp.

Campylobacter spp. is a microaerophilic, thermotolerating, curved Gram negative bacterium, with spiral shape, that is flagellated and therefore motile.

Campylobacter spp. requires a microaerophilic environment (80% N₂, 10% CO₂, 5% H₂, and 5% O₂) for growth. Three major species of *Campylobacter* are usually the cause of campylobacter enteritis or so-called campylobacteriosis. These are *C. jejuni*, *C. coli* and *C. lari*, with *C. jejuni* being the most common although in the developing countries *C. upsaliensis* is also important cause of campylobacteriosis (Phillips, 1995). These species are known as thermophilic campylobacters because they grow well at temperatures in the range 42-43°C and do not grow at 25°C and below. *Campylobacter* species are ubiquitous in the broiler farm environment and several routes of transmission to broiler chicks have been suggested (Workman et al., 2008). O'Sullivan et al. (2000) surveys show that 30–100% of poultry harbor *Campylobacter* as normal flora of the intestinal tract which indicates a need to identify prevalent organism types in flocks and trace their epidemiology.

Routine identification of cultured *Campylobacter* species is based on biochemical testing. *C. jejuni* produces catalase, is sensitive to nalidixic acid, resistant to cephalothin, and can hydrolyse hippurate as well as indoxyl acetate. *C. coli* on the other hand have the same characteristics as *C. jejuni* except for hydrolysis of hippurate for which *C. lari* is negative. *C. lari*, also catalase, could be sensitive or resistant to nalidixic acid, resistant to cephalothin and both negative to hydrolyse hippurate and indoxyl acetate. *C. upsaliensis* is catalase negative or slightly positive,

both sensitive to naladixic acid and cephalothin and both negative to hydrolysis of hippurate and indoxyl acetate (ISO, 2006).

2.2 Public health importance of *Campylobacter* spp.

Campylobacteriosis and salmonellosis in the European Union (EU) are the two most frequently reported bacterial foodborne illnesses in humans. A total of 175,561 human cases of campylobacteriosis were reported in the EU25 in 2006, being the most frequently reported zoonosis (EFSA, 2011a). Indeed, in North America, Europe and Japan, campylobacteriosis is one of the principal food-borne bacterial disease and the consumption of poultry meats and/or by-products is suspected to be the main cause of the disease (Suzuki and Yamamoto, 2009). *C. jejuni* is the most frequently isolated bacterial pathogen in human gastroenteritis in developed countries (Saito et al., 2005). The major source of human infection is undercooked chicken, raw or unpasteurized milk, and cross-contamination from the environment, particularly in kitchens (Friedman et al., 2004b). Among the foods that carry these microorganisms, the chicken is outstanding (Fonseca et al., 2006, Phillips, 1995).

In the United States, in 2008, a total of 18,499 laboratory-confirmed cases of infection in FoodNet surveillance areas were identified. Among the number of infections and incidence per 100,000 populations, 5,825 *Campylobacter* cases (12.68%) was reported. Moreover, in the United States, they conducted a population-based case-control study to determine risk factors for sporadic *Campylobacter* infection (CDC, 2009). During a 12-month study, 1316 patients were enrolled with culture-confirmed *Campylobacter* infections from 7 states. Thirteen percent of patients had traveled abroad. In multivariate analysis of persons who had not traveled, the largest population attributable fraction (PAF) of 24% was related to consumption of chicken prepared at a restaurant. The PAF for consumption of non-poultry meat that was prepared at a restaurant was also large (21%); smaller proportions of illness were associated with other food and nonfood exposures. Efforts to reduce contamination of poultry with *Campylobacter* should benefit public health (Friedman et al., 2004a).

Guillain-Barre' syndrome (GBS) is another public health concern associated with *Campylobacter* infection. As laboratories started to isolate *Campylobacter* from stool samples of patients over the past two decades, there have been numerous reports of GBS following *Campylobacter* infection (Nachamkin et al., 1998). According to Center for Disease Control and prevention (CDC), infection with the bacterium *Campylobacter jejuni* is one of the most common risk factors for GBS. *C. jejuni*-related GBS is typically acute motor axonal neuropathy (AMAN), however, after *C. jejuni* infection, other reports illustrated various cases of the demyelinating subtype of GBS (acute inflammatory demyelinating polyneuropathy [AIDP]) (Kuwabara et al., 2004).

