CHAPTER IV

RESULTS

Comparison of Two Different Size Filters for Recovering C.jejuni from Inoculated Distilled Water

The result was shown in Table 7. The 0.2-um membrane filter was better for concentration C. jejuni than another one. The smaller pore size does not let any bacterium passing through this membrane. The inoculum from 1.83×10^5 to 1.83×10^6 C. jejuni cells per ml could be detected by either 0.2 um-membrane or 0.45 um-membrane filter on both culture methods. At the inoculum of $< 1.83 \times 10^4$ c.f.u/ml on the 0.45 um membrane filter that laid face down onto B.B.A,no C. jejuni was isolated. When the inoculum size was 1.83×10^{-1} c.f.u./ml (18. 3 c.f.u/100 ml) and the 0.45-um membrane filter was used by placing in Doyle's enrichment broth with the filter further, there was no isolated organisms. The inoculum size was 1.83×10^{-1} c.f.u/ml with the 0.2-um pore membrane filter recovered C.jejuni better than the other. The data also suggested that similar results were obtained whether the 0.2 um-membrane filter was placed directly on plates or placed in enrichment broth with the filter further. Since there are not only single type of onganisms but also several types of enteric bacteria habour in canals, therefore all the organisms will be concentrated on the surface of the 0.2 um-membrane

filter and overgrown on BBA. In this situation, enrichment the 0.2 um-membrane filter in Doyle's procedure was chosen for the isolation of Campylobacter spp. from the canals in this study.

Isolation rate of Campylobacter spp. from canals

A total of the 156 samples were examined for Campylobacter spp. in three duration of time; July(1988) to September (1988), November (1988) to January (1989), and February(1989) to April (1989). The 116 Campylobacter species strains were isolated from the 156 water samples. The Campylobacter isolates were characterized by Table 8 and 9. They were 74 C. cryaerophila strains and 42 C. cryaerophila - like organism strains.

During these times, C. cryaerophila was isolated from 23 (44.23%), 27 (51.19%) and 24 (46.15%) and also C. cryaerophila-like organism from 15 (28.85%), 10 (19.23%) and 17 (32.69%) of the 52 samples in each period of time respectively as shown in Table 8 and Fig. 10. The isolation rate of both Campylobacter species during those time were not significantly difference ($P > 0.05$).

The canal-sites of Campylobacter species positive isolation were shown in Fig. 7,8 and 9 while the canal-marks of Campylobacter negative isolation in all durations were,

061 Klong Mahanak at Chakra Padipong Road Cross.

091 Klong San Sap at Din Daeng-Asoka Road Cross.

093 Klong San Sap at Bang Kapi Bridge.

094 Klong San Sap at Bang Chan estate.

095 Klong San Sap at Minburi.

123 Klong Bang Sue at Paholyothin Road Cross.

321 Klong Rat-Burana at Suksawad Road Cross.

401 Klong Bang-Kruay at Bang-Kruay Bridge.

During July to September (1988), the negative isolation of Campylobacter spp. were

082 Klong Sam Sen at Sam Sen Road Cross.

113 Klong Prem Prachakorn at Bang Sue Market.

121 Klong Bang Sue at Piboon Songkram Bridge.

201 Klong Dao Kanong at Taksin Road Cross.

232 Klong Bangkok Yai at Charoen Pars Bridge.

281 Klong Bang Na at Sukumvit Road Cross.

During November (1988) to January (1989) Campylobacter spp. were not recovered from

031 Klong Wat Rajabopit at Tre Tong Road Cross.

043 Klong Bang Lum Poo at Bang Lum Poo Water Gate.

063 Klong Mahanak behind Department of Drainage and Sewage B.M.A.

141 Klong Chong Nonsi at Ratchadapisek Road Cross.

201 Klong Dao Kanong at Taksin Road Cross.

211 Klong Bang Kun Tien at Terd Thai Road Cross.

During February to April (1989), Campylobacter spp. were not seen from,

012 Klong Lord at Gate near Rachinee School.

015 Klong Lord at Pra Pin Klao Bridge.

043 Klong Bang Lum Poo at Bang Lum Poo Water Gate.

Phenotypic Biochemical Characteristic of the Isolated C. cryaerophila and C. cryaerophila-like organisms

The result of differential tests were shown in Table 9. All strains grew aerobically at 37°C and microaerophilically (85% N_2, 10% CO_2 and 5% O_2) at 25°C and 37°C but not at 42°C. The growth in the presence of 1% glycine and 3.5% NaCl was variable. All strains were positive for catalase and nitrate reduction tests. They were unable to produce H_2S and also non hippurate-hydrolysis. The sensitivity to nalidixic acid (30 ug) disc was varied and most of the strains were resistant to cephalothin (30 ug) disc with the exception of only one sensitive strain. The C. cryaerophila-like organisms were differentiated from C. cryaerophila by positive urease test.

Table 1 Comparison of the taxonomic classification synonyms of the genus *Campylobacter**

Classification of the genus *Campylobacter*				
	Taxonomic classification synonyms according to:			
Approved List of Bacterial Name, ICSB 1980	Bergey's Manual (Smibert 1974)	Veron and Chatelain (1973)	King (1957)	Florent(1959) or Jones(1931)
I. *Campylobacter fetus* ss. *venerealis*	I. *C. fetus* ss. *fetus*	*C. fetus* ss. *venerealis* *C. fetus* ss.biotype *intermedius*	*Vibrio fetus*	*V. fetus* ss. *venrealis* (Florent)
Campylobacter fetus ss. *fetus*	*C. fetus* ss. *intestinalis*	*C. fetus* ss. *fetus*	*Vibrio fetus*	*V. fetus* ss. *intestinalis* (Florent)
II. *Campylobacter jejuni*	*C. fetus* ss. *jejuni*	*C. jejuni*, *C. coli*(?)	"Related vibrios"	*V. jejuni* (jones)
III. *Campylobacter coli*	II. *C. fecalis*	-	-	-
IV. *Campylobacter sputorum* ss. *sputorum*	III.*C. sputorum* ss. *sputorum*	*C. sputorum*	*V. sputorum*	-
Campylobacter sputorum ss. *bubulus*	*C. sputorum* ss. *bubulus*	*C. bubulus*	*V. bubulus*	-

