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by

SALEHA ABDUL AZIZ

Thesis Submitted in Fulfilment of the Requirements for
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DEDICATED TO MY HUSBAND,

MY FIVE CHILDREN AND MY MOTHER

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And Most Merciful

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LIST OF ABBREVIATIONS

Am	Ampicillin
CE	Competitive exclusion
cfu	Colony forming unit
Cm	Chloramphenicol
CO_2	Carbon dioxide
°C	Degree Celsius
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra-acetate
Em	Erythromycin
FBP broth	Ferrous-(Meta)Bisulphite-Pyruvate broth
fla	Flagellin
g	Gram
Gm	Gentamicin
h	Hour
kb	Kilobase
Kn	Kanamycin
l	Litre
M	Molar
MCE	Mucosal competitive exclusion
MDa	MegaDalton
mg	Milligram
mins	Minutes
ml	Millilitre
mm	Millimetre

mM	MilliMolar
MPN	Most probable number
N	Normal
N_2	Nitrogen
NaCl	Sodium chloride
nm	Nanometre
O_2	Oxygen
pH	Hydrogen-ion concentration
ppm	Parts per million
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
Sm	Streptomycin
TBE	Tris-buffer- EDTA
Tc	Tetracycline
ug	Microgram
ul	Microlitre
VNC	Viable, non-culturable
V/cm	Volts / centimetre
%	Percent

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

CAMPYLOBACTER JEJUNI IN BROILER CHICKENS IN SELANGOR, MALAYSIA

By

SALEHA ABDUL AZIZ

Chairman : Professor Dr. Abdul Latif Ibrahim

Faculty : Veterinary Medicine and Animal Science

In realising the importance of the poultry industry in Malaysia and the public health implication of *C.jejuni* in man, a study was undertaken to determine the prevalence, antibiotic resistance and plasmid profiles of *C.jejuni* isolated from chickens and to identify factors associated with the risk of colonization or infection by the organisms in chickens.

It was shown in this study that 72.6% and 81.9% of the commercial broiler and village chickens respectively were colonized with *C.jejuni*. Of the seventy six *C.jejuni* strains tested, all (100%) were found to be resistant to tetracycline while resistance to other antibiotics, namely ampicillin, chloramphenicol, erythromycin, gentamicin, kanamycin and streptomycin, ranged from 25 - 75%. This study also showed a rather large number, that is, 42% of *C. jejuni* isolates were resistant to nalidixic acid although a distinguishing feature of this species is its susceptibility to nalidixic acid. It was found that there was no correlation between the presence of plasmid and resistance to antibiotics. This is because in some isolates which showed antibiotic resistance, no plasmid was detected. An analysis of the

plasmid profiles of seventy one *C.jejuni* isolates, 59% harboured 1-4 plasmids, demonstrating 14 different patterns with the sizes ranging from 4.5 to 70.3 kb. This study also demonstrated that colonization of chickens with *C.jejuni* occurred at two to three weeks of age. The study could not identify clearly the factors which acted as the sources of *C.jejuni* in chickens. However, colonization is found to be associated with poor farm management and hygienic practices. Farms which practise good management and hygienic procedures tend to have low rate of colonization by *C.jejuni*. Such practices include the use of treated water, control of pests and flying birds from entering poultry houses and restriction in the movement of personnel.

This is the first time that a detailed study on *Campylobacter* in chickens was conducted, particularly on factors associated with colonization by *C. jejuni*. It can be concluded from this study that *C. jenuni* is prevalent and widespread in chicken populations in Malaysia. During slaughtering of these chickens and during processing, poultry carcasses and offals may become contaminated with *C. jejuni.* Consequently if such poultry meat and offals are undercooked or caused cross contamination of ready-to-eat food, then upon consumption of such foods causes *Campylobacter* enteritis in man. Hence, it is recommended that farmers and poultry processing plant operators be made aware of the importance of campylobacters to human health and to implement appropriate measures to control the infection in poultry.

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CAMPYLOBACTER JEJUNI PADA AYAM PEDAGING DI SELANGOR, MALAYSIA

Oleh

SALEHA ABDUL AZIZ

Pengerusi : Professor Dr. Abdul Latif Ibrahim

Fakulti : Kedoktoran Veterinar dan Sains Peternakan

Memandangkan betapa pentingnya industri ayam itik di Malaysia dan penyakit yang dapat ditimbulkan oleh *C.jejuni* pada manusia, suatu kajian dijalankan untuk menentukan prevalens, ketahanan antibiotik dan profil plasmid *C.jejuni* yang diasingkan daripada ayam dan untuk mengenalpasti faktor-faktor yang berkaitan dengan risiko pengkolonian atau jangkitan oleh organisma tersebut pada ayam.