2.3 Prevalence and associated risk factors of *Campylobacter* in broiler

Contamination rates of *Campylobacter* differ widely among countries. In 26 European Union member states, a study showed that the chicken carcass contamination rate was 75.8% on average, but ranged from 4.9% to 100% (EFSA, 2010).

In Japan, the average prevalence of *Campylobacter* contamination in retail poultry meats and by-products was approximately 60%, although frozen poultry and ground poultry meats showed lower prevalence. This contamination level is comparable to those observed in North America and Europe. Such high prevalence of *Campylobacter* contamination in retail poultry implies the necessity of the sanitary handling of poultry products (Suzuki and Yamamoto, 2009).In Ireland, isolation by the ISO method found 52.7% of samples to be positive, but overgrowth by contaminants was frequently evident (Madden et al., 2011).

In many countries, a majority of retail poultry meats and by-products were contaminated with *Campylobacter* spp. *C. jejuni* was usually the dominant *Campylobacter* species isolated from retail poultry and *C. coli* was less commonly isolated, though the ratio of *C. coli* to *C. jejuni* was significantly different among the

countries. Nonetheless, in Thailand and South Africa, *C. coli* was the main *Campylobacter* species isolated from retail poultry (Suzuki and Yamamoto, 2009).

The gastrointestinal tracts of chickens are easily colonized with *C.jejuni*. During chicken carcasses processing, the organisms can be spread either from the huge load of bacteria within the gastrointestinal tract or from the load of *C. jejuni* on the birds' feathers and skin. This vast bacterial load results in substantial contamination of processed carcasses (Bungay et al., 2005). The amount of *Campylobacter* in the gastrointestinal tract of broiler chickens at slaughter can go beyond 7.0 log₁₀ cfu/g of caecal contents (Rosenquist et al., 2006). Fecal-oral spread is likely because chicken are coprophagic (Bungay et al., 2005).

Guerin et al.(2010) reported that results from studies showed that sampled before and after scalding or chilling, or both, showed that the prevalence of *Campylobacter* generally decreased instantly after the stage (scalding: 20.0 to 40.0% decrease; chilling: 100.0% decrease to 26.6% increase). After defeathering and evisceration, the prevalence of *Campylobacter* increased (10.0 to 72.0%) and (15.0%), respectively. The prevalence after washing was contradictory among studies (23.0% decreases to 13.3% increase).

Results of a study where they used a risk assessment model for *Campylobacter* in broiler meat, show that, on average, there is a continuous decrease in the level of *Campylobacter* on the carcasses and the meat due to repeated inactivation and removal of *Campylobacter* during processing, as a consequence of submersion, washing, defeathering, skinning, storage, etc. (Nauta et al., 2007). Moreover, results show that cross-contamination during processing and food handling leads to a spreading of *Campylobacters* over the units (birds, carcasses, fillets) involved, and as a consequence the prevalence of contaminated units may increase, whereas the variability between units decreases (Nauta et al., 2005).

In Berlin, Germany, they investigated *Campylobacter* spp. on the surface and within chicken breast fillets. 100 fresh retail chicken breast fillets (skinless and

deboned) were analyzed by means of a rinse sample for surface and 55 fillets for internal pathogen contamination using 10 g meat. Prevalence was 87% on the surface and 20% in the deep tissue. The mean number of *Campylobacter* on the surface of the fillets was 1,903 CFU, with a median of 537 CFU and a maximum of 38,905 CFU. *Campylobacter* counts inside the tissue were <1 CFU g⁻¹ meat (mean = 0.24 CFU, median = 0.15 CFU, maximum = 0.74 CFU) (Luber and Bartelt, 2007).

Strict implementation of biosecurity in primary production and GMP/HACCP throughout slaughter may lessen colonization of broilers with *Campylobacter*, and contamination of carcasses. Moreover, the use of fly screens, restriction of slaughter age, or discontinued thinning may further reduce consumer risks but have not yet been tested extensively. After slaughter, a 100% risk reduction can be reached by irradiation or cooking of broiler meat on an industrial scale. More than 90% risk reduction can be obtained by freezing carcasses for 2-3 weeks. A 50-90% risk reduction can be achieved by freezing for 2-3 days, hot water or chemical carcass decontamination (EFSA, 2011b).

