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A Thesis Submitted to the Faculty of Medicine
University of the Witwatersrand, Johannesburg
for the Degree of Doctor of Philosophy.

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DEDICATION

This thesis is dedicated to my wife Peggy and my children, Jodi, Peta Ann and Bobbie for their encouragement, understanding and love at all times, and to Jeremy Neville.

ABSTRACT

In two studies on gastroenteritis in Soweto, *Campylobacter jejuni* was recovered from 31 and 32 per cent of infants under nine months of age and from 3 and 7 per cent of healthy controls ($p = <0.05$ and $p = < 0.01$ respectively). Although isolation rates were equally high in the nine months to two year diarrhoeal age groups there was no statistically significant difference between these and the control children. These results indicate that in a high prevalence population resistance to campylobacter enteritis develops early in life.

Children in Soweto, when infected, usually excreted *C. jejuni* in their faeces for one to three weeks but continued to shed the organism intermittently for prolonged periods. Serotyping of the isolates showed that both reinfection and prolonged intermittent excretion occurred and were significantly more common in children under three years of age than in adults.

In a rural study three per cent of school children excreted *C. jejuni* intermittently for more than a year. As serotyping was not available at the time, it was not clear whether reinfection or prolonged excretion of the same strain occurred.

The same *C. jejuni* serotypes could be isolated consecutively from different members of households, strongly suggesting person-to-person transmission. However, this happened infrequently and three *C. jejuni*-excreting mothers failed to infect their newborn infants. *C. jejuni* was recovered at high frequency from fowl and dog faeces and offal in Soweto. Non-human sources, together with the finding that the same serotype is often excreted intermittently in faeces, suggest an alternative explanation for the sequential isolation of *C. jejuni* from persons within households.

Subsequent to the studies in Soweto, a high prevalence of *C. jejuni* infection was identified in Bangladesh and India. In view of the high frequency of asymptomatic infections in children in these populations, the role of *C. jejuni* as a cause of diarrhoea in developing countries needs to be more clearly defined, especially in relation to host immunity. The Soweto studies were the first to indicate that while young infants are susceptible to *C. jejuni* infection and are likely to develop diarrhoea, resistance to clinically overt *C. jejuni* infection develops rapidly with increasing age.

This is to certify that the dissertation "*Campylobacter jejuni* infections in a high prevalence population" presented for the degree of Doctor of Philosophy in Medicine at the University of the Witwatersrand, Johannesburg, is my own work and has not been presented at another university.

N.J. Richardson

Neville John Richardson
M.Sc. (Witwatersrand).

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1. Detection of enteric campylobacteriosis in children. Bokkenheuser, V.D., Richardson, N.J., Bryner, J.H., Roux, D.J., Schutte, A.B., Koornhof, H.J., Freiman, I., and Hartman, E. 1979. Journal of Clinical Microbiology 9: 227-232.

2. Etiology of infantile enteritis in South Africa. Koornhof, H.J., Robins-Browne, R.M., Richardson, N.J. and Cassel, R. 1979. Israel Journal of Medical Sciences 15 : 341-347.

3. Campylobacter infections in Soweto (letter). Richardson, N.J., and Koornhof, H.J. 1979. South African Medical Journal 55: 73-74.

4. Summer diarrhoea in African infants and children. Robins-Browne, R.M., Still, C.S., Miliotis, M.D., Richardson, N.J., Koornhof, H.J., Freiman, I., Schoub, B.D., Lecatsas, G., and Hartman, E. Archives of Disease in Childhood 55: 923-928, 1980.

5. Primary isolation of *Campylobacter fetus* subsp *jejuni*. Richardson, N.J., Koornhof, H.J., and Bokkenheuser, V.D. 1981. American Journal of Medical Technology 48: 197-199.

6. Long-term infections with *Campylobacter fetus* subsp *jejuni*. Richardson, N.J., Koornhof, H.J., and Bokkenheuser, V.D., 1981. Journal of Clinical Microbiology 13 : 846-849.

7. Age related susceptibility to *Campylobacter jejuni* in a high prevalence population. Richardson, N.J., Koornhof, H.J., Bokkenheuser, V.D., Mayet, Z., and Rosen, E.U. 1983. Archives of Diseases of Childhood 58 : 616-619.

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Dr. John H. Bryner for the phage typing of and immunofluorescent studies of *C. jejuni* strains recorded in Chapter 5.

Many workers in the field of campylobacteriosis, not mentioned by name, through discussions and encouragement assisted directly and indirectly with the present studies.

It is hoped that the investigations recorded here will contribute in a small way to the vast reservoir of knowledge on campylobacter enteritis accumulated in recent years and that the candidate's friends and colleagues will accept this thesis as a tribute to their inspiration.

TABLE OF CONTENTS

	Page
Abstract	i
Declaration	ii
Acknowledgements	iii
Table of contents	v
List of Figures	xii
List of Tables	xv
Introduction	1
Chapter 1 Recognition of campylobacters as significant animal and human pathogens	4
1.1 Animal pathogen	4
1.2 Human pathogen	5
1.2.1 Early history	5
1.2.2 Recognition of the diversity of the microaerophilic "vibrios" causing disease in man	6
1.2.3 Systemic infections caused by campylobacters	8
1.2.3.1 Cerebrospinal meningitis	8
1.2.3.2 Bacteraemia in children	9
1.2.3.3 Disseminated infections in adults	9
1.2.4 Recognition of the importance of "related vibrios"	10
References	12
Chapter 2 Taxonomy of *Campylobacter*	15
2.1 Classification of the genus *Campylobacter*	15
2.1.1 Historical background	15
2.1.2 The *Campylobacter* species and subspecies controversy	16
2.2 Biochemical differentiation of campylobacters	16
2.3 Current taxonomic status of the thermophilic campylobacters	20
References	22
Chapter 3 General characteristics of *Campylobacter jejuni*	24
3.1 Morphology	24
3.1.1 Wet preparation under phase contrast microscopy	24

		Page
	3.1.2 Stained preparations ----------	24
	3.1.3 Electron microscopy ----------	24
3.2	Cultural methods and requirements for isolation ----------	24
	3.2.1 Gaseous requirements ----------	24
	3.2.2 Basic media requirements ----------	28
	3.2.3 Selective media ----------	29
	3.2.4 Enrichment procedures ----------	30
	3.2.5 Transport media ----------	31
	3.2.6 Maintenance and storage ----------	31
3.3	Antigenic structure and serology ----------	31
	3.3.1 Serotypes of the *C.* [illegible] subspecies	32
	3.3.2 Serotyping and serology of *C.jejuni*	33
3.4	The antibiotic susceptibility of *C. jejuni*	34
	References ----------	36
Chapter 4	The primary isolation of *Campylobacter jejuni/coli* from faecal specimens ----------	42
4.1	Assessment of the growth conditions for *C. jejuni* ----------	42
	4.1.1 Materials and methods ----------	42
	4.1.1.1 Sources of *C. jejuni*------	42
	4.1.1.2 Cultural techniques -----	42
	4.1.1.3 Media ----------	42
	4.1.1.4 Gas mixture ----------	43
	4.1.1.5 Temperature tolerance ----	43
	4.1.1.6 Colony counts ----------	43
	4.1.1.7 Estimation of colony sizes and identification -------	43
	4.1.2 Results ----------	43
	4.1.2.1 Evaluation of media by colony counts ----------	43
	4.1.2.2 Evaluation media by colony diameter ----------	44
	4.1.3 Discussion on the effects of growth conditions on the isolation of *C. jejuni* ----------	44
	4.1.3.1 Source of blood ----------	44
	4.1.3.2 Media ----------	44

		Page
	4.1.3.3 Gaseous requirements----	49
	4.1.3.4 Incubation ----------	49
	4.1.3.5 Duration of incubation--	49
4.2	Isolation procedures ----------	50
	4.2.1 Materials and methods ----------	50
	4.2.1.1 Source of specimens-----	50
	4.2.1.2 Media ----------	50
	4.2.1.3 Filtration technique----	51
	4.2.1.4 Culture procedures ----	51
	4.2.1.5 Quantitation of *C. jejuni* in faeces ----------	51
	4.2.2 Results if the isolation procedures	52
	4.2.2.2 Quantitative assessment of faecal excretion of *C. jejuni* ---------- ----	52
	4.2.3 Discussion of the isolation procedures	52
	4.2.3.1 Evaluation of isolation procedures----------	52
	4.2.3.2 Quantitative assessment of faecal excretion of *C. jejuni*	59
	4.3 Concluding remarks ----------	60
	References ----------	61
Chapter 5	The isolation of *C. jejuni* from babies under two years of age with or without gastro-enteritis - a preliminary study ----------	63
	5.1 Introduction ----------	63
	5.2 Materials and methods ----------	63
	5.2.1 Selection of children ----------	63
	5.2.2 Faecal specimens ----------	64
	5.2.3 Clot cultures ----------	64
	5.2.4 Isolation of *Salmonella* and *Shigella* from stool specimens ----------	64
	5.2.5 Isolation of *C. jejuni* ----------	64
	5.2.5.1 Media ----------	64
	5.2.5.2 Cultural technique ------	64
	5.2.6 Identification and characterization of *C. jejuni* isolates ----------	65

			Page
		5.2 6.1 Morphology ----------	65
		5.2.6.2 Aerotolerance ----------	65
		5.2.6.3 Biochemical identification procedures ----------	65
		5.2.6.4 Temperature tolerance-----	65
		5.2.6.5 Immunofluorescence techniques	65
		5.2.6.6 Agglutination of isolates--	66
		5.2.6.7 Phage typing ----------	66
		5.2.6.8 Antibiotic susceptibility testing ----------	66
	5.3 Results ----------		67
	5.3.1 Isolates from stool specimens -----		67
	5.3.2 Clinical features ----------		68
	5.3.3 Treatment ----------		68
	5.3.4 Identification of *C. jejuni* -------		69
		5.3.4.1 Biochemical and tolerance tests ----------	69
		5.3.4.2 Agglutination patterns-----	69
		5.3.4.3 Direct immunofluorescence--	70
		5.3.4.4 Phage typing ----------	71
		5.3.4.5 Antimicrobial susceptibility	72
	5.4 Discussion ----------		72
	5.4.1 Significance of *C. jejuni* in human faeces ----------		72
	5.4.2 Species identification of *C. jejuni*--		74
	5.4.3 Antigenic patterns ----------		75
	5.4.5 Antibiotic susceptibility ---------		75
	References ----------		76
Chapter 6	Age-related susceptibility to *Campylobacter jejuni* infection in a high prevalence population ----------		79
	6.1 Introduction ----------		79
	6.2 Materials and methods ----------		79
	6.2.1 Patients ----------		79
	6.2.2 Controls ----------		80
	6.2.3 Media and bacteriological techniques		80
	6.3 Results ----------		81
	6.3.1 Recovery of bacterial pathogens from stool specimens ----------		81

Page

6.3.2 Age-specific faecal excretion of *C. jejuni* ---------- 81
6.3.3 Clinical picture ---------- 81
6.3.4 Breast feeding ---------- 87
6.3.5 *C. jejuni* excretion after acute gastroenteritis ---------- 87
6.4 Discussion ---------- 87
6.4.1 Age-related susceptibility to infection ---------- 87
6.4.2 Evidence for herd immunity --------- 91
6.4.3 Clinical features ---------- 93
6.4.4 Effect of breast feeding on *C. jejuni* colonization ---------- 93
References ---------- 94

Chapter 7 Possible sources of *C. jejuni* in Soweto ---------- 96
7.1 Sources of *C. jejuni* reported in the literature ---------- 96
7.1.1 Waterborne outbreaks ---------- 96
7.1.2 Milk as a vehicle of *C. jejuni* enteritis outbreaks ---------- 96
7.1.3 Outbreaks associated with chicken ---------- 97
7.1.4 The role of red meat as a source of campylobacter enteritis ---------- 97
7.1.5 Evidence of direct spread from animals to birds and man ---------- 98
7.1.6 Human transmission of *C. jejuni* ---------- 98
7.2 Isolation of *C. jejuni* from fowl and dog faeces and from bovine offal and tripe ------ 98
7.2.1 Materials and methods ---------- 99
7.2.2 Results ---------- 99
7.2.3 Discussion ---------- 99
References ---------- 101

Chapter 8 Long-term infections with *C. jejuni* ---------- 105
8.1 Introduction ---------- 105
8.2 Materials and methods ---------- 105
8.2.1 Donors ---------- 105
8.2.2 Faecal samples ---------- 106

			Page
		8.2.3 Isolation of *C. jejuni* ----------	106
		8.2.4 Salmonellae, shigellae and parasites	106
	8.3	Results ----------	108
		8.3.1 Campylobacterios s ----------	108
		8.3.2 Salmonellae, shigellae and parasites ----------	113
	8.4	Discussion ----------	113
		8.4.1 Reinfection versus carriers ----	113
		8.4.2 Risk of long-term colonization	115
	References ----------		118
Chapter 9	The human reservoir and person-to-person transmission of *C. jejuni* in Sowetan children----		119
	9.1	Introduction ----------	119
	9.2	Children and methods ----------	120
		9.2.1 Age related faecal excretion of *C. jejuni* in Sowetan children----	120
		9.2.1.1 Age groups in children--	120
		9.2.1.2 Entry criteria ---------	120
		9.2.1.3 Sampling procedure -----	120
		9.2.1.4 Bacteriological examination of faeces ----------	120
		9.2.1.5 Serotyping of *C. jejuni*	121
		9.2.1.6 Information sheet on clinical and epidemiological ----------	122
		9.2.2 Prevalence of *C. jejuni* and evidence of person-to-person transmission in Sowetan families ----------	122
		9.2.2.1 Entry criteria for family studies ----------	123
		9.2.2.2 Sampling procedure for family studies ---------	123
		9.2.2.3 Bacteriological examinations and serotyping ---------	123
	9.3	Results ----------	123
		9.3.1 Age related excretion of *C. jejuni*	123

Page

9.3.2 Correlation of some environmental, social and clinical factors with faecal excretion of *C. jejuni* ----- 142

9.3.3 Results of family studies ---------- 142

9.3.3.1 *C. jejuni* serotypes and other bacterial pathogens in Family number 35 ------ 142

9.3.3.2 Chronological order of *C. jejuni* isolations in family studies ---------- 146

9.3.3.3 Isolation rates and prolonged excretion of *C. jejuni* in families ---------- 160

9.3.3.4 Age-related faecal excretion of *C. jejuni* in families--- 163

9.3.4 Patterns and duration of *C. jejuni* excretion ---------- 163

9.4 Discussion ---------- 169

9.4.1 Prevalence of *C. jejuni* and other intestinal pathogens in Soweto------ 169

9.4.2 Age-related excretion of *C. jejuni* 169

9.4.3 Pattern and duration of *C. jejuni* excretion according to age --------- 170

9.4.4 Evidence of person-to-person transmission in households in Soweto----- 172

References ---------- 175

Chapter 10 Campylobacter infection in a population with a high endemicity. General discussion and concluding remarks. ---------- 178

10.1 High prevalence of *C. jejuni* in Soweto - historical perspective ---------- 178

10.2 Age-related susceptibility to *C. jejuni* infection ---------- 178

10.3 Duration and patterns of *C. jejuni* excretion- 180

10.4 Intra-family spread ---------- 180

10.5 Sources of *C. jejuni* infection ---------- 181

10.6 Unresolved problems - Scope for further studies ---------- 181

Page

10.6.1 Resistance to *C. jejuni* diarrhoea in high prevalence populations ----- 181

10.6.2 Patterns and duration of *C. jejuni* excretion ---------- 182

10.6.3 Sources of infection and person-to-person transmission ---------- 182

10.7 Concluding remarks ---------- 182

References ---------- 184

Page

10.6.1 Resistance to *C. jejuni* diarrhoea in high prevalence populations ----- 181

10.6.2 Patterns and duration of *C. jejuni* excretion ---------- 182

10.6.3 Sources of infection and person-to-person transmission ---------- 182

10.7 Concluding remarks ---------- 182

References ---------- 184

LIST OF FIGURES

			Page
Chapter 3	3.1	Appearance of *C. jejuni* under phase contrast microscopy ----------	25
	3.2	Microscopic appearance of *C. j juni* stained with carbol-fuschin ----------	26
	3.3	Electron micrograph of *C. jejuni*, showing bipolar flagella ----------	26
Chapter 4	4.1	Effect of media composition, temperature and gaseous environment on the diameter of colonies of *C. fetus* subspecies *jejuni* (with 95% confidence intervals) ----------	45
	4.2	Influence of temperature on colony sizes of *C. jejuni* seeded on various media and incubated in a gas mixture of 5% O_2, 10% CO_2 and 85% N_2	46
	4.3	Influence of temperature on colony sizes of *C. jejuni* seeded on various media and incubated in a gas mixture of 19% O_2, 5% CO_2 and 76% N_2	47
	4.4	Colony sizes of *C. jejuni* seeded on various media and incubated at 42°C in two gas environments ----------	48
	4.5	Effect of faecal organisms on isolation of *C. jejuni* on selective media ----------	58
Chapter 6	6.1	*C. jejuni* in babies with and without diarrhoea	84
	6.2	Natural *C. jejuni* infection----------	90
Chapter 8	8.1	Collection and processing of specimens at Tlaseng school ----------	107
	8.2	Proposed duration of spontaneous bacteriological recovery from *C. jejuni* infections among Tlaseng children based on the recovery rate reported by Karmali and Fleming (1979) and our findings ----------	117
Chapter 9	9.1	Spread of *C. jejuni* in family 35 ----------	147
		9.2.1 Family Study 1 - Family 18 --------	149
		9.2.2 Family Study 1 - Family 90 --------	150
		9.2.3 Family Study 1 - Family 128 --------	151

		Page
Chapter 9	9.2.4 Family Study 1 - Family 130 ------	152
	9.2.5 Family Study 1 - Family 131 ------	153
	9.3.1 Family Study 11 - Family 101 -------	154
	9.3.2 Family Study 11 - Family 110 -------	155
	9.3.3 Family Study 11 - Family 119 -------	156
	9.3.4 Family Study 111- Family 103 -------	157
	9.4.2 Family Study 111 - Family 127 -------	158
	9.4.3 Family Study 111 - Family 129 -------	159

LIST OF TABLES

			Page
Chapter 2	2.1	Historical terminology of some campylobacters ----------	17
	2.2	Current classification of genus *Campylobacter*	18
	2.3	Common characteristics of the main species of campylobacters ----------	19
	2.4	Differentiation of enteropathogenic campylobacters ----------	21
Chapter 4	4.1	Recovery of *C. jejuni* from 78 positive human faecal specimens ----------	53
	4.2	Number of *C. fetus* organisms per gram (net weight) in faeces from diarrhoeal and non-diarrhoeal (control) babies ----------	54
	4.3	Composition of isolation media (per litre) used by Bokkenheuser and Mosenthal (KF, BL, BU) and Richardson (BCA, SK). ----------	56
	4.4	Quantitation of *C. jejuni* from chicken caeca by filtration and selective media --------	57
Chapter 5	5.1	Isolates from stool specimens ----------	67
	5.2	Children with *C. jejuni* as the sole pathogen	68
	5.3	Biochemical characterization of 39 presumptive *C. jejuni* strains recovered from diarrhoeal and non-diarrhoeal children ----------	69
	5.4	Agglutination of 21 isolates ----------	70
	5.5	Species identification of 39 isolates by direct immunofluorescence ----------	70
	5.6	Species identification of 25 isolates by indirect fluorescence ----------	71
	5.7	Lytic activity of *C. fetus* phages on 41 isolates ----------	71
	5.8	Antibiotic susceptibility of isolates -----	73
Chapter 6	6.1	Faecal excretion of *Campylobacter jejuni* and other bacterial pathogens in Sowetan children ----------	82
	6.2	Age-specific faecal excretion of *Campylobacter jejuni* ----------	83

			Page
Chapter 6	6.3	Clinical manifestations in % of 20 children with diarrhoea, infected with *C. jejuni* only ----------	85
	6.4	Influence of bacterial pathogens on dehydration ----------	86
	6.5	Effect of breast feeding on *Campylobacter jejuni* associated diarrhoea among 18 months old children ----------	88
	6.6	Minimal duration of excretion of *C. jejuni* in babies after acute gastroenteritis ------	89
Chapter 7	7.1	Isolation of *C. jejuni* ----------	99
Chapter 8	8.1	Seasonal incidence of *C. jejuni* and persistence of infection in 73 children ----------	109
	8.2	Intestinal pathogens by age and sex --------	110
	8.3	Seasonal variations in prevalence of infective agents ----------	111
	8.4	Antibiotic sensitivity results - Tlaseng ---	112
	8.5	Seasonal prevalence of faecal parasites ---	114
Chapter 9	9.1	Isolation of bacterial pathogens in Sowetan children following an acute attack of diarrhoea ----------	124
	9.2	Faecal excretion of *C. jejuni* in Sowetan children ----------	126
	9.3	Patterns of mixed infections, *C. jejuni* excretion alone and prolonged excretion of *C. jejuni* ----------	127
	9.4	Duration of excretion of *C. jejuni*, *Salmonella* and *Shigella I* <4 month age group ----------	128
	9.5	Duration of excretion of *C. jejuni*, *Salmonella* and *Shigella II* 5-12 month age group -------	129
	9.6	Duration of excretion of *C. jejuni*, *Salmonella* *Shigella III* 13-24 month age group -----	131
	9.7	Duration of excretion of *C. jejuni*, *Salmonella* and *Shigella IV* 25-36 month age group ------	133
	9.8	Pattern and duration of faecal excretion according to serotype and age ----------	135
	9.9	Persistence of infection with *C. jejuni* for - 3 weeks ----------	136

			Page
Chapter 9	9.10	Duration of *C. jejuni* excretion according to serotype	137
	9.11	*C. jejuni* excretion feeding habits and pet dog	138
	9.12	Examples of persistence of *C. jejuni* serotypes	143
	9.13	Examples of persistence of *C. jejuni* serotypes	144
	9.14	Influence of various factors of excretion or re-infection of *C. jejuni*	145
	9.15	Correlation of mixed infections, *C. jejuni* excretion alone and prolonged excretion of *C. jejuni* with poor nutrition, dehydration and family size	146
	9.16	Summary of findings in Family Study I - Index case with *C. jejuni*-associated diarrhoea	161
	9.17	*C. jejuni* excretion in second and third family studies	162
	9.18	Faecal excretion of *C. jejuni* in families	164
	9.19	Faecal excretion of *C. jejuni* according to age	165
	9.20	*C. jejuni* excretion according to age in households	166
	9.21	Duration of *C. jejuni* excretion assuming intermittent shedding occurs, according to age (all *C. jejuni* excretors, including those in family studies)	167
	9.22	Duration of episodes of *C. jejuni* excretion according to age	168

INTRODUCTION

Events leading up to the studies comprising this thesis.

In 1970 Dr. Victor Bokkenheuser who, after he had left the South African Institute for Medical Research in 1959, maintained close links with the author of this thesis, described ten new cases of *Vibrio fetus* infection in man (Bokkenheuser 1970). These cases, eight of which were from New York City, occurred over a period of two and a half years. Bokkenheuser compared his findings with the few infections recorded earlier in the literature - 64 cases in 20 years. Together with other authors (Spink 1957; King and Bronsky 1961; Ellis, Hageman and Marzi 1965; Willis and Austin 1966), he speculated that infections in man were far more prevalent than previously realised and that our inability to recognise more cases was due to deficient laboratory techniques.

Continuing his studies on *V. fetus* infections in man, Bokkenheuser devised an indirect bacterial haemagglutination test to determine antibody levels to this group of organisms (Bokkenheuser 1972). He used a heat-stable NaOH-hydrolysis-extracted antigen derived from a human isolate of *Vibrio fetus* and regarded the test to be highly specific and reproducible. Of interest were the results of testing seven pools of gamma-globulin from various parts of the world which he and T. Dunston reported at the VI Congresso Latino Americano De Microbiologica, Caracas, 1974. (Bokkenheuser and Dunston, 1975). Those from South Africa (eight batches) yielded consistently high antibody titres to *Vibrio fetus* (subsequently the antigens used were shown to represent *Campylobacter fetus* subsp *fetus*) whereas protein content, rubella antibody levels and poliomyelitis antibody levels were comparable in all sera. At the time *V. fetus* subsp *venerealis* reclassified as *Campylobacter fetus* subsp *fetus* (Sebald 1976) or *V. fetus* subsp *venerealis* (Véron and Chatelain, 1973) was believed to be the causative organism of sexually transmitted diseases in cattle. Also, relatively little was then known about antigenic relationships between the various microaerophilic vibrios. Because the high *V. fetus* titres in South African gamma-globulin preparations suggested a relatively high prevalence of infections caused by this group of organisms in South Africa, a study was undertaken to

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Events leading up to the studies comprising this thesis.

In 1970 Dr. Victor Bokkenheuser who, after he had left the South African Institute for Medical Research in 1959, maintained close links with the author of this thesis, described ten new cases of *Vibrio fetus* infection in man (Bokkenheuser 1970). These cases, eight of which were from New York City, occurred over a period of two and a half years. Bokkenheuser compared his findings with the few infections recorded earlier in the literature - 64 cases in 20 years. Together with other authors (Spink 1957; King and Bronsky 1961; Kilo, Hageman and Marzi 1965; Willis and Austin 1966), he speculated that infections in man were far more prevalent than previously realised and that our inability to recognise more cases was due to deficient laboratory techniques.

Continuing his studies on *V. fetus* infections in man, Bokkenheuser devised an indirect bacterial haemagglutination test to determine antibody levels to this group of organisms (Bokkenheuser 1972). He used a heat-stable NaOH-hydrolysis-extracted antigen derived from a human isolate of *Vibrio fetus* and regarded the test to be highly specific and reproducible. Of interest were the results of testing seven pools of gamma-globulin from various parts of the world which he and T. Dunston reported at the VI Congresso Latino Americano De Microbiologica, Caracas, 1974. (Bokkenheuser and Dunston, 1975). Those from South Africa (eight batches) yielded consistently high antibody titres to *Vibrio fetus* (subsequently the antigens used were shown to represent *Campylobacter fetus* subsp *intestinalis* whereas protein content, rubella antibody levels and poliomyelitis antibody levels were comparable in all sera. At that time *V. fetus* subsp *venerealis* reclassified as *Campylobacter fetus* subsp *fetus* (Smibert 1974) or *C. fetus* subsp *venerealis* (Véron and Chatelain, 1973) was believed to be the causative organism of sexually transmitted diseases in cattle. Also, relatively little was then known about antigenic relationships between the various microaerophilic vibrios. Because the high *C. fetus* titres in South African gamma-globulin preparations suggested a relatively high prevalence of infections caused by this group of organisms in South Africa, a study was undertaken to

recover *V. fetus* from the vaginas of pre-natal women near Pretoria. Of 184 samples, none yielded a growth of "vibrios" (Bokkenheuser and Doneton 1975).

Undeterred by the unsuccessful attempts to isolate microaerophilic "vibrios" from humans in South Africa, it was decided at Dr. Bokkenheuser's request and with active encouragement, to evaluate a technique described by Dekeyser and his colleagues (Dekeyser, Gossuin-Detrain, Butzler and Sternon, 1972) for the isolation of microaerophilic "vibrios", by then reclassified as campylobacters (Hugh and Feeley 1972), from the faeces of babies in South Africa. Dekeyser and his Belgian co-workers used this filtration technique and were the first to isolate "related vibrios" (*C. fetus* subsp *jejuni*, *C. jejuni/coli*, *C. jejuni*) from the stools of two nurses with diarrhoea (Dekeyser, Gossuin-Detrain, Butzler and Sternon, 1972). That a similar organism was recovered from the nurses's blood strongly supported the role that the isolated "vibrio" might have played in causing gastro-enteritis. This same team one year later reported the isolation of "related vibrios" from 5 per cent of 800 infants and children and from 4 per cent of adults (Butzler, Dekeyser, Gossuin-Detrain and Dehaen 1973).

With the knowledge that "related vibrios" had been recovered from diarrhoea cases, that South African human gamma-globulin preparations showed high antibody levels to campylobacters and the fact that gastro-enteritis was a major problem among infants and young children in South Africa and elsewhere, studies were initiated in 1976 to establish the possible role of those organisms in gastro-enteritis in Johannesburg.

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1. RECOGNITION OF CAMPYLOBACTERS AS SIGNIFICANT ANIMAL AND HUMAN PATHOGENS.

1.1 Animal Pathogen.

In 1897, Bang recovered a pathogenic agent, later known as *Brucella abortus*, and showed that it was responsible for contagious abortion in cattle - a problem which had troubled farmers for years. McFadyean and Stockman in 1909 confirmed Bang's observation but found in addition that a species of "vibrio" for which they proposed the name *Vibrio fetus ovis* was responsible for abortion in sheep. (MacFadyean and Stockman 1913). The potential of this "vibrio" to cause abortion was confirmed when inoculation of the organism into pregnant cows and ewes resulted in loss of their foetuses.

Six years later, Smith and Taylor (1919) in the United States isolated an organism which they at first called a spirillum from a high percentage of cases of infectious abortion in cattle. They observed that these bacteria were present in short comma as well as long spiral forms and suggested that the generic term "Vibrio" appeared appropriate. They were astute enough to realise that these organisms resembled closely the "vibrios" described by McFadyean and Stockman (1913) and proposed the name *Vibrio fetus* N. sp. to include the organisms recorded by both groups of workers. In the same year, abortion in sheep due to "vibrio" organisms was recorded independently by Carpenter (1919).

Following these early reports, *Vibrio fetus* was for a long time regarded as an organism causing disease which only concerned the veterinarian. It was then and still is recognised as an organism causing appreciable economic loss to the farmer. In cattle it is considered to be the cause of venereal disease, being carried asymptomatically on the prepuce of the bull. In sheep, however, the source of *V. fetus* causing abortion is endogenous, the bacteria forming part of the normal intestinal flora. Infection also occurs in pigs, goats, chickens and turkeys.

Using biochemical and serological tests, it became apparent that organisms reported as *V. fetus* really constituted two different groups justifying separate species or subspecies recognition. Smith and Taylor (1919) found that "vibrios" isolated from aborted bovine foetuses were serologically distinct as were isolates of ovine origin tested by Blakemore

and Cledhill (1946). Blastridge and his colleagues (1951) also reported serological differences in *V. fetus* cultures originating from sheep and cattle respectively. Similar differences in "vibrio" strains of bovine and ovine origin were noted by Marsh and Firehammer (1953). It was however only in 1959 that *V. fetus* was convincingly divided by Florent into the two well characterized subspecies which he labelled *V. fetus* subsp *venerealis* and *V. fetus* subsp *intestinalis* respectively.

The first claim that a "vibrio" may be the causative agent of dysentery in cattle was made by Jones, Dreurt and Little in 1931. They named this organism *Vibrio jejuni*. In 1948 a "vibrio" allegedly causing swine dysentery was named *Vibrio coli* by Doyle [illegible]8). The taxonomic status of this organism and how it is related [illegible] *jejuni* will be discussed in Chapter [illegible].

1.2. Human Pathogen

The history of *C. fetus* as a human pathogen may conveniently be divided into four periods:-

(i) The early history when relatively little was known about the diversity and classification of the organisms (1913-1956)

(ii) the recognition of the diversity of the micro-aerophilic "vibrios" (1957-1970)

(iii) the emergence of campylobacters as a cause of systemic infection in man

(iv) recognition of the taxonomic status and clinical importance of the "related vibrios" (*C. fetus* subsp *jejuni*, [illegible] *jejuni*, [illegible] *jejuni*

1.2.1. Early history

Curtis in 1913, was the first to describe motile curved vibrio-like organisms found in leukorrheal discharges of the human vagina. The bacilli were usually sparsely scattered among other organisms and their role in maintaining leukorrhoea was unclear. Early attempts to obtain pure cultures failed. In two cases however, curved bacteria were found in large numbers, one case following instrumental abortion and the other in a patient with puerperal sepsis after a full-term pregnancy. These organisms could only be isolated under strict anaerobic conditions and died with the introduction of small amounts of oxygen. Phillips and Taylor (1982) pointed out that several workers over the years reported the isolation of these curved anaerobic rods from patients with non-

specific vaginitis. More recently an association between the presence of these and other anaerobic organisms and *Gardnerella vaginalis* has been noted (Phillips and Taylor 1982). These authors also pointed out that there may be two morphological groups depending on whether only one polar flagellum is present or several (two to eight polar or subpolar flagella). The taxonomic status of these bacteria remains to be elucidated.

In 1946 a large outbreak of gastro-enteritis in two adjacent penal institutions involved 357 inmates (Levy 1946). Their illness was serious enough to warrant hospitalization. Although laboratory attempts to culture the organisms from stools failed, specimens prepared from mucoid material from stools showed vibrio-like bacteria on microscopic examination. Of 39 patients whose blood was cultured, 13 yielded a "spirillum" morphologically similar to the "vibrios" seen in the faeces. Studies on isolates which were unfortunately lost on subculture before the organism could be fully characterized, showed that it closely resembled the "*Vibrio jejuni*" recovered from cattle by Jones, Orcutt and Little (1931). Milk was suspected as being the vehicle for the outbreak. It was Vinzent, Dumas and Picard in France, in 1947, who first reported a case of human abortion caused by a *Vibrio fetus* organism. Unlike the situation in animals, this organism is regarded as a rare cause of abortion in man. Ward (1948) from England a year later, cultured *Vibrio fetus* from a pustule on the cheek of a laboratory worker who was studying the organism.

1.2.2. Recognition of the diversity of the microaerophilic "vibrios" causing disease in man.

Although *V. fetus* (MacFadyean and Stockman 1909; Smith and Taylor 1919), *V. jejuni* (Jones 1931) and *V. coli* (Doyle 1948) had already been accepted as separate entities in the 1930's and were included in the seventh edition of Bergey's Manual of Determinative Bacteriology in 1957, the biochemical and cultural differences were vague and not clearly defined, while the existence of *V. fetus* subsp *intestinalis* was not yet recognized (Blair, Berman, Brandon and Breed, 1957). It was only in 1959 that Florent suggested the division of *V. fetus* into two subspecies *venerealis* and *intestinalis* (Florent, 1959). Most of the taxonomic studies up to that stage were performed on strains recovered from animals.

The first meaningful attempts to classify the microaerophilic "vibrios" of importance in man was made in 1957 by the renowned Elizabeth King who was associated with the then Communicable Disease Center in Atlanta, Georgia and had access to a wide range of organisms submitted from different laboratories in the United States. She not only suggested the separation of thermophilic "vibrios" which grew well at 42°C from the other microaerophilic "vibrios" but also correlated them with clinical disease in man (King, 1957).

Reviewing 19 isolates of "vibrios" from blood cultures from human cases, King described in 1957 four strains which she called "related vibrios". These strains failed to grow at 25°C and grew more luxuriantly at 42°C than at 37°C. It is interesting that all four "related vibrios" were isolated from blood cultures of children, three of whom were under 4 months of age while the fourth patient was five years old. Furthermore, all the children had diarrhoea, three severe and two with bloody stools. The fourth patient passed five to six loose but not watery stools per day.

In the other 15 cases of *V. fetus* infections reviewed by King (1957), fever, usually undulating, was the only symptom common to all. Chills occurred in ten patients, headache in five, weight loss in four and five had some liver involvement. Four patients had either nausea or diarrhoea. All these 15 isolates showed the usual temperature requirements for the growth of *V. fetus*, i.e. a good growth at 37°C and 25°C and very little at 42°C. The author speculated that the "related vibrios" isolated from the children with diarrhoea may be closely related to the *Vibrio fetus* organisms described by Jones and his co-workers in 1931 and Levy in 1946. She too described isolates of *Vibrio bubulus* and pleaded for clarification of the relationship between these vibrios, which, with the exception of *V. bubulus* had, until then, been listed under one species, *V. fetus*.

King, in 1962, again reviewed the clinical histories of patients from whom *V. fetus* had been isolated. These now totalled 29 strains, the majority of which having been isolated from blood cultures. In addition 11 isolates, also from blood cultures, are found to be "related vibrios". The other sites from which *V. fetus* organisms were recovered were spinal, synovial, subdural and chest fluid, a thigh abscess and several different specimens from an abortion case.