*Rettig 1979, Ohashi 1982, Karmali and Skirrow 1985

Table 2 Phenotypic characteristics of Campylobacter species*

Organisms	Growth tests							Biochemical reaction									Susceptibility to	
	25°C	36°C	42°C	1 % glycine	1.5 % NaCl	3.5 % NaCl	MaC Conkey	Oxidase	Catalase	Nitrate reduction	H_2S in TSI	Rapid H_2S in FBP	DNA hydrolysis	Hippurate hydrolysis	0.04% TTC	Urea hydrolysis	Nalidixic acid	Cephalothin
C.jejuni	-	+	+	+	-	-	+	+	+	+	-	d	d	+	+	-	S	R
C.coli	-	+	+	+	-	-	+	+	+	+	d	-	d	-	+	-	S	R
C.laridis	-	+	+	+	+	-	+	+	+	+	-	+	d	-	-	-	R	R
C.fetus SS.fetus	+	+	d	+	-	-	+	+	+	+	-	-	-	-	-	-	R	S
C.fetus SS.venerealis	+	+	-	-	-	-	+	+	+	+	-	-	-	-		-	R	S
C.sputorum SS.faecalis	+	+	+	+	+	+	+	+	+	+	+	+	-	-		-	R	S
C.hyointestinalis	d	+	d	+	-	-	+	+	+	+	+	-	-	-	-	-	R	S
"C.cinaedi"	-	+	d	+	-	-	-	+	+	+	-	-	-	-	+	-	S	S
"C.fenelliae"	-	+	d	+	-	-	-	+	+	-	-	-	-	-	+	-	S	S
C.pylori	-	+	-	+	-	-	-	+	+	-	-	-	-	-		+	R	S
C.cryaerophila	+	+	-	-	+	d	d	+	+	+	-	-	d	-	-	-	d	d
C.sputorum SS.sputorum	-	+	+	+	+	-	+	+	-	+	+	+	-	-		-	R	S
C.sputorum SS.bubulis	d	+	d	+	+	+	-	+	-	+	+	d	-	-		-	d	S
C.mucosalis	-	+	+	-	-	-	+	+	-	+	+	+	-	-		-	S	S
C.concisus	-	+	-	-	-	-	+	+	-	+	+	+	-	-		-	R	R
"C.upsaliensis"	-	+	+	+	-	-	-	+	-/w	+	-	-		-	-	-	S	S

* = Morris and Patton 1985, Taylor and Blaser 1987, Barrett et al. 1988.

d = variation depend on strains.

"..." = Proposed names not officially approved by the International Committee on Systemic Bacteriology are in quote.

Table 3 Biotyping scheme for C. jejuni, C. coli and C. laridis according to Lior (Lior 1984)

Tests	C. jejuni				C. coli		C. laridis	
	Biotypes				Biotypes		Biotypes	
	I	II	III	IV	I	II	I	II
Hippurate hydrolysis	+	+	+	+	-	-	-	-
Rapid H_2S test	-	-	+	+	-	-	+	+
DNA hydrolysis	-	+	-	+	-	+	-	+

Table 4 Comparison of four methods to isolate Campylobacter species in 331 specimens obtained from 100 children (Taylor et al. 1987)

Isolation method	NO. (%) of isolates
Before enrichment	
Filter onto non selective media	101 (74)
Direct onto selective media	52 (38)
After enrichment	
Filter onto non selective media	92 (67)
Direct onto selective media	39 (29)

Table 5 List of canals and sites of water sample collection

Name of canals	Code of samples and sites of Collection	
1. Klong Lord	012	Gate near Rachinee School
	015	Pra Pin Klao Bridge
2. Klong Wat Tep Tida	021	Behind the Bangkok City Hall 1
3. Klong Wat Rajabopit	031	Tre Tong Road Cross
4. Klong Bang Lum Poo	042	Infront of Nana Market
	043	Bang Lum Poo Water Gate
5. Klong Ong Ang	051	Ong Ang Water Gate
6. Klong Mahanak	061	Chakra Padipong Road Cross
	063	Behind Department of Drainage and Sewerage, BMA
7. Klong Pradung Krung Kasem	072	Krung Kasem Pump Station
	075	Teves Market
8. Klong Sam Sen	081	Maepra Temple
	082	Sam Sen Road Cross
	085	Behind Din Daeng Flat
9. Klong San Sap	091	Din Daeng-Asoka Road Cross
	093	Bang Kapi Bridge
	094	Bang Chan estate
	095	Minburi
	096	San Sap Gate

Name of canals	Code of samples and sites of Collection	
10. Klong Tan	101	Klong Tan Pump Station
11. Klong Prem	113	Bang Sue Market
Prachakorn	114	Bang Ken Jail
12. Klong Bang Sue	121	Piboon Songkram Bridge
	122	Paholyotin Center
	123	Paholyotin Road
13. Klong Sathon	131	YMCA
14. Klong Chong Nonsi	141	Ratchadapisek Road Cross
15. Klong Lad Prao	181	Soi Sena Nikom 1
	182	Pibool Upatam School
16. Klong Bang Nam Chon	191	Taksin Road Cross
17. Klong Dao Kanong	201	Taksin Road Cross
18. Klong Bang Kun Tien	211	Terd Thai Road Cross
19. Klong Pasi Jaroen	221	Wat Rang Bua School
20. Klong Bangkok Yai	232	Charoen Pars Bridge
21. Klong Rama VI	241	Rama VI Engineering School
22. Klong Mon	251	Charan Sanit Wong Road
23. Klong Bangkok Noi	262	Suwan Naram School
24. Klong Pra-Kanong	271	Pattanakarn Bridge
	273	Pra-kanong Water Gate
25. Klong Bang Na	281	Sukumvit Road Cross
26. Klong Pai-Singto	301	Mahat-Thai Temple
27. Klong Chaeng-Ron	311	Suksawad Road Cross
28. Klong Rat-Burana	321	Suksawad Road Cross

Name of canals	Code of samples	and sites of Collection
29. Klong Bang-Prakok	331	Suksawad Road Cross
30. Klong Bang-Pa-Kaew	341	Suksawad Road Cross
31. Klong Samrong	371	Sukumvit Road
32. Klong Bang-Kruay	401	Bang Kruay Bridge
33. Klong Mahasawat	411	Wat Chaiya-Pruk Bridge
34. Klong Tavee-Wattana	421	Pinklao-Nakorn Chaisri
35. Klong Sanam-Chai	431	Lao Temple
36. Klong Pravate	462	Infront of Lan Boon Temple

