Ain Shams University
Faculty of Medicine

CAMPYLOBACTER INFECTIONS IN MAN

A thesis
submitted for partial fulfilment of Master Degree
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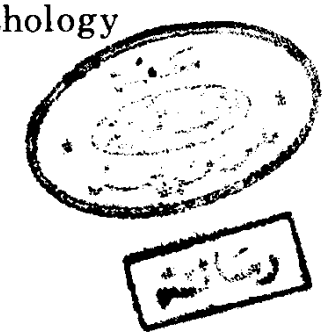

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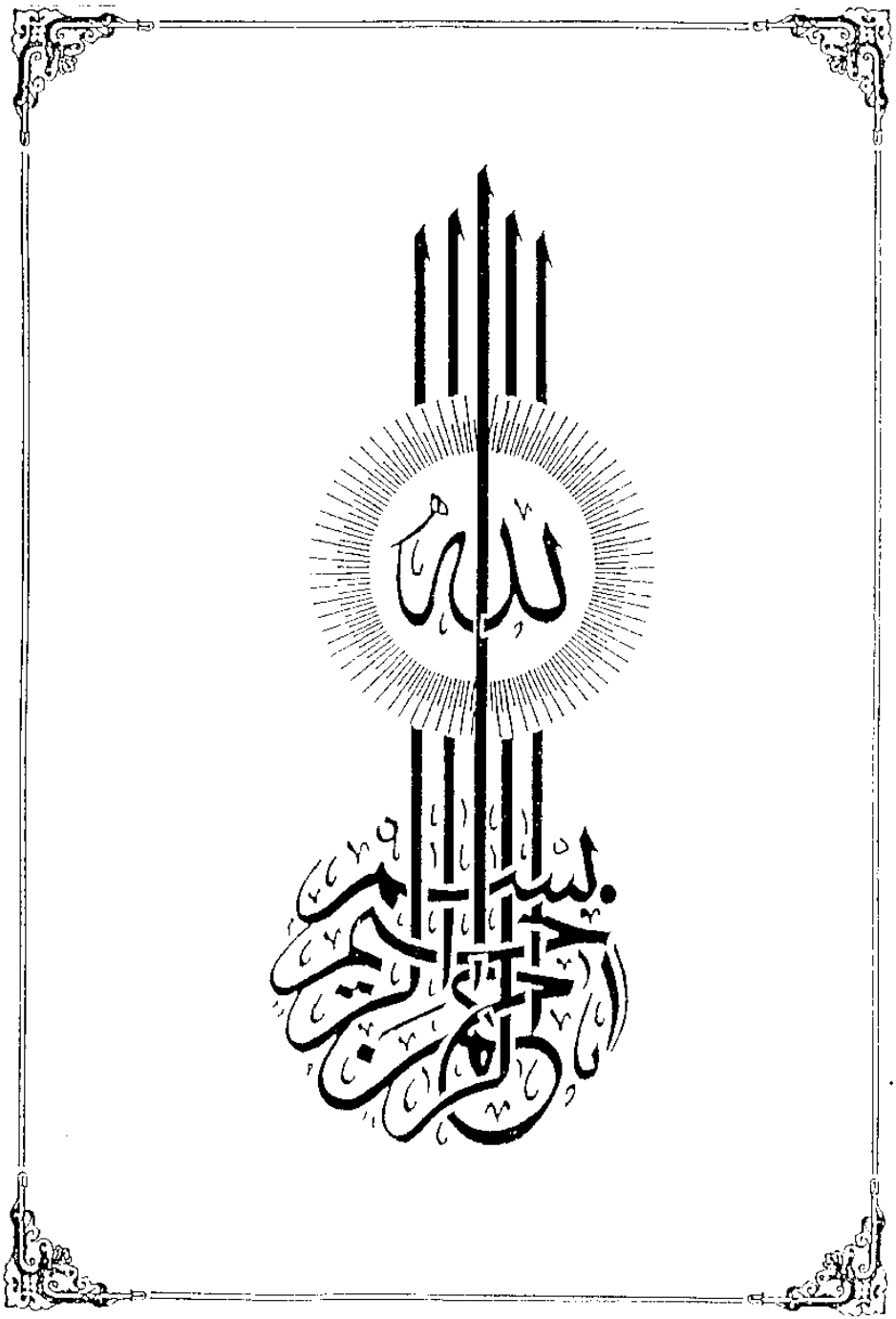

CONTENTS

	Page
Introduction	1
Historical background	3
Microbiology	7
Morphology	7
Ultrastructure	8
Metabolism	9
Biochemical characters	10
Cultural characters	11
Oxygen requirements	11
Optimum temperature	13
Culture media	13
Colonial morphology	16
Maintenance procedures	17
Classification and nomenclature	18
Typing of Campylobacter strains	22
Phenotyping	23
Biotyping	30
Serotyping	36
Phage typing	40
Pyocine typing	41
Plasmid fingerprinting	41
Clinical aspects	42
Systemic campylobacteriosis	43

Page

Campylobacter enteritis 56

Infective dose 57

Incubation period 57

Clinical presentation 58

Duration of illness 61

Post infection excretion of organisms 62

Pathology 63

Pathogenesis 65

Complications 67

Laboratory diagnosis 72

Direct microscopy 72

Culture 74

Serological diagnosis 77

Epidemiology 85

Reservoirs of infection 85

Transmission to man 92

Prevalence and incidence 101

Geographic 101

Age and sex 107

Seasonality 109

Occupation 109

Treatment 111

Prevention 115

Summary 118

Bibliography 120

Arabic summary

INTRODUCTION

Organisms belonging to the genus Campylobacter are now recognized as a new class of agents that are infectious to human beings.

