UNIVERSITI PUTRA MALAYSIA

OCCURRENCE OF ARCOBACTER SPECIES IN FARM CHICKEN AND CHICKEN MEAT AT RETAIL OUTLETS IN MALAYSIA

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MASTER OF SCIENCE
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By

AMARE BERHANU LEMMA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of the Master of Science

November 2009

DEDICATED TO:

MY LATE SISTER YETNAYET BERHANU,

MY LATE GRAND MOTHERS ASEGEDECH AMARE,

AND TAEHAYNESH GUANGUL,

AND

MY LATE GRAND FATHER LEMMA SHIFERAW.

ALSO DEDICATED TO:

MY BELOVED FATHER BERHANU LEMMA,

MY MOTHER FIRDAWOK TEFERA,

MY BROTHER SAMUEL BERHANU,

MY SISTER HANNA BERHANU,

OTHER FAMILY MEMBERS AND FRIENDS.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of the Master of Veterinary Science

OCCURRENCE OF *ARCOBACTER* SPECIES IN FARM CHICKEN AND CHICKEN MEAT AT RETAIL OUTLETS IN MALAYSIA

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AMARE BERHANU LEMMA

November 2009

Chairperson : Professor Dr. Saleha Abdul Aziz, PhD

Faculty : Veterinary Medicine

The genus *Arcobacter* comprises the former "aerotolerant campylobacters" that are recognized recently as emerging, potential food- and water-borne pathogens. Like their closely related campylobacters, arcobacters are major contaminants of poultry products rendering poultry in general and chicken in particular significant risk factors to humans. A number of protocols have so far been described to detect *Arcobacter* on food although their apparent deficiencies and the likely underestimation therein of the actual rate of contamination are well emphasized. Furthermore, there is a general scarcity of data on the occurrence of the bacteria in the food chain in Malaysia. The present study was, therefore, undertaken to evaluate the performances of existing *Arcobacter* isolation protocols for maximum detection of naturally contaminated chicken meat, to determine the occurrence of *Arcobacter* at two levels (chicken meat at retail outlets and chickens in the farms) and to identify the species of the isolates.

Forty-eight retail chicken meat samples were subjected to three established protocols [modified Lammerding (method I), Steele and Mc Dermott (method II), Houf (method III)], and an 'in-house' method (method IV) comprising the enrichment procedure of Houf and the plating media and techniques specified in the modified Lammerding protocol. Method II failed to recover the organisms from any of the samples but *Arcobacter* were detected by the other protocols (methods I, III, and IV). The *Arcobacter* isolates were identified by a multiplex PCR as *Arcobacter butzleri* from 30 (62.5%) chicken meat samples. By comparison, the 'in-house' protocol (method IV) recovered the bacteria from a greater number of samples (24 of 48) than the modified protocol of Lammerding (12 of 48) ($p= 0.019$), or the protocol of Houf (17 of 48) ($p= 0.046$). Each of the methods involved some degree of false negative results with the high frequency of positive samples detected by the 'in-house' protocol (50%). This suggested that the 'in house' method offer a more accurate estimate of the actual occurrence of *Arcobacter* on food than either of the parent protocols (method I: 25%; method III: 35.42%) thereby making it a good alternative to consider whenever available resources proscribe the simultaneous use of multiple methods. It is recommended that further verification and/or validation of the 'in-house' protocol be made prior to its adoption. To determine the frequency of occurrence of *Arcobacter* and the species distribution, fresh/'warm' (n= 61) and chilled (n= 62) chicken meat parts were purchased from various retail outlets in Selangor. Forty eight of the 123 (prevalence: 39.02%; range: 0 – 88.2%) chicken

meat parts selected from five sampling sites (retail outlets) were found to be *Arcobacter*-positive with *A. butzleri* as the only species isolated. The fresh/'warm' chicken meat portions had a higher contamination frequency (41%) than the chilled chicken meat parts (37.1%) although the difference was not statistically significant (χ^2= 0.2; p= 0.655). A farm-level survey was also conducted to assess the presence of *Arcobacter* species in live chicken so as to determine whether intestinal carriage of the bacteria accounted for its high rate of occurrence on the chicken meat portions. The bacteria were not detected in any of 210 cloacal swabs collected from six chicken farms in Southern (Melaka and Johor) and Central (Selangor) regions of peninsular Malaysia probably supporting the views that poultry are less likely hosts for *Arcobacter*. Environmental factors such as water may play the role for the presence of *Arcobacter* on chicken meat.