Table 6 Schedule of canals monitoring

Months of collection	Sequence of collection week	Water samples (according to code)				
February	1st	012	015	021	031	043
July	2nd	075	082	241	182	123
November	3rd	091	281	101	081	085
	4th	131	301	141	063	
March	1st	211	221	232	251	262
August	2nd	042	051	072	061	401
December	3rd	113	121	181	114	
	4th	273	271	462		
April	1st	311	321	331	341	
September	2nd	191	201	371	096	122
January	3rd	093	094	095		
	4th	411	421	431		

Table 7 Comparison of two different size filters for recovering C. jejuni from inoculated distilled water

Dilution	Inoculum of C.jejuni (c f u/ml)	Recovery with filter :			
		0.45-um-pore filter		0.2-um-pore filter	
		Face down[a]	After enrichment[b]	Face down[a]	After enrichment[b]
10^{-1}	1.83×10^{6}	+	+	+	+
10^{-2}	1.83×10^{5}	+	+	+	+
10^{-3}	1.83×10^{4}	-	+	+	+
10^{-4}	1.83×10^{3}	-	+	+	+
10^{-5}	1.83×10^{2}	-	+	+	+
10^{-6}	1.83×10	-	+	+	+
10^{-7}	1.83	-	+	+	+
10^{-8}	1.83×10^{-1}	-	-	+	+

a. The filter was placed directly on Brucella agar plate so that the surface with bacteria was in direct contact with the surface of the agar

b. After 18-24 h incubation in Doyle's medium, the subculture was dropped through the 0.45 um membrane filter.

Table 8 The incidence of Campylobacter species from the water samples (canals)

Organisms	July-September	November-January	February-April
	No.of isolates/ N.of samples (% isolation)	No.of isolates/ No.of samples (% isolation)	No.of isolates/ No.of samples (% isolation)
C.cryaerophila	23/52 (44.23)	27/52 (51.19)	24/52 (46.15)
C.cryaerophila like organism	15/52 (28.85)	10/52 (19.23)	17/52 (32.69)

Table 9 Phenotypic biochemical characteristics of isolated Campylobacters

Tests	C.cryaerophila		C.cryaerophila-like organism	
	Total No.of tested isolates=74 strains		Total No.of tested isolates=42 strains	
	No. of (+)test (%)	No.of (-)test (%)	No.of (+)test (%)	No.of (-)test (%)
Growth at/or in				
-25°C	74 (100)	-	42 (100)	-
-36°C	74 (100)	-	42 (100)	-
-42°C	-	74 (100)	-	42 (100)
-1% Glycine	7 (9.46)	67 (90.54)	8 (19.05)	34 (80.95)
-1.5% NaCl	74 (100)	-	42 (100)	-
-3.5% NaCl	67 (90.54)	7 (9.46)	32 (76.19)	10 (23.81)
-Mac Conkey agar	68 (91.89)	6 (8.11)	38 (90.48)	4 (9.52)
-Aerobe (37°C)	74 (100)	-	42 (100)	-
Biochemical tests:				
Oxidase test	74 (100)	-	42 (100)	-
Catalase test	74 (100)	-	42 (100)	-

Tests	C.cryaerophila		C.cryaerophila-like organism	
	Total No.of tested isolates=74 strains		Total No.of tested isolates=42 strains	
	No. of (+)test (%)	No.of (-)test (%)	No.of (+)test (%)	No.of (-)test (%)
Nitrate reduction	74(100)	-	42(100)	-
H_2 in TSI	-	74(100)	-	42(100)
Rapid H_2S in FBP	-	74(100)	-	42(100)
DNA hydrolysis	22(29.73)	52(70.27)	14(33.33)	28(66.67)
Hippurate hydrolysis	-	74(100)	-	42(100)
tolerance to 0.04% TTC	-	74(100)	-	42(100)
urease test	-	74(100)	42(100)	-
Susceptibility to:	sensitive	resistant	sensitive	resistant
Nalidixic acid (30 ug)	27(36.49)	47(63.51)	14(33.33)	28(66.67)
Cephalothin (30 ug)	1(1.35)	73(98.65)	-	42(100)