In less than a decade they have emerged from obscurity as a veterinary pathogen to recognition as a leading cause of enteritis in humans.

It is now known that there are several species of Campylobacter but the major pathogen of humans is C. jejuni together with C. coli which are responsible for an acute gastroenteritis in children and adults (Karmali and Fleming, 1979). C. fetus subsp. fetus is less often involved in human disease being responsible for systemic infections in immuno-compromised hosts (Guerrant et al., 1978).

Studies in the present decade indicate that infection with Campylobacter spp. is more important and widespread than it was believed. Rettig (1979) reported that their incidence in gastrointestinal infections is comparable to or even exceeds that of Salmonella and Shigella.

The aim of this work is to present a review of the spectrum of human diseases associated with the genus Campylobacter, to discuss the relevant taxonomic and

microbiologic aspects of this genus as well as the epidemiology, pathogenesis, complications, methods of diagnosis, treatment and control of this infection.

HISTORICAL BACKGROUND

Campylobacter organisms were first isolated by Smith and Taylor (1919) from aborted bovine fetal fluids. At that time, they were classified as Vibrios since they are of similar shape and exhibit the rapid darting type of motility characteristic of Vibrios. They were called Vibrio fetus because they were associated with infectious infertility and abortion in cattle and sheep.

In 1931, Jones et al., observed the association of Campylobacter with diarrheal disease when they attributed winter dysentery in calves to infection with a vibrio they called "Vibrio jejuni".

In 1944, Doyle described a similar organism associated with swine dysentery in pigs, he later named it "Vibrio coli".

The first recorded cases of Campylobacter infection in man was a milk-born outbreak of acute diarrheal illness which was reported by Levy in 1946. He examined 73 stool specimens microscopically, of which 31 specimens revealed motile vibrio-like organisms but such bacteria could not be grown on stool culture. Blood cultures were made from 39 patients of whom 13 cultures grew a "spirillum", but it could not be isolated on solid media to be fully iden-

tified. Levy reported that it appeared to be quite similar to "Vibrio jejuni" isolated by Jones et al., in 1931 from bovine diarrheic cases.

In 1947, Vinzent et al., isolated Vibrio fetus for the first time from a person;and over the next 10 years this organism was occasionally isolated from blood, cerebrospinal fluid and other body fluids and from abscesses (Bokkenheuser, 1970). Most of the affected patients were elderly or were debilitated by alcoholism, malignant disease, diabetes mellitus or cardio-vascular disease. For this reason, Vibrio fetus was considered to be an opportunist, as it caused systemic illness predominantly in compromised hosts.

However, in 1957, Elizabeth King recognized that there were 2 groups of Vibrio fetus which were indistinguishable morphologically but were different in the optimum temperature of growth. She called the group that grew best at 42°C and failed to grow at 25°C : "Related Vibrios" in contradistinction from the "Typical Vibrio fetus" group which grew well at 25°C and 37°C but not at 42°C. She noticed that although the "Related Vibrios" were isolated from blood cultures, yet in each case the patient had a preceding diarrheal illness. Therefore she postulated that the "Related Vibrios" might be responsible for acute

diarrheal illness but could not be isolated from fecal specimens because they were slow growing and fastidious and were overgrown by coliform organisms.

Florent (1959) studied Vibrio fetus and divided the species into 2 subspecies:

1- Vibrio fetus subspecies venerealis: was the cause of vibrionic abortion in cattle and was venereally transmitted. It was negative for hydrogen sulphide production and did not grow in 1% glycine or 3.5% sodium chloride.

2- Vibrio fetus subspecies intestinalis: was the cause of abortion in sheep and sporadic abortion in cattle. It was not considered venereally transmitted. Contaminated food and water were suspected of being the transmitting agents. It was negative or weakly positive for hydrogen sulphide production. It grew in 1% glycine but not in 3.5% sodium chloride.

In 1963, Sebald and Véron reported that "Vibrio fetus" and "Related Vibrios" differed fundamentally from true Vibrios in the growth and biochemical characteristics and in the DNA base composition. True Vibrios are strictly aerobic, ferment selected sugars with acid production, can grow in 3% sodium chloride and have Guanine +

Cytosine content of 48% relative to the total base content. While Vibrio fetus and Related Vibrios are microaerophilic, neither ferment nor oxidize sugars and have Guanine + Cytosine content of 30-35%. In light of these major differences, a new genus "Campylobacter" was proposed to include "Vibrio fetus" and "Related Vibrios" (Campylos in the greek meaning curved and bakterion, a rod). Campylobacter fetus was proposed as the type species of the genus.