This is the first large scale study on the occurrence of *Arcobacter* species in the Malaysian food production chain and the first to establish the distribution of species. Overall, the findings of the study indicated arcobacters to constitute a major proportion of the chicken meat microbiota rendering this product a significant risk factor for human acquisition of the organisms.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluhan untuk ijazah Master Sains Veterinar

KEHADIRAN SPESIS *ARCOBACTER* PADA AYAM DI LADANG DAN DAGING AYAM DI PASARAN DI MALAYSIA

Oleh

AMARE BERHANU LEMMA

November 2009

Pangerusi: Profesor Dr. Saleha Bt Abdul Aziz

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Genus *Arcobacter* terdiri daripada “aerotolerant *Campylobacter*” (*Campylobacter* tahan udara) dan organisma berkaitan yang baru-baru ini dikenal sebagai patogen makanan dan air berpotensi baru muncul. Seperti kampilobakter yang mana mereka berhubung rapat, arkobakter merupakan kontaminan utama pada produk unggas, menyebabkan unggas pada amnya dan ayam khususnya merupakan faktor risiko yang bererti keatas manusia dibandingkan dengan makanan lain. Sebilangan protokol telah dihuraikan untuk mengesan *Arcobacter* pada makanan walaupun terdapat kekurangan dalam kaedah dan juga anggaran yang kurang dalam kadar sebenar kontaminasi. Ditambah dengan kekurangan data mengenai kehadiran bakteria dalam rantaian makanan di Malaysia, maka projek penyelidikan ini dilaksana untuk menilai prestasi tiga kaedah dalam mengesan *Arcobacter* pada ayam, menentu kadar kontaminasi pada daging

ayam di pasaran dan pada ayam di ladang dan mengenalpasti spesies *Arcobacter* yang diasingkan.

Tiga protokol, yang sedia ada (kaedah “modified ” Lammerding (kaedah I), Steel dan McDermott (kaedah II) dan Houf (kaedah III)) serta kaedah “in house” (kaedah IV) yang terdiri daripada prosedur pengkayaan yang mengguna protokol pengasingkan *Arcobacter* oleh Houf dan media serta teknik kultur menurut protocol “modified” Lammerding dilaksanakan ke atas 48 sampel daging ayam daripada pasar untuk mengesan *Arcobacter*. Kaedah II tidak dapat mengesan kehadiran *Arcobacter* tetapi kaedah I, III dan IV dapat mengasingan *Arcobacter* yang dikenalpasti sebagai *Arcobacter butzleri* dengan menggunakan teknik *multiplex* PCR berjumlah 30 (62.5%). Kaedah IV dapat mengasing lebih banyak *Arcobacter* (24 daripada 48 sampel) dibandingkan dengan kaedah Lammerding (12 daripada 48) ($p= 0.019$) dan kaedah Houf (17 daripada 48) ($p= 0.046$). Walaupun setiap kaedah ada darjah kegagalan dalam mengesan semua sampel yang terkontaminat, sampel yang positif yang dapat dikesan oleh kaedah “in house” (50%) membuat kaedah ini lebih menepati dalam memberi anggaran kehadiran sebenar *Arcobacter* dalam makanan dibandingkan dengan protokol lain (kaedah I= 25%, kaedah III= 35.4%). Oleh itu kaedah IV merupakan satu alternatif yang baik apabila menggunakan lebih daripada satu protokol. Disarankan bahawa verifikasi dan/atan validasi keatas kaedah “in house” dilaksanakan sebelum digunakan.

Untuk menentukan frekuensi kehadiran *Arcobacter* dan sebaran spesis, dikaji daging ayam segar (n= 61) dan daging ayam dingin (n= 62) yang terdapat di pasaran sekitar Selangor. Empat puluh lapan daripada 123 (prevalens: 39.02%, julat: 0-88.2%) sampel daging ayam di beli daripada lima tempat pensampelan untuk mengesan kehadiran *Arcobacter* didapati positif untuk *Arcobacter* dan kesemuanya dikenalpasti sebagai *A. butzleri*. Daging ayam segar didapati mempunyai frekuensi kontaminasi yang lebih tinggi (41%) berbanding daging ayam dingin (37.1%) walaupun perbezaannya secara statistik tidak bererti (χ^2= 0.2; p= 0.655). Tinjauan tahap ladang dilaksanakan bagi menilai kehadiran *Arcobacter* pada ayam menentukan sama ada bakteria dalam usus yang menyebabkan kadar kehadiran yang tinggi pada daging ayam. *Arcobacter* tidak dapat dikesan pada 210 swab kloaka ayam yang diperolehi daripada enam ladang ayam di kawasan selatan (Melaka dan Johor) dan di kawasan tengah (Selangor) Malaysia. Penemuan ini menyokong pendapat bahawa ayam mungkin bukan perumah bagi *Arcobacter*. Faktor persekitaran, seperti air, mungkin memainkan peranan dalam kehadiran *Arcobacter* pada daging ayam.