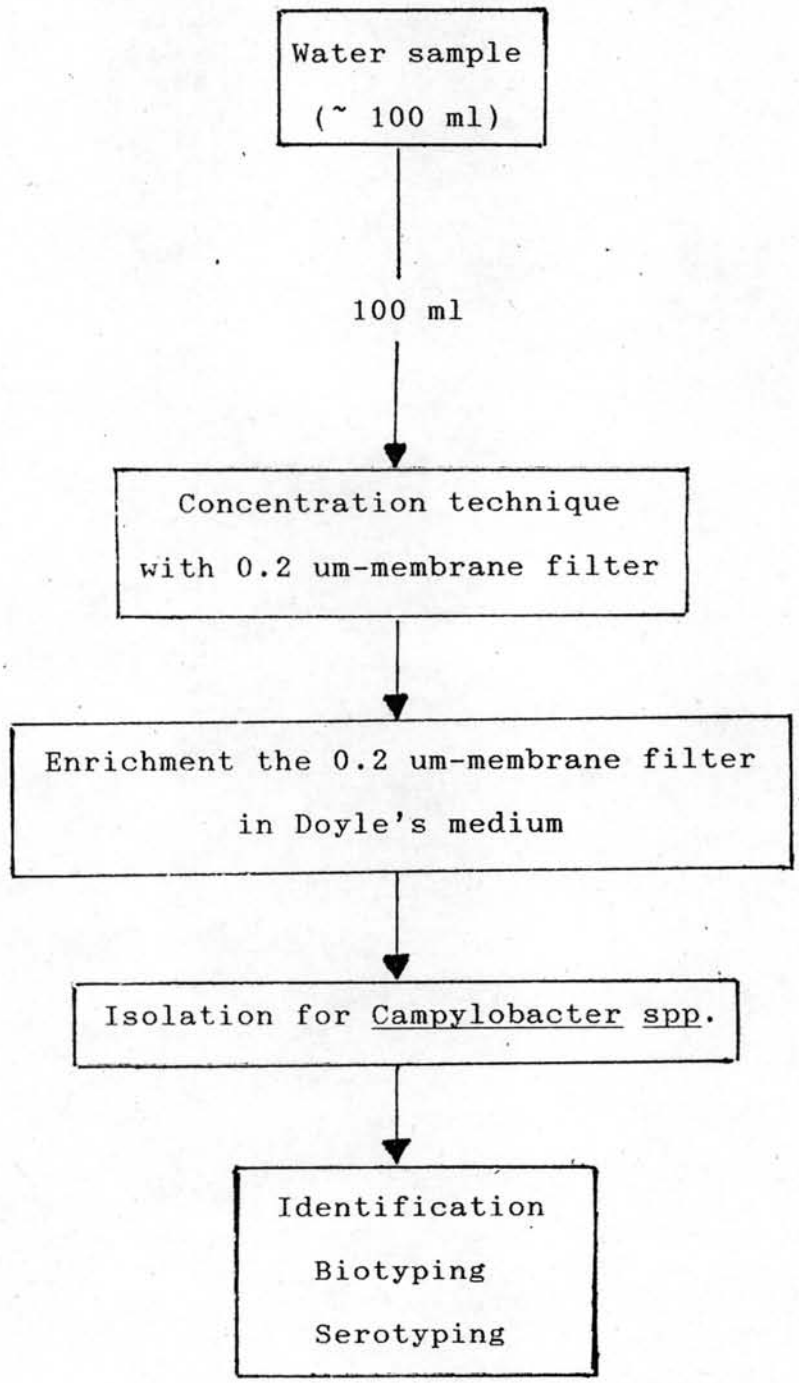

Fig.1 Processing of water samples.

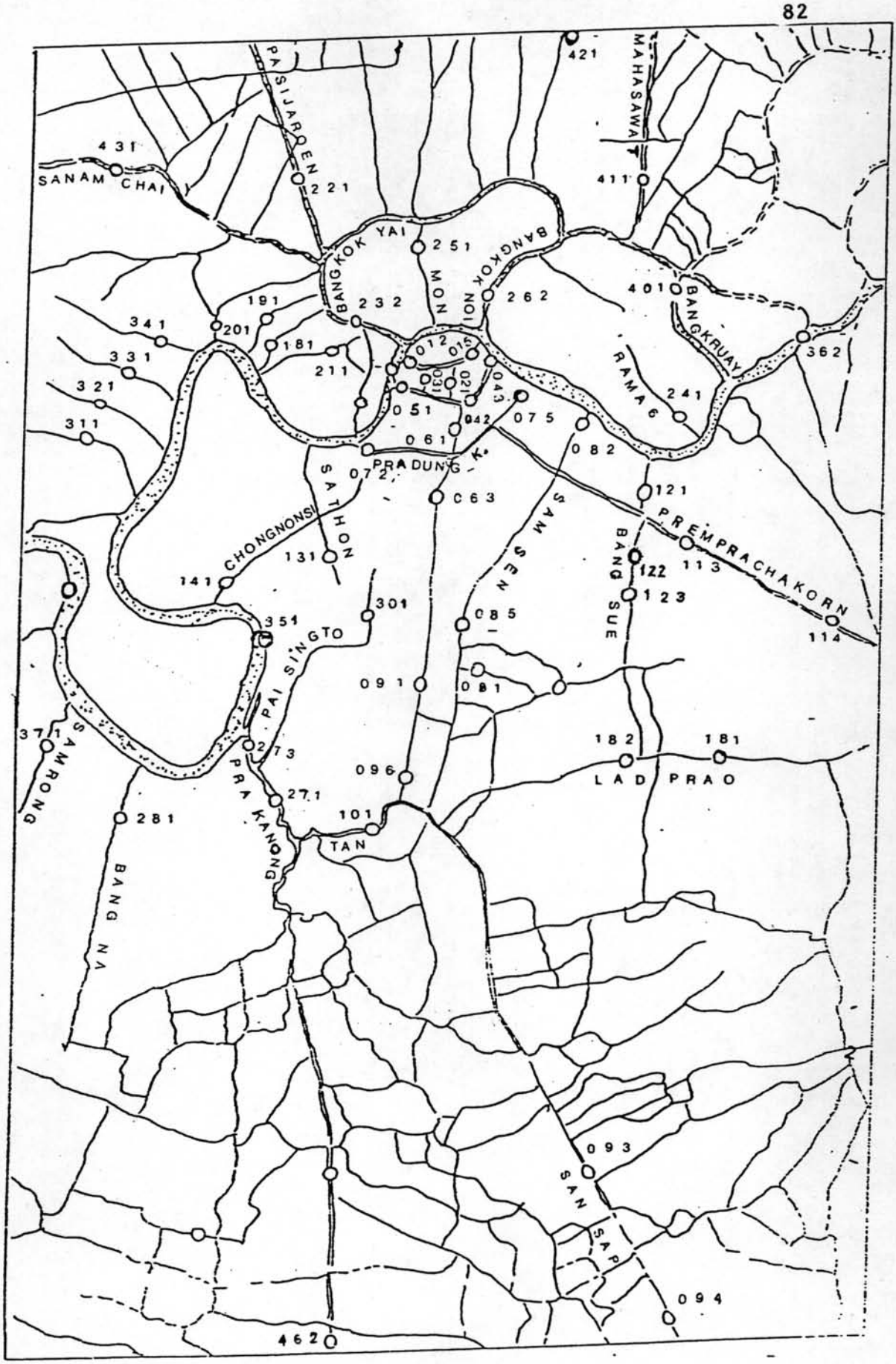

Fig. 2. Map of canals and sample sites(O) in Bangkok Metropolitan Area

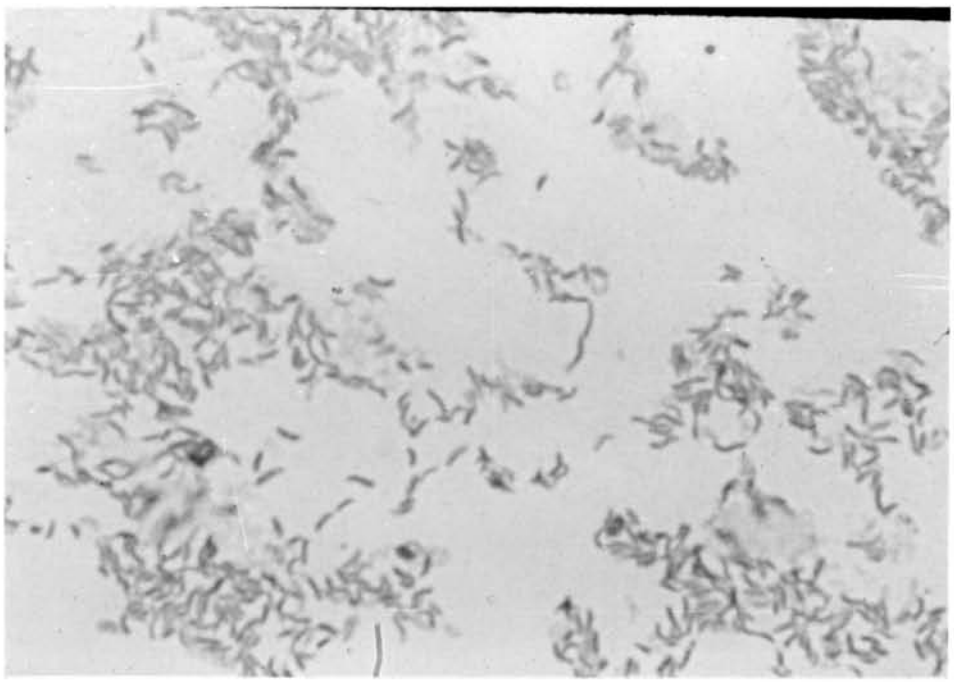

Fig 3 Microscopic Morphology of Campylobacter (1,000X)