One patient from whom a "related vibrio" was isolated is worthy of mention. He was a chicken farmer who was an alcoholic with cirrhosis of the liver and hypertension. He became very dehydrated because of severe

diarrhoea and died in shock. Autopsy revealed that the jejunum and the proximal half of the ileum were haemorrhagic and congested but there were no colon lesions, a similar type of pathology described by Jones, Orcutt and Little (1931) in cattle with dysentery caused by *V. jejuni*. King speculated that the organisms causing avian infectious hepatitis in chickens described by Peckham in 1958, dysentery in cattle and vibrionic dysentery in humans were the same.

Although Florent suggested that *V. fetus* should be subdivided into two subsp *V. venerealis* and *V. intestinalis* which he had characterized biochemically, as many blood culture isolates from patients with systemic infections were subsequently only identified, by other workers, to the species level. Thus in 1963 Kilo and Hageman described a case of septic arthritis and bacteraemia caused by *V. fetus*. Eden in 1966 reviewed eight reported cases of human infections with the organism in pregnancy and discussed its role as a cause of perinatal mortality. By 1970, the number of cases reported had risen to 74. These were reviewed by Bokkenheuser (1970) and included 10 cases from which he had isolated *V. fetus*.

1.2.3. Systemic infections caused by campylobacters

Bokkenheuser (1970) was the first author to comprehensively review and put into perspective the importance of systemic campylobacter infections in man. It was subsequently shown that *C. fetus* subsp *intestinalis* is responsible for the vast majority of these disseminated infections in man (Guerrant, Lahita, Washington, Jnr., and Roberts 1979). Systemic campylobacteriosis in man may conveniently be discussed under the headings cerebrospinal meningitis, bacteraemia in children and disseminated infections in adults.

1.2.3.1. Cerebrospinal meningitis

Almost all the patients in this group are infants, often born prematurely or with congenital defects of the central nervous system (Eden 1966; Willis and Austin 1966). The syndrome may develop as early as the day of birth. The onset usually presents with a low grade fever, poor appetite, weight loss and watery stools with blood. Two to seven days later there may be signs of meningeal involvement. Cerebrospinal fluid and sometimes blood yield a growth of campylobacter, most often *C. fetus* subsp *intestinalis*. The prognosis is serious and mortality is 50 per cent

(Eden 1966). At least two cases due to *C. jejuni* in infants have been described (Eden 1962; Thomas, Chan and Ribeiro; 1980).

1.2.3.2. Bacteraemia in children

Relatively few cases have been reported in this group. (King 1957, 1962, Smith, Marymont Jnr., and Schweers, 1976; Wyatt, Younoszen, Anuras and Myers 1977; Guerrant, Lakita, Washington Jnr. and Roberts 1978; Rettig 1979). Malnutrition appears to be an associating factor (Schewitz and Roux 1978). The onset is gradual with diarrhoea and the stools are often streaked with blood and mucus. The child may vomit, have anorexia and fever and in severe cases weight loss and dehydration occur. Blood cultures collected in the febrile phase may be positive. In children *C. jejuni* is most commonly associated with bacteraemia but *C. fetus* subsp *intestinalis* is also common. (King 1957; Guerrant, Lakita, Washington Jnr. and Roberts 1978; Rettig 1979). Complete recovery follows antibiotic or symptomatic treatment, or there may be spontaneous cure.

1.2.3.3. Disseminated infections in adults

Most of the patients are debilitated and there is a two to one male to female ratio. The more common disposing factors are cardio-vascular diseases, chronic alcoholism, malignancies, hepatorenal and endocrinological disorders and immuno suppressive therapy (Bokkenheuser 1970; Guerrant, Lakita, Washington Jnr. and Roberts 1978; Rettig 1979).

Complications are rare and the infection is usually quite benign although in pregnant women it may lead to foetal death, abortion, premature delivery and necrotic infarcts of the placenta (Rettig 1979).

Diagnosis is made by blood cultures in more than 90 per cent of the cases. A few strains have been recovered from the pericardium, pleura, peritoneum, joints and placenta (Bokkenheuser 1970; Guerrant, Lakita, Washington Jnr. and Roberts 1978; Rettig 1979).

Most of the isolates in cases of systemic campylobacter infections are *C. fetus* subsp *intestinalis* and using an indirect haemagglutination technique, antibodies to this organism can be demonstrated in about two thirds of the cases. Reciprocal titres have been found to range from 1 600 - 6 400 in early convalescent phase to 40 - 320 up to six months after bacteriologic cure (Bokkenheuser 1972).

In a review of 102 human bacteraemia infections caused by campylobacters, Rettig (1979) recorded several clinical patterns. These vary from transient asymptomatic bacteraemia to fulminant fatal sepsis.

Prolonged bacillaemia persisting for as long as eight to thirteen weeks with spontaneous relapses and remissions were also encountered while ten cases, nine of them male and five with pre-existing heart disease had endocarditis. He also stressed that thrombophlebitis may be a presenting sign and found this complication in nine of the 102 cases. Other reported sites of infections in systemic campylobacteriosis compiled by Rettig include hepatitis, pericarditis, peritonitis, salpingitis, septic arthritis, lung abscess and chest wall abscess.

1.2.4 Recognition of the importance of "related vibrios"

The description by King (1957) of the four "related vibrio" strains was followed by a report from Wheeler and Borchers (1961) of four infants with acute diarrhoea from whom "related vibrios" had been isolated from blood cultures. In the light of our present knowledge, it is interesting that in the home of one of the infants, the family puppy had developed intermittent vomiting and bloody diarrhoea which began a few days after spaying. The dog often curled up on the bed with the baby and there was considerable contact between them during the dog's illness. A diagnosis of 'worms' was made but treatment was deferred. In the same year Middelkamp and Wolf (1961) reported a similar isolate, also from an infant with diarrhoea. It seemed then that almost all the patients from whom "related vibrios" had been isolated from blood, suffered from a very definite diarrhoea. In all reports the clinical appearance was similar to the disease caused by the organism *Vibrio jejuni* described by Jones, Orcutt and Little (1931) in cattle and Levy (1946) in human patients many years before.

Although Cooper and Slee (1971) recovered a campylobacter which they called a *Vibrio fetus* from the stool of a patient with lymphoma, the first breakthrough in isolating "related vibrios" (*C. fetus jejuni*, *C. jejunitis*, *C. jejuni*) from the stool came in 1972 when Dekeyser and his Belgian colleagues using the brilliant green medium of Florent (1956) and a selective medium devised by Plumer, Duwall and Shepler (1962), filtered diluted faeces through a 0.65 um membrane filter. The faeces came from two nurses with diarrhoea. A blood culture from one of them also yielded "related vibrios". The same team,(Butzler, Dekeyser, Detrain and Dehaen 1973) using the filtration technique, found that 5.1 per cent of children and 4.0 per cent of adults with diarrhoea and 1.3 per cent of asymptomatic children excreted these organisms. The application of this technique revealed the possible importance of "related vibrios" as a cause of gastro-enteritis. At the time of these

investigations the methods used for identification of "related vibrios" and *C. fetus* subsp *jejuni* (Smibert 1974) did not allow for the separation of *C. coli* from two *C. jejuni* biotypes described by Skirrow and Benjamin (1980).

The development of a selective medium which was relatively simple and which excluded the filtration step, by Skirrow in 1977, facilitated significantly the isolation of campylobacters from specimens with mixed bacterial populations. A spate of publications on infections caused by the group of "vibrios" variously termed *C. fetus* subsp *jejuni*, *C. jejuni/coli* or more specifically *C. jejuni* as the cause of diarrhoea appeared in the medical literature, often to the extent of these organisms outnumbering salmonellae and shigellae. There were also subsequently indications that in the tropics and developing countries, campylobacter infections may be extremely common. However, their significance in the spectrum of diarrhoeal diseases which are responsible for such a high morbidity and mortality in those parts of the world is still uncertain as many healthy infants without diarrhoea harbour and excrete the organism (see Chapters 5, 6, 9 and 10).

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2. TAXONOMY OF *CAMPYLOBACTER*

2.1. Classification of the genus *Campylobacter*

2.1.1. Historical Background

Smith and Taylor in 1919 changed the name of the organism they isolated from aborted calves from "spirillum" to *Vibrio fetus*. They reasoned that there were more single comma-shaped cells than spirilla and the bacteria were similar to the vibrios described by Metchnyeon and Stockman in 1913. Thus all comma-shaped organisms were given the generic title *Vibrio* with *V. cholerae*, Pacini 1854 as the type species (Feeley 1966).

In 1931 Jones, Orcutt and Little isolated a vibrio from cattle with intestinal complications (winter scours) and named it *Vibrio jejuni*, while in 1944 a vibrio isolated from the intestine of pigs with swine dysentery was named *Vibrio coli*. In 1959 Vincent divided *V. fetus* into two subspecies. *V. fetus* subsp *venerealis* was the cause of vibrionic abortion in cattle and was venerally transmitted, while *V. fetus* subsp *intestinalis* caused sporadic abortion in cattle but was not considered venerally transmitted. Microaerophilic vibrios from humans with diarrhoea were isolated (from the bloodstream) by King (1957; 1962).

In 1963 Sebald and Véron showed that there were basic differences between the microaerophilic *V. fetus* group and the "true" vibrios such as *V. cholerae* and *V. parahaemolyticus* particularly in biochemical reactions, antigenic characteristics and most conclusively the guanosine + cytosine content of their respective DNA's. The G + C content of the DNA from *V. fetus* was found to be 33 - 35 mol per cent while that of the classical vibria (*V. cholerae*) was 47.2 ± 0.7 mol per cent. Because of these differences Sebald and Véron proposed a new genus - *Campylobacter* with *C. fetus*, previously *V. fetus* (Smith an Taylor) as the type species. The name campylobacter (curved rod) was derived from the Greek campylo and bacter. In April 1972 the Sub-committee of Taxonomy of Vibrios reported to the International Committee on Nomenclature of Bacteria and proposed that *Vibrio fetus* (Smith and Taylor 1919) and related microaerophilic vibrios to be removed from the genus *Vibrio* (Pacini). They further pointed out that Sebald and Véron proposed that *V. fetus* be transferred to the genus *Campylobacter* with *C. fetus* as the type species

of the genus. Following this announcement the genus *Campylobacter* gained widespread acceptance in preference to that of *Vibrio* as the designation for the microaerophilic curved rods.

2.1.2. The *Campylobacter* species and subspecies controversy

A further reclassification of the genus by Véron and Chatelain took place in 1973. *C. fetus* subsp *fetus* was the same as Florent's *V. fetus* subsp *intestinalis* while *C. fetus* subsp *veneralis* was identical to Florent's *V. fetus veneralis*. *C. jejuni*/*coli* represents King's "thermophilic vibrios". Smibert in 1974 adopted yet another system - *C. fetus* subsp *intestinalis* and *C. fetus* subsp *veneralis* (Véron and Chatelain) being called *C. fetus* subsp *fetus*. *C. fetus* subsp *jejuni* represents the *C. jejuni*/*coli* group of Véron and Chatelain and the "related vibrios" of King. Table 2.1 summarizes the designation that various medical and veterinary important species have been given.

In an attempt to clarify the problems of nomenclature the approved list of bacterial names was published in 1980 (Skerman, McGowan and Sneath 1980). The principal terminology used at the present time is outlined in table 2.2. This classification system was adopted at a recent international workshop on campylobacter infections in Reading (Skirrow and Véron 1982).

2.2. Biochemical differentiation of campylobacters

As shown in Table 2.2 the genus is divided into two groups, one producing catalase and the other not. The catalase-negative campylobacters *C. sputorum* subsp *sputorum* (Loesche, Gibbons and Socransky 1965; Smibert 1974) and *C. sputorum* subsp *bubulus* (Florent 1953; Véron and Chatelain 1973; Smibert 1974) have not been shown to be pathogenic to man and animals. A third subspecies *C. sputorum* subsp *mucosalis* (Lawson, Rowland and Woodine 1975) is associated with intestinal adenomatosis of pigs. The various characteristics of the main species are shown in Table 2.3.

Of the catalase positive campylobacters, *C. fetus* subsp *fetus* (Smibert) produced a venereal disease in cattle often resulting in infertility and occasionally abortion. In many cases substantial financial losses to farmers occur. *C. fetus* subsp *intestinalis* (Smibert) is often found in the gut of sheep and cattle. It is pathogenic to both man and animals, causing disseminated infections and occasionally enteric infections and sporadic abortion.

TABLE 2.1 : HISTORICAL TERMINOLOGY OF SOME CAMPYLOBACTERS

King (1957)	Florent (1959)	Véron and Chatelain (1973)	Sulbert (1974)
Vibrio fetus	*V. fetus* subsp *intestinalis*	*C. fetus* subsp *fetus*	*C. fetus* subsp *intestinalis*
	V. fetus subsp *venerealis*	*C. fetus* subsp *venerealis*	*C. fetus* subsp *fetus*
"Related vibrios"		*C. jejuni* nov	*C. fetus* subsp *jejuni*

TABLE 2.2 : Current Classification of Genus *Campylobacter* (Skerman, McCowan and Sneath 1980)

catalase positive group	*C. fetus* (+ 25°C, - 43°C)	subsp. *venerealis* (subsp. *fetus*[a])
		subsp. *fetus* (subsp. *intestinalis*[a])
	"thermophilic group"	*C. jejuni* - biotype 1 biotype 2
	(*C. fetus* subsp *jejuni*[a]) (-25°C, + 43°C)	*C. coli*[b] NARTC +[b]
catalase negative group	*C. sputorum*	subsp. *sputorum*
	"thermotolerant group"[c]	subsp. *bubulus*[d]
		subsp. *mucosalis*[d]

[a]Smibert (1974), +nalidixic acid-resistant thermophilic campylobacter - Skirrow and Benjamin (1980b)

[b]NARTC = Nalidixic-acid-resistant thermophilic campylobacters (Skirrow and Benjamin, 1980b)

[c]Sandstedt, Ursing and Walder, 1983.

[d]Lawson and Rowland, 1974.

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		subsp. *fetus* (subsp. *intestinalis*[a])
	"thermophilic group"	*C. jejuni* - biotype 1 biotype 2
	(*C. fetus* subsp *jejuni*[a]) (-25°C, + 43°C)	*C. coli*[b] NARTC +[b]
catalase negative group	*C. upsaliensis*	subsp. *upsaliensis*
	"thermotolerant group"[c]	subsp. *bubulus*[d] subsp. *mucosalis*[d]

[a]Smibert (1974), +nalidixic acid-resistant thermophilic campylobacter - Skirrow and Benjamin (1980b)

[b]NARTC = Nalidixic-acid-resistant thermophilic campylobacters (Skirrow and Benjamin, 1980b)

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catalase negative group	*C. sputorum*	subsp. *sputorum*
	"thermotolerant group"[c]	subsp. *bubulus*[d]
		subsp. *mucosalis*[d]

[a]Smibert (1974), +nalidixic acid-resistant thermophilic campylobacter - Skirrow and Benjamin (1980b)

[b]NARTC = Nalidixic-acid-resistant thermophilic campylobacters (Skirrow and Benjamin, 1980b)

[c]Sandstedt, Ursing and Walder, 1983.

[d]Lawson and Rowland, 1974.

TABLE 2.3 : Common characteristics of the main species of *Campylobacter*
(Holdeman, Cato and Moore 1977)

	C. fetus subsp *jejuni*	*C. fetus* subsp *intestinalis*	*C. fetus* subsp *fetus* (*venerealis*)	*C. sputorum* subsp *sputorum*	*C. sputorum* subsp *bubulus*
Oxidase	+	+	+	+	+
Catalase	+	+	+	–	–
Sugar fermentation	–	–	–	–	–
Nitrate reduction	+	+	–	+	+
H_2S-SIM	–	–	–	+	+
H_2S-PbAc paper	+/-	+/-	-W	+	+
Cystine (1%) growth	+	+	–	+	+
NaCl (3.5%) growth	–	–	–	–	+
25°C growth	–	+	+	+	V
42°C growth	+	–	–	–	V
Nalidixic acid, 30 µg disc	S	R	R		

S - Sensitive
R - Resistant
W - Weak
V - Variable

2.3. Current taxonomic status of the thermophilic campylobacters

The various names by which *C. fetus* subsp *jejuni* (Smibert) has been called have been alluded to earlier. In the American classification system (Smibert), all thermophilic (+43°C to 25°C) campylobacters are placed in a single subspecies (*C. fetus* subsp *jejuni*) while in the French nomenclature (Véron and Chatelain 1973) they are divided into the two species *C. jejuni* and *C. coli* , names derived from the *Vibrio jejuni* of Jones, Orcutt and Little (1931) isolated from calves and the *V. coli* of Doyle (1948) isolated from pigs. Following the application of the hippurate hydrolysis test to the classification of *C. fetus* subsp *jejuni* (Smibert), Skirrow and Benjamin (1982) showed that *C. fetus* subsp *jejuni* (Smibert) was hippurate positive (as judged by the development of a purple colour after ninhydrin addition) while *C. coli* was negative. Based on H_2S production on the iron-containing medium of Hoffmann, Krieg and Smibert (1979) and susceptibility to nalidixic acid using 30ug discs, a further differentiation of *C. fetus* subsp *jejuni* into two biotypes (Skirrow and Benjamin 1980a) was possible. (Table 2.6). A nalidixic acid resistance thermophilic group of campylobacters which produced H_2S in this system was subsequently named *Campylobacter lanienae* (Benjamin, Leaper, Owen and Skirrow 1983). Even more recently, Sandtedr, Ursing and Walder (1983) described campylobacters which are thermotolerant and have no or weak catalase activity (Table 2.2).

Since these tests have only recently become available, many of the earlier isolates obtained during these studies (pre-1981) will not have been typed by this scheme and therefore where appropriate the term *C. jejuni/coli* will be alluded to in order to denote thermophilic campylobacters. These organisms may be part of the normal flora of lower animals and are also the principal organisms isolated in the studies described in this thesis. The required biochemical characterization to exclude *C. coli* was only done in studies recorded from Chapter 6 onwards and because *C. coli* was rarely found from the clinical material investigated subsequent to the investigations of Chapters 4 and 5, the taxon *C. jejuni* will be used in this thesis to denote *C. fetus* subsp *jejuni* or *C. jejuni/coli* strains. This usage is in accordance with views expressed at an international workshop on *Campylobacter* infections, held at the University of Reading in March 1981 (Skirrow and Véron, 1982).

TABLE 2.4 : Differentiation of Enteropathogenic Campylobacters (Skirrow and Benjamin 1980).

	C. fetus subsp. *fetus*	*C. jejuni* biotype 1	*C. jejuni* biotype 2	*C. coli*	NARTC[a]
Growth at 25°C	+	−	−	−	−
Growth at 43°C	−	+	+	+	+
Nalidixic acid (30 µg/disc)	R[b]	S	S	S	R
Hippurate hydrolysis	−	+	+	−	−
H_2S in iron medium	−	−	+	−	+

(a) Nalidixic-acid-resistant group of thermophilic campylobacters *(C. laridis)*

(b) R - Resistant; S - Sensitive

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3. GENERAL CHARACTERISTICS OF *CAMPYLOBACTER JEJUNI*

3.1. phology

3.1.1. Wet preparation under phase contrast microscopy

The short forms of bacteria from young colonies on solid or from liquid media are extremely active with a characteristic oscillating or "stop-go motility with frequent changes of direction. They may appear either as a single curved bacterium, two together which resemble a sea-gull in flight, or several together (spiral). The loss of motility is a forerunner of a gradual degeneration to coccoidal forms, particularly on solid media; these are generally presumed not viable. The variant forms are shown in Figure 3.1.

3.1.2. Stained preparations

Using 2.0 percent carbol-fuschin as a counter stain, campylobacters are Gram-negative curved rods 1.5 - 3.5 µm long by 0.2 - 0.4 µm thick. As with the wet preparation the organisms appear singly, in pairs (sea-gull or S-shaped), or spirally as a chain of single curved cells (Figure 3.2).

3.1.3. Electron microscopy

As seen in the electronmicrograph (Fig.3.3) each cell has a long, single polar flagellum two to three times its length. A few cells may have one flagellum at each end and occasionally more than one (Smibert 1978).

3.2. Cultural methods and requirements for isolation

3.2.1. Gaseous requirements

The microaerophilic campylobacters, as the term implies are organisms that require a reduced oxygen content and increased carbon dioxide for growth. The relative sensitivity of campylobacters to oxygen (Ware, Colley and Park 1977; George, Hoffman, Smibert and Krieg 1978) is probably due to excessive vulnerability to superoxide anions and free radicals (George, Hoffman, Smibert and Krieg 1978: Hoffman, George, Krieg and Smibert 1979). This is the case in spite of the fact that campylobacters generate superoxide dismutases at concentrations typical of aerotolerant bacterial species (Ware, Colley and Park 1979; Hoffman, George, Krieg and Smibert 1979). Supplementation of basal media with substances such as blood, haemin, haematin, dihydroxyphenyl compounds and iron salts in order to neutralize

Figure 3.1 Appearance of *C. jejuni* under phase contrast microscopy

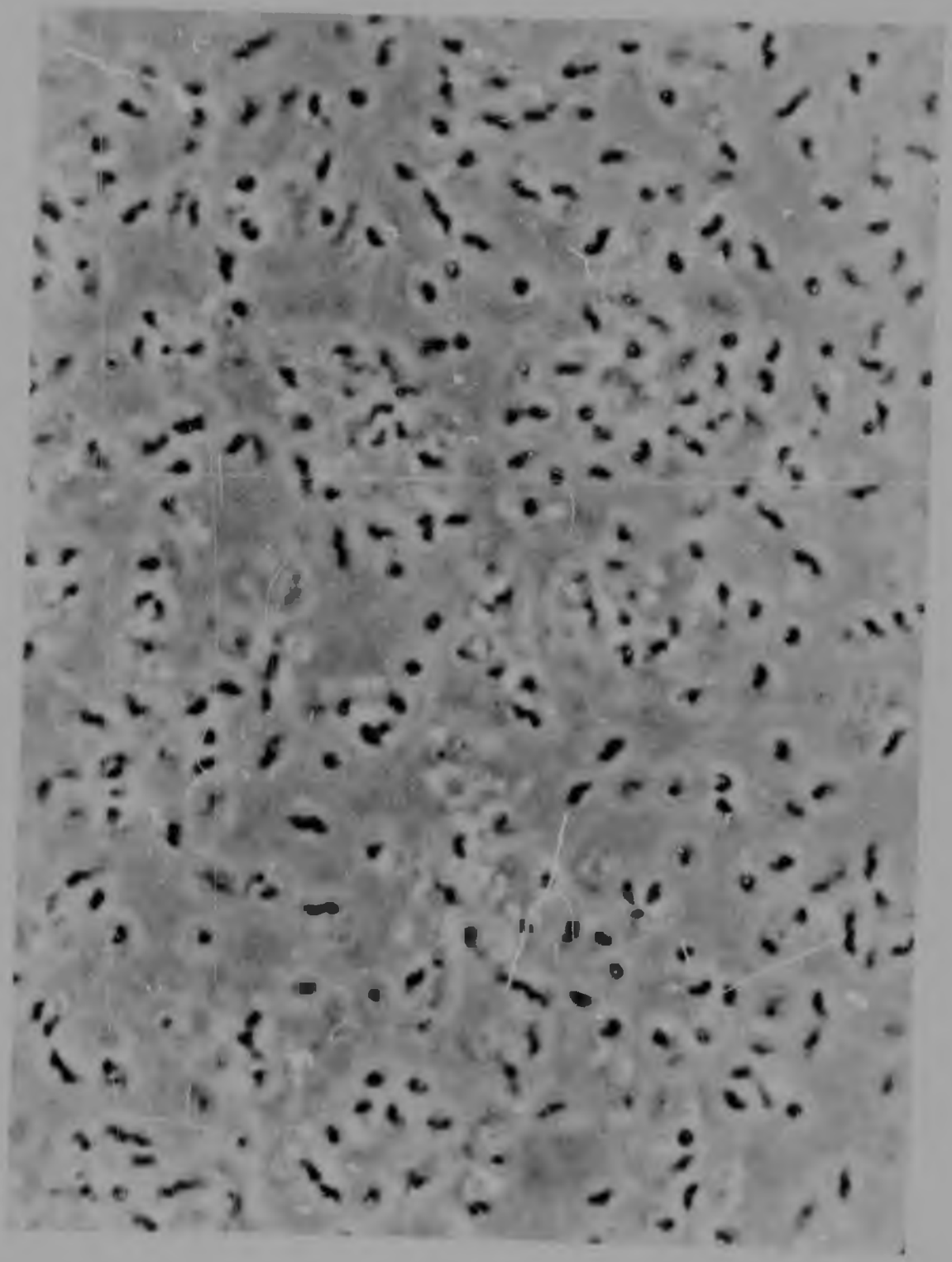


Figure 3.2 Microscopic appearance of *C. jejuni* stained with carbol-fuschin

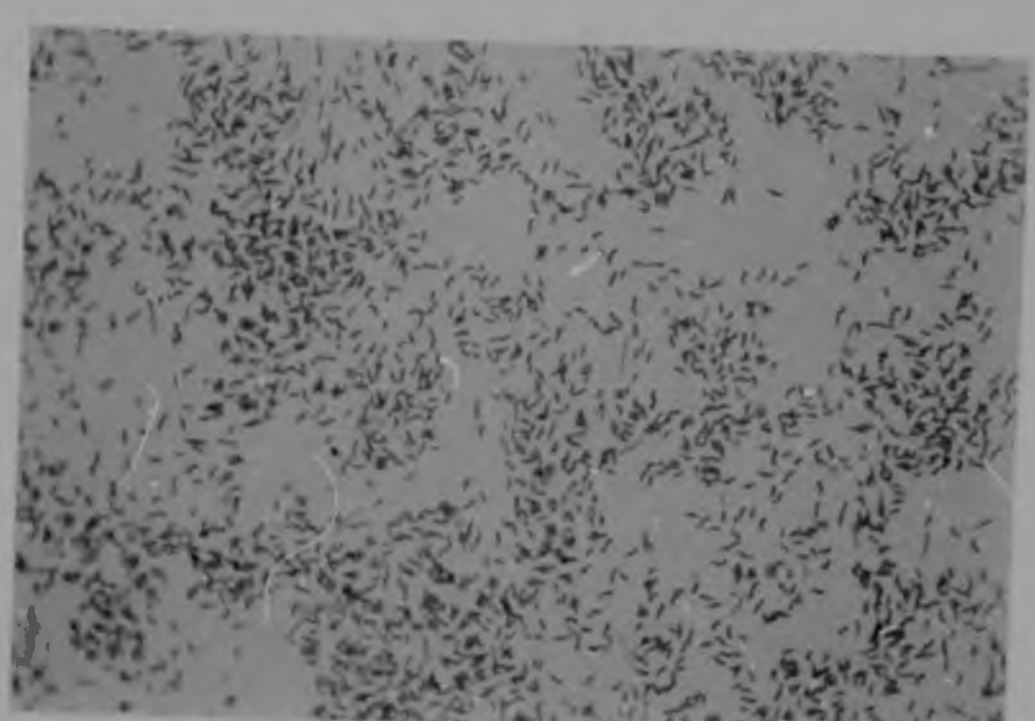
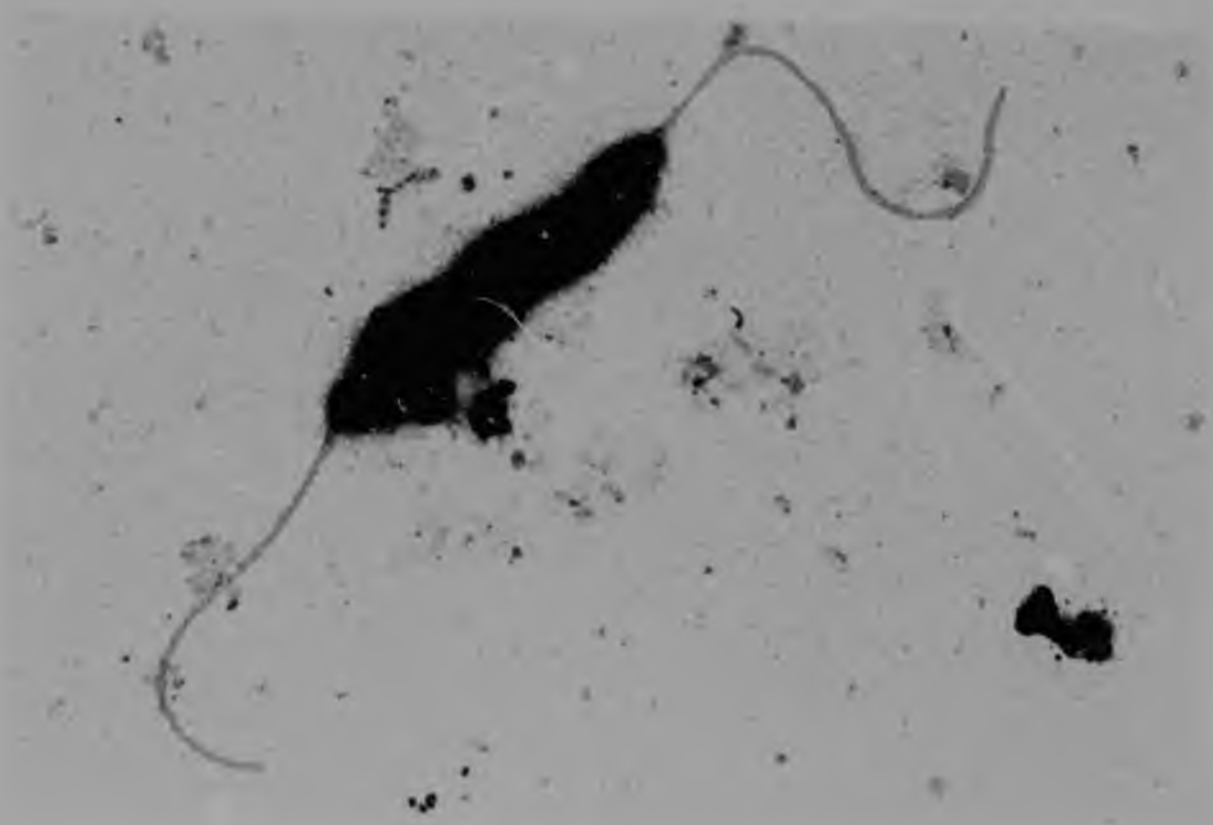


Figure 3.3 Electron micrograph of *C. jejuni*, showing bipolar flagella



superoxide anions and other oxygen derivatives should therefore be beneficial for the growth of these organisms.

Over the years a variety of concentrations of oxygen, carbon dioxide and nitrogen have been used for the cultivation of campylobacters. Based on the atmospheric requirements of *Brucella abortus*, early workers favoured the addition of carbon dioxide for the growth of these organisms which were then known as "spirilla" or "vibrios" (Smith 1918; Smith and Taylor 1919). Reich, Mosse and Wilson (1956) found a considerably better growth in an environment of 6 per cent oxygen in helium than in air. Kiggins and Plastridge during the same year reported an optimum growth of *Vibrio fetus* of bovine origin in an atmosphere of 5 per cent O_2 and 10 per cent CO_2. This was irrespective of whether or not the pressure within the jar was adjusted to 96 per cent of atmospheric pressure by the addition of nitrogen.

More recently workers were able to isolate campylobacters with relative ease in concentrations of CO_2 ranging from 5-10 per cent in air (MacDonald and Mautner 1970; Guerrant, Lakita, Washington, Jnr., and Roberts 1978; Taylor, Weinstein and Bryner 1979; Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979), and in an atmosphere obtained in a candle jar (Hinton 1959; [illegible]ert 1965). The partial evacuation of anaerobe jars and subsequent replacement with CO_2 to produce an atmosphere of 5 per cent O_2, 5-10 per cent CO_2, and 85-90 per cent N_2 is popular (MacDonald and Mautner 1970; Berg, Jutila and Firehammer 1971; Veron and Chatelain 1973; Blaser, Berkowitz, LaForce, Cravens, Reller and Wang 1979).

In a comprehensive study, George, Hoffman, Smibert and Krieg (1978) showed that 17/38 strains of *C. fetus* subsp *intestinalis* grew on brucella agar incubated in air with 2.5 per cent CO_2. When the medium was supplemented with 0.025 per cent (wt/vol) each of ferrous sulphate ($FeSO_4.7H_2O$), sodium metabisulphite and sodium pyruvate (FPB), 25/38 strains grew in the same atmosphere. Reduction of the oxygen content to 6 per cent resulted in colony formation of 34/38 strains on brucella agar and 37/38 on FBP agar. One strain was sensitive to even 6 per cent oxygen and required oxygen tension of 1 per cent or less. Thermophilic campylobacters were more tolerant to oxygen. When incubated on brucella agar in 2.5 per cent CO_2 in air, 13/18 *C. jejuni* strains grew on FBP agar in the same atmosphere and on brucella agar when incubated in 6 per cent oxygen, 2.5 per cent CO_2 and the balance nitrogen.

In recent years various gas mixtures have been used after partial evacuation of air in anaerobic jars. Hydrogen/carbon dioxide gas generating kits (Gas generating kit Oxoid Ltd; Gaspak, Becton Dickinson) have been recommended using a two-jar method (Simmons 1977). Alternatively special kits (Campylobacter gas generating kit, Oxoid Ltd., BBL CampyPak II, Becton Dickinson) are available for use in a jar with a catalyst. This method obviates evacuation of jars since the active catalyst will cause the hydrogen generated to be consumed, thus eliminating the risk of explosion.

Most of the *C. jejuni* reported in this thesis were isolated from stool specimens in an atmosphere of 10 per cent CO_2 in air which gives a reduced oxygen tension of 18 per cent. A study showing the difference in size of *C. jejuni* colonies in two different gas atmospheres will be reported later (see Chapter 4).

3.2.2. Basic Media Requirements

Over the years, campylobacters have been isolated and sub-cultured on a variety of media. Likening the organism to the requirements of the brucella group, some workers used brucella agar (Spink 1957; Smibert 1978). Others used blood agar, with the source of blood coming from humans (Hinton 1959; Hallett, Botha and Logan 1977), horses (Veron and Chatelain 1973; Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979) sheep (Dekeyser, Rossuin-Detrain, Butzler and Sternon 1972; Butzler, Dekeyser, Detrain and Dehaen 1973; Blaser, Berkowitz, LaForce, Cravens, Feller and Wang 1978), bovines (White and Walsh 1970; Border, Firehammer and Myers 1974) and rabbits (King 1957; 1962), in concentrations of either 5, 10 or 15 per cent.

Various basal nutrient broths have in recent years been recommended for addition to solid isolation media. These include Blood Agar Base No. 2, Oxoid CM271 (Skirrow 1977), thioglycolate agar (Lauwers, de Boeck and Butzler 1978) tryptose agar (Difco) or Columbia agar base (Oxoid)(Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979). These bases will again be referred to when the selective media are discussed.

As described under "gaseous requirements", George, Hoffman, Smibert and Krieg (1978) found brucella agar with ferrous sulphate ($FeSO_4.7H_2O$) sodium pyruvate and sodium metabisulphite additives (FBP) to be beneficial to the growth of campylobacters. FBP supplementation is probably not required in media containing lysed blood when cultures are

incubated at 42°C, but offers a significant improvement at an incubation temperature of 37°C (Luechtefeld, Reller, Blaser and Wang 1982).

The moisture content of the medium was shown by Buck and Kelly (1981) to have a profound effect on the size and morphology of colonies of *C. jejuni*. Following drying of the media at 30°C for 24h and 48h the colonies were smaller and more circular with defined edges when dried for 48h compared with slightly larger and moist colonies after a 24h period of drying. Fresh media produced flat watery colonies with an irregular shape and a tendency to spread.

3.2.3. Selective media

The first indication of selective media being used for the isolation of campylobacters came from Plumer, Duwall and Shepler in 1962. They used media containing antibiotics to which *C. fetus* subsp *venerealis* strains were resistant to isolate the organisms from bulls that were *C. fetus* subsp *venerealis* carriers. Dekeyser, Gossuin-Detrain, Butzler and Sternon (1972) used this medium when they first isolated "related vibrios" (*C. jejuni*) from the stools of two nurses, using a filtration technique. The medium contained bacitracin, polymixin B sulphate, novobiocin and cycloheximide. An additional plate used by these workers contained brilliant-green in the medium. This technique had previously been suggested by Florent (1956) to inhibit Gram-positive organisms when searching for *C. fetus* subsp *venereclis* and *C. fetus* subsp *intestinalis* in animals.

