



UNIVERSITI PUTRA MALAYSIA

**CHARACTERIZATION OF THERMOPHILIC CAMPYLOBACTER SPP.
IN COMMERCIAL BROILER CHICKEN FROM FARM TO RETAIL**

JOHN TANG YEW HUAT

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By

JOHN TANG YEW HUAT

**Thesis submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfillment of the Requirements for the
Degree of Doctor of Philosophy**

October 2010



**Dedicated to beloved wife (Sapphire), parents, and brothers for their
endless love and support**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Farinazleen Mohamad Ghazali, PhD

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The public health importance of thermophilic *Campylobacter* in man and limited data available in Malaysia led the present study to determine the prevalence of thermophilic *Campylobacter* from farm to retail and its potential of cross-contamination to cooked food. *Campylobacter* isolates were tested for their resistance and Multiple Antibiotic Resistance (MAR) index was determined. Currently, *Campylobacter jejuni* (physiology and metabolism) have not been fully understood. Thus, *C. jejuni* ATCC 33560 physiology and metabolism was being investigated using Phenotypic MicroArray (PM) technology to provide insight of *C. jejuni* carbon source



utilisation and sensitivity towards osmolytes and pH. Prevalence of *Campylobacter* in chickens from farm to retail was determined using plating and Polymerase Chain Reaction (PCR) method. This study found no *Campylobacter* detected in cloacal swabs from chickens in farms practising close-house system but occurred at 95.0% in farms practising open-house systems. Prevalence of *Campylobacter* in chicken samples from conventional slaughterhouse and processing plant was 84.0% and 94.0%, respectively while *Campylobacter* contamination in chicken samples at wet market and supermarket was 78.0% and 92.0%, respectively. These showed chicken products are highly contaminated and it would be informative to know the *Campylobacter* load in chickens sold at retail level. *Campylobacter* load in chicken's parts is determined using MPN-PCR method. Current investigation showed chicken meats and chicken by-products were contaminated with *Campylobacter* with their numbers ranged from <3 to 4600 MPN/g. In this study, chicken keels, chicken livers and gizzards harboured higher concentration of *Campylobacter* at 10^3 - 10^4 MPN/g compared to other chicken parts at 4.3%, 33.3% and 9.2%, respectively. Such high numbers of *Campylobacter* in chicken parts pose risk of human ingestion through contaminated food. Thus, *C. jejuni* cross-contamination from raw to cooked chickens was investigated. *Campylobacter jejuni* was



found to contaminate cooked food regardless the food was left at room temperature ($28\pm 0.5^{\circ}\text{C}$) or taken directly from boiling water ($100\pm 0.5^{\circ}\text{C}$). Scored cutting boards were found to trap and transfer greater number of *C. jejuni* compared to untreated or new cutting boards. Scored cutting board made from rubberwood showed greater risk of cross-contamination than scored cutting board made from polyethylene. There was no significant difference ($P>0.05$) between two types of cutting boards in untreated condition with regards to the number of *C. jejuni* transferred to cooked samples. Brief contact between raw chicken with cutting board was sufficient to contaminate the cutting board with *C. jejuni*. Hot samples rapidly lose heat ($72-76^{\circ}\text{C}$) after taken from boiling water with the cutting process accelerated the heat loss. The findings suggested there are possibilities of *Campylobacter* being ingested by human through cooked chickens if proper hygienic practise is not practised in kitchen. Thus, it would be important to determine the antibiotic susceptibility profiles of *Campylobacter* isolates from chickens that might be ingested by consumers as this will determine the effectiveness of antibiotic regimen prescribed for infected individuals. Antibiotic susceptibility test was carried out on 126 *Campylobacter* isolated from retail commercial broiler chickens. *Campylobacter* showed high resistance to erythromycin (97.6%) and

tetracycline (92.9%) while low resistance to gentamicin (35.7%) and nalidixic acid (35.7%). *Campylobacter* showed resistance towards all groups of antibiotics tested, namely beta-lactam (87.7%), cephalosporins (69.8%), quinolones (65.3%), chloramphenicols (84.9%), macrolides (97.6%), aminoglycosides (68.8%) and tetracyclines (92.9%). Of 126 *Campylobacter* spp. isolates, 0.8% showed resistant to five or less antibiotics, 60.3% showed antibiotic resistance to between six to ten antibiotics and 38.9% isolates showed resistance to more than 10 antibiotics. All isolates of *Campylobacter* spp. showed MAR index more than 0.20 which indicated there are abuse in antibiotic usage in chicken farming. In addition to antibiogram information, the present study also aimed to investigate the physiology and metabolism of *Campylobacter* that enable it to survive all the processes despite its fastidious characteristics and sensitivity to environmental stresses. The present study employed Phenotypic MicroArray (PM) technology to investigate *C. jejuni* metabolism and physiology. Phenotypic MicroArray (PM) profiles *C. jejuni* ATCC 33560 showed utilization of carbon sources from amino acids and carboxylates but not from sugars. *Campylobacter jejuni* ATCC 33560 is sensitive to NaCl at 2% and above but showed survival in a wide range of food preservatives (sodium lactate, sodium phosphate, sodium benzoate, ammonium sulphate and sodium nitrate). No phenotype