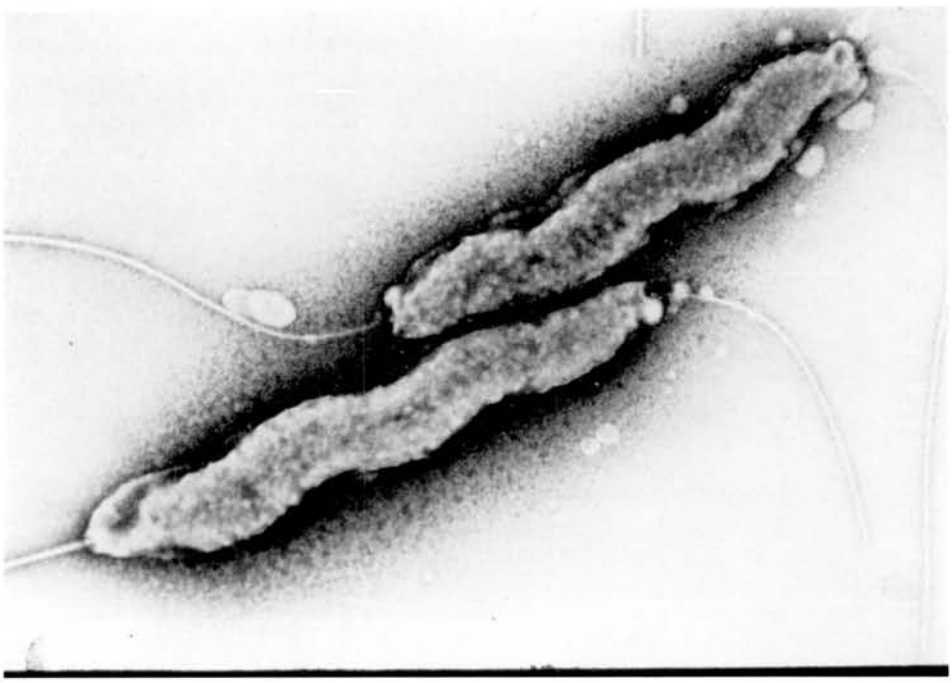

Fig 4 Electron Micrograph of Campylobacter (11,000X) showing a single poloar flagellum at both ends

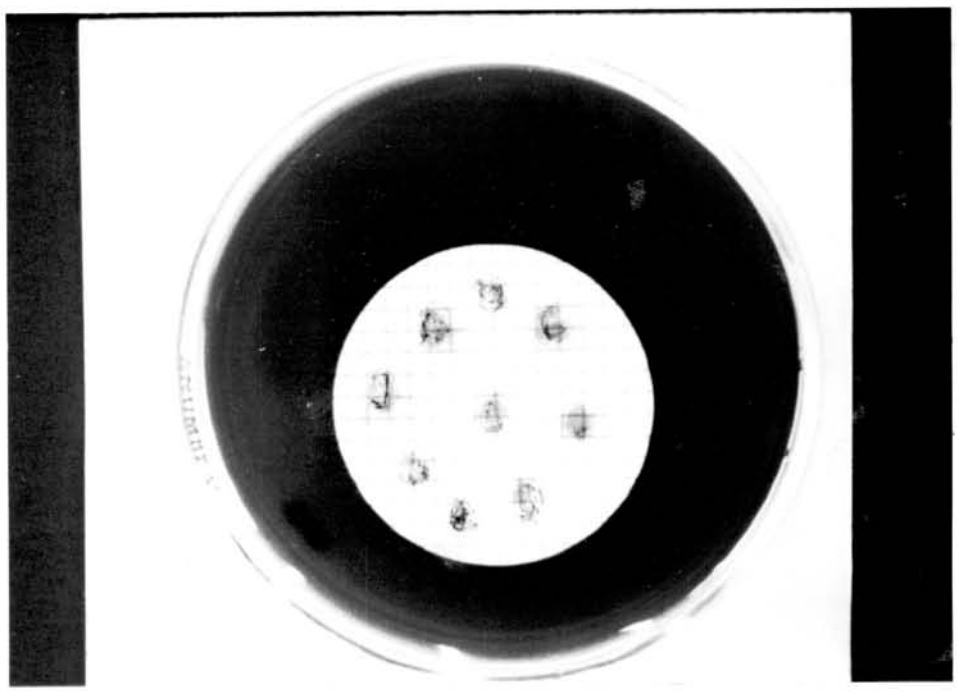

Fig 5 Drops of Doyle's medium on surface of the filter on Brucella blood agar.