Skirrow's selective medium enables stools to be streaked directly onto the plate (Skirrow 1977). The medium contains Oxoid Blood Agar Base No. 2 with 5% lysed horse blood and 10% µg/mℓ of vancomycin, polymixin B sulphate 2.5 I.U/mℓ and trimethoprim lactate 5µg/mℓ. In this medium, lysed horse blood is required for neutralization of trimethoprim antagonists. Vancomycin is incorporated into the medium to inhibit Gram-positive bacteria while polymixin B and trimethoprim prevent growth of *Proteus* and other members of the *Enterobacteriaceae* family.

Butzler's selective medium is made up of thiogycolate agar with 15 per cent sheep blood and bacitracin 2.5 I.U/mℓ, novobiocin 5 µg/mℓ, cycloheximide 50 µg/mℓ, colistin 10 units/mℓ and cefazolin 15µg/mℓ (Lauwers, De Boeck and Butzler 1978; Butzler and Skirrow 1979). This medium is stated to be more selective than Skirrow's. The small colonies obtained on Butzler's medium as described originally may be obviated by the use of 1.5 per cent as opposed to 3 per cent agar concentration.

incubated at 42°C, but offers a significant improvement at an incubation temperature of 37°C (Luechefeld, Roller, Blaser and Wang 1982).

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3.2.3. Selective media

The first indication of selective media being used for the isolation of campylobacters came from Plumer, Duwall and Shepler in 1962. They used media containing antibiotics to which *C. fetus* subsp *venerealis* strains were resistant to isolate the organisms from bulls that were *C. fetus* subsp *venerealis* carriers. Dekeyser, Gossuin-Detrain, Butzler and Sternon (1972) used this medium when they first isolated "related vibrios" (*C. jejuni*) from the stools of two nurses, using a filtration technique. The medium contained bacitracin, polymixin B sulphate, novobiocin and cycloheximide. An additional plate used by these workers contained brilliant-green in the medium. This technique had previously been suggested by Florent (1956) to inhibit Gram-positive organisms when searching for *C. fetus* subsp *venerealis* and *C. fetus* subsp *intestinalis* in animals.

Skirrow's selective medium enables stools to be streaked directly onto the plate (Skirrow 1977). The medium contains Oxoid Blood Agar Base No. 2 with 5% lysed horse blood and 10% µg/mℓ of vancomycin, polymixin B sulphate 2.5 I.U/mℓ and trimethoprim lactate 5µg/mℓ. In this medium, lysed horse blood is required for neutralization of trimethoprim antagonists. Vancomycin is incorporated into the medium to inhibit Gram-positive bacteria while polymixin B and trimethoprim prevent growth of *Proteus* and other members of the *Enterobacteriaceae* family.

Butzler's selective medium is made up of thioglycolate agar with 15 per cent sheep blood and bacitracin 2.5 I.U/mℓ, novobiocin 5 µg/mℓ, cycloheximide 50 µg/mℓ, colistin 10 units/mℓ and cefazolin 15µg/mℓ (Lauwers, De Boeck and Butzler 1978; Butzler and Skirrow 1979). This medium is stated to be more selective than Skirrow's. The small colonies obtained on Butzler's medium as described originally may be obviated by the use of 1.5 per cent as opposed to 3 per cent agar concentration.

Although *C. jejuni* grows at 37°C, selectivity is increased if modifications of the selective media are incubated at 42-43°C. Better growth is also obtained at this temperature compared with 37°C.

Yet another selective medium described by Wang, Blaser and Cravens (1978)included amphotericin B to inhibit yeasts in the stool and cephalothin as well as other antibiotics used in Skirrow's medium. Using this medium, cephalothin was shown to reduce normal enteric flora without loss of *C. jejuni* (Blaser, Berkowitz, LaForce, Cravens, Reller and Wang 1979). As with the other two selective media, the organisms grow well at 42°C.

Investigators at the Mayo Clinic, Gilchrist, Grewell and Washington Jnr. (1981) compared two selective media with brucella agar as the base and containing 10 µg vancomycin per mℓ, 5 µg of trimethoprim per mℓ, 8 I.U. of polymixin B per mℓ and 5 µg of cephalothin per mℓ (Medium C-2) as opposed to the C-3 medium which contained 10 µg of vancomycin per mℓ, 5µg of trimethoprim per mℓ, 2.5I.U of polymixin B per mℓ and 1µg of cephalothin per mℓ, as well as a broth enrichment procedure using "Campy-thio" described by Blaser, Berkowitz, LaForce, Cravens, Reller and Wang (1979). There was little difference in the isolation rates but mixed cultures were more common on C-2 and C-3 media than with the enrichment procedure, but growth was much more rapid on the C-2 and C-3 media. For this reason and because of the simplicity of direct plating compared with a two-step enrichment procedure, the authors preferred direct plating on C-2 or C-3 medium. These authors also showed that the incubation temperature (42°C or 35°C) will effect the activity of colistin and that this should be taken into consideration when the composition of selective media is considered and that lower concentrations of colistin (and polymixin B) should be used when the media are incubated at 42°C.

3.2.4. Enrichment procedures

Several enrichment procedures have been reported which have given good preliminary results (Blaser, Berkowitz, LaForce, Cravens, Reller and Wang 1979; Bruce, Zochowski and Fleming 1980; Lander and Gill 1980). The various formulations combine selective agents, mainly those already used in solid selective media, with a good basal nutrient broth and defibrinated blood (usually horse). Although they may increase isolation rates slightly the disadvantages of an extra procedure with the concommitant extra cost and loss of time mitigate somewhat against their routine use.

3.2.5. Transport media

Ideally faecal samples should be plated on appropriate media and be placed in a suitable atmosphere within two to three hours after collection. If this is not possible the use of a transport medium is advocated.

In an evaluation of five transport media for campylobacters Lior and Krol (1980) found plain Cary-Blair medium and Cary-Blair with agar the best, followed by Stuart s medium with charcoal, Amies' modification of Stuart's medium and buffered glycerol (the latter ga e a poor performance).

The enrichment medium described by Lander and Gill (1980) which suppresses unwanted bacteria and fungi has also been recommended for transportation of specimens.

3.2.6. Maintenance and storage

Campylobacters are notoriously vulnerable to "bench" conditions and die readily unless stored in appropriate maintenance media or subcultured regularly. It is claimed that cultures may be stored satisfactorily at room temperature for up to six months in Tarrozi's liver broth (Ullmann 1979).

A semi-solid yeast extract aspartate nutrient broth (YNAAB) consisting of bacteriological peptone (Oxoid) 5g, Lab Lemco (Oxoid) 4g, yeast extract (Difco) 1g, sodium chloride 5g, potassium L- aspartate 2g, agar (Oxoid technical agar No. 3) 2g and demineralized water 1ℓ, with adjustment of the pH to 7.4 is recommended for both short and long-term maintenance of campylobacter cultures. (Skirrow 1980, personal communication). The organisms remain viable for up to one week when regularly opened for subculturing. For long-term storage cultures are kept in YNAAB at 37°C. Alternatively cultures may be stored in liquid nitrogen or as long as a year or at -20°C for 6 months in FBP broth made up in glycerol instead of water, [Nutrient broth No. 2 (Oxoid CM67) made up in 850 mℓ water and 150 mℓ glycerol, agar P 2g, $Fe_2SO_4.7H_2O$ 0.5g, sodium metabisulphite 0.5g and sodium pyruvate 0.5g .] King stored her stock cultures for as long as two years at -40°C using growth obtained from a 24-hour blood agar slant suspended in 0.5mℓ of sterile defibrinated rabbit blood, placed in a small vial and quick-frozen in alcohol and dry ice (King 1957). Although freeze-drying of campylobacter cultures has not been universally successful, Ullman (1979) claimed prolonged survival for several years.

3.3. Antigenic structure and serology

The antigenic diversity and interrelationships between the various campy-

lobacters are complex and still incompletely understood. The five species of the *Campylobacter* genus possess O and H (Veron and Chatelain, 1973), and K antigens but most of the present knowledge is based on the heat stable (mainly polysaccharide O) antigens (Dennis 1959; Ristic and Brandly 1959; Ristic and Walker 1959; Winter 1966). Relatively little is known about other, including the flagellar antigens.

With regard to the antigenic relationships between the *Campylobacter* species and subspecies Berg, Jutila and Firehammer (1971), based on heat stable antigens and a slide agglutination technique, assigned serotype A to *C. fetus* subsp *venerealis* (including "*C. fetus intermedius*") and some strains of *C. fetus* subsp *intestinalis*, serotype B to other *C. fetus* subsp *intestinalis* strains and serotype C to *C. jejuni/coli*. Apart from these thermostable antigens the authors described multiple thermolabile antigens.

Little information is available on cross reactions between the thermophilic and non-thermophilic campylobacters or between the catalase positive and negative members of the genus. However, King (1957) reported little antigenic cross-reactivity between "related vibrios" and non-thermophilic *C. fetus*, neither was there evidence of major cross-antigenicity with *C. sputorum* subsp *bubulus* isolates.

3.3.1 Serotypes of the *C. fetus* subspecies

Most strains of *C. fetus* subsp. *venerealis* and some of the strains of *C. fetus* subsp *intestinalis* belong to the same serotype (designated 0:1) while probably the remainder of *C. fetus intestinalis* strains belong to serotype 0:2 (Mitsherlich and Liess 1958; Véron and Chatelain 1973). The serotypes A and B of Morgan (1959) and Berg, Jutila and Firehammer (1971) are probably identical to serotypes 0:1 and 0:2 respectively. Véron and Chatelain (1973) tested 26 strains belonging to *C. fetus* subsp *venerealis* and *C. fetus intestinalis* and found that of six *C. fetus* subsp *venerealis* strains, five belonged to serotype 0:1 and one was not agglutinable. Five strains of *C. fetus* subsp *intestinalis* belonged to serotype 0:1 and eight to serotype 0:2 while one strain failed to agglutinate with either of the two serotype antisera.

As mentioned in Chapter 1 most human bacteraemic infections are caused by *C. fetus* subsp *intestinalis* and it is therefore not surprising that, based on somatic antigens, Winkenwerden (1966) and White and Walsh (1970) classified human bacteraemic strains into two distinct serogroups. Bokkenheuser (1972) found that five of eight culture-positive patients had

haemagglutination titres between 1 : 320 and 1 : 3200 against a representative human strain. Two of three seronegative patients were immunocompromised (with leukaemia and lymphosarcoma) while none of the 184 healthy male control patients had significant titres.

3.3.2 Serotyping and serology of *C. jejuni*

Conventional approaches have to date been applied for the serotyping of *C.jejuni* strains. Thermostable antigens obtained by heating the organisms at 100°C for varying periods were used to prepare antisera and unknown strains were typed by passive haemagglutination (Abbott, Dale, Eldridge, Jones and Sutcliff 1980; Penner and Hennessy 1980; Lauwers, Vlaes, and Butzler 1981), tube agglutination (Abbott, Dale, Eldridge, Jones and Sutcliff 1980; Itoh, Saito, Yanagawa, Sakai and Ohashi,1982) and bactericidal (Abbott, Dale, Eldridge, Jones and Sutcliff 1980) techniques. Typing based on thermolabile antigens involved immunising rabbits with formalized whole cell suspensions in some instances(Lior, Woodward, Edgar and La-Roche 1981) and absorption of the sera with heat-treated homologous strains in order to leave antibodies to the heat-labile antigens only. Serotyping was done by slide agglutination of live organisms (Lior,Woodward, Edgar and La Roche 1981; Rogol, Sechter, Braunstein and Gerichter 1982) or tube agglutination of formalized suspensions (Abbott, Dale, Eldridge, Jones and Sutcliff 1980; Itoh, Saito, Yanagawa, Sakai and Ohashi 1982, Lambe and Ferguson 1982; Rogol, Sechter, Braunstein and Gerichter 1982). Lambe and Ferguson (1982) also used direct immunofluorescence to demonstrate thermolabile antigens of *C. jejuni*.

From the abovementioned efforts it is clear that *C.jejuni* has a complex antigenic structure with many serotypes involving both heat-stable O-antigens and heat-labile flagellar and possibly other surface antigens. Undoubtedly further work involving K-antigens will give additional information on the antigenic diversity of *C.jejuni*.

For the serological detection of antibodies in patients with campylobacter enteritis Watson, Kerr and MacFadyean(1979)reported on the use of formalized cultures which showed typical H-type agglutination in 69.6per cent of 66 sera. Watson and Kerr (1982) also used an indirect immunofluorescent technique with live organisms representing two local isolates and achieved an overall positivity rate of 81 percent. Complement fixation with saline suspension heated at 56°C for 30 minutes gave positive results in 62 per cent of 40 culture-positive patients.

Using a pool of glycoprotein antigens from ten strains, Svedhem, Gunnarsson and Kaijser (1982) immunized rabbits subcutaneously in

Freund's complete adjuvant. With the serum obtained, broad cross reactivity between the inoculated strains was demonstrated. Glycoprotein antigens from two of the ten strains and a glycoprotein from an epidemic strain were then used to demonstrate IgM and IgG antibodies in sera of patients and blood donors by means of a diffusion-in-gel enzyme-linked immunosorbent assay (DIG-ELISA). Based on their results, the authors suggested that this test is both effective and simple and could form the basis for the serological diagnosis of *jejuni* enteritis.

Using direct immunofluorescence, workers at the Centers for Disease Control, Atlanta recently defined 10 serotypes of *C. jejuni* and also found 82% of 316 strains to belong to a serogroup characteristic of the species. The same authors also described two serogroups of *C. coli* and *C. fetus* respectively (Herbert, Hollis, Weaver, Steigerwalt, McKinney and Brenner 1983).

It is clear that, in spite of intensive research and the good progress already made, much work has still to be done to elucidate the full antigenic complexity of *C. jejuni*. Thereafter the preparation of suitable antigens and the development of more specific and sensitive techniques should result in more satisfactory tests for the serological diagnosis of the campylobacterioses.

3.4 The antibiotic susceptibility of *C. jejuni*

Several studies to determine the susceptibility of *C. jejuni* strains to a wide range of antibiotics using minimal inhibitory concentration (MIC) techniques have been reported (Chow, Patten and Bednorz 1978; Vanhoof, Gordts, Dierick, Colaens and Butzler 1980; Ahonkhai, Cherubin, Sierra, Bokkenheuser, Shulman and Rosenthal 1981 and Karmali, DeGrandis and Fleming 1981). From these *in vitro* data it is clear that *C. jejuni* is highly susceptible to erythromycin (MIC range 0.06 - 2.0 µg/ml), rosaramicin, gentamicin and other aminoglycosides, tetracyclines, chloramphenicol, furazolidone, clindamycin and metronidazole and much less susceptible to the β-lactam-antibiotics with the exception of N-formimidoyl thienamycin which demonstrated a MIC range of 0.01 - 0.03 µg/ml, as opposed to 2.0 - 8.0 µg/ml for ampicillin and penicillin G, 2.0 - 4.0 µg/ml for cefotaxime and 4.0 - 32 µg/ml for moxolactam. The organisms are resistant to vancomycin, cephalothin and the "first generation" cephalosporins, colistin, trimethoprim and rifampicin. Several of these drugs have been used in selective media used for the isolation of *C. jejuni* from faeces. (Skirrow 1977,

Lauwers, De Boeck and Butzler 1978).

Erythromycin, based on *in vitro* data, is currently regarded as the drug of choice for the treatment of *C. jejuni* infections although tetracycline, gentamicin and chloramphenicol have also been used successfully (Butzler and Skirrow 1979; Karmali and Fleming 1979). However, in a recent double-blind study erythromycin therapy did not affect the clinical course of the disease significantly compared with placebo-treated controls but was successful in shortening the duration of excretion of *C. jejuni* in the stools of treated patients (Anders, Lauer, Paisley and Reller 1982). It is of interest that erythromycin-resistant strains of *C. jejuni* have been documented (Vanhoof, Vanderlinden, Dierich, Lauwers, Yourasowsky and Butzler 1978; Vanhoof, Gerdts, Dierich, Coignau and Butzler 1980; Walder and Forsgren 1978; Walder 1979) but the incidence of such strains is low. The same situation occurs in respect of tetracycline resistance in *C. jejuni* which has been shown to be transmissible (Taylor, DeGrandis, Karmali and Fleming 1980).

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4. THE PRIMARY ISOLATION OF *CAMPYLOBACTER JEJUNI/COLI* FROM FAECAL SPECIMENS

As indicated in the previous chapter (Section 3.2), the effect of media composition, gaseous environment, temperature and duration of incubation on the primary isolation of microaerophilic campylobacters has been the subject of much controversy. With the emergence of *C. jejuni* as an important cause of diarrhoea, it was considered opportune to assess some factors affecting its recovery in the laboratory. In this chapter the effect of growth conditions on the isolation of *C. jejuni* will be assessed, as well as the efficacy of selective media compared with a filtration technique.

4.1 Assessment of the growth conditions for *C. jejuni*

4.1.1 Materials and Methods

4.1.1.1. Sources of *C. jejuni*

Of the nine strains of *C. jejuni* used in the study two were recent isolates from stools of children with diarrhoea and seven were recovered from fowl faeces.

4.1.1.2. Cultural techniques

About 1 g of faeces was shaken in 9.0 mℓ phosphate buffered saline (pH7.2) and centrifuged for 5 minutes at 600 x g. A small volume of approximately 5 mℓ of the supernatant was passed through a prefilter system containing membranes of 8.0 µm and 1.2µm pore sizes respectively and a 0.65 µm sterile membrane (Millipore Corporation, Bedford, Massachusetts). The filtrate was then further diluted to 1/100, and from this dilution, four plates of each of several media being evaluated were seeded with a 200mℓ Oxford pipetter, spread with a glass rod and incubated for 48 hours.

With the filtration technique and further dilution of the filtrate, almost invariably the overwhelming proportion of colonies developing on the non-selective plates were *C. jejuni*. This procedure usually gave a sufficient number of suitably spaced colonies on the various media.

4.1.1.3 Media

Media consisting of 39 g of Columbia agar base (Oxoid, Ltd., Wade Road, Basingstoke, Hampshire) in one litre of distilled water were supplemented with the following citrated additives:

(a) 10 per cent horse blood

(b) 5 per cent horse blood

(c) 5 per cent sheep blood

(d) 5 per cent horse blood heated to 75-80°C to form chocolate agar.

The remaining two media were:

(e) 51 g cysteine heart agar (Difco Laboratories, Detroit, Michigan), 5 mg novobiocin (Albamycin)(Upjohn International Inc., Kalamazoo, Michigan), 1 mℓ of 0.01% brilliant green, 10% citrated horse blood and distilled water to one litre; and

(f) 45 g tryptose-brucella agar (Difco Laboratories, Detroit, Michigan) 10% citrated horse blood and distilled water to one litre.

4.1.1.4 Gas mixtures

(a) 5% O_2; 10% CO_2 and 85% N_2 was piped into Torbal jars at a rate of 200 mℓ per minute for 48 hours. A continuous flow of gas was necessary to avoid a build up of moisture which caused spreading of the colonies.

(b) 5% CO_2 in air.

4.1.1.5 Temperature tolerance

Seeded plates were incubated in both gas environments at 37°C and 42°C.

4.1.1.6 Colony counts

Two specimens of fowl faeces were processed by filtration, diluted and using the Miles and Misra (1938) technique, seeded on the six different media for subsequent counting of colonies.

4.1.1.7 Estimation of colony sizes and identification

Using a 10 x objective and a calibrated micrometer eye piece, the diameter of 25 colonies from each plate was measured after 48 hours incubation. To avoid erroneous readings due to possible competition for nutrients by bacteria, only colonies at least 1 mm apart were measured. Differences in colonial diameters were evaluated by the Students' t-test.

A representative of each group of measured colonies was identified by conventional methods (Holdeman, Cato and Moore 1977)

4.1.2 Results

4.1.2.1 Evaluation of media by colony counts

Whether incubated at 37°C or 42°C in an atmosphere of 5 % O_2, 10% CO_2 and 85% N_2 or in 19% O_2, 5% CO_2 and 76% N_2, no apparent differences in the number of colonies developing were obtained in preliminary experiments. In view of problems associated with the reliability of the techniques

it was decided that continuing colony counting of isolates for the evaluation of different media would be unrewarding.

4.1.2.2 Evaluation of media by colony diameter

Effects of the composition of the medium, the incubation temperature and the gaseous environment on the size of the colonies of wild strains of *C. jejuni* from nine sources are summarised in Figure 4.1. The colony diameter was increased -

(a) by an incubation temperature of 42°C rather than 37°C ($p<0,01$) (Figures 4.2 and 4.3).

(b) on Columbia agar base ($p<0.05$) compared with the other agar bases (Figure 4.1).

(c) by reduced oxygen tension when the medium was supplemented with 5% sheep blood ($p<0.02$). Whether the medium contained 5% or 10% blood or whether the blood had undergone heat treatment (chocolate agar) had little bearing on the diameter of the colonies especially when incubated at 37°C and with reduced oxygen tension. (Figure 4.2). Sheep blood appears to be marginally superior to horse blood. Growth on chocolate agar is better when incubation takes place at 42°C in reduced oxygen tension (Figure 4.4).

4.1.3 Discussion on the effects of growth conditions on the isolation of *C. jejuni*

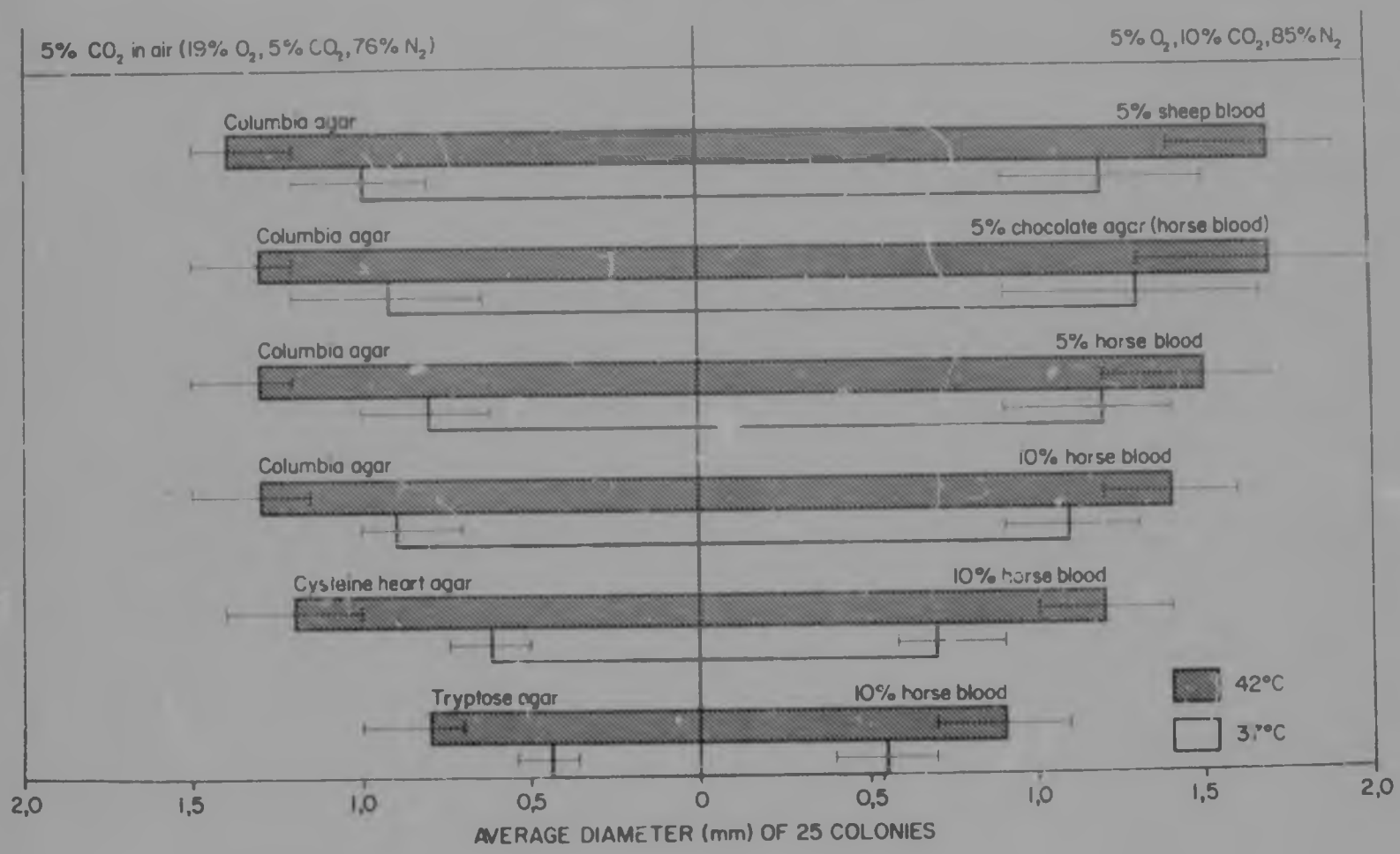
4.1.3.1. Source of blood

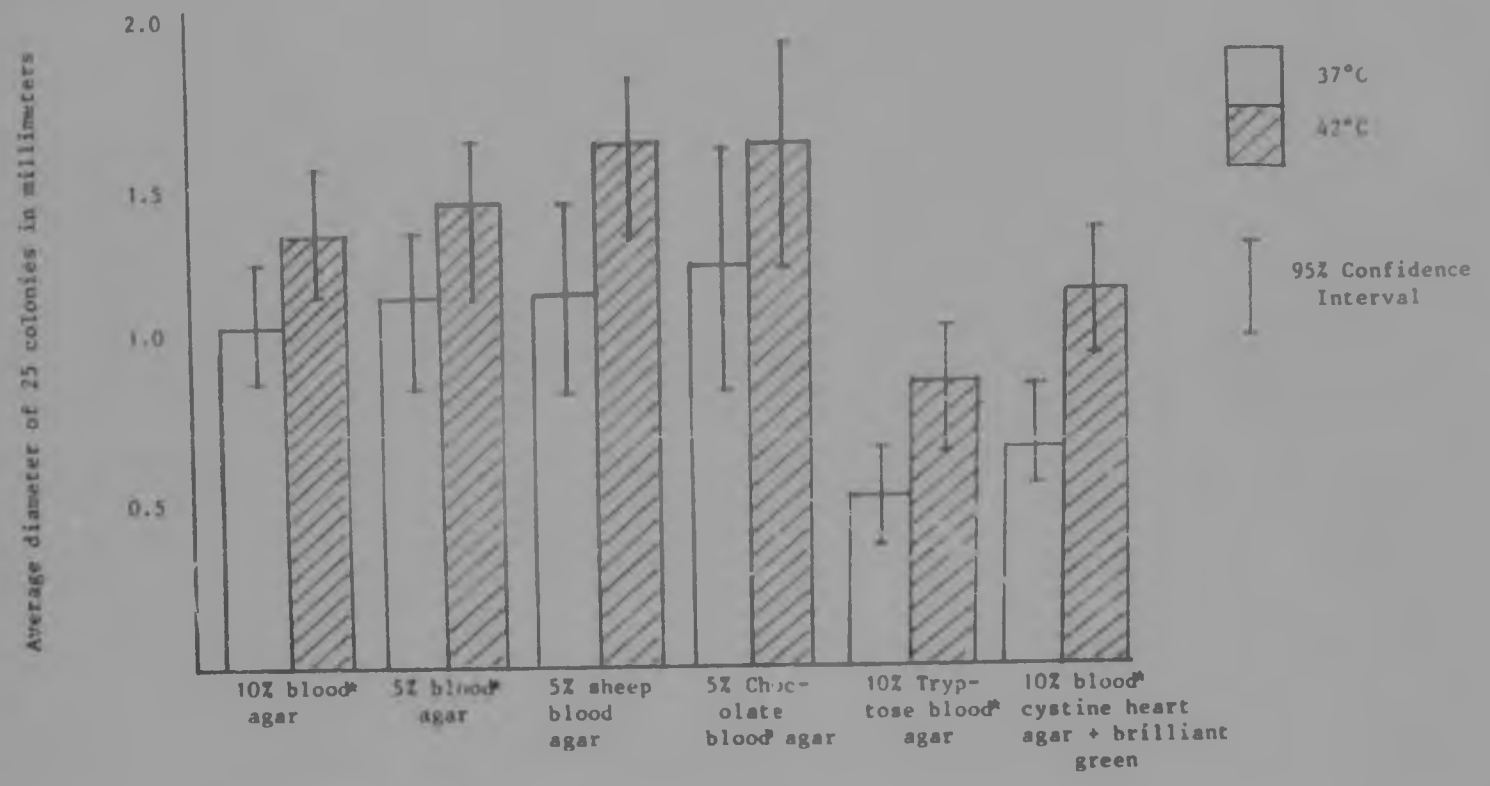
Blood from many sources has been incorporated in media, including horse (Véron and Chatelain 1973) and sheep (Blaser, Berkowitz, La Force, Cravens, Reller and Wang 1979). Although, as pointed out in Section 4.1.2.2, minor differences were obtained in our experiments, neither the number of colonies nor the colonial diameter differed materially whether horse or sheep blood was used or whether these supplements were present in concentrations of 5% or 10%. Likewise heat treatment of horse blood only marginally increased the size of colonies. These factors therefore do not appear to be of overriding importance for routine diagnostic purposes.

4.1.3.2. Media

Of the three nutrient bases evaluated, all supplemented with 10% horse blood, larger colonies developed on Columbia agar than on cysteine heart or on tryptose-brucella agar.

Figure 4.1 : Effect of media composition, temperature and gaseous environment on the diameter of colonies of *C. jejuni* (with 95% confidence intervals)





*Horse blood

Figure 4.2 Influence on temperature on colony sizes of *C. jejuni* seeded on various media and incubated in a gas mixture of 5% O_2, 10% CO_2 and 85% N_2.

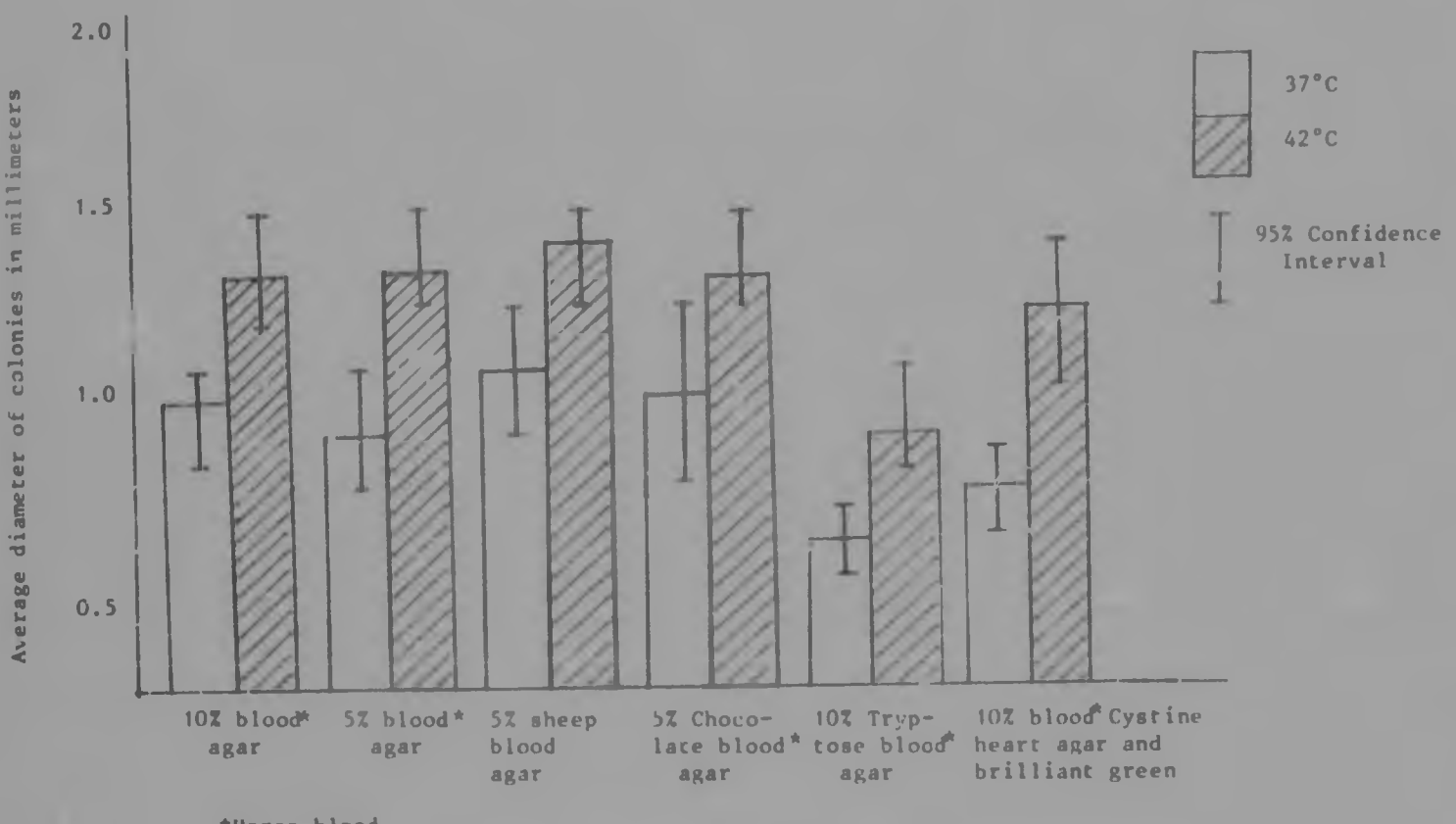


Figure 4.3. Influence of temperature on colony sizes of *C. jejuni* seeded on various media and incubated in a gas mixture of 19% O_2, 5% CO_2 and 76% N_2

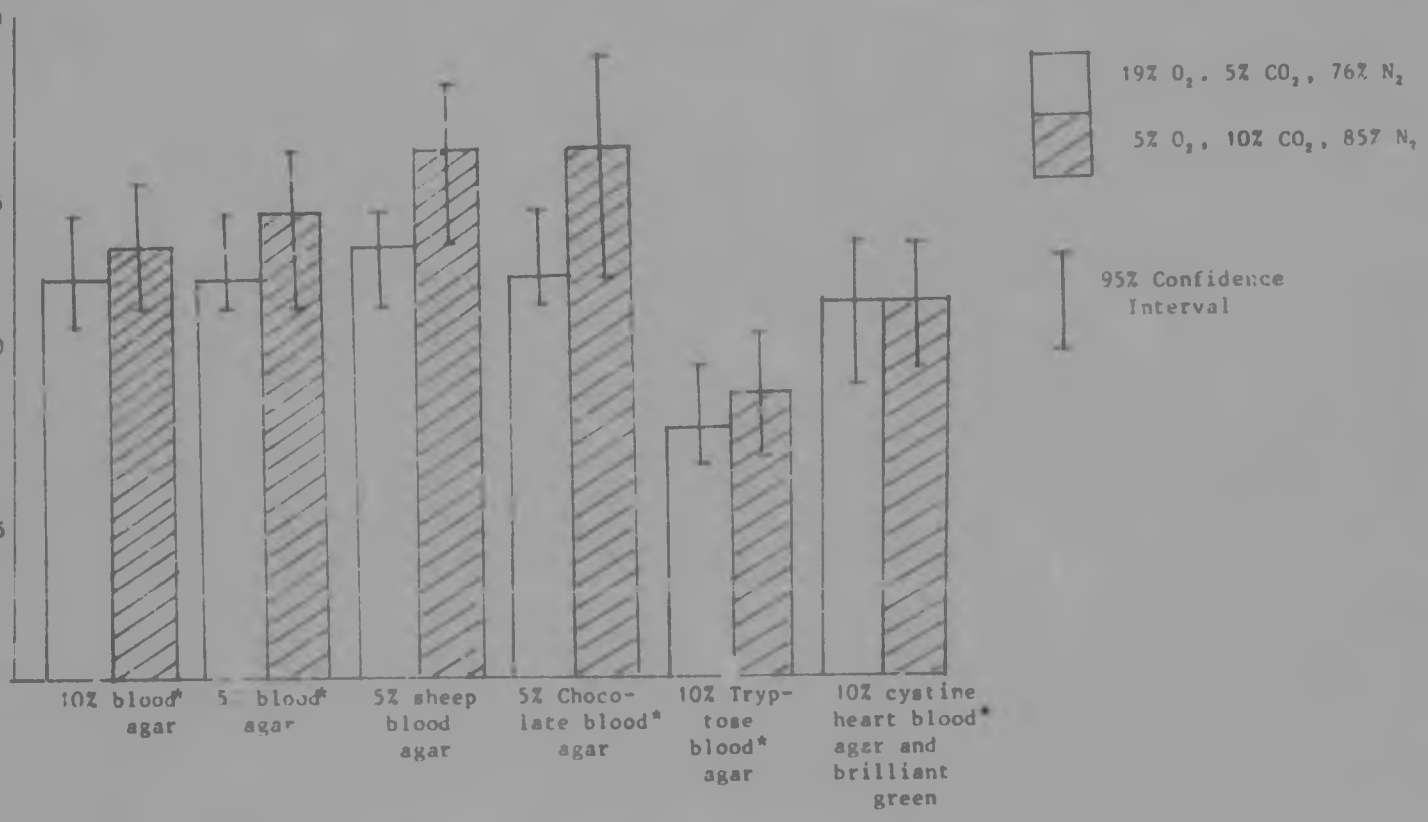


Figure 4.4. Colony sizes of *C. jejuni* seeded on various media and incubated at 42°C in two gas environments

4.1.3.3 Gaseous requirements

The gaseous requirements of *C. fetus* are determined by both the composition of the media and the nature of the subspecies. For example, Guerrant, Lakita, Washington and Roberts (1978) isolated *C. fetus* subsp. *intestinalis* on 5% sheep blood agar in an aerobic atmosphere supplemented with 5% CO_2. In a comprehensive study, George, Hoffman, Smibert and Krieg (1978) showed that 17 of 38 strains of *C. fetus intestinalis* grew on brucella agar incubated in air with 2.5% CO_2. When the medium was supplemented with 0.025% each of $FeSO_4 . 7H_2O$, sodium metabisulphate and sodium pyruvate (FBP agar), 25 of 38 strains grew in the same atmosphere. Reduction of the oxygen content to 6% resulted in colony formation in 34 of 38 strains on brucella agar and 37 of 38 strains on FBP agar. One strain was sensitive to even 6% oxygen and required an oxygen tension of 1% or less.