loss was observed in carbon source plates when *C. jejuni* was incubated at suboptimal temperature. Phenotype loss of *C. jejuni* ATCC 33560 was observed in sodium chloride (1%), sodium sulphate (2-3%), sodium formate (1%), sodium lactate (7-12%), sodium phosphate pH 7 (100 mM and 200 mM), ammonium sulphate pH 8 (50 mM), sodium nitrate (60 mM, 80 mM and 100 mM), sodium nitrite (10 mM), and growth in pH 5. These results suggested that *C. jejuni* is still metabolically active at suboptimal growth temperature. This is the first study conducted in Malaysia to study the prevalence of *Campylobacter* in commercial broiler chickens from farm to retail, its cross contamination in food preparation as well as its physiology and metabolism. It can be concluded from this study that farm biosecurity is important to control *Campylobacter*. Despite the absence of *Campylobacter* in close-house farms, *Campylobacter* contamination in chickens at slaughterhouses and retails was still prevalent. Farmers, poultry processing plant operators and consumers should be aware of the importance of *Campylobacter* to human health and how to prevent its infection.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN *CAMPYLOBACTER* SPP. TERMOFILIK PADA AYAM
PEDAGING DARI LADANG KE TAHAP PENJUALAN**

Oleh

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Oktober 2010

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Kepentingan implikasi *Campylobacter* termofilik ke atas kesihatan manusia serta data yang terhad di Malaysia mendorong kajian ini dilaksanakan untuk menentukan prevalen *Campylobacter* termofilik dari ladang ke tahap jualan serta potensi *Campylobacter* untuk kontaminasi makanan masak. Isolat *Campylobacter* diuji kerintangan terhadap antibiotic-antibiotik untuk menentukan tahap rintangan dan indeks Rintangan Pelbagai Antibiotik (RPA). Buat masa kini, fisiologi dan metabolisme *C. jejuni* belum diketahui sepenuhnya. Oleh hal yang demikian, fisiologi dan metabolisma *C. jejuni*



ATCC 33560 dikaji dengan menggunakan teknologi “*Phenotypic MicroArray (PM)*” untuk memberi maklumat *C. jejuni* ATCC 33560 dalam penggunaan sumber karbon serta sensitiviti terhadap osmolit and pH. Prevalen *Campylobacter* dalam ayam dari ladang ke tahap jualan ditentukan dengan menggunakan kaedah plat dan *Polymerase Chain Reaction (PCR)*. *Campylobacter* tidak dikesan dalam swab kloaka ayam yang diusahakan dalam ladang sistem rumah tertutup tetapi sebanyak 95.0% swab kloaka adalah positif dalam ladang sistem rumah terbuka. Prevalen *Campylobacter* dalam sampel ayam dari rumah penyembelihan konvensional dan kilang pemprosesan pada 84.0% dan 94.0% masing-masing manakala kontaminasi *Campylobacter* turut berlaku pada sampel pasar pagi (78.0%) dan *supermarket* (92.0%). Keputusan ini menunjukkan produk ayam mempunyai kontaminasi yang tinggi dan adalah penting untuk mengetahui bilangan *Campylobacter* yang hadir dalam ayam yang dijual pada peringkat jualan. Dalam kajian ini, keputusan menunjukkan kontaminasi *Campylobacter* daging serta bahan sampingan ayam berlaku dari <3 hingga 4600 MPN/g. Kajian ini menunjukkan dada, hati dan tonkeng ayam mempunyai konsentrasi *Campylobacter* (10^3 - 10^4 MPN/g) yang lebih tinggi berbanding dengan sampel ayam yang lain dengan peratusan 4.3%, 33.3% dan 9.2% masing-masing. Bilangan *Campylobacter* yang tinggi dalam bahagian ayam