2.4 Detection of *Campylobacter* spp. in poultry

Qualitative method for the detection of *Campylobacter* spp. involved enrichment step in Bolton broth at 37 °C for 4 to 6 h followed by 44 to 48 h at 41.5 °C in microaerobic conditions. Charcoal-cefoperazonedesoxycholate- agar (CCD agar) or modified charcoal cefoperazone deoxycholate agar (mCCD agar) plates will then be streak with the incubated broth and the plates will then be incubated at 41.5 °C for 44 to 48 h in microaerobic conditions for the isolation and confirmation of *Campylobacter* spp. (Garin et al., 2012, ISO, 2006).

On the other hand, semi-quantitative detection according to ISO 10272-3 requires four stages. Firstly, enrichment in Bolton broth, which is a selective medium, where in this stage, the test portion and decimal dilutions thereof are inoculated or diluted in Bolton broth and homogenized. The enrichment medium is incubated at 37

°C for 4 to 6 h followed by incubation at 41.5 °C for 44 to 48 h. Secondly, isolation and selection for confirmation where from the cultures obtained in the previous method, the selective solid medium modified charcoal cefoperazone deoxycholate agar (mCCD agar) is inoculated, incubated at 41.5 °C in a microaerobic atmosphere and inspected after 44 to 48 h to detect the presence of colonies presumed to be *Campylobacter* spp. because of their characteristics. Lastly, confirmation stage where the colonies to be *Campylobacter* spp. are subcultured on a non-selective Columbia blood agar, then confirmed by means of microscopic examination and appropriate biochemical tests and antibiotic sensitivity tests.

There are number of official protocols offered in the world for detecting *Campylobacter* spp. from food products, such as ISO method (ISO 10272), FDA-CFSAN (BAM) method, UK (PHLS) method. In addition to these official protocols, numerous other methods were reported in many research papers (Suzuki and Yamamoto, 2009). But conventional detection and confirmation methods for *Campylobacter* spp. are time-consuming and tiresome (Ng et al., 1997). The conventional culture test required more than 3 to 4 days to isolate and identify *C. jejuni* and *C. coli* in the Preston enrichment cultures (Yamazaki et al., 2009).

Nonetheless, the developed multiplex polymerase chain reaction method (mPCR) is a reasonably low-cost and efficient means to detect *Campylobacter* spp. in chicken meat after 24 h of enrichment. Furthermore, suspected colonies of those bacteria on the selective media used in conventional culture methods may hasten the identification by the developed mPCR (Alves et al., 2012). Lately, molecular epidemiology has also been useful to compare the isolates from the environment with those from the chickens (EFSA, 2011). It was also proven in a study that loop-mediated isothermal amplification (LAMP) assay for detection of chicken meat samples naturally contaminated with *Campylobacter jejuni* and *Campylobacter coli* is a powerful tool for rapid, sensitive, and practical detection of *C. jejuni* and *C. coli* (Yamazaki et al., 2009).

2.5 Antimicrobial resistance of *Campylobacter* spp.

Antimicrobial resistance can extend illness and compromise treatment of patients with bacteremia. The rate of antimicrobial-resistant enteric infections is highest in developing countries, where the use of antimicrobial drugs in humans and animals is moderately unrestricted. The continuing practice of indiscriminate use of antibiotics makes clinical management of campylobacteriosis cases more difficult which result to an increasing rate of human infections caused by antimicrobial resistant strains of *C. jejuni*. In developed countries, national surveillance systems planned at gathering data on antimicrobial resistances among zoonotic bacteria. It is considered by food safety authorities as of main concern and has been lately implemented (EFSA, 2011c). From 5 major cities in Africa (Dakar in Senegal, Yaounde in Cameroon), Oceania (Noumea in New Caledonia), the Indian Ocean (Antananarivo in Madagascar) and Asia (Ho Chi Minh City (HCMC) in Vietnam, 546 isolates (546/633, 86.3%) were tested for antibiotic susceptibility. Resistance of the isolates to erythromycin (11%), ampicillin (19%) and ciprofloxacin (50%) was detected and the city where antibiotic resistant rates were the highest (95%, $p=0.014$) was HCMC (Garin et al., 2012). In the study conducted in France, resistance was common to tetracycline (64.5%), erythromycin (47.9%), and nalidixic acid (23.5%). Thirty seven out of 1257 *C. coli* (2.9%) were resistant to both erythromycin and ciprofloxacin, preferred drugs for treatment of invasive human campylobacteriosis. EU also reported that in isolates of *Campylobacter* from meat and animals, resistance was frequently identified to ciprofloxacin, nalidixic acid and tetracyclines at levels of 33 % to 78 %, while much lower levels were reported to erythromycin and gentamicin (EFSA, 2011a).