Kajian ini merupakan kajian pertama yang agak besar dalan mengkaji kehadiran *Arcobacter* dalam rantaian pengeluaran makanan di Malaysia dan dalam menentukan sebaran spesis. Secara keseluruhan, penemuan kajian ini menujukkan arkobakter merupakan bahagian besar mikrobiota daging ayam dalan rantaian pengeluaran daging ayam di Malaysia, menyebabkan daging ayam merupakan faktor risiko utama bagi manusia memperoleh bakteria tersebut.

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I certify that a Thesis Examination Committee has met on 11th November, 2009 to conduct the final examination of Amare Berhanu Lemma on his thesis entitled "Occurrence of *Arcobacter* Species in Farm Chicken and Chicken Meat at Retail Outlets in Malaysia" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date: 8 April 2010

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 not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

AMARE BERHANU LEMMA

Date:

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CHAPTER 1

INTRODUCTION

Emerging food-borne pathogens, most of which are considered zoonotic, constitute a major health hazard in both developed and developing nations. Within the last few decades, several microbial agents unrecognized previously as harmful have emerged as important food-borne pathogens (Zessin, 2006). Among the well recognized emerging food-borne pathogens, campylobacters have evolved as the leading causes of diarrhea in humans in all parts of the world accounting annually, for instance, in the United States of America (USA) alone approximately 2.5 million illnesses (12.4% of all defined food-borne illnesses) and 124 deaths (Lan and Kopecko, 2003).

Although campylobacters still remain to be the major food-borne pathogens of prime public health concern, other '*Campylobacter*-like' organisms are also being increasingly recognized in recent years as causative agents of important human and animal illnesses. The relatively recently described *Arcobacter* species belonging to the family *Campylobacteraceae* is one such group of organisms that are currently recognized as emerging food-borne and water-borne pathogens. The organisms are indicated to play a greater role in human and animal illnesses than had been previously appreciated (Ho *et al*., 2006a; Snelling *et al*., 2006; Lehner *et al*., 2005).

Since their first isolation from aborted bovine fetuses in 1977, studies worldwide have reported the presence of ten *Arcobacter* species with six species namely, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirowii*, *Arcobacter cibarius*, *Arcobacter mytili*, and *Arcobacter thereius* of animal origin and four found in different aquatic habitats (Collado *et al*., 2009; Houf *et al*., 2009; Kim *et al*., 2009). They cause human infections by the oral route through consumption of contaminated water and food (Houf and Stephan, 2007; Ho *et al*., 2006a; Phillips, 2001b). Poultry meat in particular is repeatedly and categorically indicated to harbor the bacteria at a significantly greater frequencies than any other food commodities at higher stages (retail) of the food production chain implicating poultry in general, and chicken in particular, as the main risk factor to humans (Ho *et al*., 2006a; Lehner *et al*., 2005; Phillips 2001b).

From the public health point of view, *Arcobacter butzleri* is regarded as an important human pathogen and is reported to cause human illness which manifests primarily as enteritis and septicemia (Houf and Stephan, 2007; Ho *et al*., 2006a; Snelling *et al*., 2006). The organism has already caused outbreaks of gastroenteritis in North America (Rice *et al*., 1999) and Europe (Vandamme *et al*., 1992a) in addition to a number of sporadic cases worldwide (Lehner *et al*., 2005; Phillips, 2001b). *Arcobacter butzleri* has also been isolated frequently from food animals such as cattle, small ruminants (sheep and goats), and pigs, food of animal origin such as milk, beef, pork, and chicken, as well as from various water sources all leading to its categorization as a “serious hazard to human health” by the International Commission on Microbiological

Specifications for Foods (ICMSF) (Atabay *et al*., 2006; On *et al*., 2003). Despite these notable recognitions, overall scientific knowledge on arcobacters, particularly those in the food chain, is very limited and little is known on the global contribution of the different species in the causation of human illnesses due largely to the absence of a standard method for their detection and identification (Ho *et al*., 2006a; Snelling *et al*., 2006; Phillips, 2001a). Furthermore, public awareness of the bacteria is generally limited. The aim of this study was, therefore, to evaluate and compare the performances of three cultural protocols for isolation of *Arcobacter* species with the view to identify the best method of detecting the bacteria on naturally contaminated chicken meat. Additionally, it was also the aim of this study to generate prevalence data on the occurrence of *Arcobacter* species at two levels, farm and retail, in the Malaysian chicken production chain.

Specifically, the objectives of this study are:

1. to evaluate the performances of existing *Arcobacter* isolation protocols for maximum detection of naturally contaminated chicken meat;
2. to determine the rate of contamination of chicken meat (fresh and chilled) with *Arcobacter* species at retail outlets (wet markets and supermarkets) and to identify the species; and,
3. to assess the presence of *Arcobacter* species in broiler chickens at different poultry farms.

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