C. jejuni was more tolerant to oxygen: 13 of 18 strains grew on brucella agar incubated in 2.5% CO_2 in air; all strains grew of FBP with 2.5% CO_2 (George, Hoffman, Smibert and Krieg 1978). These results lend support to the recommendation by Kiggins and Plastridge (1956), Véron and Chatelain (1973) and Blaser, Cravens, Powers and Wang (1978), of using the popular mixture of 5% O_2, 10% CO_2 and 85% N_2. In our experiments only colonies grown on 5% sheep blood agar benefitted from a reduced oxygen tension. On horse blood agar there was no significant difference in the size of colonies whether 5 or 10% oxygen was used, provided 5-10% CO_2 was added to the mixture.

4.1.3.4 Incubation temperature

As pointed out by King (1977), "related vibrios" (*C. jejuni*) grow better at 42°C than at 37°C but the number of colonies hardly varies. Thus, if an incubation temperature of 37°C is used routinely, smaller colonies must be expected.

4.1.3.5 Duration of incubation

Recommendations as to the duration of incubation of primary cultures vary from 1 to 4 days (King 1957; Smith, Marymont Jnr., Schweers 1976). We found that colonies were visible and recognizable after 48 hours incubation. However, under all cultural conditions, larger colonies could be obtained at 72 hours, although after longer incubation periods colonies often consisted of non-viable coccoidal forms.

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4.2 Isolation procedures

Since the introduction of the filtration method (Dekeyser, Gossuin-Detrain, Butzler and Sternon 1972) various selective media which are considered to be less laborious have been widely used for the isolation of *C. jejuni* from faeces. As a high isolation rate had been obtained with the filtration method (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979) it was decided to compare the recovery of *C. jejuni* from human faecal specimens on Skirrow's medium with that of the filtration procedure. At the same time, in a collaborative study with the author of this thesis, Bokkenheuser and Mosenthal compared the filtration technique with three different commercially available selective media using human faeces obtained from 8 patients and 23 unfiltered and 23 filtered specimens of caecal contents from chickens. In addition they seeded faeces with *C. jejuni* for further quantitative evaluation of the procedure. The results of these studies will be discussed in detail in section 4.2.3.1.

4.2.1 Materials and methods

4.2.1.1 Sources of specimens

Stool specimens from 78 children under two years of age, most of whom had diarrhoea and who were all excreting *C. jejuni* in their faeces, were used. The children all came from Soweto and the *C. jejuni* strains were recovered during aetiological studies conducted in Johannesburg (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979; Richardson, Koornhof, Bokkenheuser, Mayet and Rosen 1983). Specimens were transported from Soweto to Johannesburg at the prevailing temperature and processed within four hours of collection.

4.2.1.2 Media

The composition of the blood agar medium (BCA) using Columbia agar base (Oxoid Ltd.,) used in the filtration technique was as follows:

Columbia agar base (Oxoid Ltd)	39 g
Citrated horse blood	100 mℓ (10%)
Distilled H_2O to	1ℓ

Skirrow's medium (Skirrow 1977) contained Blood agar Base No. 2. (Oxoid)

	40 g
Lysed horse blood	70 mℓ
Vancomycin (Lilly Laboratories)	10 mg
Polymyxin B sulphate (Wellcome)	2500 IU
Trimethoprim lactate (Roche)	5 mg

4.2.1.3 Filtration technique

In order to obtain a consistency suitable for filtration, 1 to 2 of soft or 4 mℓ of liquid faeces was diluted in about 10 mℓ of buffered saline (pH 7.2) to form a thin slurry. After vigorous shaking, the suspension was centrifuged at 1000 x g for 10 minutes, and 3-4 mℓ of supernatant fluid was then transferred to a syringe for membrane filtration.

The filtration apparatus consisted of two 25 mm chambers. The non-sterile upper compartment contained two filter membranes (Millipore Inc., Bedford, Mass.) with 8.0µm and 1.2 µm porosity respectively, and the lower sterile chamber contained a membrane with a 0.6 µm pore size. The prefilters were only used when faecal suspensions were considered to be too heavy to pass readily through the 0.65µm membrane. Usually only the large pore size prefilter was used. The first few drops were discarded; then 0.2 mℓ of filtrate was spread over the surface of BCA plates which were incubated at 37°C in an atmosphere of 10% CO_2 in air. The plates were examined after 48 to 72 hours. Colonies morphologically resembling *C. jejuni* were selected and sub-cultured for further identification (see Chapter 5).

4.2.1.4 Culture procedures

The filtrates were inoculated onto the BCA plates and incubated as described in 4.2.1.3. Skirrow's medium was either inoculated with faeces directly or, on a separate plate with the filtrate after membrane filtration. Incubation was at 42°C in an atmosphere of 10% CO_2, 18% O_2 and 72% N_2 . The number of colonies of bacteria other than those of *C. jejuni* was noted.

4.2.1.5 Quantitation of *C. jejuni* in faeces.

One gram of faeces from each of 11 patients with diarrhoea and 9 non-diarrhoeal excretors of *C. jejuni* was mixed with 9 mℓ buffer (pH 7.2), agitated on a Vortex shaker and centrifuged at 600xg for five minutes. The supernatent was decanted into a sterile tube and shaken. A 3.5 mℓ aliquot was filtered according to the technique described above (4.2.1.3.) and several ten-fold dilutions. Volumes of 50µℓ and 20µℓ of the first three dilutions were spread on BCA plates and incubated for 48 hours. Typical *C. jejuni* colonies were counted and representative colonies were selected for identification.

4.2.2 Results of the isolation procedures

4.2.2.1 Evaluation of isolation procedures

The rates achieved by the respective isolation techniques are given in Table 4.1. Of 78 faecal specimens 92% were positive by filtration technique using BCA and 62% by direct plating on SK ($p<0.01$) (McNemar's test). However, six cases missed by filtration were detected by direct plating. The filtration experiments also demonstrate that Skirrow's medium was significantly more inhibitory to primary cultures of *C. jejuni* than the non-selective BCA ($p<0.01$). As could be expected inoculation of Skirrow's medium with raw faeces yielded a higher percentage of positive results than did inoculation with the corresponding filtrate.

4.2.2.2 Quantitative assessment of faecal excretion of *C. jejuni*

Quantitation of *C. jejuni* in the stools of 11 diarrhoeal patients following filtration gave counts ranging from 5×10^{1} to 7×10^{6} per gram of faeces with a mean of 3.2×10^{4}. The corresponding figure in 9 non-diarrhoeal children showed a range of 6.6×10^{2} to 6.1×10^{4} faeces. There was no significant difference in the *C. jejuni* counts between the diarrhoeal and non-diarrhoeal children (Table 4.2) as assessed by the U-test of Mann-Whitney (Siegal 1956).

Appreciably more colonies not belonging to the *Campylobacter* genus were detected on Skirrow's medium than on BCA plates following filtration which contained only occasional contaminating faecal organisms.

4.2.3 Discussion of the isolation procedures

4.2.3.1 Evaluation of isolation procedures

As the studies of Bokkenheuser and Mosenthal referred to earlier were part of a collaborative study with the author of this thesis and they have a direct bearing on the investigations presented in this chapter, their findings will be given in some detail.

Similar to the present study, Bokkenheuser and Mosenthal obtained more positive isolates of *C. jejuni* from the faeces of patients using the filtration technique than when two commercially available media were used. The filtration procedure produced eight positives while two commercial media yielded seven and six campylobacter isolates respectively. The media concerned were Butzler's (BU) medium (Lauwers, de Boeck and

TABLE 4.1. RECOVERY OF *C.JEJUNI* FROM 78 POSITIVE HUMAN FAECAL SPECIMENS.

	Filtration BCA		Skirrow's Medium Seeded Directly		Skirrow's Medium Seeded with Filtrate	
C.jejuni +ve	72[a]	(92%)	48[b]	(62%)	40[c]	(51%)
C.jejuni -ve	6	(8%)	30	(38%)	38	(49%)

For composition of media, see Table 4.2.

McNemar's test of significance

a vs b: $p < 0.01$

a vs c: $p < 0.01$

b vs c: $p < 0.05$

TABLE 4.2. NUMBER OF *C. JEJUNI* ORGANIMS PER GRAM (WET WEIGHT) IN FAECES FROM DIARRHOEAL AND NON-DIARRHOEAL (CONTROL) BABIES

DIARRHOEAL BABIES		NON - DIARRHOEAL BABIES (CONTROL)	
No. of Baby	*C. jejuni*[a] per gram	No. of Baby	*C. jejuni* per gram
Z 64	1.8×10^5	J 71	2.0×10^3
Z 65	3.5×10^5	J 72	2.1×10^4
Z 71	3.9×10^3	J 74	1.2×10^4
Z 78	9.1×10^3	J 76	4.4×10^4
Z 83	2.2×10^4	J 79	3.6×10^3
Z 89	2.5×10^3	J 80	6.1×10^4
Z 90	7.0×10^6	J 84	6.6×10^2
Z 91	5.4×10^3	J 91	2.5×10^4
Z 93	3.1×10^3	J 93	8.0×10^2
Z 102	4.0×10^4		
Z 111	2.0×10^4		

No significant difference (Wilcoxon's Sum of Rank's test)
(U-test of Mann-Whitney)

[a] Colony forming units/gram of faeces

Butzler 1978) and one (KF) described by Karmali and Fleming (1979). All eight *C. jejuni* isolates were recovered from another commercial medium (BL) described by Blaser, Berkowitz, La Force, Cravens, Reller and Wang (1979). The composition of these media are given in Table 4.3.

Although six of the 78 *C. jejuni*-positive stool specimens recovered on Skirrow's medium were missed by the filtration technique, a markedly better isolation rate was achieved with the latter method. In a separate experiment using a *C. jejuni* suspension in broth, a ten-fold decrease in the number of *C. jejuni* colonies developing on BCA plates following filtration was obtained when compared with direct plating of the same culture suspension without filtration. Furthermore in quantitative studies with caecal material from chickens Bokkenheuser and Mosenthal found the BL, KF and BU selective media to be much more sensitive than filtration (Table 4.4).

Further information on the sensitivity and specificity of selective media was obtained by Bokkenheuser and Mosenthal in experiments with human faeces seeded with predetermined quantities of *C. jejuni*. Pure suspensions of *C. jejuni* yielded similar numbers of colony-forming units whether inoculated on to chocolate agar without antibiotics or on to the selective media. The sensitivity of these media was reduced by 50 to 75 percent when *C. jejuni* was mixed with faecal flora and there was an inverse relationship between the concentration of non-*C. jejuni* organisms and the sensitivity of the selective media (Figure 4 5). The specificity of the three media tested was similar. Common concomitant faecal bacteria found on the selective plates were *E.coli* (BL BU, KF), *Proteus* and *Pseudomonas* (BU), streptococci (BL,KF) and yeasts (KF). With the filtration technique, as was the case in the present study, hardly any competitive faecal organisms were encountered.

The abovementioned findings clearly show that the concomitant faecal organisms affect the sensitivity of selective media for *C.jejuni* adversely but less so than is the case with the filtration technique. The better performance of the latter technique compared with Skirrow's medium in aetiological studies in Johannesburg appears to be mainly due to differences in specificity of the two techniques as a result of the interference of concomitant intestinal bacteria with the recovery of *jejuni*. This interference was more marked in the case of Skirrow's medium.

TABLE 4.3. COMPOSITION OF ISOLATION MEDIA (PER LITRE) USED BY BOKKENHEUSER AND MOSENTHAL (KF, BL, BU) AND RICHARDSON (BCA, SK).

	Blood Columbia Agar (BCA)	Skirrow's Medium (SK)	Karmali and Fleming Medium (KF)	Blaser Medium (BL)	Butzler's Medium (BU)
Columbia Agar	39g		42.5g		39g
Brucella Agar				43g	
Blood Agar Base No.2 (Oxoid)		40g			
Horse Blood	100ml	70ml	70ml		70ml
Sheep Blood				100ml	
Novobiocin					2.5mg
Vancomycin		10mg	10mg	10mg	
Polymixin B Sulphate		2500 IU	30000 IU	2500 IU	
Trimethoprim lactate		5mg		5mg	
Cephalothin				15mg	
Amphotericin B				2mg	
Bacitracin					12500 IU
Colistin sulphate					5000 IU
Cephazolin sodium					7.5mg
Cyclohexamide					25mg

TABLE 4.4. QUANTITATION OF *C.JEJUNI* FROM CHICKEN CAECA BY FILTRATION AND SELECTIVE MEDIA

(Bokkenheuser and Mosenthal)

CAECAL MATERIAL		FILTRATION[a] (Choc. agar)	KF[a]	BL[a]	BU[a]
Unfiltered N = 23	Range	4.2 - 7.9[b]	5.3 - 8.5	5.6 - 8.4	5.0 - 8.3
	Geom. Mean	6.3[b]	7.0	7.1	6.6
	Rel. Sens. of Methods	1[c]	1	5.5	2
Filtered N = 23	Range	4.2 - 7.9	4.3 - 7.9	4.0 - 7.8	4.3 - 7.9
	Geom. Mean	6.3	6.2	6.3	6.0
	Rel. Sens. of Media	2	1.5	2	1[c]

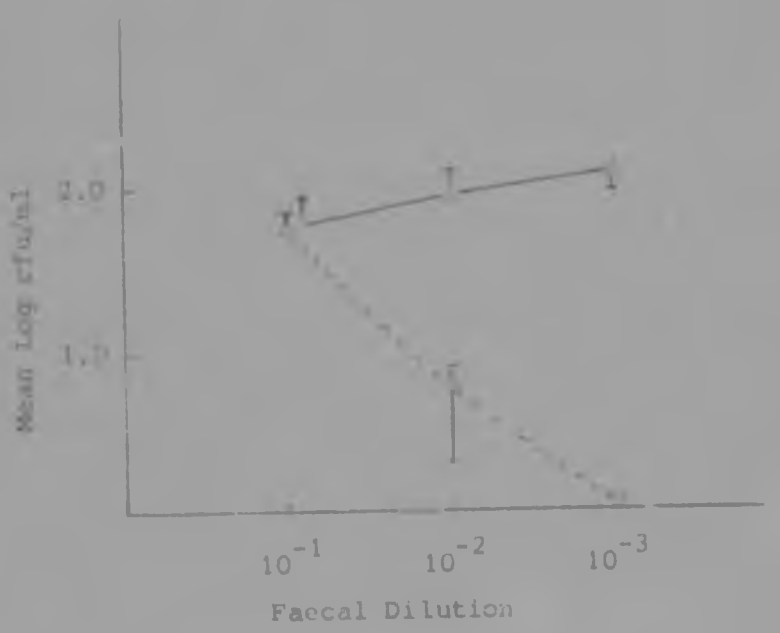
a. For identification and composition of media and methods, see Table 4.3.

b. Log_{10} *C.jejuni*/g caecal contents

c. The relative sensitivity is calculated by assigning 1 to the lowest geometric mean.

FIGURE 4.5. EFFECT OF FAECAL ORGANISMS ON ISOLATION OF *C.JEJUNI* ON SELECTIVE MEDIA. (Blokkenheuser and Mosenthal).

Dilutions of fresh human faeces admixed with a fixed number of *C.jejuni* (2.5×10^3/ml)



o - - o Competing faecal organisms

Δ——Δ *C.jejuni*

Another possible factor which has not been clearly resolved in these studies is the effect of temperature on the isolation of *C. jejuni* from human faeces. In the aetiological studies in Johannesburg the incubation temperature for the filtration procedure was 37°C while that for Skirrow's medium was the recommended 42°C. *C. jejuni* may have become adapted to the normal human body temperature and 37°C may therefore be an appropriate t rature for the primary isolation of *C. jejuni* from human faeces, especially when processed and incubated soon after collection. It is interesting to note that Janssen and Helstad (1982) found that although the incubation of faecal samples in Skir ow's medium yielded significantly more isolates of *C. jejuni* at 42°C than at 35°C, this difference disappeared when gross delays were avoided and the samples were processed on the day of collection. Also, Skirrow's medium incubated at 35°C will allow the growth of more faecal contaminants than incubation at 42°C and this may have been an important reason why Skirrow's medium performed poorly at 35°C in the Janssen and Helstad study. This problem is much less applicable to the filtration tech que with ncubation at 37°C, because hardly any faecal contaminants are present.

4.2.3.2 Quantitative assessment of faecal excretion of *C. jejuni*

Caution should be exercised when interpreting the quantitative comparison of *C. jejuni* isolations from patients with enteritis and control subjects without diarrhoea. The filtration technique is obviously not sufficiently sensitive for such a study. Painstaking dilution methodology without the use of filtration or selective media would be the most reliable approach for quantitative studies but would technically be extremely difficult and laborious. Other unknown factors in quantitative comparisons of this kind are the kinetics of *C. jejuni* excretion during the various stages of a diarrhoeal episode and during intermittent shedding of *C. jejuni* in non-diarrhoeal excretors. For a meaningful comparison, specimens produced at the peak excretion period during diarrhoea should be compared with a series of specimens from *C. jejuni* excretors without diarrhoea. Such studies would be cumbersome and were not contemplated for this thesis.

4.3 Concluding remarks

The initial actiological studies on gastro-enteritis in Soweto in which the filtration technique was used preceded the evaluation of techniques for the isolation of *C. jejuni* from faeces recorded in this chapter. However, the filtration procedure yielded sufficiently good results to warrant its continued use in further studies presented in this thesis. This decision was also considered to be justifiable as the consistent use of the same methodology would render comparison of data more meaningful.

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5. THE ISOLATION OF *C. JEJUNI* FROM BABIES UNDER TWO YEARS OF AGE WITH AND WITHOUT GASTRO-ENTERITIS - A PRELIMINARY STUDY

5.1. Introduction. Events leading up to study.

As described in the introduction Bokkenheuser and Dunston (1975), in a serological survey, using a haemagglutination technique showed that antibody titres to *C. fetus* subsp *intestinalis* were four times higher in preparations of commercial human gamma globulin obtained from South Africa than in batches from other parts of the world. Previously it has been observed that cases of disseminated *C. jejuni* infection are often associated with diarrhoea. (King, 1962 Kilo, Hageman and Marzi, 1965). Furthermore, five cases had been reported in which both blood and stool cultures yielded *C. jejuni* (Schewitz and Roux 1978; Butzler, Dereume, Barbier, Smekens and Dekeyser 1977).

These findings together with the fact that many cases of infantile gastro-enteritis in South Africa were still of unknown aetiology, prompted a search for campylobacters in the faeces of children with and without diarrhoea. At the same time such a study provided an opportunity to characterize faecal isolates from Sowetan children.

5.2. Materials and Methods

5.2.1. Selection of children

The investigation was conducted in the gastroenteritis ward of the Paediatric Division of the Baragwanath Hospital during the summer of 1977.
The hospital serves the residents of Soweto on the outskirts of Johannesburg.
Stool specimens were obtained from 78 black children, 0 to two years old with acute gastroenteritis. Specimens from non-diarrhoea cases were collected from napkins of children of the same age attending the clinics. According to their mothers, they had not had diarrhoea for at least two weeks and had not received antibiotics recently. Because of administrative difficulties it was not possible to collect specimens from the controls at the same time as the diarrhoea cases.

The children were examined clinically and notes were made of their nutritional status (Alleyne, Hay, Picou, Stanfield and Whitehead 1977) and of the degree of dehydration (mild: sunken eyes or impaired turgor; moderate: sunken eyes and fontanelle and poor turgor; severe: features of

moderate dehydration associated with shock and/or acidosis).

5.2.2. Faecal specimens

Within two hours of arrival in the hospital, faecal specimens were obtained from the children. In some cases , mechanical stimulation of the rectum was required. The specimens were transported to Johannesburg at the prevailing temperature and processed within four hours of collection. Special arrangements were made for the transportation of faecal specimens from the non-diarrhoeal control infants and children from the clinics to the laboratory and t' ere also processed within four hours.

5.2.3. Clot cultures

Blood samples were taken from all patients with gastro-enteritis. After the serum had been removed, the clot was incubated at 37°C overnight in 10 mℓ of streptokinase broth (meat infusion broth, one litre; sodium taurocholate, 5g; streptokinase [illegible] Laboratories, Pearl River, N.Y.) in a 10% CO_2 atmosphere. The mixture was then transferred to Todd-Hewitt broth (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated further at 37°C in 10% CO_2. Subcultures on to 10% horse blood agar were made after 10 days.

5.2.4. Isolation of *Salmonella* and *Shigella* from stool specimens

Salmonellae and shigellae were isolated, identified to species level and serotyped with conventional techniques. Enteropathogenic *Escherichia coli* were characterised by conventional analysis of the O antigens of *E. coli* strains.

5.2.5. Isolation of *C. jejuni*

5.2.5.1. Media. Three media were used routinely:

(a) 45g of tryptose agar (Difco Laboratories, Detroit, Mich.), 100 mℓ of citrated horse blood, and distilled water to 1 litre (BTA);
(b) 51g of cystine heart agar (Difco Laboratories), 100 mℓ of citrated horse blood, 8 mg of novobiocin (Albamycin, Upjohn, Kalamazoo, Mich.), 1 mℓ of 0.1% brilliant green, and distilled water to 1 litre (BCHA);
(c) 39g of Columbia agar base (Oxoid Ltd), 10% citrated horse blood, and distilled water to 1 litre (BCA).

5.2.5.2. Cultural technique

To obtain a consistency suitable for filtration, 1 to 2 g of soft or 4 mℓ

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of liquid faeces were diluted in about 6 mℓ of buffered saline (pH 7.2) to form a thin slurry. After vigorous shaking, the suspension was centrifuged at 600 x g for 10 minutes. From three to four mℓ of supernatant fluid was transferred to a syringe for membrane filtration (0.65 µm; Millipore Corp., Bedford, Mass.). The first few drops were discarded; then 0.2 mℓ of filtrate was spread over the surface of BTA and BCHA plates which were then incubated at 37°C in an atmosphere of 10% CO_2 in air. The plates were examined daily for 5 days. Colonies morphologically resembling *C. jejuni* were selected and subcultured for further examination.

5.2.6. Identification and characterization of *C. jejuni* isolates

5.2.6.1. Morphology

In the Gram stain 0.2% carbol fuschin was employed as the counterstain. Due to the low refractory index of the organisms, motility was examined by phase-contrast or dark field microscopy.

5.2.6.2. Aerotolerance

Duplicate BCA plates were seeded and incubated for 3 days, one under aerobic conditions and the other in a 10 per cent CO_2 atmosphere.

5.2.6.3. Biochemical identification procedures

The strains were identified to subspecies level by conventional methods(Holdeman Cato and Moore 1977). Briefly, Albimi broth (Pfizer Diagnostics, New York) was supplemented with 0.15% agar and one of the following: 1% glucose, 1% glycerine, 3.5% NaCl, 8% glucose or (to detect H_2S production) 0.02 or 0.05 % cystine. BCA plates supplemented with cephalothin (3µg/mℓ), or triphenyltetrazolium chloride (0.4 mg/mℓ) were inoculated for an extended identification profile. (Charlier, Dekeyser, Florent, Strobbe and De Ley 1974)

5.2.6.4. Temperature tolerance

Replicate plates of BCA were seeded and incubated under microaerophilic conditions (7% O_2, 81% N_2, 6% CO_2, and 6% H_2) at 25, 37 or 42°C for 2 days.

5.2.6.5. Immunofluorescence techniques

The direct test was performed with fluorescein isothiocyanate-conjugated rabbit antisera prepared against one strain *C. fetus* subsp *fetus*,6 strains of *C. fetus* subsp *intestinalis* representing both antigenic types, one strain of *Campylobacter sputorum* subsp *bubulus*, and three strains of *C. jejuni* (one of animal and two of human origin). Air dried smears of bacterial

suspensions were fixed for 10 minutes in 95% ethanol, rinsed in distilled water, and dried. The slides, flooded with conjugate, were incubated in moist chambers for 30 minutes, rinsed and dried. Cover slips were mounted with phosphate-buffered glycerol (pH 8.6).

Indirect immunofluorescence determinations were performed with rabbit antiserum and fluorescein-conjugated goat and anti-rabbit gamma globulin (Cherry, Goldman and Carsky 1960; Herbert, Pelham and Pittman 1973; Herbert 1974). Rabbit antisera were prepared against *C. fetus* subsp *fetus* (A-1) *C. fetus* subsp *intestinalis* (A-2 and B), and *C. jejuni* (C). The slides were prepared and stained by conventional techniques.

Control slides incorporated with all batches, revealed good staining with homologous bacterial strains Heterologous strains did not stain.

5.2.6.6. Agglutination of isolates

The strains were grown on Albimi agar (Pfizer Diagnostics) and harvested in buffered saline (pH 6.8) containing 0.3% formaldehyde. One drop of a suspension previously adjusted to 10 times 0.6 optical density unit at 550nm, was mixed on a glass plate with 50 µℓ of hyperimmune rabbit serum. After gentle rotation of the plate for 3 minutes the [illegible] was read in a Bang's box (a lighted box with a glass top). The strains were tested for autoagglutinability in a mixture of normal serum and saline.

5.2.6.7. Phage typing

Phages I to IV, originally recovered from *C. fetus* subsp *fetus* were propagated in *C. fetus* subsp *intestinalis*. These phages lysed strains of both subspecies but not *C. jejuni*. Phage C, obtained from and propagated in *C. jejuni*, lysed strains of the latter species only. The test was formed by John H. Bryner according to previously published methods (Bryner, Ritchie, Booth and Foley 1973).

5.2.6.8. Antibiotic susceptibility testing

Minimal inhibitory concentration determinations were performed on plates containing graded concentrations of the different antimicrobial agents. Sulphonamide antagonistic-free agar (Mast Laboratories, Ltd., Liverpool, England) was used throughout and was supplemented with 0.5% lysed horse blood for sulphonamide and cotrimoxazole minimum inhibitory concentration estimations. The plates inoculated with 0.01mℓ of a suspension containing approximately 10^6 bacteria per mℓ were incubated at 37°C in a 10% CO_2 atmosphere for 2 days.

Antibiotic susceptibility was also tested by a disc diffusion method

on 5% horse blood agar plates (Oxoid Columbia base) with an inoculum obtained from 48h cultures in Todd-Hewitt broth (Oxoid) adjusted to a density corresponding to 0.5 MacFarland No. 1. barium sulphate standard. For sulphonamide and cotrimoxazole susceptibility testing, a smaller inoculum containing about 10^3 bacteria per mℓ (Hawkins and Green 1945) was used on sulphonamide antagonistic-free plates containing 0.5% lysed horse blood. Plates were inoculated with a sterile cotton swab on a wooden applicator and incubated as described above. The disc contents of the different antimicrobial agents are given in Table 5.3. Inhibition zones were measured in millimeters.

5.3. RESULTS

5.3.1. Isolates from stool specimens

C. jejuni was recovered from 35% of children with diarrhoea and from 16% of asymptomatic children (Table 5.1). The incidence was related neither to age nor to sex. The difference was most pronounced in the 0 to 8 month age group (32% positive for *C. jejuni* in the group with diarrhoea compared with 4% of the non-diarrhoeal). By the time the children were 9 to 24 months of age this difference no longer existed (39 and 44% in diarrhoea and non-diarrhoeal groups respectively). The faecal samples, in addition, yielded growth of shigella in one case, salmonella in 10 cases and in 36 cases serologically established enteropathic *E. coli* were isolated.

TABLE 5.1. ISOLATES FROM STOOL SPECIMENS

Clinical status	Age (Months)	No of children	% Positive with			
			C. jejuni	Salmonella	Shigella	EPEC *
Diarrhoea	0-8	47	32	0	2	38
	9-24	31	39	16	0	23
	Total	78	35	6	1	32
No diarrhoea	0-8	45	4	2	0	13
	9-24	18	44	22	0	28
	Total	63	16	8		17

* Enteropathogenic *E. coli*

5.3.1.1 Subjects yielding *C. jejuni* only

C. jejuni was the sole bacterial pathogen in the faeces of 34% of cases with diarrhoea (Table 5.2).

TABLE 5.2 CHILDREN WITH *C. JEJUNI* AS THE SOLE PATHOGEN

Clinical Status	Age (Months)	No. of children	No. positive(%)
Diarrhoea	0-8	29	9(31)[a]
	9-24	21	8(38)
	Total	50	17(34)
No diarrhoea	0-8	38	2(5)[a]
	9-24	10	4(40)
	Total	48	6(13)

[a] $\chi^2 = 7.92$; $P < 0.05$

The recovery rate was similar in younger and older diarrhoeal children. This compared with a prevalence of *C. jejuni* in non-diarrhoeal children of 13%, increasing from 5% in the youngest to 40% in the oldest group. The younger children are of particular interest. In this group *C. jejuni* was isolated from 31% of those with diarrhoea when compared with 5% from the asymptomatic children, a highly significant difference ($\chi^2 = 7.92$; $P < 0.05$).

In one child, with diarrhoea (V54), similar strains of *C. jejuni* were recovered from the stool and blood clot. For many children the present illness was their first experience with gastro-enteritis.

5.3.2 Clinical features

The stools of children with *C. jejuni* associated diarrhoea usually were very soft to watery and often were bile stained and contained flakes of mucus. A history of vomiting was elicited in the vast majority of cases In 2/3 of the cases, the temperature ranged from normal to 38°C. Hyperpyrexia was not observed. Mild dehydration was common; severe dehydration and/or malnutrition occurred in 10% of the children.

There was no clear correlation between diet and infection. It is noteworthy, however, that *C. jejuni* was recovered from the stools of twins who were breast fed only.

C. jejuni was isolated from 17% of the children with diarrhoea on the first day of illness and from 67% within the first week.

5.3.3 Treatment

On admission, electrolyte imbalance of dehydrated children was immediately corrected. Antibiotics were withheld in uncomplicated cases. Penicillin G

or ampicillin was given to 17 of 28 children who were infected with *C. jejuni* and showed respiratory tract complications. Candidiasis was treated with nystatin.

All infected patients, including the child with bacteraemia, made an uneventful recovery, in most cases within 3 to 6 days.

5.3.4. Identification of *C. jejuni*

Because of their similarity, isolates from diarrhoeal and non-diarrhoeal cases are considered together.

5.3.4.1. Biochemical and tolerance tests

All isolates were H_2S positive on cystine media, tolerant to 1% glycine, and capable of growing at 42°C but not at 25°C (Table 5.3). The majority of strains were catalase positive, nitrate reducers, and resistant to cephalothin (3 µg/mℓ) and nalidixic acid (3µg/mℓ)

TABLE 5.3. BIOCHEMICAL CHARACTERIZATION OF 39 PRESUMPTIVE *C. JEJUNI* STRAINS RECOVERED FROM DIARRHOEAL AND NON-DIARRHOEAL CHILDREN

Test	No. of strains positive
Catalase	38
H_2S in cystine 0.02%	35
H_2S in cystine 0.05%	39
NO_3 reduction	36
Growth in 1% glycine	39
Growth in 3.5% NaCl	0
Growth on tetrazolium agar	3
Growth in 8% glucose	0
Growth in thiol broth	39
Growth on cephalothin	33
Growth on nalidixic agar	35
Growth at 42 and 37°C but not at 25°C	39

5.3.4.2. Agglutination patterns

Most of the isolates were agglutinated by antiserum to *C. jejuni* strain 958 (Table 5.4). A few strains also reacted weakly with antisera to *C. fetus* subsp *fetus* and *C. fetus* subsp *intestinalis*. Strain K35 agglutinated strongly with the two latter anti-sera but not at all with antiserum to *C. jejuni*. Four strains were not agglutinated by any of the antisera.

TABLE 5.4. AGGLUTINATION OF 21 ISOLATES

Antisera to	No. of strains showing agglutination			
	None	+	++	+++
C. fetus subsp *fetus* [a]	15	1	2	1[b]
C. fetus subsp *intestinalis* [c]	14	3	3	1[b]
C. jejuni [d]	6	3	10	2

[a] Serotype A-1 strain 1289
[b] Isolate K35
[c] Serotype B, strain 1083
[d] Serotype C, strain 958

5.3.4.3. Direct immunofluorescence

Cellular staining of the isolates was noted, but flagellar staining, which occurred with many antisera, was ignored. Fluorescein-conjugated antisera to human isolates of *C. jejuni*, PB 1/175 and PB 1/68, reacted with 39 and 32 of 39 isolates, respectively (Table 5.5).

TABLE 5.5. SPECIES IDENTIFICATION OF 39 ISOLATES BY DIRECT IMMUNOFLUORESCENCE

Conjugated antisera to	No. of strains showing fluorescence		
	None	Weak	Good
C. sputorum subsp. *bubulus* (1 strain)	39		
C. fetus subsp *fetus* (1 strain)	39		
C. fetus subsp *intestinalis* (6 strains)	34	4	1
C. jejuni (porcine; AF/58/3)	2[a]	13	24
C. jejuni (human; PB 1/68, PB 1/175)	0	14	25

[a] Isolates V77 and K35.

A conjugate of antiserum to *C. jejuni* (AF/58/3) of porcine origin reacted with 37 of 39 strains. Five isolates were stained with one of the six *C. fetus* subsp *intestinalis* conjugates, but no two strains reacted with the same conjugate; conjugates to *C. fetus* subsp *fetus* and *C. sputorum* subsp *bubulus* did not stain any of the strains. K35 was one of the two

isolates that failed to react with *C. jejuni* (AF/58/3) conjugate.

The results of the direct fluorescence test were essentially confirmed by the indirect method (Table 5.6). However, reactions were weaker and more scattered.

TABLE 5.6. SPECIES IDENTIFICATION OF 25 ISOLATES BY INDIRECT FLUORESCENCE

Antisera to	No. of strains showing indirect fluorescence		
	None	Weak	Good
C. jejuni subsp *fetus*[a]	19	5	1
C. fetus subsp *intestinalis*[b]	20	4	1
C. jejuni[c]	10	10	5

[a]Serotype A-1, strain 1289
[b]Serotype B, strain 1083
[c]Serotype C, strain 958

5.3.4.4. Phage typing

Phage C, lytic for many strains of *C. jejuni*, but not for *C. fetus* subsp *fetus* and *C. fetus* subsp *intestinalis*, lysed 30 of 41 isolates (Table 5.7).