Telah diperlihatkan dalam kajian ini bahawa 72.6% ayam pedaging komersil dan 81.9% ayam kampung telah mengalami pengkolonian oleh *C.jejuni*. Antara tujuh puluh enam isolat *C.jejuni* yang diuji, kesemuanya (100%) didapati tahan terhadap tetrasiklin sedangkan ketahanan terhadap antibiotik lain, yaini ampisilin, kloramfenikol, eritromisin, gentamisin, kanamisin dan streptimisin, berkisar antara 25 - 75%. Kajian ini juga menunjukkan bahawa sebilangan yaini 42% dari isolat *C. jejuni* didapati tahan terhadap asid nalidisik. Walaupun suatu ciri khas spesies ini adalah kerentanan

terhadap asid nalidisik. Daripada kajian ini didapati bahawa tiada korelasi antara kehadiran plasmid dan ketahanan terhadap antibiotik. Ini disebabkan dalam beberapa isolat yang menunjukkan ketahanan antibiotik, tiada plasmid yang dapat dikesan. Suatu analisis profil plasmid keatas tujuh puluh satu isolat *C.jejuni* mendapati 59% mengandungi 1-4 plasmid, menunjukkan 14 corak dengan saiz plasmid berkisar antara 4.5 hingga 70.3 kb. Kajian ini juga memperlihatkan bahawa pengkolonian oleh *C.jejuni* pada ayam terjadi pada umur antara dua hingga tiga minggu. Kajian ini tidak dapat mengenalpasti dengan jelas faktor-faktor yang bertindak sebagai sumber *C.jejuni* pada ayam. Walau bagaimanapun didapati bahawa pengkolonian berkait rapat dengan pengurusan ladang yang kurang baik dan amalan yang kurang bersih. Ladang yang mengamalkan pengurusan yang baik dengan mempraktikkan amalan kebersihan cenderung mengalami kadar pengkolonian yang rendah. Amalan-amalan tersebut meliputi penggunaan air bersih yang didisinfek, mengawal haiwan perosak dan burung daripada memasuki rumah / reban ayam dan mengawasi pergerakan pekerja.

Ini adalah kali pertama kajian yang sedemikian terperinci mengenai *C.jejuni* pada ayam dijalankan, terutama mengenai faktor-faktor yang berkaitan dengan pengkolonian oleh *C.jejuni*. Daripada kajian ini dapat disimpulkan bahawa *C.jejuni* didapati tersebar luas dalam populasi ayam di Malaysia. Semasa penyembelihan ayam tersebut dan semasa pemprosesan, karkas ayam dan ofal dapat mengalami kontaminasi dengan *C. jejuni*. Selanjutnya sekiranya daging ayam dan ofal kurang dimasak atau terjadi pengkontaminatan ke atas makanan sedia-untuk-dimakan, maka keadaan ini dapat menyebabkan enteritis

Campylobacter pada manusia. Dengan ini disyorkan pada para penternak dan pengusaha kilang pemprosesan ayam untuk mengambil perhatian mengenai kampilobakter dan akibat yang dapat ditimbulkan keatas kesihatan manusia dan untuk melaksanakan langkah-langkah yang sesuai lagi berkesan untuk mengawal jangkitan *C.jejuni* pada ayam.

CHAPTER 1

INTRODUCTION

Campylobacters were first recognized about 70 years ago; but it is only within the last 10 years that these organisms, in particular *Campylobacter jejuni*, have emerged and being appreciated because they have now been recognised as among the most frequent causes of bacterial diarrhoea in man (Franco, 1988; Butzler and Oosterom, 1991). Several studies have indicated that the acute gastrointestinal infection in man caused by *C.jejuni*, exceeds the rates of illness caused by both *Salmonella* and *Shigella* species (Stern and Kazmi, 1989). In surveys of diarrhoeal diseases in England and United States of America, *Campylobacter* species were isolated from about 5% of the patients with diarrhoea, considerably higher than for *Salmonella* species which were isolated from 2 to 3% of patients while isolations of *Shigella* species were from 1% of patients (Taylor, 1992). In developing countries of Asia, Africa and Latin America, *Campylobacter* isolation rates have ranged from 10 to 20% in surveys carried out on children with diarrhoea (Taylor, 1992).

Within the *Campylobacter* family, there are about seven species that are recognised as having clinical importance in man (Butzler and Oosterom, 1991); five of these species are commonly found in animals (Table I). *Campylobacter jejuni* and *C.coli* are the most frequently isolated species from human patients with enteric diseases or enterocolitis (Butzler and Oosterom, 1991).

Table 1

***Campylobacter* species in animals of public health importance**

Species	Animal hosts	Site(s) from which usually isolated	Disease in man
C.jejuni	poultry, sheep, dog, cat, cattle, swine, rabbit	faeces	fever, enteritis, systemic infection.
C.coli	poultry, swine	faeces	enteritis
C.lari	poultry	faeces	enteritis
C.upsaliensis	cat, dog	faeces	diarrhoea
C.fetus	cattle, sheep	blood, various body fluids	systemic infection, abortion, perinatal sepsis and meningitis, enteritis.