menyebabkan risiko infeksi kepada manusia melalui makanan yang terkontaminasi. Oleh hal yang demikian, kajian dilakukan untuk mengetahui kemungkinan kontaminasi silang *C. jejuni* dari ayam mentah ke ayam masak. *C. jejuni* didapati mampu mengkontaminasi makanan yang telah dimasak dalam keadaan suhu bilik ($28\pm 0.5^{\circ}\text{C}$) dan keadaan panas (diambil terus dari air didih ($100\pm 0.5^{\circ}\text{C}$)). Papan potong yang dicalarkan didapati memerangkap dan memindahkan lebih banyak *C. jejuni* berbanding dengan papan potong yang tidak dicalarkan ataupun baru. Papan potong calar yang dibuat daripada “*rubberwood*” menunjukkan risiko kontaminasi yang lebih tinggi berbanding dengan papan potong yang dibuat daripada “*polyethylene*”. Bilangan *C. jejuni* yang dipindahkan kepada makanan masak adalah tidak signifikan ($P>0.05$) antara kedua-dua papan potong yang tidak calar. Kontak sementara antara ayam mentah dengan papan potong adalah memadai untuk mengkontaminasi papan potong dengan *C. jejuni*. Ayam masak panas hilang haba ($72-76^{\circ}\text{C}$) dengan cepat seurus selepas dikeluarkan daripada air didih dan proses pemotongan mempercepatkan kehilangan haba. Hasil kajian ini menunjukkan kemungkinan *Campylobacter* dimakan oleh manusia melalui makanan sekiranya amalan kebersihan tidak diamalkan dalam dapur. Oleh itu, adalah penting untuk mengetahui profil sensitifiti antibiotik isolat

Campylobacter dari ayam yang mungkin dimakan oleh manusia kerana ini akan menentukan keberkesanan antibiotik yang diberi kepada individu yang dijangkiti. Sensitiviti antibiotik diuji ke atas 126 isolat *Campylobacter* spp. dari ayam daging pada tahap jualan. *Campylobacter* spp. menunjukkan rintangan tinggi terhadap eritromisin (97.6%) dan tetrasiklin (92.9%) serta rintangan rendah terhadap gentamisin (35.7%) dan asid nalidisik (35.7%). *Campylobacter* spp. menunjukkan rintangan terhadap semua kumpulan antibiotik yang diuji seperti beta-laktam (87.7%), sefalosporin (69.8%), kuinolon (65.3%), kloramfenikol (84.9%), makrolid (97.6%), aminoglikosid (68.8%) dan tetrasiklin (92.9%). Daripada 126 isolat *Campylobacter* spp., 0.8% menunjukkan rintangan antibiotik kepada lima atau kurang antibiotik, 60.3% menunjukkan rintangan kepada enam hingga sepuluh antibiotik dan 38.9% menunjukkan rintangan kepada lebih sepuluh antibiotik. Semua isolate *Campylobacter* spp. menunjukkan indeks RPA melebihi 0.20. Selain maklumat antibiogram, kajian ini turut bertujuan untuk mengkaji fisiologi dan metabolisme *Campylobacter* yang membolehkannya untuk mandiri dalam pelbagai proses sedangkan sifatnya yang sensitive terhadap tekanan. Kajian ini menggunakan teknologi *Phenotypic MicroArray (PM)* untuk mengkaji metabolisme dan fisiologi *Campylobacter*. Profil "*Phenotypic MicroArray (PM)*" *C. jejuni* ATCC 33560 menunjukkan penggunaan sumber



karbon dari asid amino dan karbosilat tetapi bukan daripada gula. *C. jejuni* ATCC 33560 adalah sensitif terhadap NaCl pada 2% ke atas tetapi menunjukkan kemandirian dalam pengawet makanan pada julat yang luas (sodium laktat, sodium fosfat, sodium benzoat, amonium sulfat dan sodium nitrat). Tiada kehilangan fenotipik diperhatikan dalam plat sumber karbon apabila *C. jejuni* dieramkan pada suhu suboptimal. Kehilangan fenotipik *C. jejuni* ATCC 33560 diperhatikan dalam sodium klorid (1%), sodium sulfat (2-3%), sodium format (1%), sodium laktat (7-12%), sodium fosfat pH 7 (100 mM dan 200 mM), sodium nitrit (10 mM), dan pertumbuhan dalam pH 5. Keputusan ini menunjukkan *C. jejuni* masih aktif secara metabolic pada suhu suboptimal. Ini adalah kajian pertama dijalankan di Malaysia untuk mengkaji kehadiran *Campylobacter* dalam ayam pedaging komersil dari ladang hingga ke tahap jualan, kontaminasi silang serta fisiologi dan metabolismenya. Kajian ini boleh dirumuskan bahawa biosekuriti ladang adalah penting dalam kawalan *Campylobacter*. Akan tetapi, kontaminasi *Campylobacter* pada rumah penyembelihan dan peringkat jualan masih tinggi. Oleh hal yang demikian, peladang, operator plan pemprosesan ayam serta pengguna harus menyedari kepentingan *Campylobacter* terhadap kesihatan manusia dan bagaimana untuk menghindari daripada dijangkiti.



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APPROVAL

I certify that an Examination Committee has met on 6 October 2010 to conduct the final examination of John Tang Yew Huat on his degree thesis entitled "Prevalence, enumeration and characterization of thermophilic *Campylobacter* spp. in commercial broiler chicken from farm to retail" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Doctor of Philosophy. Members of the Examination Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is nor concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

JOHN TANG YEW HUAT

Date: 6 October 2010



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