TABLE 5.7. LYTIC ACTIVITY OF *C. jejuni* PHAGES ON 41 ISOLATES

Phage[a]	None	No. of strains showing lysis		
		1-49 Plaques	>50 Plaques	Complete
I	38	3	0	0
II	37	3	1	0
III	39	1	1	0
IV	39	2	0	0
C	11	17	9	4

[a]Phage types: phages I, II, III and IV were derived from *C. fetus* subsp *fetus* or *C. fetus* subsp *intestinalis* and are active against these subspecies, but do not attack *C. jejuni*. Phage C was derived from

C. jejuni strain 651/917 and was active against many but not all *C. jejuni* strains from aborted foetuses; the C phage does not attack *C. fetus* subsp *fetus* or *C. fetus* subsp *intestinalis*.

A few isolates were also lysed by phages I to IV, considered to be specific for strains of *C. jejuni* subsp *fetus* and *C. fetus* subsp *intestinalis*. Spontaneous plaque formation occurred with many of the phage-resistant isolates. Phages recovered from these plaques lysed *C. jejuni* NADC 917.

5.3.4.5. Antimicrobial susceptibility

The minimum inhibitory concentrations and measurements of inhibition zones around discs are summarized in Table 5.8. According to both techniques, the isolates were susceptible to most commonly used antimicrobial agents but resistant to benzylpenicillin, cephalothin, colistin and trimethoprim.

5.4. DISCUSSION

5.4.1. Significance of *C. jejuni* in human faeces

The significantly higher prevalence of *C. jejuni* in 0 to 8 month old children with diarrhoea than in the asymptomatic children strongly suggests that the organism is a causative agent of diarrhoea in very young children (Table 5.1). This contention may well be valid for children in developing countries where there is a higher incidence of *C. jejuni* infections. Further epidemiological studies with suitably matched controls and evidence of seroconversion are, however, required to firmly establish the role of *C. jejuni* in infantile gastroenteritis (See Chapter 6).

In asymptomatic children, the prevalence of *C. jejuni* increased dramatically after 9 months of age. With a prevalence of this magnitude it is to be expected that *C. jejuni* will be isolated from a number of patients with diarrhoea in whom it is not the causative agent of the illness. At this stage it is not clear whether the organisms are indicative of a transient infection to which there is no apparent host reponse, or whether they have colonized the gut on a more permanent basis.

The high incidence of asymptomatic *C. jejuni* infections observed in this series contrasts strikingly with the lower incidence reported from England (Skirrow 1977) and Belgium (Butzler, Dereume, Barbier, Smekens and Dekeyser 1977). Whether these differences are real or attributable to differences in methodology is debatable.

TABLE 5.8. ANTIBIOTIC SUSCEPTIBILITY OF ISOLATES

Antibiotic	No. of isolates tested	MIC's (µg/ml) for cumulative percentage of susceptible strains			Disc susceptibility test		Interpretation
		95%	50%	10%	Mean zone diameter (mm)	Drug dose per disc (g)	
Penicillin G	37	8	4	1	8	6	R
Ampicillin	35	4	1	0.25	33	10	S
Cephalothin	37	>128	64	32	6	30	R
Terracycline	37	0.5	0.25	0.06	48	30	S
Chloramphenicol	38	8	2	1	46	30	S
Erythromycin	35	0.25	0.06	0.03	49	15	S
Streptomycin	38	2	1	0.5	41	10	S
Gentamicin	38	5	2.5	0.125	44	10	S
Clindamycin	38	0.25	0.125	0.06	42	2	S
Colistin	36	16	8	1	9	10	R
Sulphamethoxazole	38	32	8	4	18	50	I
Cotrimoxazole	38	32	8	4	21	TMP 1.25:SMX 23.75[b]	I
Trimethoprim	20	>256	>256	>256	6	1.25	R

[a] R, Resistant, S, susceptible, I, intermediate susceptibility

[b] TMP, Trimethoprim, SMX, sulphamethoxazole

5.4.2. Species identification of *C. jejuni*

The individual strains recovered in this investigation share sufficient characteristics with . *jejun* to make their identity unequivocal. However, they show considerable variation biochemically, antigenically and on phage typing (Tables 5.3 to 5.7). The characteristics that most accurately predicted the designation '. *jejuni* are production of catalase and H_2S; growth in 1% glycine and at 42°C; and inhibition of growth by 3.5% NaCl and 85 glucose. Tests for inhibition by nalidixic acid or triphenyltetrazolium chloride (Charlier, Dekeyser, Florent, Strobbe and DeLey 1974) at the concentrations used were of little help. Unfortunately the methods used in this study differ significa tly from those .hat were recommended subsequently for the biotyping of . *jeuni* and for d fferentiating between . *jejuni* and '. strains (Skirrow and Benjamin 1982). Thus the nalidixic acid concentrations (Skirrow and Benjamin 1982) of 3 µg/mℓ incorporated in the agar plates was far too low for comparison with 30 µg nalidixic acid-containing discs and the H_2S production in Albimi medium with or without the presence of cystine is not comparable with the technique using iron containing medium Hoffman, Krieg and Smibert 1979) as prescribed by Skirrow and Benjamin (1982). Furthermore a hippurate hydro. is test which is essential for the differentiation of the *C. jejuni* b ypes from . was not performed. Subsequent studies (see Chapter 6) have shown, however, that the vast majority of Sowetan strains are *C. jejuni*, biotype I (nalidixic-acid sensitive, hippurate hydrolysis positive and absence of H_2S production on the iron-containing medium recommended by Skirrow and Benjamin in 1982).

5.4.3. Antigenic patterns

Although antigenic analysis was done with antisera against different strains of the subspecies, the direct immunofluorescence technique gave the most consistent reactions with . *jejuni* antisera. However, the tests indicated that several isolates possess antigenic determinants common to the two pathogenic subspecies of . *fetus* as well as to *C. jejuni* (Butzler 1973). Further evidence of heterogenicity of strains of . *jejuni* is exemplified by isolate K35, which shares antigenic determinants with *C. fetus* and . subsp *intestinalis* but is lysed by Phage C.

The results recorded here are consistent with the findings of King (1957) who found only minor cross reactionsbetween the thermophilic campylobacters

("related vibrios")and other campylobacters (vibrios) including *C. sputorum* subsp [illegible]. They precede but are in keeping with the recently proposed typing schemes based on thermostable antigens of Penner and Hennessy (1980), Lauwers, Vlaes and Butzler (1981) and heat labile antigens of Lior, Woodward Edgar and La-Roche (1981.)

5.4.4. Phage typing

Until now only a single [illegible] available for typing of *C. jejuni*. Phages recovered from isolates with spontaneous plaque formation may enhance our capability to further characterise strains of *C. jejuni*, but at this stage [illegible] be in its infancy.

5.4.5. Antibiotic susceptibility

The antibiotic susceptibility results are similar to those published by other workers prior to [illegible] [illegible]nhauser, Richardson, Bryner, Roux, Schutte, [illegible] 1979; Chow, Patten and Bednorz 1978; Vanhoof, [illegible], [illegible]owsky and Butzler 1978) and subsequently Walder 1979; Vanhoof, [illegible]rdts, Dierickx, Coignau and Butzler 1980; Karmali, DeGrandis and [illegible] 198[illegible], Ahonkhai, Chembin, Sierra, Bokkenheuser, Shulman and Mosenthal 1981).

With regard to the [illegible]ibility tests, despite good overall correlation between the minimum inhibitory concentration and disc diffusion methods, several discrepancies were encountered with individual strains which on repetition incriminated the disc diffusion technique as less reliable. This technique, [illegible] in the present study, is therefore [illegible] routine use.

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Until now only a single [illegible] phage has been available for typing of *C. jejuni*. Phages recovered from isolates with spontaneous plaque formation may enhance our capability to further characterize strains of *C. jejuni*, but at this stage phage typing of this organism should be considered to be in its infancy.

5.4.5. Antibiotic susceptibility

The antibiotic susceptibility results are similar to those published by other workers prior to this study (Bokkenheuser, Richardson, Bryner, Roux, Schultz, Koornhof, [illegible] and [illegible] 1979; Chow, Patten and Bednorz 1978; Vanhoof, Vanderlinden, [illegible], Lauwers, Yourasowsky and Butzler 1978) and subsequently Walder 1979; Vanhoof, Gordts, Dierickx, Coignau and Butzler 1980; Karmali, DeGrandis and Fleming 1981; Ahonkhai, Cherubin, Sierra, Bokkenheuser, Shulman and Mosenthal 1981).

With regard to the disc susceptibility tests, despite good overall correlation between the minimum inhibitory concentration and disc diffusion methods, several discrepancies were encountered with individual strains which on repetition incriminated the disc diffusion technique as less reliable. This technique, as applied in the present study, is therefore considered to be not sufficiently reliable for routine use.

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6. AGE RELATED SUSCEPTIBILITY TO *CAMPYLOBACTER JEJUNI* INFECTION IN A HIGH PREVALENCE POPULATION

6.1 Introduction

In developing countries, intestinal *Campylobacter jejuni* infections are common in young children (Butzler 1973; De Mol and Bosmans 1978; Bokkenheuser, Richardson, Bryner, Schutte, Roux, Koornhof, Freiman and Hartman 1979; De Mol, Brasseur, Lauwers, Zissis and Butzler 1980; Blaser, Glass-Huz, Stroll, Kibriya and Alim 1980; Berry, Gracey and Bamford 1981; Billingham 1981; Prasanna and Mathan 1982). In studies in Zaire, (Harvey 1980), Ruanda (De Mol and Bosmans 1978), South Africa (Bokkenheuser, Richardson, Bryner, Schutte, Roux, Koornhof, Freiman and Hartman 1979), Bangladesh (Blaser, Glass, Huq, Stroll, Kibriya and Alim 1980), and the Gambia (Billingham 1981), an important association between the presence of *C. jejuni* in the stool and diarrhoea has been shown. In the South African study *C. jejuni* was isolated in 32% of infants less than nine months old with diarrhoea compared with 4% of controls. In contrast, in children 9-24 months old, the rate of isolation of *C. jejuni* from the faeces of children with diarrhoea was similar to that of controls(Bokkenheuser, Richardson, Bryner, Schutte, Roux, Koornhof, Freiman and Hartman 1979). These findings prompted a more critical investigation of the age at which children convert from a diarrhoea-susceptible to a diarrhoea-refractory response to *C. jejuni* in the intestinal tract. Concurrently the duration of excretion of *C. jejuni* after acute attacks of diarrhoea was evaluated and the protective effect of breast feeding on the outcome of infection determined. The main clinical features of *C. jejuni* associated enteritis were also noted and the aetiological role of other bacterial pathogens in diarrhoea in these children investigated.

6.2 Materials and Methods

6.2.1 Patients

Clinical data on 111 children with diarrhoea admitted to the Baragwanath Hospital near Johannesburg were recorded. Stool specimens were obtained for culture and processed within 4 hours of admission. Mothers of babies in whom *C. jejuni* was isolated were asked to bring them back three weeks later for further stool examination. Children with positive stool cultures on this occasion were checked weekly until negative results

Controls

each patient with diarrhoea was matched for age and sex with a control baby. These apparently healthy babies came with their mothers to family planning or childcare clinics. We excluded children who had suffered any illness, including diarrhoea, within the previous week. Information on feeding was recorded. Faeces were collected from the napkins, transported to the laboratory in Johannesburg and within 4 hours

o.2.3. Media and bacteriological techniques

media were used for the isolation of *C. jejuni*. The composition per litre medium was : BCA-39 g Columbia agar base (Oxoid Ltd., Basingstoke, England) and 100 mℓ citrated horse blood; BCHA - 31 g cystine heart agar (Difco Laboratories, Detroit, USA), 100 ml citrated horse blood, 5 mg novobiocin (Upjohn, Kalamazoo, Michigan, USA), and 0 mℓ 0.5% brilliant green in distilled water (Florent 1956); and Skirrow's medium (Skirrow 1977) - 40g Oxoid blood agar base No. 2, 0-70 mℓ lysed horse blood, 10 mg vancomycin, 2 500 IU polymixin B sulfate and 5 mg trimethoprim lactate (Oxoid Ltd.) Skirrow's medium was streaked directly with a loopful of faeces and incubated at 42°C. In addition all three media were seeded with 0.2mℓ each of a filtrate prepared as described (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Voornhof Freiman and Hartman 1979; Dekeyser, Gossuin-Detrain, Butzler, and Sternon 1972; Butzler, Dekeyser, Detrain, Dehaen 1973) and incubated at 37°C. All media were incubated in an atmosphere of 10% CO_2, 18% O_2, and 72% N_2 for 48 hours. Colonies morphologically resembling *C. jejuni* were subcultured and identified according to Hoʻdeman, Cato and Thermoresistance, sensitivity to nalidixic acid, hippurate hydrolysis (Harvey 1980) and H_2S production (Skirrow and Benjamin 1980) were used for identification and biotyping of isolates.

Conventional techniques were used for the isolation and identification (including serotyping) of salmonellas and shigellas. Five *Escherichia coli* colonies per sample of faeces were tested for antigens associated with enteropathogenicity (EPEC) by using commercially available EPEC antisera (Hoechst, Behring-Werke, Germany; Wellcome Research Reagents, England), for enteroinvasiveness by Séreny's test (EIEC) and for heat stable and heat labile enterotoxin producing strains (ETEC) as

by Robins-Browne, Still, Miliotis, Richardson, Koornhof, Freiman, Schoub and Lecatsas (1980).

6.3. RESULTS

6.3.1. Recovery of bacterial pathogens from stool specimens

C. jejuni was isolated from 31% of children with diarrhoea regardless of their age group (Table 6.1) and in 19% of the controls It was the sole pathogen in 15% and 14% respectively in the diarrhoea and control groups. The 13 isolates available for biotyping according to hydrolysis hippurate belonged, with one exception,to biotype None of the isolates proved to be *Campylobacter coli*.

The incidence of salmonellas and shigellas was 4% or less in all groups. EPEC strains were recovered from 43% of children with diarrhoea and from 23% of controls (P<0.01). ETEC strains of *E.coli* were detected in 12 patients with diarrhoea and five controls No EIEC was found

6.3.2. Age specific faecal excretion of *C. jejuni*

There was a statistically significant difference (P<0.01) between the isolation rate of *C. jejuni* from infants with diarrhoea and from controls up to age eight months(Table 6.2) This was also true when children excreting *C.jejuni* as the sole bacterial pathogen were considered (P <0.02). This trend changed conspicuously once children reached the age of nine months, and between one and two years of age there was a steady decline in the number of children with *C. jejuni*-associated diarrhoea. In contrast, in the control group the prevalence of *C. jejuni* in the faeces increased markedly after nine months of age (Figure 6.1) The finding of fewer cases of *C. jejuni* -associated diarrhoea after age one year coincides with a general decline in the number of patients in this group admitted to hospital with diarrhoea.

6.3.3 Clinical picture

The clinical presentation of *C. jejuni* diarrhoea in our investigation was relatively mild: bloody stools, shock, acidosis, and hyperpyrexia were rare. Moderate dehydration was, however, common and moderate malnutrition was frequently detected especially among weaned children. Few of the younger and most of the older children had suffered earlier attacks of gastroenteritis Clinical recovery was generally rapid and most children were discharged in seven days without specific antimicrobial treatment Details of the clinical presentations are shown in Tables 6 and 6

TABLE 6.1. FAECAL EXCRETION OF *CAMPYLOBACTER JEJUNI* AND OTHER BACTERIAL PATHOGENS IN GAMBIAN CHILDREN

GROUP	AGE (Months)	NUMBER	*C.JEJUNI* AS SOLE BACTERIAL PATHOGEN	*C.JEJUNI* PLUS OTHER BACTERIAL PATHOGENS*	BACTERIAL PATHOGENS OTHER THAN *C.JEJUNI*	NO BACTERIAL PATHOGENS
Diarrhoea	0 - 8	60	10	8	13	21
	9 - 24	51	7	9	11	24
	TOTAL	111	17	17	32	45
Controls	0 - 8	60	1	7	16	30
	9 - 24	51	14	3	12	28
	TOTAL	111	15	10	18	68

*Mainly enteropathogenic *Escherichia coli*; some children harboured 2 or more pathogens.

TABLE 6.2. : AGE SPECIFIC FAECAL EXCRETION OF [illegible]

AGE GROUP (MONTHS)	0 - 4	5 - 8	9 - 12	13 - 16	17 - 20	21 - 24
Number investigated*	2 x 24	2 x 36	2 x 20	2 x 12	2 x 14	2 x 5
[total] [illegible] isolations						
Diarrhoeal patients, No.(%)	9 (38)	9 (25)	8 (40)	2 (17)	5 (36)	1 (20)
Controls, No.(%)	3 (13)	1 (3)	2 (10)	1 (8)	4 (29)	2 (40)
[illegible] as sole pathogen						
Diarrhoeal patients, No.(%)	6 (25)	4 (11)	5 (25)	1 (8)	1 (7)	0 (0)
Controls, No.(%)	0 (0)	1 (3)	6 (30)	3 (25)	3 (21)	2 (40)

*The diarrhoea and control groups were equally matched and the numbers are expressed as 2 x the number of each of the respective matched groups.

Figure 6.1. *C. JEJUNI* IN BABIES WITH AND WITHOUT DIARRHOEA

*No of babies in each group

TABLE 6.3 CLINICAL MANIFESTATIONS IN % OF 20 CHILDREN WITH DIARRHOEA, INFECTED WITH *C. JEJUNI* ONLY

AGE GROUP (MONTHS)	NUMBER OF CHILDREN	DIARRHOEA	VOMITING	TEMPERATURE			DEHYDRATION	MALNUTRITION	CONSISTENCY OF STOOL			1ST EPISODE OF DIARRHOEA
				37+	38+	39 - 40+			LIQUID	SOFT	MUCOID	
2 - 8	11	100	91	86	14	0	100	36	49	31	18	91
9 - 24	9	100	100	89	11	0	100	67	67	11	22	67

In the whole group (11 children) Not breast fed - 67%

Breast fed + supplementary - 27%

Breast fed only - 7%

No previous medication - 73%

Two-thirds brought to hospital within 3 days of illness.

TABLE 4.— DIFFERENCE IN BACTERIAL ISOLATES ON REHYDRATION — PERCENTAGES IN BRACKETS.

	Age Groups (months)	Total	Severe	Moderate	Mild	None	Not stated
[illegible]	0 – 8	18	1 (6%)	11 (72)	4 (22)	—	—
	9 – 24	14	—	12 (86)	2 (14)	—	1
EPEC	0 – 8	13	1 (8)	10 (78)	2 (15)	—	1
	9 – 24	11	—	11 (100)	2 (18)	1 (9)	3
ETEC	0 – 8	5	1 (20)	3 (60)	1 (17)	—	1
	9 – 24	5	—	3 (60)	1 —	—	1
Salmonella	0 – 8	1	—	1	—	—	—
	9 – 24	1	—	1	—	—	—
Shigella	0 – 8	1	—	—	1	—	—
	9 – 24	1	—	—	1	—	—
No isolates of bacterial pathogens	0 – 8	31	—	13(62)	7 (33)	—	1
	9 – 24	24	1 (4)	12(50)	10(42)	—	1

a - Two patients died of unrelated causes.

EPEC - Enteropathogenic *E.coli*

ETEC - Enterotoxigenic *E.coli*

6.3.4 Breast feeding

Patients aged up to eight months in whom *C. jejuni* was the only bacterial pathogen isolated were investigated for the protective effect of partial or complete breast feeding. Table 6.5 shows that breast feeding did not prevent infection with *C. jejuni*. Considering infants aged up to eight months old as a group, a similar analysis (not tabulated) revealed that 31 of 60 infants with diarrhoea and 45 of 60 control infants were breast fed, indicating that breast feeding prevents some cases of diarrhoea caused by agents other than *C. jejuni* ($P = 0.01$).

6.3.5 *C. jejuni* excretion after acute gastroenteritis

Eleven children with diarrhoea excreted *C. jejuni* from three to 12 weeks and one for at least 40 weeks (Table 6.6). After the acute attack, faecal samples from this patient were examined weekly from week three to week 10 and then fortnightly for the next ten weeks (Figure 6.2). The organism was isolated on all but two occasions and mixed infections with EPEC, salmonellas, or shigellas were observed seven times. Diarrhoea occurred during week 15 and week 29 and at the last episode the baby was treated with erythromycin for five days. The diarrhoea subsided, faeces were negative for *C. jejuni* five weeks later, but the organism reappeared during week 40 (Figure 6.2). Control babies were not available for follow up cultures. As the isolates from these children were not serotyped at the time, we do not know to what extent reinfection played a role in the prolonged recovery of *C. jejuni* from their faeces.

6.4 DISCUSSION

6.4.1 Age related susceptibility to infection

These findings strongly reinforce earlier observations that *C. jejuni* infection is widespread in black Soweran children (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979). They also confirm that faecal excretion of the organism in early infancy (<9 months of age) is almost always associated with diarrhoea ($P<0.02$).

The decline in *C. jejuni* associated diarrhoea observed with increasing age occurred despite the persistently high excretion rates of

TABLE 6.5 .EFFECT OF BREAST FEEDING ON *CAMPYLOBACTER JEJUNI* ASSOCIATED DIARRHOEA AMONG 8 MONTHS OLD CHILDREN.

GROUP	WHOLLY/PARTIALLY BREAST FED		BOTTLE FED	
	Total	*C.jejuni* infected*	Total	*C.jejuni* infected*
Diarrhoea (n=31)+	16	5	15	5
Controls (n=41)+	32	1	9	0

*Only children excreting *C. jejuni* as sole pathogen are included.

+Excludes children infected with intestinal pathogens other than *C.jejuni*.

TABLE 6.6. MINIMAL DURATION OF EXCRETION OF *C. JEJUNI* IN BABIES AFTER ACUTE GASTROENTERITIS.

No.of Babies	Duration of Excretion (in weeks)
1	3
1	4
2	6
1	7
2	9
1	11
1	12
1	40

Figure 5.2. Prolonged excretion of *C. jejuni* in a baby

- ▶ *C. jejuni*
- ▷ salmonella (SAL), shigella (SH), EPEC (EP)
- ○ *C. jejuni* not isolated
- ▌ diarrhoea
- ▏ no diarrhoea
- Rx treatment with erythromycin

C. jejuni in the 9 months age group (Table 6.1). The same trend was observed in Bangladesh where a 2% random sample of patients at a "diarrhoea hospital" showed a decline in the isolation rates of *C.jejuni* in children with diarrhoea from 21% in infants under 1 year of age to 9.5% in patients aged between 1 and 2 years (Blaser, Glass, Huq, Stroll, Kibriya and Alim, 1980).

Why many older children in Soweto and developing countries do not develop diarrhoea while harbouring *C.jejuni* is not clear. Earlier studies in the Sowetan community indicated a high exposure of familes to *C.jejuni* through eating chicken, which is the cheapest meat available to them, and their custom of keeping fowls in their backyards. Bovine intestine is also a cheap and popular source of food while many families keep pet dogs that are commonly fed on poultry scraps. We isolated *C.jejuni* in 26 of 30 (86%) samples of fowl faeces, 4 of 30 (13%) samples of bovine intestine, and 12 of 33 (36%) of dog faeces collected in Soweto (Richardson and Koornhof, 1979). In a controlled study in Sweden an association between the preparation and consumption of chicken and campylobacter enteritis was shown (Norkrans and Svedhem, 1982). In the Swedish study sick pets transmitted the disease to 2 patients and 3 definite instances of secondary cases were noted. We also found household spread of *C.jejuni* in Soweto and have recently observed repeated reinfections of different serotypes of *C.jejuni* in Sowetan children who excrete *C.jejuni* for prolonged periods (Chapter 9). The extensive exposure of this population to *C.jejuni* may well hold the key to the development of resistance to clinically manifested infections in older children and may well be a manifestation of herd immunity.

6.4.2. Evidence for herd immunity

For effective and lasting herd immunity, both a high exposure rate to the infecting agent and the ability of the population to mount an effective immune response are required. The large number of children excreting *C.jejuni* in the under 2 years age group reflects extensive exposure to the potential immunizing agent. The almost identical faecal excretion rates in the 9 months to 2-year old diarrhoeal and control groups provide convincing evidence for the development of resistance against clinically overt campylobacter enteritis. This resistance may very well be immunologically based. However, evidence of herd immunity to enteric campylobacteriosis in Soweto is largely circumstantial at this stage and

in the 9 months age group (Table 6.2). The same trend was observed in Bangladesh where a 2% random sample of patients at a "diarrhoeal hospital" showed a decline in the isolation rates of *C.jejuni* in children with diarrhoea from 21% in infants under 1 year of age to 9.5% in patients aged between 1 and 2 years (Blaser, Glass, Huq, Stroll, Sarkar and Alim, 1980).

Why many older children in Soweto and developing countries do not develop diarrhoea while harbouring *C.jejuni* is not clear. Earlier studies in the Soweto community indicated a high exposure of familes to *C.jejuni* through eating chicken, which is the cheapest meat available to them, and the tradition of keeping fowls in their backyards. Bovine intestine is also a cheap and popular source of food while many families keep pet dogs that are usually fed on poultry scraps. We isolated *C.jejuni* in 26 of 30 (87%) samples of fowl faeces, 4 of 30 (13%) samples of bovine intestine, and 14 of 74 (19%) of dog faeces collected in Soweto (Richardson and Goodhart, 1981). In a controlled study in Sweden an association between chicken preparation and consumption of chicken and campylobacter enteritis was found (Norkrans and Svednem, 1982). In the Swedish study sick pets transmitted the disease to 2 patients and 3 definite instances of secondary cases were noted. We also found household spread of *C.jejuni* in Soweto and have recently observed repeated reinfections of different serotypes of *C.jejuni* in Soweto children who excrete *C.jejuni* for prolonged periods (Chapter 9). The extensive exposure of this population to *C.jejuni* may well hold the key to the development of resistance to clinically apparent infections in older children and may well be a manifestation of herd immunity.

Evidence for herd immunity

Evidence for lasting herd immunity, both a high exposure rate to the immunizing agent and the ability of the population to mount an effective immune response are required. The large number of children excreting *C.jejuni* in the 1 month age group reflects extensive exposure to the presumed immunizing agent. The almost identical faecal excretion rates in the 1 month to 1 year old diarrhoeal and control groups provide considerable evidence for the development of resistance against clinically apparent campylobacter infection. This resistance may very well be immunologically based. However, evidence of herd immunity to enteric campylobacter infection in Soweto is largely circumstantial at this stage and

serological studies involving both specifi IgA and IgG in intestinal secretions and humoral IgG and IgM are required to provide more substantial immunological evidence for an extensive community-wide immunity. The possible role of cell-mediated immunity also needs to be determined. We need to know more about the basis of immunity to *C.jejuni* infection including which antigens (e.g.membrane protein, cell wall lipopoly saccharide or even flagellar molecules) are involved and also whether or not immunity is related to specific serotypes based on existing typing systems. Until these aspects have been elucidated, meaningful community studies would probably be premature.

One aspect in the design of this study renders interpretation of the findings in relation to possible herd immunity difficult. It could be argued that the control children have been free of diarrhoea for at least 4 weeks rather than the 2 weeks used in this study, to exclude residual faecal excretion subsequent to an acute episode (Karmali and Fleming, 1979). This 4 week period is also relevant to the findings in the present study which, similar to those of Karmali and Fleming (1979)indicate that approximately 70% of convalescent patients with diarrhoea cease to excrete *C.jejuni* within 4 weeks. Although a longer diarrhoea-free period in control children would have been advantageous it would be extremely unlikely for a sufficiently large number of these children to have developed an acute *C.jejuni* enteritis more than 4 weeks prior to their acceptance for this study. Despite these cogent arguments the concept of herd immunity to *C.jejuni* infection remains conjecture. Although extremely likely the presence of herd immunity in Soweto and other high endemicity regions still has to be established on firm immunological grounds.

The clearance of *C. jejuni* from the intestinal tract, irrespective of whether the individual has enteritis or not, also deserves further study. In a preliminary unpublished investigation of asymptomatic children in Soweto, 4 of 36 (11%) of 2-4 year old and 1 of 19 (3%) of 4-6 year old children excreted *C.jejuni*.

A similar trend of lower excretion rates of *C.jejuni* related to age was encountered in rural school children at Tlaseng (see Chapter 8) where, following the examination of 285 faecal samples during 5 visits, 9 of 60 (15%) of children in the 6-8 year age group and only 1 of 60 (1.7%) of 13-16 year olds (243 faecal samples) intermittently excreted *C.jejuni*.

These findings suggest the development of a more efficient intestinal clearing mechanism of *C.jejuni* in Sowetan children with increasing age. Alternatively, a greater resistance to intestinal colonization is being manifested in older children. The duration of excretion of *C.jejuni* in different age groups will be discussed in Chapter 9.

6.4.3. Clinical features

The episodes of diarrhoea in our infants excreting *C.jejuni* were only moderately severe and the absence of frank blood in their stools was unexpected, although variable results on the prevalence of this feature have been reported in the published reports. (Anonymous 1977; Karmali and Fleming 1979; Pai, Sorger, Lackman, Sinai and Marks 1979; Blaser, Berkowitz, LaForce, Cravens, Reller and Wang, 1979). Data from Britain suggests that blood in the stools of infants aged from 1 month to 1 year may be appreciably less common than in older patients (Anonymous, 1977).

6.4.4. Effect of breast feeding on *C.jejuni* colonization

The apparent lack of protection given by breast feeding against colonization in young infants needs further investigation. The numbers in this study were small and it would be premature to deny a role of specific IgA in the prevention of diarrhoea caused by this organism. Bactericidal antibodies actively produced after infection (Karmali and Fleming, 1979) may, however, be more important than those passively acquired through mother's milk. The nature of host resistance resulting in subclinical and asymptomatic infection needs elucidation and apart from immunological mechanisms, competition for, or changes in receptor sites, and other factors affecting intestinal epithelial cell physiology may be responsible.

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CHAPTER 7. POSSIBLE SOURCES OF *C. JEJUNI* IN SOWETO

Following the discovery of a very high prevalence of *C. jejuni* infections in young children in Soweto (Chapters 5 and 6) it became an important consideration to determine the sources of *C. jejuni* in this community. Based on the available information, the most likely sources would be animals, especially poultry, water and milk. In addition the large reservoir of *C. jejuni* in young Sowetan children may, either by person-to-person transmission via the faecal-oral route or by contamination of food or water, be an important source of infection. In this Chapter the sources of *C. jejuni* described in the literature will be briefly reviewed and an investigation into the presence in Soweto of this organism in fowl and dog faeces as well as in offal will be presented. The human reservoir and transmission within Sowetan households will be discussed in Chapter 9.

7.1. Sources of *C. jejuni* reported in the literature

7.1.1. Waterborne outbreaks

Several outbreaks of campylobacter enteritis from drinking water have been described. In the United States in Vermont, approximately 3 000 people of a population of 15 900 acquired *C. jejuni* enteritis (Vogt, Sours, Barrett, Feldman, Dickinson and Witherell 1982), while in Wyoming where stream water was implicated, 21 individuals were affected (Taylor and Blaser 1981) quoted by Blaser, Feldman and Wells (1982). Another outbreak occurred in Connecticut where 1300 of 7000 people at risk became ill after ingesting municipal water (Kornblatt *et al*, quoted by Blaser p.981; Blaser, Feldman and Wells 1982). In an extensively investigated study, Mentzing (1981) described a waterborne outbreak in Central Sweden in which an estimated number of more than 2 000 people of a total risk population of 14 600 were involved.

Knill, Suckling and Pearson (1982) found that *C. jejuni* was present in 30.4% of 540 water samples collected from rivers, lakes and estuaries in England but to date, no water-associated outbreaks have been described in the United Kingdom.

7.1.2. Milk as a vehicle of *C. jejuni* enteritis outbreaks

The consumption of unpasteurised milk has been implicated in several outbreaks of campylobacter-associated diarrhoea (Robinson, Edgar, Gibson,

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Matchett and Robertson 1979; Taylor, Weinstein and Bruner 1979; Blaser, Craven, Powers, LaForce, Taylor and Wang 1979; Porter and Reid 1981; Jones and Willis 1982). *C.jejuni* was usually not recovered from the milk in these outbreaks but the epidemiological evidence was incontrovertible. In one outbreak the organism was recovered from milk socks (Robinson, Edgar, Gibson, Matchett and Robertson 1979).

7.1.3. Outbreaks associated with chicken

Undercooked chicken (Schaefer, Conklin, Bunce, Storck, Arnold, Viner, Merritt, Krisch, Rothby, Currier, Wintermeyer and Davis, 1979) and chicken livers (Mouton, Lauwers, Butzler and Veltkamp 1981) have been reported as sources of *C.jejuni* infections in two separate outbreaks. *C. jejuni* has been isolated from both intestines and processed meat of chickens and turkeys (Smith and Muldoon 1974; Simmons and Gibbs 1979; Grant, Richardson, and Bokkenheuser 1980; Luechtefeld and Wang 1981). Flocks of chickens in two slaughterhouses in Yugoslavia were contaminated by *jejuni* and the organism survived for 7 days at 4°C and for up to 21 days in frozen carcasses(Mehle, Gubina and Gliha 1982.) Luechtefeld and Warg (1982) found that *C. jejuni* survived in 34% of freshly dressed turkey carcasses after overnight soaking in cooled chlorinated water. The same authors and others in separate studies showed that various wild birds are infected with *C. jejuni* (Luechtefeld, Blaser, Reller and Wang 1980; Luechtefeld and Wang ;1982).

7.1.4. The role of red meat as a source of campylobacter enteritis

C. jejuni has been isolated from the faeces of sheep, pigs and cattle (Luechtefeld and Wang 1982; Stich-Groh 1982) and from gall bladders of sheep and cattle (Bryner, O'Berry, Ester and Foley 1972). Hudson and Roberts (1982)found *C. jejuni* in 59% of pork carcasses but failed to recover it from lamb or beef. In retail stores in Britain 1.5% of minced meat samples were found to be infected with *C. jejuni*(Turnbull and Rose 1982) while a higher *C. jejuni* contamination rate of minced meat was observed in Sweden (Kaijser and Svedhem 1982).

In a *C. jejuni* outbreak associated with red meat, raw hamburger was implicated as the probable source of infection in 33 of 54 persons at risk. (Oosterom, Beckers, van Noorle Jansen and van Schothorst; 1980).

7.1.5. Evidence of direct spread from animals to birds and man

The presence of *C. jejuni* in animals and birds provides an almost unlimited source of infection (Luechtefeld and Wang 1982). Transmission of *C.jejuni* from these potential sources is implied by epidemiological data but most reports have not been substantiated by either isolation of the organism or by serotyping of the isolate concerned. Skirrow(1977), Blaser, Cravens, Powers and Wang(1978)and Norkrans and Svedhem(1982) incriminated a number of human infections to *C. jejuni*-associated diarrhoea in puppies while Svedhem and Norkrans(1980)and Skirrow, Turnbull, Walker and Young (1980), described cases of human enteritis associated with infected kittens. Severin(1982)showed a statistical correlation between contact with cage-birds and the development of campylobacter enteritis.

7.1.6. Human transmission of *C. jejuni*

Person-to-person transmission of *C. jejuni* has not previously been thoroughly investigated. Anecdotal incidents of spread of *C. jejuni* within households have been described when the index cases were young children who were incontinent of faeces (Cadranel, Rodesch, Butzler and Dekeyser 1973; Blaser, Waldman, Barrett and Erlandson 1981). Vertical transmission of *C. jejuni* from symptomatic and asymptomatic mothers to their neonates has also been described (Mawer and Smith 1979; Karmali and Tan 1980). However it is generally assumed that, at least in developed countries, person-to-person transmission rarely occurs (Leading article, Lancet 1982; Symonds 1983) because the sensitivity of *Campylobacter* to oxygen probably limits its survival (Symonds 1983). In a large waterborne outbreak in Bessington, Vermont, there was no evidence of secondary spread of *C. jejuni* enteritis from patients to contacts (Vogt, Sours, Barrett, Feldman, Dickinson and Witherell 1982) lending support to this view. However, whether this holds true for developing countries with conditions of overcrowding and poor hygiene is debatable. This problem will be addressed in Chapter 9.