The assumption of King in 1957 that the related vibrios could be an important cause of gastroenteritis in humans remained unproved, until 1972 when Dekeyser et al., applied a veterinary technique to isolate related vibrios from the stools of 5% of children with diarrhea.

Since then, selective cultural techniques have been in general use resulting in over 9000 Campylobacter isolations being reported in 1980 to the Communicable Disease Surveillance Centre, Colindale (Skirrow, 1982). The picture elsewhere is similar and Campylobacter enteritis is now emerging as one of the commonest causes of bacterial diarrhea worldwide.

MICROBIOLOGY

Morphology :

As described by Smibert (1984) in Bergey's manual, Campylobacters are slender, non-sporing, non-capsulated, spirally curved, gram-negative rods which are arranged singly, in pairs or in short chains. They appear either comma-shaped or S-shaped or gull-shaped and are about 0.5 - 5 um in length and 0.2 - 0.5 um in breadth. A single organism has one or two spirals, the length of each spiral being about 2 um and the amplitude 0.5 um. In the long forms the spirals are drawn out, so that their length is far greater than their breadth. The short forms are sharply curved while the spirals often have an obtuse angled curve.

In young cultures, the bacteria move rapidly with a very characteristic corkscrew-darting type of motility which can be observed with phase contrast or dark field microscopy. This motility occurs by means of a single polar flagellum at one or both ends of the cell. The flagella are about 2-3 times the length of the organism and are 18 nm in width. In one week old cultures, very few are motile (Topley and Wilson, 1983).

In old cultures, the growth medium becomes alkaline

(pH 8.5 - 9.0) and coccoid forms occur under these unfavorable conditions. These coccoid forms are considered by many to be degenerative forms because cultures composed mainly of coccoid forms are non-viable (Smibert, 1984).

Ultra-structure :

Electron microscopic studies done by Ritchie et al., (1966) on Campylobacter fetus have shown that:

The outer cell membrane is double layered and is loosely fitted over the cell wall with a wavy morphology.

The cross section of the cell wall is typical of Gram-negative bacilli being three-layered with an outer lipoprotein layer, a middle lipopolysaccharide layer and an inner mucopeptide layer.

The inner cytoplasmic membrane is thickened at the polar region forming a multilaminar polar membrane that is located just under the plasma membrane at both ends of the cell.

Mc Coy et al., (1975) studied the antigens on the cell surface of Campylobacter fetus ssp. intestinalis (now called Campylobacter fetus ssp. fetus). He defined an antiphagocytic surface component designated as antigen (a) which is a glycoprotein containing 4% carbohydrates. It is

a virulence factor that renders the organism resistant to phagocytosis by macrophages. A mutant strain that lacked antigen (a) and contained an antigen (c) was readily phagocytosed by macrophages. Mc Coy et al., (1976) gave evidence that antigen (a) also protects the organism from antibody-mediated immobilization.

An endotoxin present in culture fluids was described by Osborne and Smibert (1964). It is a lipopolysaccharide containing 53% total carbohydrates. It is biologically active, producing a febrile response and a generalized Schwartzman reaction in rabbits and is lethal for mice. This endotoxin has abortifacient properties; when injected intravenously in cows, it caused them to abort.

Metabolism :

Campylobacters have a strictly respiratory form of metabolism. Hoffman (1978) recorded that the energy required for growth of Campylobacters is derived from tricarboxylic acid cycle intermediates and from few amino acids such as glutamic acid and aspartic acid which can be deaminated to give α-ketoglutaric acid and oxaloacetic acid which are tricarboxylic acid cycle intermediates.

He reported that an intact tricarboxylic acid cycle was operative and demonstrated the presence of its enzymes

in Campylobacter fetus. The organism can oxidize citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate, malate and oxaloacetate.

On the other hand, carbohydrates cannot be utilized; they are neither oxidized nor fermented. According to Hoffman (1978), no oxygen uptake occurred with glucose, adonitol, arabinose, galactose, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, xylose and glycerol. Glycolytic intermediates also cannot be utilized.

Biochemical characters :

Campylobacters are rather inactive biochemically. They are all oxidase positive. They are non-saccharolytic and non-proteolytic and so carbohydrates are neither oxidized nor fermented and gelatin is not liquified. They reduce nitrates to nitrites. No lipase or urease activity is detected; urea is not hydrolysed. Pigments are not produced. No acid or neutral end products are produced. Indole production, methyl red reaction and Voges-Proskauer reactions are all negative (Smibert, 1984).

Other biochemical reactions such as catalase production, hydrogen sulphide production, hippurate hydrolysis, alkaline phosphatase activity and deoxyribonuclease