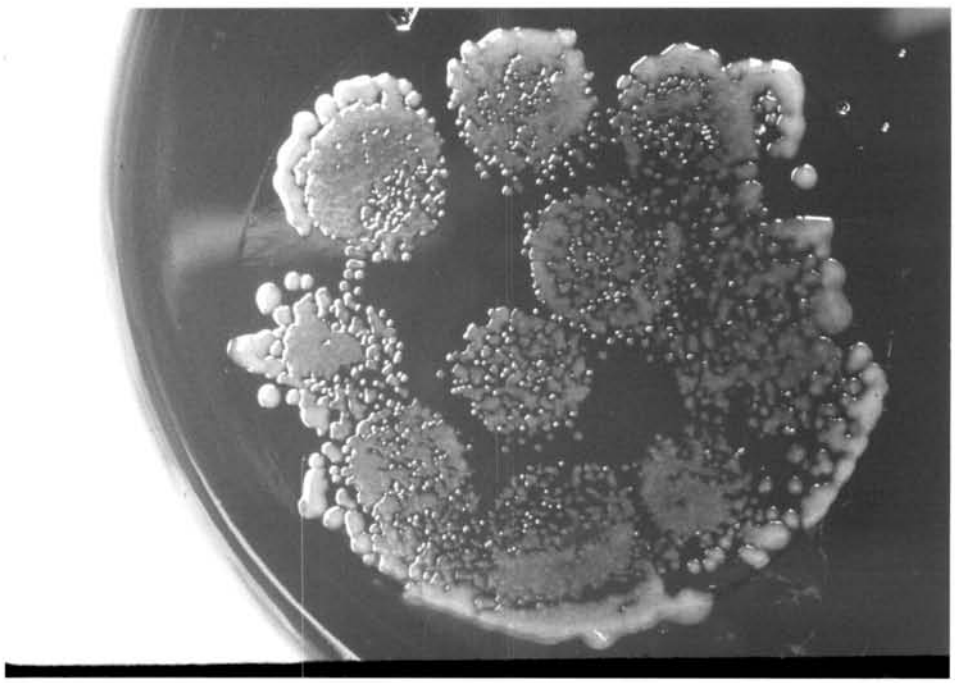

Fig 6 Campylobacter jejuni colonies on Brucella blood agar.