7.2. Isolation of *C. jejuni* from fowl and dog faeces and from bovine offal and tripe

Following the conclusion of the preliminary study (Chapter 5) on the isolation of *C. jejuni* from 34% of babies with acute gastroenteritis and 13% of asymptomatic controls, a small survey was initiated to investigate chicken faeces

and bovine tripe and intestine to find a possible source of these infections in Soweto. In addition, because of the possibility of dogs being carriers of *C. jejuni* (Blaser, Cravens, Powers and Wang 1978), freshly-voided dog faeces were collected from pavements for study.

7.2.1. Materials and methods

With the aid of the City of Johannesburg Health Department. samples of fresh fowl faeces were collected randomly from 30 coops which contained from 7 to 16 birds each. Bovine tripe and intestine was obtained from butchers' shops and street vendors.

All samples were processed within two hours of collection. Faecal specimens (approximately 2.0g) were thoroughly shaken in 9.0 mℓ of chilled phosphate-buffered saline (PBS), spun for 10 minutes at 1 000xg and filtered through a 0.65 micromillipore filter. Tripe and intestine portions were ground in PBS in Griffiths tubes and filtered. Isolation plates included plain blood agar, tryptose blood agar and brilliant green blood agar, all containing 10% horse blood. These were incubated in 10% CO_2 for up to 3 days at 37°C.

All isolates were identified and subspeciated by conventional methods (Holdeman, Cato and Moore 1977). For temperature tolerance, 10% blood agar plates were incubated under microaerophilic conditions at 25°C, 37°C and 42°C for 48 hours.

7.2.2. Results

The results of the attempts to recover campylobacter from probable sources of *C. jejuni* in Soweto are given in Table 7.1.

TABLE 7.1. ISOLATION OF *C. JEJUNI*

	C. jejuni	*C. fetus* subsp *intestinalis*	*C. fetus* subsp *fetus*
Fowl faeces from coops	26/30 (86.7%)		
Dog faeces	12/33 (36.4%)	1/33 (3.0%)	1/33 (3.0%)
Bovine intestine	4/30 (13.3%)		
Bovine tripe	1/30 (3.3%)		

7.2.3. Discussion

From the results in table 7.1. it is clear that the large number of isolates

of *C. jejuni* are present in fowl coops and dog faeces. It is interesting to note that in a similar study carried out on 46 broiler chickens from a live poultry market in New York City 39 isolates (83 per cent) of *C.jejuni* were found (Grant, Richardson, Bokkenheuser, 1980).

Fowls, bovine tripe and intestine are eaten in substantial quantities by the inhabitants of Soweto. It is estimated that 10 000 fowls are sold in Soweto per week to a population of about 700 000 persons. With an average of 5.5 persons per family, one family out of 13 eat a fowl per week These birds, usually sold live to the consumer, are killed and prepared in the homes and the intestines, highly contaminated with *C.jejuni* are a potential source of infection to the families. The same reasoning could apply to the handling of bovine tripe and intestine.In a study on epidemiological aspects of *C.jejuni* enteritis, Norkrans and Svedhem (1982) found that patients infected in Sweden had eaten chicken significantly more often than a corresponding control group. Also, the preparation of chicken contaminated with *C. jejuni* seemed to elevate the risk of contracting the disease. Similar findings supported by statistical analysis were reported by Severin (1982) in the Netherlands.

None of the dog faeces tested showed evidence of diarrhoea, which suggests that dogs are possible carriers of *C. jejuni*. As intimated by Skirrow 1977, these dogs could easily be infected by eating poultry scraps discarded by householders. Regardless of the explanation, however, dogs provide another possible reservoir for the transmission of the disease.

Whether these findings have any bearing on the high incidence of campylobacteriosis among the babies of Soweto can only be determined by further studies. Education in hygienic handling of food is a logical first step towards the prevention of enteritis caused by this organism.

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8. LONG-TERM INFECTIONS WITH *C. JEJUNI*

8.1 Introduction

Although the research reported in this chapter was conducted in a rural area, the findings are interesting and corroborate some of those encountered in Soweto. It was therefore decided to include it in this thesis. The purpose of this study was to examine the prevalence of enteric pathogens in schoolchildren living in a district of gradually improving hygienic standards. Before 1958, the drinking water in Tlaseng, a rural village, 165 km west of Johannesburg, was obtained from open, shallow surface wells shared by humans and animals (Bokkenheuser and Richardson 1960). Some 5 years later, deep wells were constructed, and in recent years the villagers were encouraged to use pit privies, rather than open fields for defaecation (Richardson, Hayden-Smith, Bokkenheuser and Koornhof 1968).

Information on the prevalence of salmonellae, shigellae and intestinal parasites is mainly of local interest and will be reported elsewhere. Included in this study was a search for *C. jejuni* to investigate the possible existence of a carrier rate in two groups of school children. This followed on from the results reported in Chapters 5 and 6 where studies showed positivity in the stools of 34 to 40 per cent of children aged nine to 24 months, whether they suffered from acute gastroenteritis or were asymptomatic. Although transient infections cannot be ruled out, it seems most reasonable to assume that the *C. jejuni* remained after an earlier acute episode which resulted in clinical, but not bacteriological cure. This investigation was undertaken to study the occurrence of *C. jejuni* excretion among apparently healthy children from an environment with a high prevalence of *C. jejuni* diarrhoea (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979).

8.2. Materials and Methods

8.2.1. Donors

A total of 120 apparently healthy children aged 5 to 8 and 13 to 16 years were chosen for this study. They were pupils of Tlaseng School. Their parents belonged to the lower socio-economic groups. In view of our previous studies on campylobacteriosis it might have been more appropriate to select younger children. However, schoolchildren are more easily available for repeated investigations. The groups represented the youngest and the eldest students. Their diet consisted primarily of porridge made from maize and

"kaffir corn" (*Sorghum vulgar*) supplemented with beans and "morogo" (wild spinach) and citrus fruits in season. Because of the high cost, pork, or beef was rarely consumed, but most families kept chickens and ate one or two a week.

8.2.2. Faecal samples

Over a 15 month period faecal specimens were collected five times (Figure 8.1); twice in winter, and once each in autumn, spring and summer. Children whose stools were positive for *C.j.juni* at any time were examined monthly for a further two months. The consistency of the stools was noted, and specimens were processed within 20 minutes of voiding.

8.2.3. Isolation of *C. jejuni*

As previously described (Chapter 5) *C. jejuni* was isolated using the filtration technique (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979) and spread on 10% horse blood agar plates. Skirrow's selective medium was streaked with faeces. The plates were stacked in stainless steel containers, flushed with a gas mixture consisting of 5% O_2, 10% CO_2 and 85% N_2, taped and transported to the laboratory in Johannesburg. The inoculated media were incubated for up to 5 days in an atmosphere of 10% CO_2 in air and examined daily from the second day. The blood agar plates were incubated at 37°C and the selective plates were incubated at 42°C.

Colonies which morphologically resembled *C. jejuni* were subcultured prior to identification. Isolates were identified (Holdeman, Cato and Moore (1977). and tested for antibiotic sensitivity (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979).

8.2.4. Salmonellae, shigellae and parasites

Desoxycholate (BBL Microbiology Systems), MacConkey agar and selenite F enrichment media were seeded with faecal material, transported to the laboratory and incubated overnight. The next morning the enrichment cultures were streaked on fresh desoxycholate agar plates. Non-lactose-fermenting colonies were tested biochemically, and those conforming to salmonellae were serotyped. Shigellae were classified according to their group antigen.

For parasitological investigation, stools were centrifuged and examined by the merthiolate-iodine-formaldehyde method (Blagg, Schloegel, Mansour and Khalaf 1955).

"kaffir corn" (*Sorghum vulgar*) supplemented with beans and "morogo" (wild spinach) and citrus fruits in season. Because of the high cost, pork, or beef was rarely consumed, but most families kept chickens and ate one or two a week.

8.2.2. Faecal samples

Over a 15 month period faecal specimens were collected five times (Figure 8.1); twice in winter, and once each in autumn, spring and summer. Children whose stools were positive for *C.jejuni* at any time were examined monthly for a further two months. The consistency of the stools was noted, and specimens were processed within 20 minutes of voiding.

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For parasitological investigation, stools were centrifuged and examined by the merthiolate-iodine-formaldehyde method (Blagg, Schloegel, Mansour and Khalaf 1955).

Figure 8.1 Collection and processing of specimens at Tlaseng School

A

B

C

D

E

8.3. RESULTS

Some children left school before the end of the survey, and others were absent on the day of collection of specimens, reducing the number of children present at all five collections to 73 (Table 8.1). Of these, 46 were 6 - 8 and 27 were 13 - 16 years old. The results of the bacterial isolations are given in Tables 8.1, 8.2 and 8.3.

8.3.1. Campylobacteriosis

C. jejuni was isolated from nine children, three girls and six boys (Tables 8.1 and 8.2). Children positive for *C. jejuni* were followed up monthly for another six months. Table 8.1. shows that eight of the infections were observed in the 46 children aged 6 to 8 years. The organism was isolated intermittently from 11 per cent for at least 9 months and from 4 per cent for more than one year. The only infected subject among the 27 older children (4 per cent) was positive for *C. jejuni* for more than a year. Two of the three long-term infected children excreted the organism at the time the study was terminated.

The prevalence of the infection was eight per cent in summer and five per cent in winter (Table 8.2).

The nine subjects from whom *C. jejuni* was isolated were positive on 24 occasions (Table 8.1) All isolates were recovered by filtration techniques, but only seven of 24 grew in primary culture on Skirrow's medium. However, in sub-cultures the selective medium supported growth of all 24 isolates.

All isolates displaying the typical morphology of campylobacters were identified as *C. jejuni*. Eighteen of the isolates grew well at 42°C, and seven grew poorly. None grew microaerophically at 25°C. All were susceptible to nalidixic acid (30ug/disc).

In an attempt to differentiate between carriers and reinfected individuals, the isolates were further characterized. All were H_2S positive (by the lead acetate hydrochloric oxidase positive and resistant to NaCl (3.5%); all but one were catalase positive. In contrast to the findings by Holdeman, Cato and Moore (1977), some of the isolates reduced nitrate , others did not. The reliability of the nitrate test as a marker for *C. jejuni* was not determined.

The antibiograms (see Table 8.4) were remarkably uniform and almost identical to those found in the initial Soweto study. (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979).

TABLE 8 SEROLOGICAL INCIDENCE OF *C. JEJUNI* AND PERSISTENCE OF INFECTION IN 73 CHILDREN

CHILD No.	AGE (years)	SEX	APRIL 1978 Autumn	JULY 1978 Winter	OCTOBER 1978 Spring	JANUARY 1979 Summer	JULY 1979 winter	SEPTEMBER 1979 Spring	OCTOBER 1979 Spring	NOVEMBER 1979 (Spring)	PERSISTENCE OF INFECTION (Months)
10	6	F	-	+	-	+	-	-	+	+	≥ 16
38	6	M	-	+	-	+	+	+	+	-	≥ 15
40	13	M	-	+	-	+	-	-	+	+	≥ 16
54	8	M	-	-	-	+	-	-	-	+	≥ 11
55	8	M	-	-	-	+	-	-	+	-	≥ 10
28	6	M	-	-	+	-	+	-		-	≥ 9
31	6	M	-	+	+	-	-	Left Village			≥ 3
19	6	F	-	-	-	+	-	-	-	-	0
22	6	F	-	-	+	-	-	-	-	-	0
% Positive*			0%	5.5%	5.5%	8.2%	2.7%	1.4%	5.6%	4.2%	

*Percentage of 73/72 children positive for *C. jejuni*

TABLE 5.2 INFECTION PATTERNS BY AGE AND SEX

Age (years)	No. of individuals	No. of specimens	Individuals infected with:						All infected individuals	
			C. jejuni		Salmonella		Shigella			
			No.	%	No.	%	No.	%	No.	%
0 - 4	60	183	9	15,0	5	8,3	7	11,7	19	31,7
13-16	60	343	1	1,7	2	3,3	4	6,7	7	11,7
TOTAL	120	526	10	8,3	7	5,8	11	9,2	26	21,7
Females	64	274	3	4,7	4	6,3	5	7,8	10	15,6
Males	56	254	7	12,5	3	5,4	6	10,7	16	28,6

TABLE 8.3 SEASONAL VARIATIONS IN PREVALENCE OF INFECTIVE AGENTS

Season	*C. jejuni* Child's number:	*Salmonella* Child's Number:	*Shigella flexneri* Child's Number:	Percentage infected with: *C. jejuni*	*Salmonella*	*Shigella flexneri*	Total infected children: No.	%
Autumn (April)	-	-	-	-	-	-	-	-
Winter (July)	(18) (31) (35) (45)	47	106	5.5	1.4	1.4	8	8.2
Spring (October)	(18) (28) (31) 72		2 19 31 102	5.5	-	5.5	7	9.6
Summer (January)	(18) (28) (36) 54 55 19	3 11 43 48		8.2	5.5	-	10	13.7
Winter (July)	(28) (38)	59	26	2.7	1.4	1.4	4	5.5
TOTAL:	16	6	6					

Total number of children = 73
From children, 28 pathogens were recovered
Ringed numbers indicate children from whom *C. jejuni* strains were isolated on more than one occasion

Pen = Penicillin
Amp = Ampicillin
Ceph = Cephalothin
Tet = Tetracycline
Chlor = Chloramphenicol
Eryth = Erythromycin
Strept = Streptomycin
Gent = Gentamicin
Clinda = Clindamycin
Sulph = Sulphonamide
Cotri = Cotrimoxazole
TMP = Trimethoprim
Met = Metronidazole
MIC = Minimum Inhibitory Concentration (mg/l)
ZS = Zone Size (mm)

Child no.	Date	[illegible]		Amp		[illegible]		Chlor		Eryth		Strept		Sent		Clinda		Colistin		Sulph		Cotri		TMP	Met	
		MIC	ZS	MIC	[illegible]	MIC	ZS	MIC	ZS	MIC	ZS	MIC	ZS	MIC	ZS	MIC	ZS	MIC	ZS	MIC	ZS	MIC		MIC	MIC	ZS
18	07/78	1	—	1	[illegible]	256	—	[illegible]	45.0	4	38.0	1	29.0	4	12.0	2	25.0	0.5	28.0	4	—	8	—	32	11	>256	ND	
18	01/79	1	8.0	0.5	25.0	64	—	0.12	62.0	1	54.0	0.06	54.0	[illegible]	52.0	[illegible]	53.0	0.06	54.0	8	—	8	—	8	14.0	256	ND	
18	01/79	0.5	9.0	0.5	26.0	64	—	0.12	47.0	2	40.0	0.06	50.0	[illegible]	52.0	0.12	44.0	0.06	42.0	8	—	8	14.0	8	16.0	>256	ND	
18	10/79	0.5	8.0	[illegible]	24.0	32	8.0	0.03	60.0	0.5	60.0	0.03	54.0	0.25	45.0	0.12	55.0	0.03	48.0	16	—	[illegible]	20.0	4	24.0	>256	4	30
28	07/79	2	—	0.12	36.0	128	—	0.06	58.0	1	54.0	0.03	50.0	0.5	43.0	0.12	44.0	0.06	43.0	1	12.0	[illegible]	22.0	4	26.0	>256	ND	
35	01/79	0.5	6.0	1	20.0	64	—	0.12	60.0	2	31.0	0.06	54.0	0.5	39.0	0.25	47.0	0.06	47.0	4	15.0	8	—	4	17.0	256	ND	
35	01/79	1	9.0	0.12	38.0	32	—	0.06	62.0	1	45.0	0.03	40.0	0.5	35.0	1.12	42.0	0.06	44.0	4	—	[illegible]	—	32	—	>256	ND	
38	07/78	2	—	0.5	30.0	16	—	0.06	50.0	1	45.0	0.03	41.0	[illegible]	22.0	0.5	43.0	0.5	27.0	8	—	2	20.0	2	22.0	>256	ND	
38	01/79	1	—	1	21.0	8	—	0.25	40.0	8	38.0	0.06	46.0	0.25	50.0	0.25	37.0	0.06	44.0	0.5	9.0	4	19.0	2	19.0	>256	ND	
38	07/79	2	—	0.12	5.0	128	—	0.06	58.0	1	51.0	[illegible]	51.0	0.25	47.0	0.03	40.0	0.06	44.0	0.5	13.0	8	23.0	4	21.0	>256	ND	
38	09/79	1	20.0	0.25	32.0	32	17.0	0.25	40.0	4	38.0	0.12	28.0	2	25.0	0.5	39.0	0.25	28.0	16	—	4	22.0	2	30.0	>256	4	29
38	10/79	1	8.0	1	25.0	16	11.0	0.03	47.0	1	47.0	0.03	55.0	0.5	38.0	0.12	47.0	0.03	4.0	32	—	16	10.0	16	16.0	>256	2	36
54	11/79	1	24.0	0.25	30.0	32	19.0	0.06	50.0	2	40.0	0.03	45.0	8	—	0.25	44.0	0.12	40.0	1	12.0	4	28.0	2	29.0	>256	2	29
55	07/78	0.5	6.0	0.5	26.0	32	—	0.12	60.0	0.5	55.0	0.03	44.0	0.5	50.0	0.12	52.0	0.03	52.0	[illegible]	—	16	—	4	19.0	128	ND	
55	10/79	1	9.0	4	30.0	16	12.0	0.03	52.0	1	53.0	0.03	56.0	0.25	47.0	0.25	51.0	0.03	44.0	16	—	8	17.0	16	14.0	256	1	38
96	07/78	2	—	1	21.0	256	—	0.12	43.0	2	51.0	0.03	40.0	0.25	26.0	0.5	33.0	0.06	40.0	0.5	10.0	16	—	32	11.0	>256	ND	
96	01/79	0.5	7.0	0.5	25.0	64	—	0.06	61.0	1	45.0	0.12	43.0	0.25	35.0	0.12	57.0	0.03	51.0	16	—	[illegible]	19.0	4	23.0	256	ND	
96	10/79	0.5	8.0	2	25.0	32	—	0.03	54.0	1	43.0	0.03	54.0	0.5	38.0	0.5	42.0	0.03	43.0	16	—	32	—	16	14.0	>256	2	36
96	11/79	1	20.0	0.25	[illegible]	32	15.0	0.03	45.0	2	42.0	0.03	40.0	2	28.0	1	37.0	0.25	37.0	1	19.0	4	24.0	2	34.0	256	2	28

[illegible] = Penicill[illegible]
Amp = Ampicill[illegible]
[illegible] = Cephalot[illegible]

[illegible] = Sulph[illegible]
Eryth = Erythromyc[illegible]
Strept = Streptomyci[illegible]

Chlor = Chloramphenic[illegible]
Clinda = Clindamycin
Sulph = Sulphonamide
[illegible] = Cotrimoxazole
TMP = Trimethoprim

M[illegible] = Metronidazole
MIC = Minimum Inhibitory Concentration (mgl^{-1})
ZS = Zone Size (mm)

Only streptomycin seemed to have potential as an antibiotic ma[illegible]. 19 isolates (five died before they could be tested), 17 showed a mini[illegible] inhibitory concentration of 0.6µg/mℓ (range 0.25 to 2µg/ml), whe[illegible] two strains gave minimum inhibitory concentration values of [illegible] and [illegible] µg/[illegible] with correspondingly small zones around the discs (Bokkenheuser, [illegible] Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979). One of th[illegible] resistant strains was isolated from child No. 18, who later yielded thr[illegible] organisms of expected streptomycin susceptibility. The most resis[illegible] strain was isolated on one occasion only - from child No. 54. [illegible] antibiogram results are given in Table 8.4.

8.3.2. Salmonellae, shigellae and parasites

Samples from six of the 73 children yielded growth of salmonellae and six yielded growth of shigellae. None of these pathogens were isolated from individuals with faecal *jejuni* infections. Approximately seven per cent of the children were infected with *Giardia lamblia*. [illegible] with *Hymenolepis nana*, *Entamoeba histolutica* and *Ascaris lumbricoides* was detected occasionally (Table 8.5). All children were asymptomatic.

8.4. DISCUSSION

8.4.1. Reinfection versus carriers

From available data it cannot be determined whether all seven individuals from whom *C. jejuni* was isolated repeatedly (Table 8.[illegible]) had become infected on more occasions or whether they were carriers. Neither biochemical reactions nor antibiograms provided reliable bacteriological markers. Unfortunately serotyping, which was still in its developing stages when this study was conducted, was not available at the time.

Epidemiological data tends to suggest that the seven long-term infected individuals were carriers: (i) in a homogenous population one would expect that multiple infections were randomly distributed and not confined to a small select group; (ii) in developed countries the organism is ubiquitous, suggesting that in developing countries it would be hard not to be constantly exposed to *C.jejuni* and (iii) intermittent excretion of pathogens is well known in typhoid carriers. It is possible that the observed intermittency reflects the limit of sensitivity of the technique for detecting *C. jejuni*. Recovery of *C.jejuni* by the filtration technique and by our selective media requires the presence of approximately 5 000 and 1 000 organisms per g of faeces respectively (see Chapter 4).

TABLE 8.5. SEASONAL PREVALENCE OF FALCAL PARASITES

Prevalence percentage

Parasite	Autumn	Winter	Spring	Summer	Mean
Hymenolepis nana	0,9	2,8	3,6	3,1	2,5
Giardia lamblia	8,5	1,0	7,1	8,3	6,5
Entamoeba histolytica	-	4,6	-	1,0	1,4
Ascaris lumbricoides	-	-	0,9	-	0,2

Thus, samples with fewer than 1 000 organisms per g of faeces are likely to yield negative results. However, despite these cogent arguments, reinfection with different serotypes was later shown to occur in Soweto (Chapter 9) and this could explain the findings in Tlaseng.

8.4.2. Risk of long-term colonization

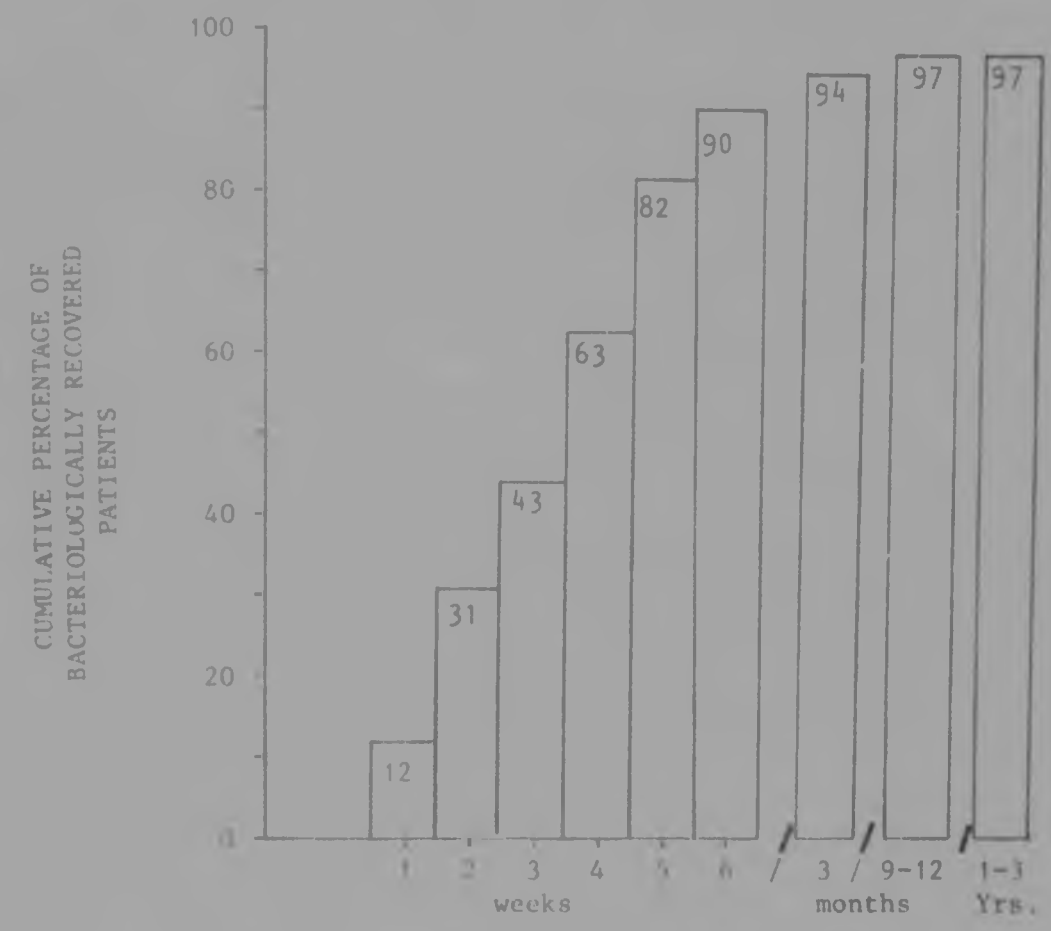
On the assumption that the long-term infected individuals are carriers, we may calculate the minimum risk of an acute infection, divided or subdivided, resulting in long-term colonization. From previous observations it is reasonable to assume that most children from developing countries have suffered at least one infection before they start school (Blaser, Glass, Huq, Stoll, Kibriya and Alim 1980 ; Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979). Among the 46 children six to eight years old, we observed five (10.9%) who were infected for at least sixteen months. The carrier rate could be higher because of the failure to detect some mild, intermittent excretors and the possibility of a less than 100% exposure to [illegible] early in life would tend to increase the observed risk of becoming a carrier. It is interesting that among the 27 older children there was only one(four per cent) long-term infected individual, suggesting that spontaneous bacteriological cure or resistance to infection occurs with time. This is consistent with the rarity of disseminated campylobacteriosis due to [illegible] in elderly, debilitated individuals in whom the predominating organism is [illegible]*nalis*(Bokkenheuser 1970).

Our findings appear to be at variance with the observations of Karmali and Fleming (1979). They found that 50% of untreated children with acute campylobacter enteritis ceased to excrete the organism within three weeks of onset, and 100% were bacteriologically cured within seven weeks. Several factors tend to reduce the differences between the surveys. Firstly, in a group of 24 children one could only expect one or two long-term colonized individuals. Secondly, as stated above, intermittent excretors are easily missed. Thirdly, all isolates of *C.jejuni* in this study were recovered by filtration technique, whereas 17 of 24 failed to grow in primary culture on Skirrow's medium. Thus, differences in findings could be attributable to differences in techniques. It is conceivable then, that the natural history of campylobacter enteritis in South Africa is best appreciated by taking both studies into account. For the purpose of the calculation it is assumed that all 73 children

in our study experienced an infection with *C.jejuni* before starting school. In that case, at least seven of 23 (10 per cent of the infected individuals would remain infected for more than three months (Table 8.1) The remaining 90 per cent were either persistently negative, or children from whom *C.jejuni* was isolated only once. It may be assumed that they achieved bacteriological clearance as described by Karmali and Fleming (1979).The results of this computation are shown in Fig. 8.2 which graphically expresses the spontaneous bacteriological recovery, as it would appear to occur under the epidemiological conditions prevailing in South Africa. It infers that at least 3% of untreated children carry the organism for a year or more. Furthermore, if less than 100% of the children were infected with *C.jejuni* before the survey, the probablity of an infection resulting in a long-term carrier state would still be higher.

Alternative explanations are possible. Firstly, the Canadian and South African populations live under vastly different conditions. Secondly, infective strains may differ in their ability to colonize the gut. Thirdly, the immune response may depend on the severity of the primary infection, clinical or subclinical. The Canadian children suffered clinically manifested *C.jejuni* enteritis (Karmali and Fleming, 1979), whereas the South African children had no recent history of diarrhoea.

Figure 8.2 Proposed duration of spontaneous bacteriological recovery from *C. jejuni* infections among Tlaseng children based on the recovery rate reported by Karmali and Fleming (1979) and our findings.



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9. THE HUMAN RESERVOIR AND PERSON-TO-PERSON TRANSMISSION OF *C. JEJUNI* IN SOWETAN CHILDREN.

9.1 Introduction

Clear evidence of the high prevalence of *C. jejuni* in Sowetan children was presented in Chapters 5 and 6 (Bokkenheuser,Richardson,Bryner,Roux, Schutte, Koornhof, Freiman and Hartman 1979; Richardson, Koornhof, Bokkenheuser, Mayet and Rosen 1983). In these chapters it was shown that, while there was no difference in the isolation rates of the 0-8 month and 9-24 month age groups of children with diarrhoea, the isolation rates among non-diarrhoeal children were significantly higher in the older age group (Table 5.1). A plausible interpretation of these findings is that older children in areas of high endemicity such as Soweto, can harbour the organism, whether or not they suffer from gastroenteritis, and begin to develop a resistance to clinically manifest infection at about 8 months of age.

It was further shown in Chapter 6, that after recovery from acute infections, 11 children continued to excrete *C. jejuni* for three to 12 weeks (Table 6.5) and, as illustrated in Fig. 6.2, another infant harboured the organism for 40 weeks. This child was either a persistent but intermittent excretor or was repeatedly re-infected with different serotypes. These results and the findings of a pilot investigation in which 11% of 2-4 year-old and 3% of 4-6 year-old children in Soweto excreted *C. jejuni* (unpublished data, see Chapter 6.4.2) indicated the need for the study and better understanding of excretion and carriage of this bacterium among infected children in a range of age groups. This is of obvious consequence to the understanding of the epidemiology transmission and reservoirs of this diarrhoeal agent. As was said in the concluding paragraphs of Chapter 6, asymptomatic excretors of *C. jejuni* could be an important source of infection for other household contacts.

In Chapter 7 it was shown that *C. jejuni* could be recovered with high frequency from chicken and dog faeces as well as from bovine offal and tripe in Soweto (Richardson and Koornhof 1979). Unfortunately serotyping facilities were not available when these studies were performed and, in any case, were not yet fully developed for epidemiological purposes. It is therefore not known whether the *C. jejuni* serotypes present in the specimens from animals and animal products were responsible for human infection in Soweto. Neither was it known whether the prolonged

excretion of *C. jejuni* encountered in children was the result of persistent shedding of the same serotype or whether repeated reinfection with different serotypes occurred. When serotyping became available to us and Dr. Sabine Lauwers of the Department of Clinical Microbiology, Free University, Brussels and Dr D M Jones, Public Health Laboratory, Withington Hospital, Manchester, U.K.. kindly agreed to type our isolates according to their heat-stable antigen schemes, it was decided to investigate the pattern and duration of excretion of *C. jejuni* serotypes in the faeces of Sowetan children in different age groups up to 3 years of age. At the same time person-to-person transmission of *C. jejuni* within households in Soweto was studied.

9.2 Children and Methods

9.2.1 Age related faecal excretion of *C. jejuni* in Sowetan children

9.2.1.1 Age groups of children

The faecal excretion of *C. jejuni* was studied in children under 3 years of age who suffered from diarrhoea and were brought to clinics in Soweto by their mothers. Approximately 10 children were investigated in each of the following age groups: 0-4 months, 5-12 months, 13-24 months and 25-36 months of age.

9.2.1.2 Entry criteria

For entry into the study the following criteria had to be met:

(a) A current acute diarrhoeal episode;

(b) Isolation of *C. jejuni* from faeces;

(c) No antibiotic therapy prescribed by the clinic doctor, nor any given during two weeks prior to entry into the study.

9.2.1.3 Sampling procedure

As far as possible, one faecal sample was investigated bacteriologically at weekly intervals until 6 consecutive specimens were negative for *C. jejuni* salmonellae or shigellae.

9.2.1.4 Bacteriological examination of faeces

(a) The filtration technique and inoculation onto Skirrow's selective medium as described in Chapter 4 and used in the studies described in Chapters 5, 6, 7 and 8 were used for the recovery of *C. jejuni* from faeces.

During the early stages of the present investigations one single

colony was removed from the *C. jejuni* plates, and was then subcultured and identified according to Holdeman, Cato and Moore (1977). Following storage in liquid nitrogen, cultures were lyophylised before being sent to Brussels and Manchester for typing. Subsequently, a sweep of 3 to 5 colonies were removed from the primary isolation plates and after culture and identification were stored in liquid nitrogen. Initially the Cary-Blair transport medium (Cary and Blair 1964) was used but subsequently the Amies medium was inoculated before being shipped by air mail to Europe and the United Kingdom. (Amies and Douglas 1965)

(b) Isolation and identification of salmonellae, shigellae and diarrhoeagenic *Escherichia coli* were performed as described by Robins-Browne, Still, Miliotis, Richardson, Koornhof, Freiman, Schoub, Lecatsas and Hartman (1980).

The techniques were conventional and although they included serotyping for enteropathogenic *E. coli* (EPEC) assay for enterotoxin production (ETEC) and testing for enteroinvasiveness (EIEC), these results will not be presented in detail in this thesis.

9.2.1.5 Serotyping of *C. jejuni*

The following serotyping techniques were used:

(a) A passive haemagglutination method described by Lauwers, Vlaes and Butzler (1981).

This technique is based on the detection of *C. jejuni* heat-stable 'O' antigens which were used to sensitize human group O rhesus-negative erythrocytes. These sensitized red blood cells were subsequently agglutinated in specific antisera raised in rabbits. The typing was performed by Dr. Lauwers of the Department of Clinical Microbiology of the Free University, Brussels.

(b) Detection of heat-stable antigens by techniques developed in Manchester (Abbott, Dale, Eldridge, Jones and Sutcliffe 1980).

These methods are based on the bactericidal activity of specific antisera raised in rabbits against *C. jejuni* strains as well as the use of passive haemagglutination (Penner and Hennessy 1980) and slide agglutination with formalized suspensions of *C. jejuni* strains. Drs D M Jones and E M Sutcliffe of the Public Health Laboratory, Withington Hospital, Manchester, performed the typing.

9.2.1.6 Information sheet on clinical and epidemiological data

Two simple information sheets were designed to cover the following aspects-

Personal: Age, sex, address, breast feeding and supplemented feeding.

General: Family size and composition, chicken consumption and keeping of chicken and dog and whether dog was fed with chicken scraps.

Clinical: Nutritional status, weight, dehydration, stool appearance (consistency, mucus, blood) and rectal temperature.

9.2.2. Prevalence of *C. jejuni* and evidence of person-to-person transmission in Sowetan families

The spread of *C. jejuni* in selected families in Soweto was studied in three different situations:

(a) Spread of infection to siblings and other members of households of very young babies who were excreting *C. jejuni* following an acute attack of gastro-enteritis. At the same time the duration of excretion of *C. jejuni* serotypes in the faeces of infected individuals was monitored (Family Study I).

(b) Following an observation that when experimentally infected baby mice transmit their *C. jejuni* organisms to their mothers the latter animals excreted *C. jejuni* for prolonged periods (Dr David Norrie), it was decided to examine human mothers in Soweto immediately post-partum for *C. jejuni* infection and then to follow possible spread within their families. After having examined the faeces of 125 women immediately after confinement, three such *C. jejuni*-excreting mothers were found. These individuals were used as index cases in the Family Study II.

(c) Control families with no history of a recent diarrhoeal episode (Family Study III) were examined according to the same protocol used for the Family Study I and Family Study II. The information sheets described in Section 6.2.1.6 were also used in the family studies.

It was originally planned to investigate six families in each category but, these studies have not yet been completed. Also, detailed serotyping results are only available on one of the twelve families that have been studied. These preliminary and uncompleted studies yielded interesting and relevant information which will be included in this thesis.

9.2.2.1 Entry criteria for family studies

The entry criteria for the three family studies were as follows:

(a) The presence in the family of an infant or young child with *C. jejuni*-associated diarrhoea as the index case, (six families studied, one of which has been completed).

(b) A mother excreting *C. jejuni* during the post-partum period as the index case (three families, limited serotyping results only).

(c) Families with young children in which no member experienced a recent episode of *C. jejuni* enteritis (three families, no serotyping results yet).

9.2.2.2 Sampling procedure for family studies

An attempt was made to collect one faecal sample weekly from every available member of the households concerned until 6 consecutive specimens from each individual member were negative for *C. jejuni*. These criteria could however not be followed rigidly as it was not possible to gather all the members of the respective families together once a week during normal working hours. Pre-school children therefore unavoidably predominated in these studies.

All samples reached the laboratory and were processed on the day of collection. Most of the specimens were freshly voided when collected by Ms Zola Khumalo who solicited faecal samples from each member of the family.