Source: Butzler and Oosterom, 1991; Varnam and Evans, 1991.

Both *C.jejuni* and *C.coli* are frequently found as commensals in the intestinal tracts of many domestic and wild mammalian and avian species, including poultry, swine, sheep, goats, cattle, dogs, cats, birds and rodents. Campylobacters have also been found in shellfish, such as clams (Butzler and Oosterom, 1991; Franco, 1988;). Among these animal reservoirs, chickens have been cited as the most significant reservoir of *C.jejuni* with isolation rates of up to 100% (Aho and Hirn, 1988).

Once the food - producing animals have campylobacters in their intestinal tracts and/or faeces, their carcasses frequently become contaminated with the organisms during processing. *Campylobacter jejuni* has been isolated from poultry meat, pork, beef and mutton (Butzler and Oosteroon, 1991). Faeces from cattle containing *C.jejuni* may contaminate milk whilst poultry faeces may contaminate eggs (Blaser, 1982). Also, the presence of campylobacters in the faeces may possibly contaminate water and soil since campylobacters may survive for several weeks in these inanimate reservoirs when ambient temperatures are low (Blaser, 1982; Franco, 1988). The organisms have also been isolated from fresh water (streams and rivers), effluent of poultry processing plants, seawater, mud and sewage sludge (Franco, 1988; National Advisory Committee, 1994). Vegetables, mushrooms and fruits can become contaminated if they come in contact with natural fertilisers, wild animals or birds or with contaminated water (Butzler and Oosterom, 1991).

Four sources appear to be the causes for nearly all cases of *Campylobacter* infection in man, namely poultry meat and products, raw milk and unpasteurized dairy products, untreated surface water and pets (Franco, 1988: Tauxe, 1992). Man may also acquire *Campylobacter* infection through person-to-person (from excreters with active infection), perinatal and childhood transmission (Franco, 1988). Compared with food, the other sources are not significant. *Campylobacter* enteritis is essentially a food-borne disease and the principal vehicle is raw or undercooked meat and poultry meat is by far the most important source, especially broiler chickens (Skirrow and Blaser, 1992). Chickens sold at retail outlets had contamination rates of 60 to 80% with *Campylobacter* counts in the region of 10^6 per fresh chicken whereas in frozen chicken carcasses, the count was about 2 $\log_{10}$ lower (Skirrow and Blaser, 1992).

It is important to differentiate between sporadic cases and outbreak-associated cases in *Campylobacter* infections in man as the epidemiology of the cases differs. Most sporadic cases were associated with poultry, largely by eating or handling poultry meat, whereas most outbreaks were due to consumption of contaminated raw milk and water (Tauxe, 1992). Thus far, sporadic cases of *Campylobacter* infection were more common than outbreak-associated cases (Tauxe, 1992).

Several studies have shown that poultry is the major source of *C.jejuni* and that poultry meat is predominantly associated with *Campylobacter* infection in human (Harris et al., 1986; Tauxe, 1992). Appropriate measures are therefore required to reduce or minimize infection of broiler chicks during

production, processing and distribution stages. In the broiler production stage, there is a need for a better understanding of the epidemiology of *C.jejuni* infection in chicks and its ecology in farm practices. With such available information, introduction of the organisms to the flocks can be minimised. To lower the incidence of carcass contamination during processing and distribution stages, the cleaning and disinfection strategies based on the hazard analysis critical control point (HACCP) approach should be effective (National Advisory Committee, 1994).

In Malaysia, the major component in poultry industry is chicken production with duck and quail production forming minor components (Ramlah, 1993). The total consumption of poultry meat (includes both chicken and duck) has increased tremendously since 1981 at 128,500 to 297,180 tonnes in 1990; in 1993, the figure stood at 390,000 tonnes of poultry meat (Department of Veterinary Services, 1992). In 1995, the demand for poultry meat was about 570,000 tonnes and by the year 2000, the figure is expected to go up to 907,806 tonnes (van der Sluis, 1995). Thus, the poultry industry in Malaysia is an economically important livestock industry with an annual output of meat and eggs amounting to RM 2.5 billion (Ramlah, 1993).

The per capita consumption of poultry meat in 1990 was 20.4 kg (DVS, 1992). As such, there is a risk of man in Malaysia acquiring *Campylobacter* enteritis through consumption or handling of poultry meat. The few studies available have shown that *Campylobacter* spp. occurred in live chickens and in chicken carcasses (Joseph et al., 1989; Ansary and Veloo, 1991; Ansary and Muchhala, 1992). The incidence of *Campylobacter* infection in man in Malaysia