2.6 Poultry industry in the Philippines

The broiler industry in the Philippines, as in most Asian countries, is more varied and less developed on several fronts, compared with its Western counterparts. First, the supply of chicken meat comes from both exotic hybrids of foreign origin (the so-called broilers) and native or village chickens (referring to domesticated

jungle fowl and the progenies of their crosses with exotic breeds, which are mostly raised under free-range systems) (Chang, 2007).

In the Philippines, poultry farms were at first characterized by the use of native breeds but have shifted to the formation of very large integrated contract farming operations, particularly in CALABARZON and Central Luzon regions. While smallholder livestock production systems remained prevailing in the regions farther from the city capital Manila, the configuration of market shares between the commercial and 'backyard' farms in the two high-growth regions shifted in sustaining commercial operations in the past years. It is common because the native chickens have reasonably low productivity (e.g., slow growth, low laying rate, and high mortality rate) and incoherent quality and supply because of less ordered production systems and management practices (Delgado et al., 2003).

As of January 1, 2012, around 47 % of the total chicken inventories were native or village chicken raised in backyard farms. Chicken grown in commercial farms were broilers and layers which accounted for 34 % and 19 %, respectively (BAS, 2012).

Broiler output has been one at the forefront of livestock production growth in the Philippines. However, growth of this industry has been concentrated in the two regions north and south of the capital city of Manila, which has the largest demand. These two regions are Central Luzon (Region III) north of the capital and CALABARZON (Region IV-A) south of the capital. These two regions contributed 48.64 % to the country's broiler inventory.

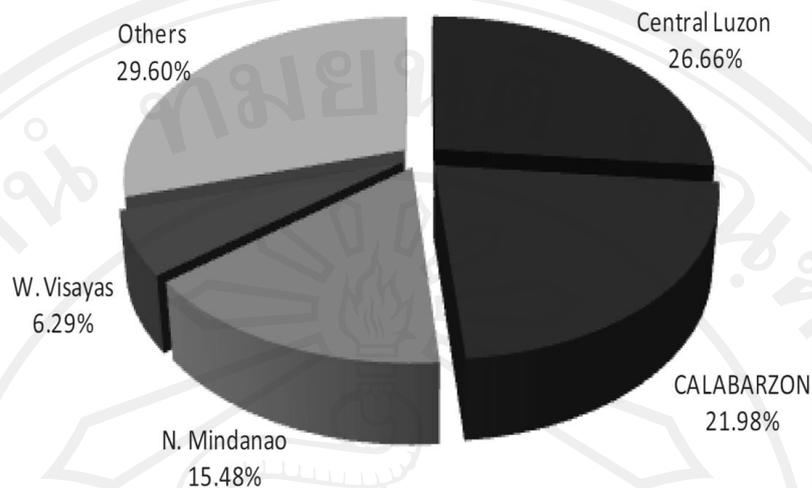


Figure 1 Distribution of broiler inventory, by region, as January 1, 2012

(source: BAS, 2012)

The substitution of native chicken breeds with modern broiler types has laid the foundation for technological change in the poultry meat sector. Grandparent and parent stock of broiler breeds are imported to produce day-old chicks for broiler raising. Nutrition is calibrated by use of carefully controlled mixed feed rations. Vaccines and antibiotics are used to guard against disease. Broilers are raised to optimal market weight, 41 to 42 days on average (Delgado et al., 2003).