9.2.2.3 Bacteriological examinations and serotyping

The laboratory processing of the faecal specimens, including serotyping of *C. jejuni* isolates identical to that as described under Sections 6.2.1.4 and 6.2.1.5.

9.3 Results

9.3.1 Age Related excretion of *C. jejuni*

The results of the isolation of bacterial pathogens from the faeces of different age groups in Sowetan children are given in Table 9.1.

Table 9.1 Isolation of bacterial pathogens in Sowetan children [illegible] acute attack of diarrhoea[a]

Age group (Months)	No. of patients	[illegible]	Salmonella	Shigella	EPEC[b]	ETEC[b]	[illegible]
0 - 4	8	12	6	0	4	1	1
5 - 12	9	30	9	2	2	1	0
13 - 24	10	31	3	0	2	3	1
25 - 36	9	32	0	3	4	6	0

a. Specimens examined at approximately weekly intervals until 6 consecutive negative results were obtained

b. Excretions not followed up

Table 9.1 Isolation of bacterial pathogens in Sowetan children following an acute attack of diarrhoea[a]

Age group (Months)	No. of patients	*Salmonella*	*Shigella*	EPEC[b]	ETEC[b]	EIEC[b]
0 - 4	8	13	6	0	4	0
5 - 12	9	30	9	2	2	1	0
13 - 24	10	31	3	0	2	3	1
25 - 36	9	32	0	3	4	6	0

a. Specimens examined at approximately weekly intervals until 6 consecutive negative results were obtained

b. Excretions not followed up

C. jejuni was recovered at a much higher frequency from these children than salmonellae and shigellae. The isolation rate of *C. jejuni* was lowest in the 0-4 months age group. As fewer samples were examined for diarrhoeagenic *E. coli*, the isolation rates of EPEC, ETEC and EIEC were not comparable with those of the other bacterial pathogens. Table 9.2 illustrates the number of children in the different age groups yielding *C. jejuni* on more than one occasion. More children over four months of age excreted *C. jejuni* repeatedly than their younger counterparts.

The patterns of mixed bacterial infections, *C. jejuni* excretion alone and prolonged excretion of *C. jejuni*, irrespective of serotype, is presented in Table 9.3. There was a tendency for longer excretion of *C. jejuni* or greater frequency of intestinal colonization by this organism in children older than four months, while a lower rate of mixed bacterial infections by *C. jejuni*, salmonellae, shigellae and diarrhoegenic *E. coli* was observed in the 0-4 months-old age group.

Using available serotyping results to distinguish between prolonged excretion of the same *C. jejuni* serotype and reinfection with different serotypes, it was found that both situations prevail in Sowetan children. (Tables 9.4, 9.5, 9.6 and 9.7) Prolonged excretion of the same serotype as well as re-infection with multiple serotypes tended to occur more frequently in children older than 4 months of age (Table 9.8). The presumed rates of re-infection with different serotypes are impressive, even in the group of children excreting *C. jejuni* for less than 3 weeks. (Tables 9.8 and 9.11). The presumed frequency of reinfection in the prolonged excretors of more than 3 weeks is no less 12 of 15 (80%) or 15 of 34 (44%) of all excretors in which serotyping findings were available (Tables 9.4 - 9.8).

Based on the repeated excretion of non-typable strains or strains belonging to serotypes that share sufficient antigenic determinants to suggest that they may represent variants of the same serotypes, another 5 may be added to the list of 5 definite excretors (Table 9.12) giving a rate of 10 of 34 (29%) of probable prolonged excretors of the same serotypes. These findings are summarised in Table 9.9. A list of patients on whose isolates serotyping was done and the respective serotypes recovered on more than one occasion are given in Table 9.10. The feeding habits and nutritional status of the patients concerned are also tabulated. (Refer page 142).

Table 9.2 Faecal excretion of *C jejuni* in Sowetan Children

Age group (in months)	No of patients	No yielding *C jejuni* once only	No yielding *C jejuni* more than once
0 - 4	8	6	2
5 - 12	9	3	6
13 - 24	10	3	7
25 - 36	9	0	9

Table 9.3
Patterns of mixed infections, *C jejuni* excretion alone and prolonged excretion of *C jejuni*

Age (months)	Mixed infections (*C jejuni* + other bacterial pathogens)	*C jejuni* alone excreted	Prolonged excretion of *C jejuni**	
			> 3 weeks	≥ 3 weeks
0 - 4	4	4	0/8	2/8
5 - 12	8	1	4/9	5/9
13 - 24	6	4	6/10	7/10
25 - 36	7	2	6/9	6/9

*Same or different serotypes

TABLE 9.4

Duration of excretion of *C. jejuni*, *Salmonella*, *Shigella* and *E. coli* I: ≤ 4 months age group.

Patient	Age (months)	Sex	Weeks Positive	*C. jejuni* serotype A[a]	*C. jejuni* serotype B[b]	*Salmonella*	*Shigella*	*E. coli* toxin, invasive, serotype
C10	3	F	0[c]	ND	ND	Nil		ST
C25	2	F	0	ND	ND	Nil		Nil
C35	3	M	0	NT	37	*S. typhimurium*		044
			1	NT	37	*S. typhimurium*		0142
			2	NT	na	*S. typhimurium*		—
			3	NT	37	*S. typhimurium*		0142
C119	1	F	0	ND	1,29	*Llandudno* (week 5)		Nil
C173	2	F	0	na	na	Nil		0117
			1	na	NT			
			3[d]	na	NT			
C253	2	F	0	2	NT	Nil		Nil
C350	3	M	0 only	na	NT	Nil		Nil
C353	1	F	0 only	na	NT	Nil		Nil

[a] = Manchester Public Health Laboratory; [b] = University of Brussels; [c] = 0 = Zero time - first isolation;

[d] = Left Soweto at this point; ND = Not done; NT = Not typable

na = not available (strain died) ST = stable toxin

TABLE 9.5

Duration of excretion of *C. jejuni*, *Salmonella*, *Shigella* and *E. coli* in 5-12 month age group

Patient	Age	Sex	*C. jejuni* isolation on weeks	*C. jejuni* Serotype A[a]	B[b]	*Salmonella* / *Shigella* isolated	*Shigella* / *Salmonella* isolation on weeks	*E. coli* toxin, invasive, serotype
C9	5	M	0[c]	4,13,50	3,25	*S. blockley*	4	Nil
			4	4,13,50	NT	—	—	
			6	3	na	—	—	
			7	NT	na	—	—	
C15	9	F	0 only	ND	ND	*S. larochelle*	3	086
C22	11	M	0	na	na	—	—	Nil
			2	na	na	*Sh. flexneri*	2	Nil
			3	4,13,50	NT	—	—	
			4	4,13,50	25	—	—	
			5	4,13	na	—	—	
						S. typhimurium	7	
C24	9	M	0	na	NT	*S. lamita*	0	Nil
			1	na	17,45	—	—	
			2	na	na	*S. lamita*	4	
C40	11	M	1	NT	na	Nil	—	0128
			2	na	17,45			
			5	na	17,45			
			11	na	17,45			
			13	na	17,45			
			32	31,2	na			

Continued over.....

TABLE 9.5 Continued.....

Patient	Age	Sex	*C. jejuni* isolation on weeks	*C. jejuni* Serotype A[a]	B[b]	*Salmonella/ Shigella* isolated	*Shigella Salmonella* isolation on weeks	*E. coli* toxin, invasive, serotype
C51	10	F	0[c]	1	37	*Sh. flexneri*	0	Nil
			1	na	NT			
			2	na	NT			
			3	5	NT			
			4	NT	5,8			
C55	11	F	0 only	ND	ND	Nil		LT
C80	8	M	0	na	NT	*S. taroshella*	0	Nil
			—	NT	NT			
			2	NT	NT	*S. schwarzengrund*	2	
						typhimurium	3	
						S. jukestown	9	
C88	5	M	0 only	na	37	Nil	Nil	Nil

[a] = Manchester Public Health Laboratory; [b] = University of Brussels; [c] = 0=Zero time - first isolation;

ND = not done; NT = Not typable; na = not available (strain died);

LT = Labile toxin

TABLE 9.6

Duration of excretion of [illegible], *Shigella* and *E. coli III* : 13-24 [illegible] age group

Patient	Age (months)	Sex	Positive isolation on weeks	Serotype		*Salmonella Shigella* isolated	*Shigella Salmonella* isolation on weeks	*E. coli* toxin, invasive, serotype
C23	16	[illegible]	[illegible]	9,37 NT	NT [illegible],11	Nil Nil	Nil Nil	Nil Nil
C35E[illegible]	16	[illegible]	[illegible]	[illegible]	37	*S. typhimurium*	[illegible]	Nil
C73	14	[illegible]	[illegible]	na	[illegible]	Nil	---	Nil
C93	13	[illegible]	[illegible]	NT	14,37,44	*S. kentucky*	[illegible]	Nil
C106	14	[illegible]	0 2 12	na NT na	NT 17 na	--- *S. senftenburg* ---	--- [illegible] ---	LT
C141	13	[illegible]	0 2 3	NT NT NT	15 NT NT	Nil	---	ST/LT 055
C169	16	[illegible]	[illegible] 2 [illegible] [illegible]	NT 1 37 NT	8 8,11,53,14 11,53 37	Nil	---	Nil
C204	20	[illegible]	[illegible] [illegible]	NT NT	8 na	Nil	---	0124(EIEC)

Continued over.....

TABLE 9.6 Continued.....

Patient	Age (months)	Sex	Positive isolation on weeks	Serotype [a]		*Salmonella/Shigella* isolated	*Shigella/Salmonella* isolation on weeks	*E. coli* toxin, invasive, serotype
C260	14	F	0	19	NT	Nil	---	Nil
			1	19	Penn 19,22			
			5	na	na			
			6	19,35,37	11,14			
			11	24	na			
			12	24	na			
C263	14	F	0	19,3	NT	Nil	---	
			3	19	37			
			4	na	na			
			5	na	na			
			6	19	37			ST 044
			7	19	9,42			
			8	19	NT			
			9	4,9	na			

[a] = Manchester Public Health Laboratory; [b] = University of Brussels; [c] = 0=zero time - first isolation

[d] = C35E - This child was a member of Family 35 (Figure 1);

ND = Not done; NT = Not typable; na = not available (strain died; ST = Stable toxin; LT = Labile toxin;

EIEC = Enteroinvasive *E. coli*.

TABLE 9.7 Duration of excretion of *C. jejuni*, *Salmonella*, *Shigella* and *E. coli IV* : 25-36 month age group

Patient	Age (months)	Sex	*C. jejuni* isolation on weeks	*C. jejuni* serotype A[a]	B[b]	*Salmonella*/ *Shigella* isolat	*Shigella* *Salmonella* isolation on weeks	*E. coli* toxin, invasive, serotype
C54	30	M	0[c]	na	na	Nil	---	Nil
			1	na	na	Nil	---	Nil
C357[d]	32	M	0	NT	37[e]	Nil	---	026
			1	na	na			026
			3	NT	15,37			0128
			5	na	5,8			0128
			6	37[e]	9,37			
			7	37	37			
			8	37	5,8			
			18	18	NT			
			20	18	1			
C152	25	M	0	3	NT	Nil	---	ST/LT
			2	3	NT			ST
			4	3	NT			
C193	25	F	0	NT	NT	Nil	---	ST
			1	3	NT			
			2	3	37			
217	32	M	0	na	na	*Sh. flexneri*	0	Nil
			3	5	6			
			7	4	na			
C231	27	M	0	NT	NT			LT
			4	5	NT	*S. panama* (week 15)		

Continued over.

TABLE 9.7 Continued.

Patient	Age (months)	Sex	*C. jejuni* isolation on weeks	*C. jejuni* serotype A[a]	B[b]	*Salmonella Shigella* isolate	*Shigella/ Salmonella* isolation on weeks	*E. coli* toxin, invasive, serotype
C285	25	F	0[c]	19	NT	Nil	—	ST
			3	na	na			S1
			5	19	15			
			6	3	NT			
			7	na	na			
			12	19	na			
			13	3,24	NT			
			14	3	NT			
			15	na	na			
C372	25	F	0	ND	ND	Nil	—	ND
			1[f]	ND	ND			
C375	25	F	0	ND	ND	*Sh. flexneri*	0	ND
			3	ND	ND		1	

[a] = Manchester Public Health Laboratory; [b] = University of Brussels; [c] = 0 indicates zero time - first isolation;

[d] C35F - This child was a member of Family 35 (figure 1); [e] Serotype 37 of Brussels - not the same as Manchester serotype 37

[f] = Left Sowto at this point

ND = Not done; NT = Not typable; na = not available (strain died); ST = Stable toxin; LT = Labile toxin

of faecal excretion according to serotype and age

No.	Duration < 3 weeks				Duration ≥ 3 weeks			
	Same/ Single serotype	Possibly[a] same serotype	Re-infection, different serotypes	Sub-total	Same serotype	Possibly[a] same serotype	Re-infection, different serotypes	Sub-total[b]
				8	0	0	0	0
				5	1	2	4	4
				5	1	3	4	5
			1	2	3	0	4	6

[a] Consecutive non-typable strains or minor differences in antigenic components of serotypes

[b] Some children who excreted the same serotype for > 3 weeks were also reinfected with additional serotypes.

[c] One children excreted *C. jejuni* twice but the isolates were not serotyped.

Table 9.9 C. jejuni studies, Soweto.

	Persistence of infection with [illegible] for >1week
Total number of persistent [illegible]	
Evidence of persistent shedding of [illegible] serotype	
(a) definite[a]	
(b) Presumptive[b]	
Evidence of reinfection/mixed [illegible] C. jejuni	
(a) Probable[c]	
(b) Possible[c]	

a) Isolates from two children were not serotyped

b) Assuming that consecutive non-typable strains are the same

c) Assuming that consecutive non-typable strains are diff[illegible]

TABLE 9.10

DURATION OF *C. JEJUNI* EXCRETION ACCORDING TO SEROTYPE

NO.	AGE (MONTHS)	TYPE	DURATION (DAYS)	FEEDING BREAST	SUPPLEMENTARY	NUTRITIONAL STATUS
C35	3	37(B)[a]	31	-	+	Average
C9	4	4,13,50(M)[b]	12	-	+	Poor
C22	11	4,13,50(M)	6	-	+	Average
C40	11	17,45(B)	81	+	+	Poor
C263	14	19(M)	40	+	+	Good
		37(B)	33			
C35 F	32	37(B)	60	NR	NR	
		37(M)	14			
		18(M)	14			
C152	25	3(M)	35	NR	NR	Good
C193	25	3(M)	7	NR	NR	Good
C285	25	19(M)	113	NR	NR	Good
B119	Mother[c]	6 (B)	18	NR	NR	Good

a) Serotype according to Brussels scheme;

b) Serotype according to Manchester scheme

c) Post-partum mother without diarrhoea

d) NR: Not recorded, children too old for breast feeding.

TABLE 9.11

C. FECAL EXCRETION, FEEDING HABITS AND PET DOG

14 MONTH AGE GROUP

NO.	AGE (MONTHS)	SAME SEROTYPE SHORT[a]	SAME SEROTYPE PROLONGED[b]	MIXED/RE-INFECTION SHORT	MIXED/RE-INFECTION PROLONGED	FEEDING BREAST	FEEDING SUPPLEMENTED	CHICKEN MEAT	DOG AS PET	NUTRITIONAL STATUS
F* C10	3	+	-	-	-	+	+	+	-	Poor
F C25	2	+	-	-	-	-	+	+	-	Average
M C35	3	-	+(4)[c]	-	-	-	+	+	-	Average
F C119	1	+	-	-	-	+	-	+	-	Average
F C173	2	+	-	-	-	+	-	+	-	Average
F C253	2	+	-	-	-	+	-	+	+	Good
M C350	3	+	-	-	-	+	-	-	-	Poor
F C353	3	+	-	-	-	+	-	NR[d]	-	Poor

a) Short: duration of excretion ≤ 3 weeks

b) Prolonged: duration of excretion >3 weeks

c) Figures in brackets indicate duration of excretion in weeks (same serotype)

d) NR: Not recorded.

* F = Female, M = Male.

TABLE 9.11 continued

C. JEJUNI EXCRETION - FEEDING BABIES AND PET DOG

5 - 12 MONTH AGE GROUP

NO.	AGE (MONTHS)	SAME SEROTYPE SHORT[a]	SAME SEROTYPE PROLONGED[b]	MIXED/REINFECTION SHORT	MIXED/REINFECTION PROLONGED	FEEDING BREAST	FEEDING SUPPLEMENTED	CHICKEN MEAT	DOG AS PET	NUTRITIONAL STATUS
*M C9	5	+	-	+	-	-	+	+	+	Poor
*F C15	9	+	-	-	-	+	+	+	-	Good
M C22	11	+	-	-	-	-	+	+	+	Average
M C24	9	-	-	+	-	-	+	+	-	Average
M C40	11	-	+(12)[c]	+	+(32)	+	+	+	+	Poor
F C51	10	-	-	-	+(6)	+	+	+	-	Good
F C55	11	+	-	-	-	+	+	NR[d]	-	Poor
M C80	8	+	-	-	-	-	+	+	-	Good
M C88	5	+	-	-	-	+	+	+	-	Good

a) Short: duration of excretion ≤ 3 weeks

b) Prolonged: duration of excretion > 3 weeks

c) Figures in brackets indicate duration of excretion in weeks (same serotype)

d) NR: Not recorded.

* F = Female, M = Male.

TABLE 9.11 continued

C. JEJUNI EXCRETION - FEEDING HABITS AND PET DOG

13 - 24 MONTH AGE GROUP

NO.	AGE (MONTHS)	SAME SEROTYPE SHORT[a]	SAME SEROTYPE PROLONGED[b]	SEROTYPE MIXED/RE-INFECTION SHORT	SEROTYPE MIXED/RE-INFECTION PROLONGED	FEEDING BREAST	FEEDING SUPPLEMENTED	CHICKEN MEAT	DOG AS PET	NUTRITIONAL STATUS
M* C23	18	-	-	-	-	-	+	+	-	NR[d]
F C35E	16	+	-	-	-	-		+	-	NR
M C73	13	+	-	-	-	-	+	+	+	Good
M C93	13	+	-	-	-	-	+	+	+	Poor
M C106	14	-	-	-	-	+(12)[c]	+	+	+	Good
M C141	13	-	-	-	+	-	-	+	+	Poor
F C169	16	-	-	+(9)	-	(?)	+	NR	+	Good
F C204	20	-	-	+(4)	+		-	+	-	Good
F C260	14	-	-	-	-	+(12)	-	+	+	Average
F C263	14	-	+(6)	+(9)	-	+(9)	+	+	+	Good

a) Short: duration of excretion ≤ 3 weeks

b) Prolonged: duration of excretion > 3 weeks

c) Figures in brackets indicate duration of excretion in weeks (same serotype)

d) NR: Not recorded.

* F = Female, M = Male

TABLE 9.11 continued

C. JEJUNI EXCRETION - CHICKEN CONSUMPTION AND PET DOG - 25 - 36 MONTH AGE GROUP

NO.	AGE Month	SAME SEROTYPE SHORT[a]	SAME SEROTYPE PROLONGED[b]	POSSIBLY SAME SEROTYPE	MIXED/REINFECTION SHORT	MIXED/REINFECTION PROLONGED	CHICKEN	DOG	NUTRITIONAL STATUS
M* C54	30	+	-	-	-	-	NR	+	Poor
F C35	32		+(9)[c]	-	-	+(20)	+	-	Average
M C152	25	-	+(5)	-	-	-	+	+	Good
F C193	25	+	-	-	+		+	-	Good
M C217	32	-	-	-	-	1(7)	+	-	NR
M C231	27	-	-	+(4)	-	+(4)	NR	NR	Good
F C285	25	-	+(6) +(8) +(14)		-	+(14)	NR	NR	Good

a) Short: duration of excretion ≤ 3 weeks

b) Prolonged: duration of excretion > 3 weeks

c) Figures in brackets denote duration of excretion in weeks (same serotype)

d) NR: Not recorded.

* F = Female, M = Male

A note of caution should be expressed in the interpretation of the findings related to re-infection. They may represent, at least in some cases, examples of initial mixed infections with more than one *C. jejuni* serotype. Because the technique of selecting from the primary isolation plates catered mainly for the identification and serotyping of single colonies, original mixed infections may have resulted in the isolation of different serotypes on subsequent occasions. Indeed, certain discrepancies in the findings of the two typing laboratories on the question of prolonged excretion with the same serotype can only be explained on the basis of mixed infections.

Examples of the serotype findings in individual children who were persistent *C. jejuni* excretors with a single serotype and/or encountered reinfections are given in Tables 9.12 and 9.13.

9.3.2 Correlation of some environmental, social and clinical factors with faecal excretion of *C. jejuni*

Table 9.14 summerises some of the information obtained in the questionnaires filled out for each child. Numbers are too low to allow statistical analyses of the trends seen but in the case of absence of breast feeding, use of supplemented feeds, admission that chicken is a frequent constituent of the family diet and ownership of a dog, somewhat marked differences are seen between the percentages of a single isolation and those of repeated isolations.

No correlation was found between mixed bacterial infections, *C. jejuni* excretion alone, and repeated excretion of *C. jejuni* with poor nutritional status, dehydration or family size (Table 9.15).

The intermittent excretion of *C. jejuni* over prolonged periods in some of the children and the episodic nature of excretion will be discussed later.

9.3.3 Results of family studies

Although several families were studied, comprehensive serotyping results were only available for Family Number 35.

9.3.3.1 *C. jejuni* serotypes and other bacterial pathogens in Family Number 35.

Detailed bacteriological findings including the serotypes of *C. jejuni* from the index case and other members examined of Family 35 are represented in Figure 9.1. There is clear evidence that *C. jejuni*

Table 9.12 Examples of reinfection/persistence of *C. jejuni* serotypes

Patient No.	Age	+ve Isolation on weeks	Serotype M[a]	Serotype B[b]	Patient No.	Age	+ve Isolation on weeks	Serotype M[a]	Serotype B[b]
C35F	32/12	0	NT	37	C260	14/12	0	19	NT
		1	NA	NA			1	9	Pen 19,22
		3	NT	15,37			5	NA	NA
		6	NA	5,8			6	19,35 37	11,14
		7	37	9,37			11	24	NA
		8	37	37			12	24	NA
		21	37	5,8					
		29	18	NT					
		31	18						

[a]Manchester typing scheme

[b]Brussels typing scheme

[c]NT denotes non-typable

[d]NA denotes not available for typing

TABLE 9.13 Examples of persistence of [illegible] serotypes

Patient number	Age (months)	Positive Insulation on weeks	Serotype M[a]	Serotype B[b]
C35	3/12	0	NT[c]	37
		1	NT	37
		2	NA[d]	NA
		3	NT	37
C40	11/12	1	NT	NA
		2	NA	17,45
		3	NA	17,45
		11	NA	17,45
		13	NA	17,45
		32	31,2	NA

[a]Manchester typing scheme

[b]Brussels typing scheme

[c]NT denotes non-typable

[d]NA denotes not available for typing

Table 9.14 Influence of various factors on duration of excretion or re-infection of *C jejuni*

		No of patients with *C jejuni* isolations from faeces		Total
		Single isolation	Multiple isolations	
Breast Feeding	Yes	8	9	17
	No	3	10	13
Supplemented feeding	Yes	8	18	26
	No	3	2	5
Chicken eaten	Yes	9	20	29
	No	1	0	1
Ownership of dog	Yes	3	8	11
	No	9	11	20
Mixed infections with salmonellae/shigellae	Yes	8	12	20
	No	4	10	14

Table 9.15 Correlation of mixed infections, *C jejuni* excretion alone and prolonged excretion of *C jejuni* with poor nutrition, dehydration and family size

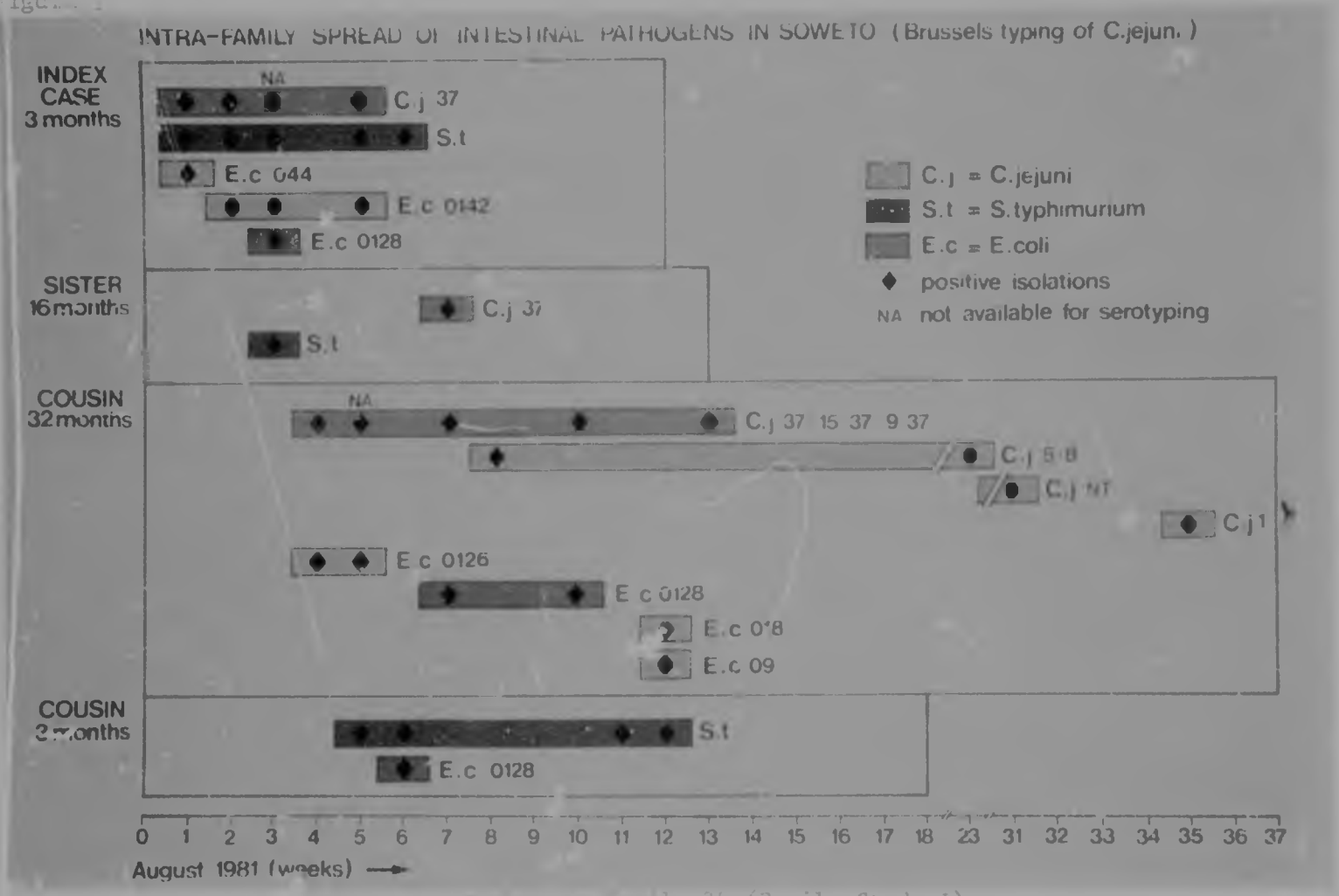
	Mixed infections (*C jejuni* + other bacterial pathogens)	*C jejuni* alone excreted	Prolonged excretion of *C jejuni*[a]
Poor nutrition	6/24	3/8	4/17
Dehydration Score			
Mild = 1 Moderate = 2 Severe = 3	28/23 (1.22)[b]	11/8 (1.38)	19/17 (1.12)
Family size Total/No of families	164/19 (8.6)[c]	52/7 (7.4)	126/17 (7.4)

[a] *Same or different serotypes

[b] Mean dehydration score derived from cumulative score and number of children

[c] Mean family size

Figure 1



Family 35 (Family Study I)

serotype 37 (Brussels) was excreted for [illegible]
case and probably persisted in his 32 month-old [illegible]
genic determinants additional to 37 were present in later [illegible]
Likewise, the 16 month-old sister was found to excrete the same
type on one occasion several weeks after the index case presented
its *C. jejuni*-associated diarrhoea and the 32 month-old cousin was
already excreting *C. jejuni* serotype 37. Interestingly, *Salmonella*
typhimurium and *E. coli* serotype 0128 which were originally excreted
by the index case, in addition to *C. jejuni* serotype 37, also subsequently appeared in the faeces of the 16 month-old sister and the 3
month-old cousin and the same *E. coli* serotype was also recovered from
the older 32 month-old cousin. Although these children frequently had
loose stools, diarrhoeal episodes could not be linked with any degree
of accuracy with excretion patterns of *C. jejuni* nor with those of
S. typhimurium and the EPEC serotypes (Figure 9.1).

9.3.3.2 Chronological order of *C. jejuni* isolations in family studies

Figures 9.2, 9.3 and 9.4 illustrate the time-relationships of *C. jejuni*
excretion in the three family studies. In the Family I study,
C. jejuni was recovered subsequent to the *C. jejuni*-associated diarrhoeal
episode in the index cases in other household members in families 35,
128, 130 and 131 but not in families 18 and 90. Considering the sequence
and time relationships of *C. jejuni* isolations, definite evidence of
person-to-person transmission was only demonstrated in Family 35 (Figure
9.1) and Family 128, possibly to three members, (Figure 9.2.3). Unfortunately due to incomplete information on *C. jejuni* serotypes, neither
person-to-person transmission nor the duration of excretion of the same
serotype could be determined in five of the six families. Of the five
serotype results to hand in the five incompletely studied families, four
concern Family 90 (see Figure 9.2.2). According to both the Brussels and
Manchester typing schemes the serotypes of isolates from three household
members of this family were all different, as was the one recovered from
the family dog. These strains were all isolated on the same day.

Two of the three post-partum mothers in the Family II study excreted
C. jejuni on three occasions over several weeks. The same serotype
according to the Brussels typing scheme was recovered twice, two weeks
apart, from the faeces of the index case of Family 119, but, subsequent isolates from this woman have not yet been typed (Figure 9.3.3).

(please turn to page 160)

FAMILY STUDY I

Family No. 18

Surveillance Period: 15.2.1982.-16.6.1982.

Fig. 9.2.1.

- Period in weeks -

AGE IN YEARS	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1/12	F	NT* ▲	△	△	△	△						△		△	△				Index case
3	M		△			△						△			△				
6	M					△							△	△				△	
Adult (Mother)	F	△	△	△									△					△	

NT* = Not typable. Brussels or Manchester

△ = *C. jejuni* not isolated

▲ = *C. jejuni* isolated

FAMILY STUDY 1.

FAMILY NO. 90.

Surveillance Period 15.3.1982. - 15.1.'8[illegible]

Fig. 9.2.2.

- Period in weeks

AGE IN YEARS	SEX	0 2 3	4 5 6	7 8 9 10 11	12 13 14 15	16 17 18 19	20 21 22 23 24	25 26 27 28	29 30 31 32	33 34	
16/12	F	NT(B)* ▲ Δ	[illegible] Δ	▲	Δ	Δ	Δ Δ Δ	Δ Δ	Δ Δ		Index case
3	F	Δ	Δ Δ	Δ Δ	Δ Δ	Δ	Δ Δ Δ Δ	Δ Δ	Δ Δ	Δ	
5	F	Δ	Δ	Δ Δ Δ	Δ Δ	Δ	Δ Δ Δ	Δ Δ			
7	M		St	Δ	Δ Δ						
10	F	Δ				Δ	Δ Δ	Δ Δ	Δ Δ		
10	F	7(M)* ▲		Δ	Δ Δ	Δ	Δ	Δ	Δ	Δ	
10	M	Δ	Δ Δ	Δ Δ	Δ			Δ			
13	F	Δ	Δ	Δ	Δ		Δ Δ	Δ	Δ Δ		
13	F	Δ	Sc Δ	Δ Δ	Δ Δ						
13	M		Δ	Δ Δ							
15	M	Δ	St Δ Δ	Δ Δ							
15	M	Δ	Δ	Δ	Δ	Δ					
15	M	Δ	Δ Δ	Δ Δ	Δ Δ	Δ	Δ				
21	M	Δ	Δ		Δ Δ	Δ					
Granny	F	43(B)* 46(M)* ▲	St Δ Δ	Δ	Δ		Δ				
Dog		Pen2.5(B) * 6.7M(M) * ▲	Δ Δ	Δ Δ			Δ	Δ	Δ Δ	Δ	

St: *Salmonella typhimurium* Sc: *Salmonella chester*

* = serotypes according to the Manchester (M) and Brussels (B) systems

Δ = *C. jejuni* not isolated

▲ = *C. jejuni* isolated

FAMILY STUDY 1

Family No. 128

Surveillance Period: 31.5.1982 - 15.11.1982

Fig. 9.7.1.

AGE IN YEARS	SEX	— Period in weeks — 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24	
4/12	F	▲ ▲ △ ▲ △ △ △ △ △ △ △ △	Index case
1/12	F	△ △ △ ▲ △ △ ▲ △ △ ▲	
12/12	M	△ △ ▲ △ ▲ △ △ △ △ △	
16/12	F	△ △ △ ▲ △ ▲ △ △ ▲ △ △	

△ = *C. jejuni* not isolated
▲ = *C. jejuni* isolated

FAMILY STUDY 1

Family No. 130

Surveillance Period: 31.5.1982. to.11.1982.

Fig. No. 6.1.1.

AGE IN YEARS	SEX	– Period in weeks –																								
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1/12	F	▲	Δ							Δ		▲	Δ	Δ		Δ		Δ	Δ	▲	▲	▲	Δ	Δ			Δ	Δ	Δ	Δ	
1/12	F	▲										Δ	▲	Δ	Δ			Δ	Δ	Δ	▲	▲	▲	Δ			Δ	Δ	Δ	Δ	
6	F																	Δ	Δ		Δ	Δ	Δ	Δ			Δ		Δ		
15	M																	Δ	Δ	Δ	*		Δ							
20	F																	Δ	Δ	Δ	Δ	Δ	Δ	Δ			Δ	Δ	Δ	Δ	
22	M																	Δ	Δ	Δ				Δ			Δ		Δ		
Dog																										Δ	Δ	Δ	Δ	

* Index case

Δ= *C. jejuni* not isolated

▲= *C. jejuni* isolated

FAMILY NUMBER 131

Figure 9.2.5

Surveillance period 19.7.1982 - 15.11.1982

- Period in weeks -

AGE IN YEARS	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1/12	M	▲			Sc ▲		△		△	△	△	△	△		△	△	△	△	
1/12	F	▲			St ▲		St ▲	▲	St △	△	△	△	△		△	△	△	△	
3	M								△	△	△	△	△		△	△	△		
3	F								△	△	△	△	△		△	△	△	△	
5	M								△			△	△				△	△	
7	F											△	△				△	△	
10	F								△			△	△		△				
26 (Mother)	F									△	△	△	△		△				
70	F								△			△	△		△				
Dog											▲		△		△	△	△	△	

Sc = *Salmonella chester* △ = *C. jejuni* not isolated
St = *Salmonella typhimurium* ▲ = *C. jejuni* isolated

FAMILY NO. II

Family No. 101

Surveillance Period: 8.2.1982. - 8.11.1982

Fig. 9.3.1.