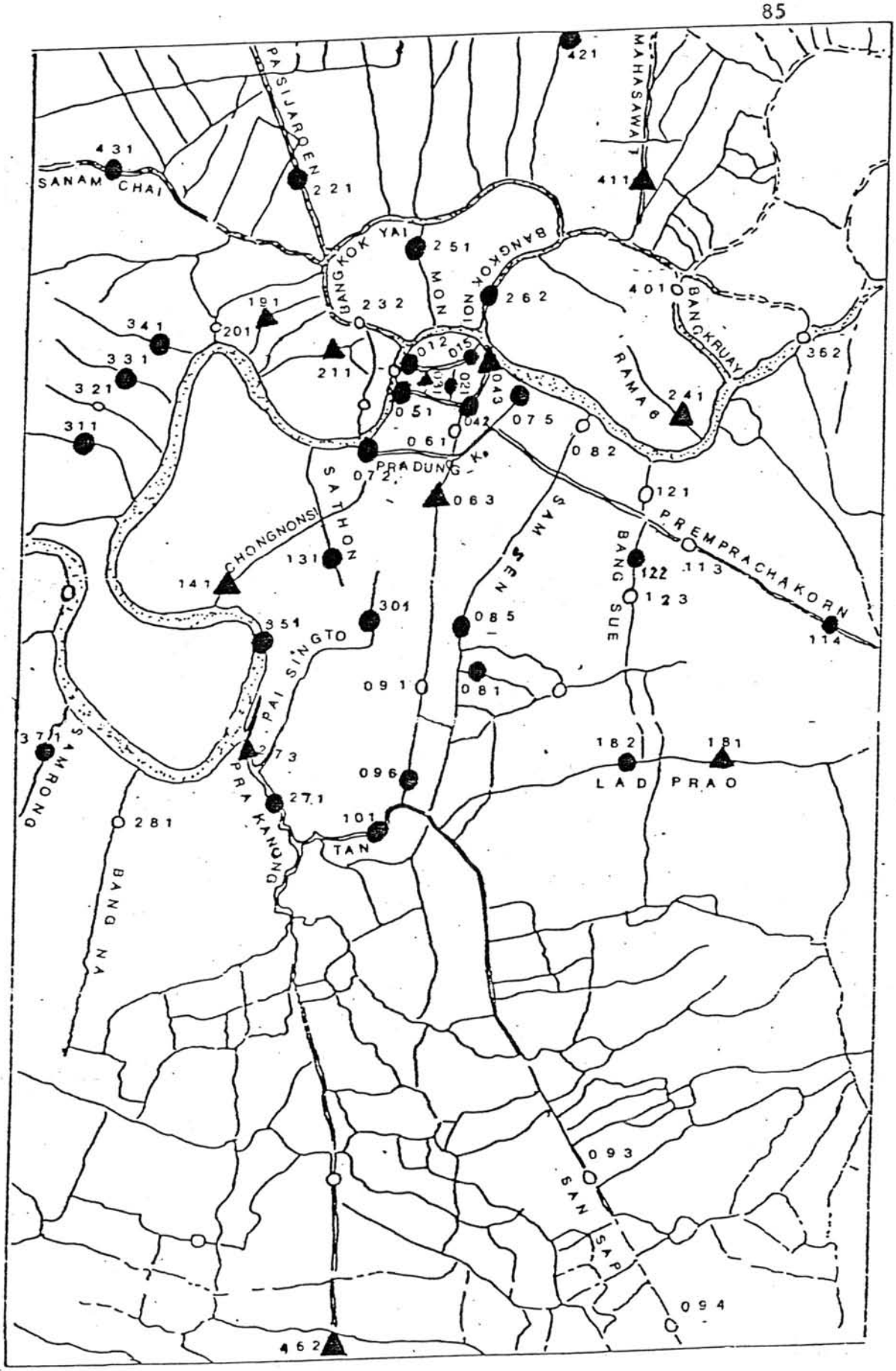

Fig. 7. Sites of Campylobacter positive isolation in July to September

● = C. cryaerophila positive isolation

▲ = C. cryaerophila-like organism positive isolation

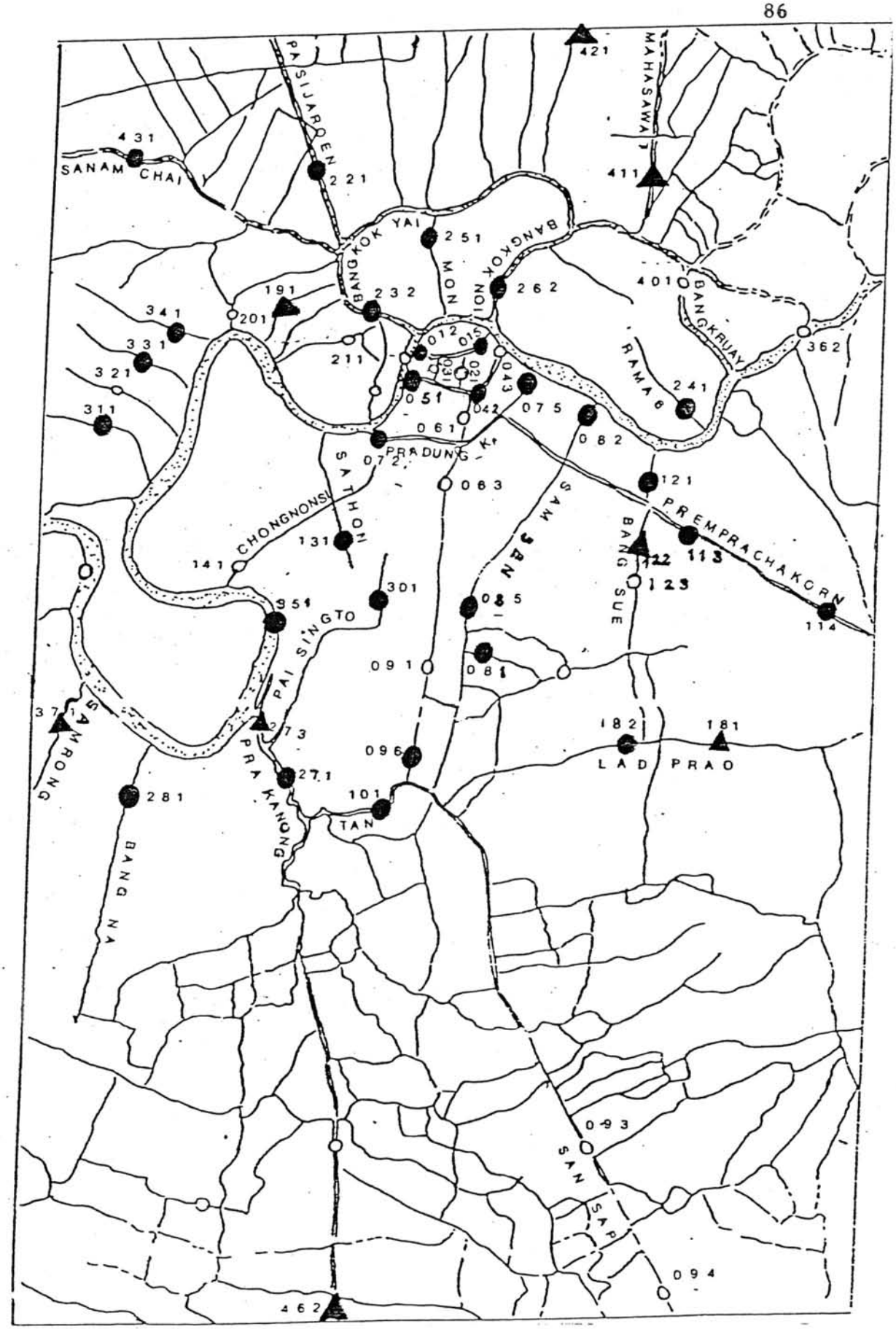

Fig. 8. Sites of Campylobacter positive isolation in November to January

● = C. cryaerophila positive isolation

▲ = C. cryaerophila - like organism positive isolation

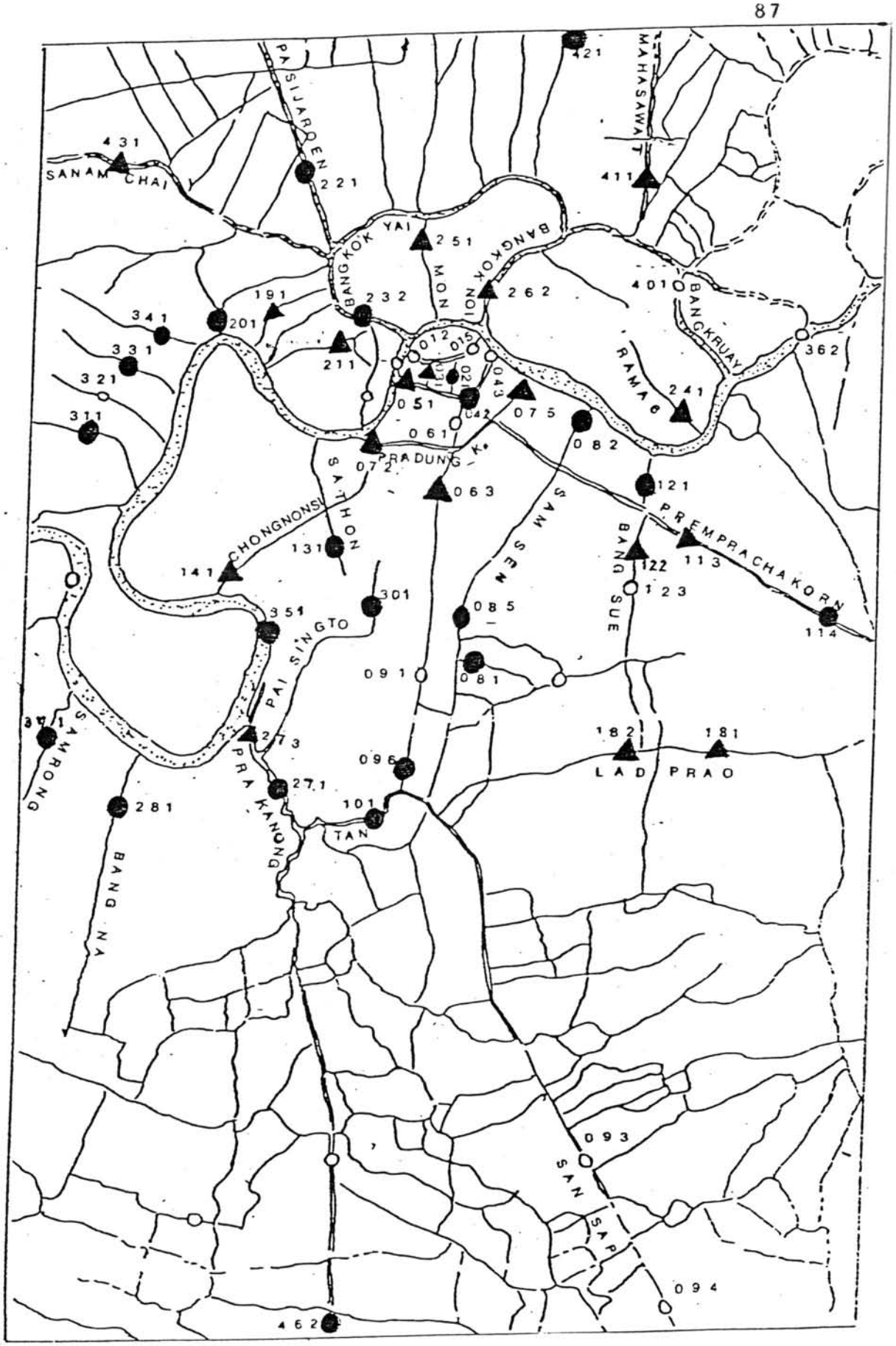