Nowadays, the broiler productions in the two regions are saturated with contract growing operations formed by large integrators. They are Swift Foods, San Miguel Pure Foods, Vitarich Corporation, Tysons Agro-Ventures, General Milling Corporation, and Universal Robina Corporation. These integrators are members of the PABI (Philippine Association of Broiler Integrators) and involved in the production and marketing of broiler chickens, the import of grandparent and parent stocks, and the manufacture and selling of commercially mixed feeds. The integrators are the key source of day-old chicks, even for independent commercial producers whose volume of operations is insufficient to maintain breeding operations. They are also a major player in the live broiler market, in which the daily reference price is set by the group on a gentleman's agreement basis (focus interview with broiler industry participants, 2002 according to Delgado et al., 2003). Even though no perfect agreement can be

determined, price-setting behavior is exercised in as far as it can be maintained. Together, they control some 80 % of the broiler market. The remainder is in the hands of independent commercial broiler producers, who maintain inventories of 20,000 to 100,000 birds, and a few smallholders, who raise 1,000 to 2,000 birds at a time.

With regard to the output market, large integrators can influence the types of products supplied by their contract growers by controlling the quality of inputs. By also exercising control over the dressing and processing of output, they can guarantee product quality for distribution to specific formal or institutional markets. Markets for dressed chicken and processed meat products are more stable than the market for live broilers (Delgado et al., 2003).

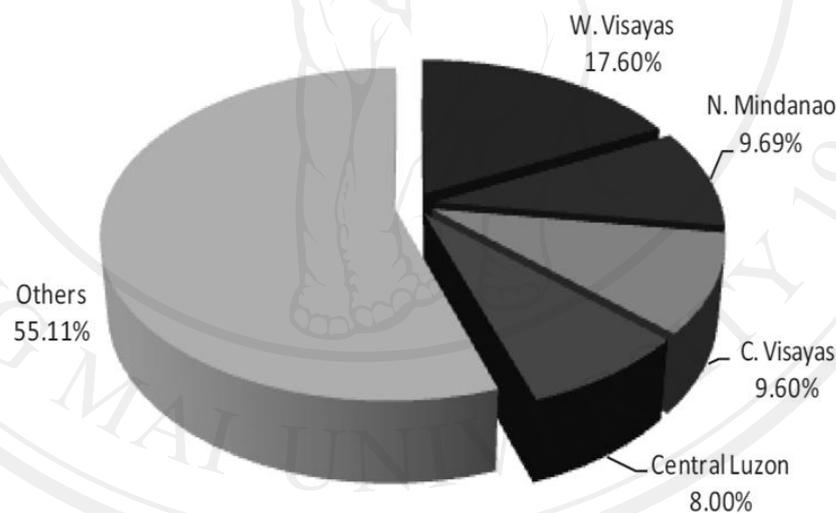


Figure 2 Distribution of native/improved chicken by region, Philippines, as of January 1, 2012 (source: BAS, 2012)

Meat quality standards in the Philippines are employed through abattoirs or dressing plants where traded meat is prepared for market, as certified by the National Meat Inspection Services (NMIS). Table 1 shows the distribution of dressing plants meeting the various standards of sanitation. There are three major categories: Class

AAA for exportable quality, Class AA for domestic trade quality, and Class A for local (municipal) trade only.

Table 1 Numbers of accredited meat establishments, by class type, May 2012
(source: BAS, 2012)

Poultry dressing plant	Numbers by class type
AAA	15
AA	69
A	8
Total	92

Output from smallholder operations undergoes processing at slaughterhouses and dressing plants that are classified at best Class AA: qualified for domestic trade but not for export.

In 2011, the average farmgate price of broilers in commercial farms was P77.27 (1.83 USD) per kilogram. It increased by 1.61 % from the 2010 average farmgate price. The lowest farmgate price was recorded during the month of October at P74.88 (1.78 USD) per kilogram. The highest price at P80.53 (1.91 USD) per kilogram was reported (BAS, 2012).