AGE IN YEARS	SEX	Period in weeks 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25	26 27 28 29	30 31 32 33	34 35
(Mother) 17	F	△ ▲ △ △ △	△	△	
3/12	F	△ △ △ △ △	△ △	△	
4	F	△ △ △ △ △ △ △		▲	
8	F	△ △ △ △		△	
10	F	△ △			
17	F	△ △ △			
Dog		△ △ △ △ △ △ △ △ △ △ △ △ ▲	△ △	△ △	△ △

△ = *C. jejuni* not isolated
▲ = *C. jejuni* isolated

Family Study II

Family Number 110

Surveillance Period 29.3.1982 – [illegible]

Fig. .3.2. – Period [illegible] weeks –

AGE IN YEARS	SEX	[illegible]	27 28 29 30	[illegible]
25(Mother)	[illegible]	[illegible] ▲ [illegible] ▲ [illegible] ▲ [illegible]	[illegible]	[illegible] Index case
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
16	F	[illegible] △ ▲	[illegible]	[illegible]

△ = *Campylobacter jejuni* not isolated
▲ = *Campylobacter jejuni* isolated

FAMILY STUDY II

FAMILY NUMBER 119

Surveillance Period [illegible] - 8.11.[illegible]

Fig. 7.1.1. Period in weeks

AGE IN YEARS	SEX	0 1	2 3 4 5	6 7 8 9 10	11 12 13 14	15 16 17 18	19 20 21 22 23	24 25 26 27	28 29 30 31	32 33
28 (Mother)	F	△	▲ 6B ▲ 60* 2(M) 3(M)	△ △	□ □	△	□ □			△
1/12	M		△	□ △	□ △		□ □			△
3	M			▲ △	□					
9	M			△	△		□ △ △			△
4	M						△ △ △			△
11	F			△ △	△					

* Serotypes according to the Brussels(B) and Manchester(M) systems.

△ = *C. jejuni* not isolated
▲ = *C. jejuni* isolated

FAMILY STUDY NO. III

Family No. 103

Surveillance Period 29.3.1982.-25.[illegible].1982

Fig. 9.4.1.

AGE IN YEARS	SEX	Period in weeks – 0 1 2 3 4	5 6 7 8 9	10 11 12 13	14 15 16 17	18 19 20 21 22	23 24 25 26
12/12	M	△ St △	△ △	△ △		△	△ △ △
27/12	F	△	△				△
4	F	△	△ △			△	△ △ △
20 (Mother)	F	△	△ △ △	△ △	△		△ △
40	M	△ △	△ △ △				
60 (Granny)	F	△ △	△ △ △	△			
Dog			△	△ △			

St - *Salmonella typhimurium* △= *C. jejuni* not isolated

FAMILY GROUP III

Family No. 22

Surveillance Period: [illegible].1981 – [illegible].1982.

Fig. 5.4.2.

– period in weeks –

AGE IN YEARS	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
3/12	F	△	△			△		△		▲										△		△		△			△		△				
13/12	F	△				△		△		▲																			△				
19	F	△	△			△			○	▲												△		△					△				
14	M	△	△		△					△																							
Granny	F	△	△					△		▲																							

△ = C. jejuni not isolated
▲ = C. jejuni isolated

FAMILY STUDY (1)

Surveillance Period [illegible] 1967 to 1.1.1982

Fig. 9.4.3

AGE IN YEARS	SEX	Period in weeks 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
[illegible]	F	
19 (Sister)	F	
10	M	

[illegible] = [illegible]
△ = [illegible] not isolated
▲ = [illegible] isolated

In two of the three families of this study, *C. jejuni* was isolated from a member other than the index case on one occasion each. In Family 119, a 3 year-old child was found to excrete *C. jejuni* about a month after the mother returned home after her confinement but it is not known whether the serotype from this boy was the same as that of his mother (Serotype 6, Brussels). In another family (Family 101) *C. jejuni* was recovered from a 4 year-old child 26 weeks after the index case started excreting *C. jejuni*. In this family a dog was found to excrete *C. jejuni* 20 weeks after the index case. In Family 110 the mother excreted *C. jejuni* during a period of at least three months of bacteriological surveillance.

In the Family III study *C. jejuni* was recovered on five occasions from members of two families, while the third family did not yield any *C. jejuni* isolates. In Family 127 four of the five members excreted *C. jejuni* during the same visit. The serotypes of these isolates are not known. (Figure 9.4.).

In summary, based on serotyping, convincing evidence of person-to-person transmission from the index case of Family 35 to two children in the household was obtained, although acquisition from other sources cannot be entirely excluded. Unconfirmed evidence of household spread was found in another two families (128 and 119) while possible but unlikely transmission may have occurred in Family 101.

9.3.3.3 Isolation rates and prolonged excretion of *C. jejuni* in families

The isolation rates of *C. jejuni* in the three family studies are summarised in Tables 9.16 and 9.17. In the Family Study I where in each household an infant or young child with *C. jejuni* associated diarrhoea was a potential source of spread within the household, *C. jejuni* was recovered from 44 of 395 specimens (11%) and from 15 of 42 individual members (36 per cent) (Table 9.16). The corresponding figures for the second family study where three post-partum mothers excreted *C. jejuni*, were 9 of 118 specimens (8 per cent) and 5 of 15 individuals (33 per cent). In the third family study where there was no obvious human *C. jejuni* source within the households, the organism was recovered from 5 of 105 specimens (5 per cent) and 5 of 14 household members (36 per cent) (Table 9.17).

Like *C. jejuni*, salmonellae were more frequently recovered in the first family study (13 of 42 individuals, 31%) than in the second and third studies which yielded 0 of 15 and 2 of 14 (14 per cent) isolations respectively. Probable prolonged excretion of *Salmonella typhimurium* (Family 131) and

(turn to page 163)

Table 9.16

Summary of findings in Family Study I:
Index case with *C jejuni* – associated diarrhoea

Family No.	No. of Specimens	No. of individuals examined[a]	*C jejuni* Positive specimens	*C jejuni* Positive individuals	*C jejuni* Excretion > 3 weeks	Excretors of salmonellae
35	48	4	13	3	2	3
128	42	4	11	4	4	0
18	22	4	1	1	0	0
130	70	6	9	2	2	0
90	144	15	4	3	1	7
131	69	9	6	2	2	3
TOTAL	395	42	44(11%)	15(36%)	11(26%)	13(31%)

[a]Only members of households from whom more than 3 faecal specimens were examined are listed.

Table 9.17

C. jejuni excretion in second and third family studies.

STUDY II Family Number	No. of specimens	No of individuals examined[a]	*C jejuni* Positive specimens	*C jejuni* Positive individuals	*C jejuni* Excretion > 3 weeks	Excretors of salmonellae
110	43	3	3	1	1	0
101	39	6	2	2	0	0
119	36	6	4	2	1	0
TOTAL	118	15	9(7.6%)	5(33%)	2(13%)	0
STUDY III						
127	31	5	4	4	0	0
103	42	6	0	0	0	1
129	30	3	1	1	0	1
TOTAL	103	14	5(4.8%)	5(36%)	0	2(14%)

[a]Only members of households from whom more than 3 faecal specimens were examined are listed.

evidence of spread of this organism, probably from a single source to several members of Family 90 were observed (Figure 9.2).

With regard to persistence of *C. jejuni* for more than 3 weeks (including intermittent excretion), 11 of 42 (26 per cent) members in the first family study excreted *C. jejuni* in their faeces for prolonged periods compared with 2 of 15 (13 per cent) in the second family study and 0 of 14 in the third study. The two persistent faecal excretors of *C. jejuni* in the second study were both index cases who excreted *C. jejuni* during the post-partum period. Apart from the three post partum mothers in the second family study, only one out of a total of five mothers in the other two family studies excreted *C. jejuni*.

9.3.3.4 Age related faecal excretion of *C. jejuni* in families

An analysis of the faecal excretion of *C. jejuni* according to age in the three family studies is represented in Tables 9.18 and 9.19. Most of the *C. jejuni* excretors in the first family study were 3 years of age or younger: 13 of 18 studied (72 per cent), as opposed to 2 of 24 (8 per cent) in the older age groups (Table 9.20). All six index cases in this study were less than 2 years of age (five were in the 0-4 months age group), giving rise to a definite bias towards *C. jejuni*-excretion in the very young (Table 9.18).

As the numbers of individuals examined in the second and third family studies were small, clear trends in the age related risk of infection or prolonged excretion of *C. jejuni* were not observed (Table 9.19). However, when the findings of all three studies are considered together, even after omiting the index cases which may have introduced bias, (see Table 9.20), there is a definite trend for children under 3 years of age to excrete *C. jejuni* more frequently (P=0.007) and also to excrete it for longer periods than the older individuals (P=.003) (Fishers exact test).

9.3.4 Patterns and duration of *C. jejuni* excretion

Based on the serotyping results (Tables 9.4, 9.5, 9.6 and 9.7 and Figure 9.1) it was shown that the same *C. jejuni* serotype could be excreted intermittently for long periods (at least 16 weeks in the case of child C285 (Tables 9.7 and 9.10). The intermittent excretion of the same serotype tended to occur in episodes lasting from one week to three weeks and in the case of one child for at least 5 weeks (C263, Table 9.6). These episodes of "continuous" excretion did not necessarily coincide with bouts of diarrhoea. Considering all the available data, including the isolation of

(turn to page 169)

Table 9.18

Faecal excretion of *C jejuni* in families

FAMILY STUDY I : Index case with *C jejuni* - associated diarrhoea

Family No.	Individual ages in years	0 - 4 mnths	5 - 12 mnths	13 - 24 mnths	25 - 36 mnths	3 - 6	7 - 16	> 16	TOTAL
35	(3/*12[a]), 3/12, 16/12, 32*/12	2(1*)	0	1(1)	1(1*)	0	0	0	4(3[2*])
128	(1*/12), 6*/12, 12*/12, 16*/12	2(2[2*])	1(1*)	1(1*)	0	0	0	0	4(4[4*])
18	(1/12), 36/12, ♀, Mother	1(1)	0	0	1	1	1	1	4(3)
130	(4*/12), 8*/12, 6,15,20,22(dog)	2(2[2*])	0	0	0	1	2	2	6(2[2*])
90	(15*/12), 36/12, 5,7,3x10,3x13,3x15, 21,4 non negative(2 dogs(1))	0	0	1(1*)	1	10	2(1)	2(1)	15(3[1*])
131	(1*/12), 1*/12, 36/12, 36/12, 5,7,10,26,70 (2 dogs(1))	2(2[2*])	0	0	0	1	2	2	9(2[2*])
	TOTAL:	8(8[7*])	1(1*)	3(3[2*])	3(1*)	11(0)	7(1)	7(1)	42(5[17*])

[a]Index cases are ringed

*Asterisks indicate *C. jejuni* excretors of >3 weeks duration (irrespective of serotype); the numbers immediately preceding the asterisks denote the number of such prolonged excretors.

( ) No of *C jejuni* excretors in brackets

Table 3.19

Faecal excretion of *C. jejuni* according to age

Family No.	Individual ages in years	0 - 4 mths	5 - 12 mths	13 - 24 mths	25 - 36 mths	3 - 5 years	6 - 16	> 16	TOTAL
FAMILY STUDY II : Index case *C. jejuni*-excreting post-partum mother									
110	1/12, 10, (25)[a]	1	0	0	0	0	1	1(1[1*])	3(1[1*])
101	12/12, 4,8,10, 17 (17) dog(1)	1(1)	0	0	0	2	2	2(1)	6(2)
119	12/12, 35/12, 6,8,11, (28)[a]	1	0	0	1(1)	2	1	1(1[1*])	6(2[1*])
	TOTAL	3(1)	0(0)	0(0)	1(1)	4(0)	4(0)	4(3[2*])	15(5[2*])
FAMILY STUDY III : No initial diarrhoeal episode									
127	15/12, 36/12, 19, 24 Gran	0	0	1(1)	1(1)	0	0	3(2)	5(4)
103	12/12, 24,12, 4,20,40,60, 1dog	0	1	1	0	1	0	3	6(0)
129	9/12, 10, 39	0	1	0	0	0	1	1(1)	3(1)
	TOTAL	0	2	2(1)	1(1)	1(0)	1(0)	7(3)	14(5)

[a]Index cases are ringed.

*Asterisk indicate *C. jejuni* excretors of > 3 weeks duration (irrespective of serotype); the numbers immediately preceding the asterisks denote the number of such prolonged excretors.

( ) No. of *C jejuni* excretors in brackets.

Table 9.33.

Cyclical excretion according to age in households

	Prevalence of excretors		Prevalence of excretors of > 3 weeks	
	Age groups		Age groups	
	≤ 3 years	> 3 years	≤ 3 years	> 3 years
Family Study I	13/18 (7/12)[a]	5/24 (2/24)	11/18 (5/12)	0/24 (0/24)
Family Study II	2/4 (2/4)	3/11 (0/8)	0/4 (0/4)	2/11 (0/8)
Family Study III	3/5 (2/5)	3/4 (3/9)	0/5 (0/5)	0/9 (0/9)
	18/27 (11/21)	8/44 (5/41)[b]	11/27 (5/21)	2/44 (0/41)[c]

[a]Figures in brackets indicate prevalence when the index cases which formed the basis for selecting the respective households are excluded.

[b]Difference significant $\chi^2 = 11.7$, $P = 0.0007$ (Yates modification)

[c]Difference significant P = 0.003 (Fisher's exact test).

Table 9.20.

C [illegible]i excretion according to age in households

	Prevalence of excretors		Prevalence of excretors of > 3 weeks	
	Age groups		Age groups	
	≤ 3 years	> 3 years	≤ 3 years	> 3 years
Family Study I	13/18 (7/12)[a]	2/24 (2/24)	11/18 (5/12)	0/24 (0/24)
Family Study II	2/4 (2/4)	3/11 (0/8)	0/4 (0/4)	2/11 (0/8)
Family Study III	2/5 (2/5)	3/9 (3/9)	0/5 (0/5)	0/9 (0/9)
	17/27 (11/21)	8/44 (5/41)[b]	11/27 (5/21)	2/44 (0/41)[c]

[a] Figures in brackets indicate prevalence when the index cases which formed the basis for selecting the respective households are excluded.

[b] Difference significant χ^2 = 11,7, P = 0.0007 (Yates modification)

[c] Difference significant P = 0.003 (Fisner's exact test)

TABLE 9.21 DURATION OF *C. JEJUNI* EXCRETION, ASSUMING INTERMITTENT SHEDDING OCCURS, ACCORDING TO AGE. (ALL *C. JEJUNI* EXCRETORS, INCLUDING THOSE IN FAMILY STUDIES.)

Age Group (years)	Number of Individuals	Number of Specimens	Cumulative Surveilance Period(weeks)	Cumulation period of excretion (weeks)	Mean Duration of excretion (weeks)
<4/12	15	151	190	66	4.4
5/12-1	10	125	191	67	6.7
13/12-2	12	151	183	80	6.6
25/12-3	11	138	216	71	6.5
>3-6	3 }	28 }	33 }	12 }	4.0 }
>6-16	4 }11	15 }112	32 }195	1 }35	1.0 }3.2
>16-70	7 }	69 }	130 }	22 }	3.1 }

TABLE 9.22 DURATION OF EPISODES[a] OF *C. JEJUNI* EXCRETION ACCORDING TO AGE

Age group (years)	No. of Children	No. of Specimens	No. of Isolations	Recovery of *C. jejuni* (per cent)	No. of Episodes	Cumulative period of Episodes (weeks)	Mean Duration of Episodes (weeks)	Range (weeks)
≤4/12	15	151	35	23%	25	33	1.3	< 1-3
5/12-1	10	125	32	26%	13	32	2.5	< 1-5
13/12-2	12	151	36	24%	20	36	1.8	< 1-10
25/12-3	11	138	34	25%	19	38	2.0	< 1-7
>3-6	3	28	1	4%	1	1	1.0	< 1-2
>6-16	3 (13)	34 (131)	4 (15)	12% (12%)	4 (17)	4 (18)	1.0 (1.1)	< 1-2
>16	7	69	10	15%	9	10	1.1	< 1-2

[a]Definition of episode: Period of continuous *C. jejuni* excretion with not more than 2 weeks separating consecutive isolations.

C. jejuni strains not typed serologically, the patterns of prolonged excretion according to age are given in Table 9.21 and the episodal nature of excretion in Table 9.22. Both the prolonged, intermittent excretion of *C. jejuni* and the episodes of excretion tended to be longer in the children under 3 years of age compared with older children and adults.

9.4 Discussion

The host of findings recorded in this chapter are mainly descriptive but several patterns have emerged that warrant consideration.

9.4.1 Prevalence of *C. jejuni* and other intestinal pathogens in Soweto

As is the case in both developed and developing countries (Bruce, Zochowski and Ferguson 1977, Rajan and Mathan 1982, Blaser, Wells, Feldman, Pollard, Allen and the collaborative diarrheal disease study group 1983, Glass, Stoll, Huq, Struelens, Blaser and Kibriva 1983, Karmali, Penner, Fleming, Williams and Hennessy 1983) . *C. jejuni* was recovered as often as salmonella or shigella or more so, in the present studies (Tables 9.1 and 9.16). The high prevalence of *C. jejuni* (25 of 71, 35 per cent) encountered in the Soweto families (Tables 9.16 and 9.17) compares with 98 of 180 (54 per cent children examined monthly over a period of nine months in Bangladesh (Glass, Stoll, Huq, Struelens, Blaser and Kibriya 1983). High isolation rates have also been encountered in other developing countries in children with diarrhoea (De Mol, Braeckeur, Lauwers, Zissis and Butzler 1980, Picciardi and Ferreira 1980, and Bellingham 1981) as well as in healthy individuals from Australian aboriginal and southern India communities in which the overall isolation rates were 16.9 and 14.8 per cent respectively (Berry, Crawley and Bamford 1981, Rajan and Mathan 1982).

9.4.2 Age related excretion of *C. jejuni*

The clear trend of a high isolation rate of *C. jejuni* in young children Tables 9.1 - 9.8 in the present study compared with older individuals (Table 9.20) is also reflected in studies in Bangladesh (Glass, Stoll, Huq, Struelens, Blaser and Kibriya 1983) and southern India (Rajan and Mathan 1982).

[illegible] ...ces in southern India and Bangladesh in [illegible] years of age were 37 per cent and 58 percent respectively. [illegible] of the bias introduced in the Sowetan family studies by the index [illegible] the results of these [illegible] stigations are not directly comparable with those of the former two studies. However, they show a similar decline in isolation rates with increasing age. In Bangladesh the percentage of children excreting [illegible] at least once during a nine-month surveillance period progressively dropped from 80 in the 6-11-month age group to 59 in the four to five-year-old children and to 40 percent in those older than five years of age. The age-specific isolation rate of *C. jejuni* in a random sample of a rural population in southern India decreased from 37 per cent in children under five years of age to 20 per cent in adults older than 18 years of age. Comparable figures in Sowetan families were 17 of 27 (63 per cent) in the under three-year-old children and 8 of 44 (18 per cent) in individuals older than three years of age or 11 of 21 (54 per cent) and 5 of 41 (12 per cent) respectively when the index cases are excluded (Table 9.20).

9.4.3 Pattern and duration of *C. jejuni* excretion according to age.

The duration of convalescent carriage of *C. jejuni* following a diarrhoeal episode is usually regarded as short and even "unimportant in epidemiology" (Lancet, 1982). The duration of excretion commonly quoted is from two to seven weeks (Karmali and Fleming 1979; Blaser, LaForce, Wilson and [illegible] Svedhem and Kaijser 1980; Blaser and Reller 1981; Symonds 1983). [illegible] problem in the published work was that intermittency of excretion was not adequately considered. Thus in a study on *Campylobacter* enteritis in Canada Karmali and Fleming (1979) examined the stools of 24 patients at approximately weekly intervals "until one or more stool cultures became negative." Unfortunately they did not specify how many further samples were cultured once they became negative. Presumably the number of additional cultures were small. Svedhem and Kaijser (1980) were fortunately more specific and indicated clearly the number of patients examined over a period of 12 weeks. Of the 55 *C. jejuni* positive patients in this study, 42 were examined at three weeks, and more than twenty up to the seventh week. However, a total of only ten samples were examined from the [illegible] to the twelfth week. Five of these being positive for

C. jejuni. During the twelfth week only one was examined and this stool was still positive. It is therefore quite conceivable that more prolonged excretion could have occurred in this, as well as in the Canadian study.

Prolonged excretion of *C. jejuni* of more than three weeks duration was encountered in aetiological studies at Baragwanath hospital and eleven patients excreted *C. jejuni* for >3 weeks and up to 40 weeks (Table 6.6). One of these children excreted *C. jejuni* continuously for 15 weeks and then intermittently for another 25 weeks. (Figure 6.2). This pattern of prolonged but intermittent excretion of *C. jejuni* was again evident in both the duration of excretion investigation and the family studies in Soweto. It is clear that most patients excrete the organism continuously for less than three weeks after an attack of acute campylobacter enteritis but that some may continue to shed it continuously for as long as 15 weeks. However, that prolonged but intermittent excretion also of the same serotype, occurs not infrequently was clearly shown in the present studies (Tables 9.4, 9.5, 9.6, 9.7 and 9.10). One two-year-old child (C285) excreted *C. jejuni* serotype 19 (Manchester) intermittently for at least 16 weeks (Tables 9.7 and 9.10). Because re-infection and almost definitely also mixed infections occur in populations with a high endemnity, only serotyping of the isolates will elucidate the true patterns of excretion, and, very importantly, also whether re-infection with a new serotype will result in diarrhoea. This aspect was not adequately addressed in the present investigation and only a carefully planned and executed longitudinal study with adequate information on the frequency of bowel movements and the consistency of stools will resolve the problem. Ideally such a study should include investigations on the development of immunity, including the determination of immunoglobulin classes in the blood and intestinal secretions of patients and carriers.

Early evidence of immunity to clinically overt *C. jejuni* infection based on high levels of IgG antibodies was obtained during a study of two clusters of milk-borne outbreaks in Minnesota and Colorado and control subjects. In the Minnesota outbreak in which unexposed persons drank raw milk, the attack rate was high and specific IgG and IgM antibodies were demonstrated. However the outbreak in Colarado involved farmers who persistently drank raw milk. Here the attack rate was low and the IgG antibody levels high in both affected and unaffected individuals (Blaser, Duncan, Osterholm, Istre and Wang 1983). On the

other hand Australian workers found that patients with intermittent or prolonged enteritis tended to mount poor antibody responses (Kaldor, Pritchard, Serpell and Metcalf 1983) while relapses and clinically manifested re-infection due to *C. jejuni* have also been described (Butzler, Dekeyser, Detrain and Dehaen 1973, Karmali, Kosoy, Newman, Tischler and Penner 1981).

9.4.4 Evidence of person-to-person transmission in households in Soweto

The high incidence of both diarrhoea-associated and asymptomatic infections in children under two years of age in Soweto (Chapters 5 and 6) and the prolonged excretion of *C. jejuni* suggested that these children could be an important reservoir of *C. jejuni* in the community and may be an important source of spread within households. This appeared to be even more likely because of overcrowding, relatively poor hygiene and the popularity of supplemented and/or artifical feeding and inadequate breast feeding. It was therefore very important to investigate person-to-person transmission of *C. jejuni* in Soweto, especially amongst young children and it was with this in mind that the family study was designed. Unfortunately only limited serotyping has thus far been completed and the picture therefore remains somewhat uncertain. Also not enough attention was paid in the present study to alternative sources of spread of *C. jejuni* within households. More specimens from food, especially chicken offal and other chicken dishes and from dogs should have been examined in the prospective family studies. The intermittent excretion of the same serotype which may in part be a reflection of relatively insensitive techniques, also bedevils interpretation of the time relationships between the excretion of *C. jejuni* from different members within the households.

In spite of the abovementioned caveats, there is strong evidence of person-to-person transmission in family 35. The index case, a 3-months-old boy with diarrhoea excreted *C. jejuni* serotype 37 (Brussels) from 1981-7-27 to 1981-8-27. On 1981-8-20 a 32 month-old cousin acquired the same serotype and subsequently excreted serotype 37,15 and later serotype 4,37 until 1981-11-22. In addition a 16 months-old sister of the index case excreted serotype 37 briefly on 1981-9-16. Although all three children may have acquired this serotype simultaneously at an early stage and excreted it intermittently, the findings do not conclusively support this possibility.

Of the 12 families studied, *C. jejuni* was recovered from at least one household in 11 of the families and from more than one member in eight. Yet evidence of possible spread of *C. jejuni* within these households was only seen in two of the six families in which the index cases were young children suffering from *C. jejuni* enteritis in two of the three families in which mothers excreted the organisms during the post-partum period and in none of the three control families. If one considers a maximum period of three weeks between excretion episodes in different members of families as a criterion for possible person-to-person transmission, such spread probably occurred in Family 35 (to two children) and may have taken place in Family 128 (to two siblings) in the first family study and in Family 119 to a three-year-old son and Family 101 to a four-year-old child in the second family study. In Families 130 and 131 the two children affected in each household appeared to have acquired the infection at the same time and this also applies to families 90 and 127 where several members apparently started excretion of *C. jejuni* at the same time. In three families (18, 110 and 129) only one member of the household excreted the organism. Thus, of the nine families in which the index cases excreted *C. jejuni*, possible spread could only have occurred in four, involving a maximum of six children (Figure 9.2). On the available evidence the frequency of person-to-person transmission appears to be surprisingly low especially if one considers that many members excreted the organism for prolonged periods. It may be significant that the presumed secondary cases in putative person-to-person transmission were invariably young children, suggesting that immune mechanisms may have been operating.

Although the present studies provide compelling evidence of person-to-person transmission the relatively low frequency appears to be in keeping with reports in the literature (Blaser and Reller 1981, Lancet, 1982, Symonds 1983). Secondary spread does not appear to occur following single source outbreaks (Blaser, Cravens, Powers, LaForce and Wang 1979, Metzing 1981, Jones, Robinson and Eldridge 1981; Robinson and Jones 1981; Osterholm, Korlath, McCullough and Judy 1982) also when specifically looked for in a water-borne outbreak in Vermont (Vogt, Sours, Barrett, Feldman, Dickinson and Wetherell 1982).

As mentioned in Chapter 7, instances of person-to-person transmission have been described in the literature (Blaser, LaForce, Wilson and Wang 1980), also amongst young children who were incontinent of faeces were concerned (Cadranel, Rodesch, Butzler and Dekeyser 1973, Blasker, Waldman, Barrett and Erlandson 1981), while vertical transmission from mothers to their newborne babies has also been reported (Mawer and Smith 1979, Karmali and Tan 1980). However, the relatively few cases reported thusfar and the present findings suggest that person-to-person spread does not occur readily. The reasons for this and a more detailed analysis of intra-family spread from different sources await further study.

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10. Campylobacter infection in a population with a high endemicity General discussion and concluding remarks.

10.1 High prevalence of *C. jejuni* in Soweto — historical perspective.

From the outset the aetiological studies conducted on patients with gastro-enteritis in Soweto, clearly showed that *C. jejuni* infections in this community behaved differently in several respects from those reported in other countries. In developed countries *C. jejuni* had been isolated from the stools of three to 14 per cent of patients who seeked medical attention but rarely from healthy persons. (Skirrow 1977; Bruce, Zochowski and Ferguson 1977; Blasker, Berkowitz, LaForce, Cravens, Reller and Wang 1979; Pai, Sorger, Lackman, Sinai and Marks 1979; Svedhem and Kaijser 1980). Even in developing countries, according to publications prior to 1980, the isolation rates of *C. jejuni* were less than ten per cent in patients with diarrhoea and absent from healthy controls (Butzler 1973; De Mol and Bosmans 1978). However, a high prevalence of *C. jejuni* in Soweto an children was reported in 1979 (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979). The isolation rates were 35 per cent in diarrhoeal babies under two years of age and 16 per cent in healthy children in the same age group. This finding was unprecedented at the time. It was only when Blaser, Glass, Huq, Stoll, Kibriya and Alim (1980) reported a 17.7 per cent isolation rate in healthy individuals and 40 percent in children nine to 24 months of age in Bangladesh that it was realized that comparable situations may exist in some developing countries including India (Rajan and Mathan 1982) and in Australian aboriginal communities (Berry, Gracey and Pamford 1981). In the Gambia (Billingham 1981) and Zaire (De Mol, Brasseur, Lauwers, Zissis and Butzler 1980) relatively high isolation rates of *C. jejuni* (13.6 per cent and 14.4 per cent respectively) were obtained in patients with diarrhoea but comparatively low rates in healthy controls (4.2 per cent and 3.0 per cent respectively).

10.2 Age related susceptibility to *C. jejuni* infection

Neither of two extensive studies in Bangladesh (Blaser, Glass, Huq, Stoll, Kibriya and Alim 1980; Glass, Stoll, Huq, Struelens, Blaser and Kibriya 1983) explained the high percentage of *C. jejuni*-infected persons that remained asymptomatic. This was in stark contrast with

the findings in developed countries. The age specific infection rates in Bangladesh failed to show significant differences between the diarrhoeal and healthy control groups, even in the < 1 year age group in which the isolation frequencies were 25 per cent and 32 per cent respectively. However, in both these studies and in Sowetan families (Chapter 9), as well as in southern India (Rajan and Mathan 1982) and the Gambia (Billingham 1981) the isolation rates of *C. jejuni* showed a progressive decline with increasing age, suggesting that a clearance mechanism, probably immunological in nature, enables older individuals in high prevalence regions to resist colonization and/or clinically overt infection by this organism. That immunological factors may play a role in the prevention of clinically manifested disease was suggested by the serological findings during two milk-borne outbreaks in Colorado and Minnesota (Blaser, Duncan, Osterholm, Istre and Wang 1983). The attack rate in the community which was chronically exposed to raw milk was much lower than that in the "virgin" population in Minnesota (26% as opposed to 54%). In the former (Colorado) community one of five *C. jejuni*-excretors were asymptomatic and two of 14 asymptomatic persons from three neighbouring farms yielded the same serotype. The attack rate in these farming communities was low but both complement fixation and IgG antibody titres were high in affected and unaffected persons.

In Sowetan children there was statistically significant evidence ($p = 0.01$) of susceptibility to *C. jejuni*-associated diarrhoea in infants under nine months of age compared with unaffected age-and sex-matched controls. In this study which showed essentially the same features as an earlier investigation (Bokkenheuser, Richardson, Bryner, Roux, Schutte, Koornhof, Freiman and Hartman 1979), 18 of 60 diarrhoeal as opposed to 4 of 60 control infants excreted *C. jejuni* (Richardson, Koornhof, Bokkenheuser, Mayet and Rosen 1983). This differer disappeared in the older children (9 - 24 months of age) of w of 51 with diarrhoea and 17 of 51 control subjects excreted the or- sm in their faeces. The mechanism of this resistance to clinically overt infection in older children remains speculative. Whether the same phenomenon of age related susceptibility applies to children in Bangladesh and other developing countries can only be resolved in age-stratified studies involving infants < 9 months of age.

10.3 Duration and patterns of *C. jejuni* excretion

Compelling evidence was presented in Chapter 9 that *C. jejuni* was not only recovered more frequently from the faeces of children ≤ 3 years of age than in other individuals but the duration of excretion was also considerably longer in the younger age group. Continuous excretion of *C. jejuni* is usually short but can be as long as 12 weeks and intermittent excretion of the same serotype may exceed 15 weeks. This aspect has not been adequately studied in other communities, nor the intermittency of shedding of a single serotype.

Re-infection based on serotyping was also shown to occur regularly in this community and probably mixed infections as well. As pointed out in Chapter 9, the real extent of re-infection and mixed infection can only be determined by identifying and typing several colonies of *C. jejuni* when each faecal sample is examined. Also, the intermittency of *C. jejuni* excretion encountered in the Soweto studies may at least to some extent, have been artificially emphasized as a result of a combination of mixed infections and the practice of selecting single colonies on plates for serotyping.

10.4 Intra-family spread

Based on serotyping of *C. jejuni* strains, fairly convincing evidence of person-to-person transmission was obtained in Family 35 (Chapter 9). However, the risk of secondary spread within households appears to be relatively low, in spite of overcrowding and poor hygiene.

Although information on person-to-person transmission in Soweto is still very incomplete, two observations may be significant. Firstly it appears that intra-family spread affects predominantly young children and secondly, three post-partum mothers in the family studies did not transmit their *C. jejuni* infections to their newborn babies. These findings are in conflict with vertical transmission from symptomatic and asymptomatic mothers reported by Mawer and Smith (1979) and Karmali and Tan (1980). However, a plausible explanation for these discrepancies may be that mothers from communities with a high endemicity would be more likely to protect their offspring, possibly through placental transfer of protective IgG antibodies, than mothers in developed countries with a low prevalence of *C. jejuni* infection.

10.5 Sources of *C. jejuni* infection

Although a high prevalence of *C. jejuni* was observed in fowl and dog faeces as well as in bovine and tripe in Soweto (Richardson and Koornhof 1979), little is known about the role of strains recovered from such potential sources in producing disease in man. For meaningful studies, the isolates should not only be carefully identified to exclude other campylobacters e.g. thermotolerant strains with no or weak catalase activity recently described by Sandstedt, Ursing and Walder (1983) in dogs, but serotyping would be essential. Considerable success has been obtained with the tracing of sources of infection, also in outbreaks, by means of serotyping of strains. (Blaser, Penner and Wells 1982; McMyne, Penner, Mathias, Black and Hennessy 1982; Blaser, Duncan, Osterholm, Istre and Wang 1983; Pearson, Barlett, Page, Jones, Lauder, Lior and Jones 1983; Pearson, Palmer, Lior and Jones 1983; Stalder, Isler, Stutz, Salfinger, Lauwers and Vischer 1983). The great diversity of serotypes recovered from patients (Abbott, Dale, Eldridge, Jones and Sutcliffe 1980; Lauwers, Vlaes and Butzler 1981; Blaser, Penner and Wells 1982; Lior, Woodword, Edgar, Laroche and Gill 1982; Penner, Hennessy and Congi 1983; Lastovica and Penner 1983; Herbert, Hollis, Weaver, Steigerwalt, McKinney and Brenner 1983; Herbert, Penner, Hennessy and McKinney 1983; Karmali, Fenner, Fleming, Williams and Hennessy 1983) suggests a multiplicity of sources. Soweto provides an excellent opportunity to study the possible role of several known sources of *C. jejuni* in the transmission of this organism to man and its perpetuation in a high prevalence population.

10.6 Unresolved problems - Scope for further studies

As the studies on campylobacter infections in Soweto progressed and the results of investigations performed elsewhere in the world in high prevalence populations, notably Bangladesh and India, became known, it was evident that many similarities exist in the epidemiology of campylobacteriosis in these countries. However, several aspects require further study.

10.6.1 Resistance to *C. jejuni* diarrhoea in high prevalence populations

The most important feature in the epidemiology of this disease in communities which a high endemicity is the large number of asymptomatic

excretors of *C. jejuni*, especially in children. This implies the presence of mechanisms of host resistance to clinical manifested enteritis which are probably immunological in nature. Similar mechanisms may also be responsible for the lower isolation rates in older individuals in these countries but differences in hygienic habits may play an important role in this respect. Studies on the nature of age-specific resistance to *C. jejuni* infection in Soweto should receive a very high priority. Serology involving detection of antibodies to heat-labile and heat-stable antigens including possibly surface protein antigens (Logan and Trust 1982; Svedhem, Gunnarsson and Kaijser 1982;Logan and Trust 1983; Rautelin and Kosunen 1983) should be attempted and the possible role of cell-mediated immunity considered.

10.6.2 Patterns and duration of *C. jejuni* excretion

The extent of re-infection and mixed *C. jejuni* infections should be further investigated by studying more colonies per faecal sample. This may also help to elucidate intermittency of *C. jejuni* excretion and the question of prolonged excretion and chronic carriage of *C. jejuni* serotypes. In Soweto these studies should be concentrated on young children and very careful monitoring of bowel habits of these children should be performed in order to correlate acquisition of new *C. jejuni* serotypes with clinical manifestations in patients. Ideally such studies should include serology, and, if feasible, involve the isolates from the persons concerned.

10.6.3 Sources of infection and person-to-person transmission

Family studies should continue and be re-designed to prospectively address not only the faecal excretion of *C. jejuni* serotypes but also the non-human sources of infection. *C. jejuni* strains isolated from such sources should be typed and correlated with faecal excretion patterns. Food consumed by household members should be carefully monitored for the presence of *C. jejuni*. Special attention should be given to chicken offal which has not yet been extensively studied.

10.7 Concluding remarks

Soweto with its high prevalence of *C. jejuni* infections, has provided a unique opportunity for the study of the epidemiology of campylobacteriosis. It may therefore serve as a convenient prototype for other

communities where the infection is highly endemic. The accessibility of patients, children and families to microbiologists, epidemiologists and clinicians could serve as an ideal milieu for the continued study of remaining problems in this field. The many diverse aspects of campylobacter infections in this community warrant further intensive investigation with the application of the most modern techniques and concepts. To this end, collaborative studies with a multidisciplinary approach should ideally be initiated.

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