Fig. 9. Sites of Campylobacter positive isolation in February to April

● = C. cryaerophila positive isolation

▲ = C. cryaerophila-like organism positive isolation

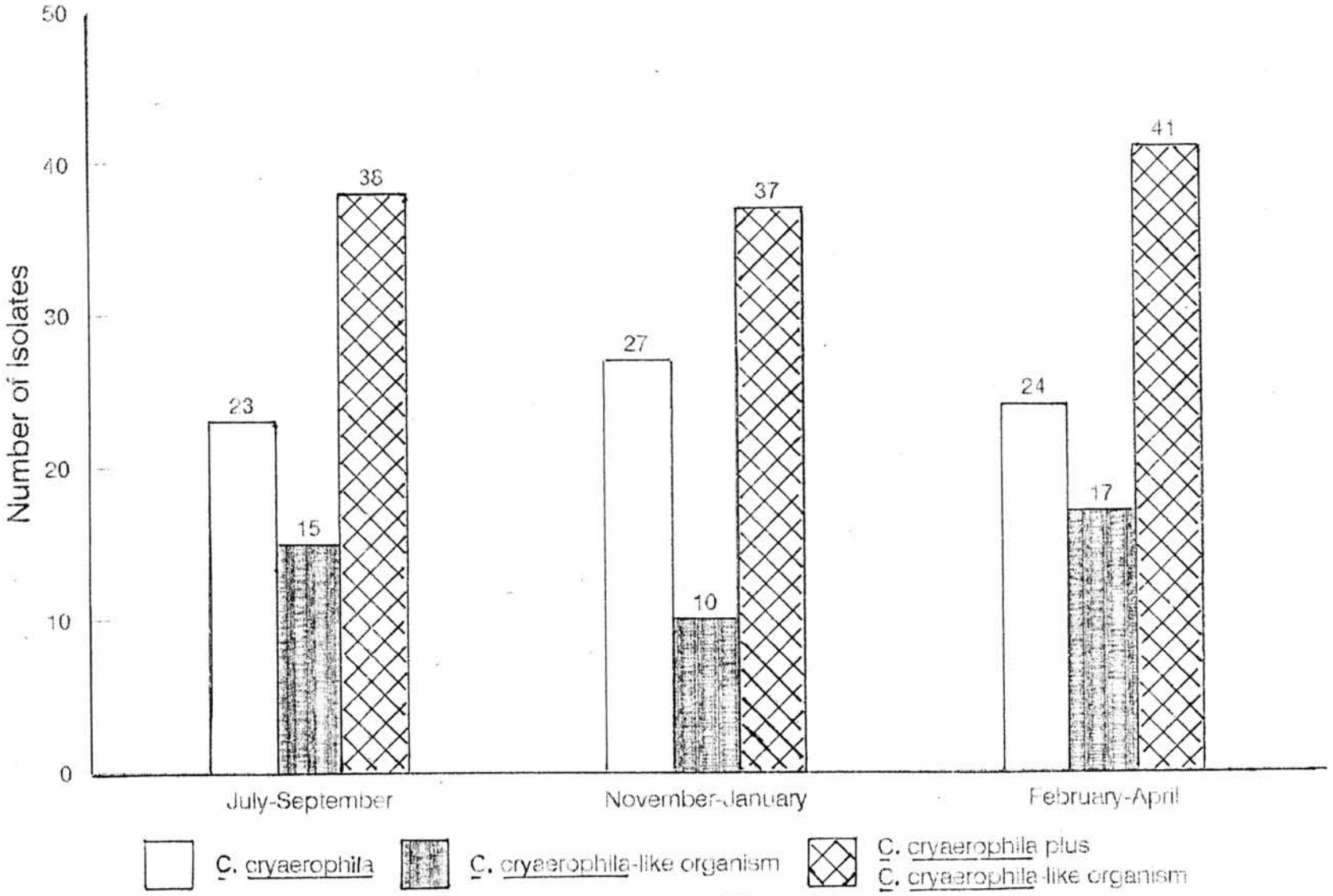

Fig. 10. Seasonal variation of Campylobacter isolates

Fig 11 Colonial appearance of C. jejuni on Brucella blood agar

Fig 12 Colonial appearance of of C. cryaerophila on Brucella blood agar

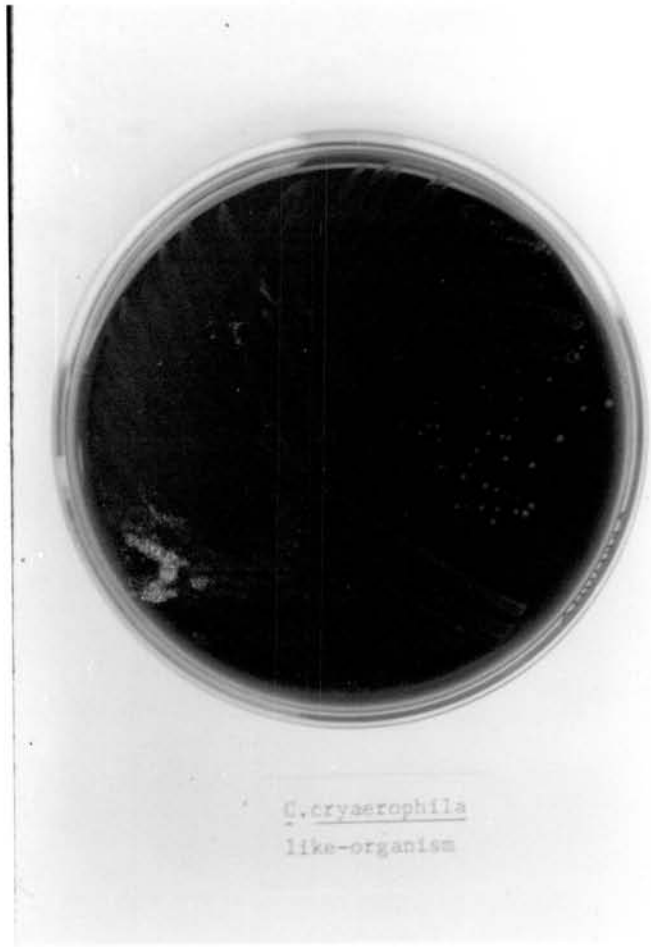

Fig 13 Colonial appearance of C. cryaerophila-like organism on Brucella blood agar