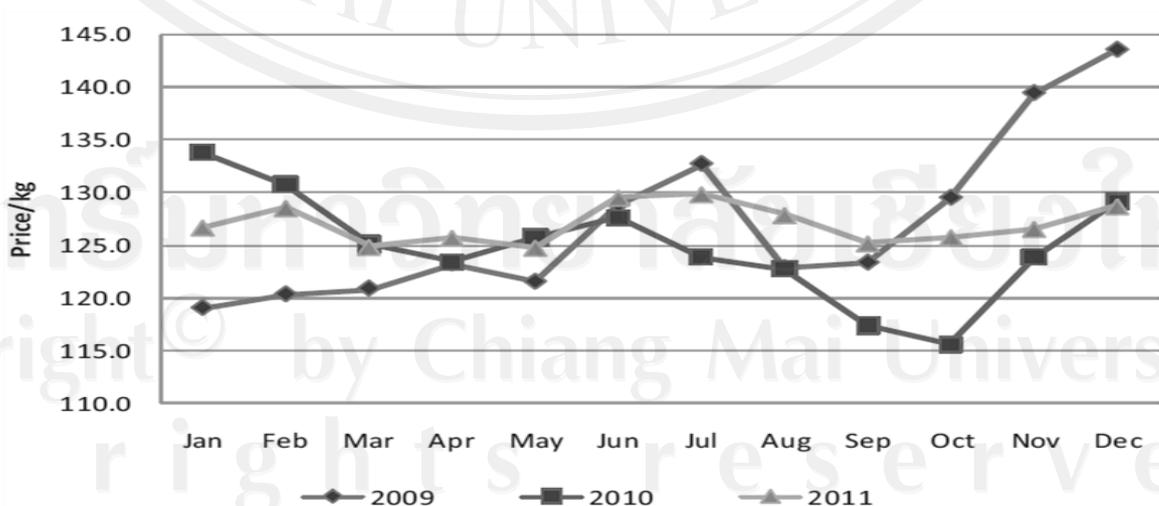


Figure 2 Broiler Monthly Farmgate Prices (Lwt.)*Philippines, 2009-2011 (source: BAS, 2012)

Metro Manila, the regular retail price for fully dressed chicken was P126.92 (3.01 USD) per kilogram. There was a 1.65 percent increase in comparison with 2010 retail price. The highest retail price was registered during the month of July at P129.78 (3.08 USD) per kilogram, while the lowest price of P124.67 (2.96 USD) per kilogram was observed in May.

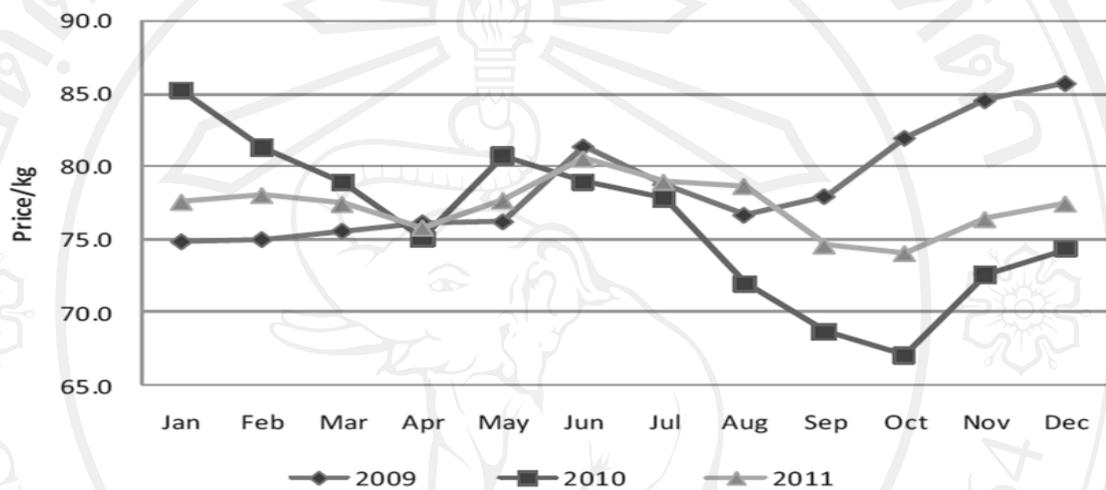


Figure 3 Fully Dressed: Monthly Retail Prices, Metro Manila, 2009-2011

(source: BAS, 2011)

Report shows in 2011 that the Philippines is 90% self-sufficient on dressed chicken. During this year, the province of Nueva Ecija dressed of more the 14 million birds in dressing plants. In addition, the utilization per capita kilogram per year of the country on dressed chicken is 10kg in the past 5 years (BAS, 2011).