CHAPTER V

DISCUSSION

From Table 7, when the 0.45 um pore membrane filter was used for concentration of C. jejuni at the inoculum size of $1.83X10^5$ c.f.u/ml and placed directly face down, C. jejuni could be detected. At the inoculum less than 1.83×10^5 (1.83×10^{-1} to 1.83×10^4), C. jejuni were not recovered when the membrane filter was placed directly onto B.B.A. whereas enrichment the filter in Doyle's medium with the filter further could recovered C. jejuni. Since this organism could motile through the 0.45-um pore membrane filter to another side of membrane filter and also into filtrate (with the suction pressure), therefore, after enrichment in Doyle's broth the (small) number of C. jejuni would increase enough to be detected again. When the 0.2-um-membrane filter was used, C. jejuni could not pass through it. All the organisms would be concentrated on the surface of this membrane and the organisms could be detected from both culture methods. Since there are several types of bacteria in canals, therefore, the use of the 0.2-um membrane filter for concentration of water sample and enrichment this membrane in Doyle's medium with the filter further was suitable method for isolation of Campylobacter spp. The Doyle's enrichment broth was effective in recovering as few as 0.1 cell of Campylobacter per gram of food (Doyle and Roman 1982). Furthermore, the Doyle's

broth has been reported to be useful to increase the isolation rate especially in carrier in the study of Taylor and colleagues (Taylor et al. 1987 and 1988). In addition, the enrichment broth may induce the non-culturable-phase (NCP) cells of the organisms to culturable-phase cell before plating. When the organisms were introduced into stream water or oligotrophic environment, they would rapidly decline to NCP and might not be detectable on conventional agar (Rollins et al. 1985).

In this study it was designed to use the 0.2-um-membrane filter for concentration all bacteria and use a-0.45 um-membrane filter method on BBA. for isolation of Campylobacter from the Doyle's medium instead of direct plating onto selective agar. Taylor et al. (1987) demonstrated that there was nearly a twofold increase in Campylobacter isolation rate when the filter was used compared with standard plating on selective media. The advantage of the membrane filter method was the filter eliminated other enteric bacteria, high rates of detection,a relative large amount of specimen could be inoculated onto the plate. Moreover, the plates (BBA) did not contain antibiotics therefore it was suitable for the detection of some antibiotic sensitive Campylobacters. Finally the medium (BBA) was simple to prepare and required less quality control than the medium containing several antibiotics. However, the disadvantage of this filter method was that it might fail to detect small numbers of

Campylobacters (so enrichment in Doyle's medium before using the filter method will resolve this problem), and the cost was higher than the conventional method.

The use of antibiotic-containing media for the isolation Campylobacters have been effective and successful (SKirrow 1977, Blaser et al.1979, Butzler and Skirrow 1979), but the disadvantage are either more difficult to prepare than the ordinary blood agar or the errors in the quantity of inhibitory antibiotics being added could adversely affect the performance of the medium (Steele and McDermott 1984). Moreover some Campylobacter strains were missed by using this direct method with antibiotics especially cephalothin or colistin incorporated in media.

Incubation temperature at 37°C would be used instead of incubation at 42°C because Campylobacters could be detected in high rates at 37°C on media without antibiotics by using membrane filter method as in the study of Steele and McDermott (1984). In addition, it was reported that some Campylobacters might be inhibited by incubation at 42°C (Fennell et al. 1984, Ng et al. 1985, Steele et al. 1985, Tee et al. 1987).

Microaerophilic condition may be obtained by using either GasPak or a gas mixture (85% N_2, 10%CO_2 and 5% O_2). The cost of GasPak is much higher than the gas mixture in Thailand, so the latter was used.

There were reports and evidences indicated that the contaminated water was the vehicle of Campylobacter infections's outbreak but Campylobacter organisms were not recovered from the incriminated water sources (Mentzling 1981, Vogt et al. 1982, Blaser et al. 1983-a, Palmer et al. 1983, Sack et al. 1986) with the exception of the studies of Taylor et al. (1983) and Knill et al. (1982). Taylor and colleagues, in 1983 showed that backcountry surface water (in Rocky Mountain) had been an important source of C. jejuni. C. jejuni was isolated from 14(2%) of 586 water specimens. For the study of Knill et al. in 1982, Campylobacter spp. were isolated from over 50% of the water samples collected from fresh and esturine sites on three river systems in West Hampshire, Southampton. Both authors used the membrane-filtration technique. The 100 ml of water was filtered through a 0.45 um-pore size filter and the filter was placed face up onto antibiotic selective media. Plates were incubated at 42°C in a microaerophilic atmosphere.

In this study, neither C. jejuni or C. coli were isolated from canals. The isolated Campylobacters were C. cryaerophila and C. cryaerophila like organism. The Campylobacter isolation rate was as high as 41(78.85%) in during February to April 1989 (Fig 11) which was not statistical significantly difference from the other durations. Therefore no seasonal variation was found in isolation rate of Campylobacter organisms.

The information about temperature and dissolved oxygen in tested water samples were obtained from Technical Division, Department of Drainage and Sewage, Bangkok Metropolitan Administration as the following.

Temperature and dissolved oxygen of tested canal samples in Bangkok Metropholitan area.

Duration&year	Temperature (°C)		Dissolved oxygen (mg/ml)	
	range	average	range	average
July to September 1988	25-31	28.5	0-2.7	0.28
November 1988 to January 1989	24.5-31	27.9	0-4.8	1.06
February to April 1989	28-32	30.5	0-4.3	0.52

This data could go a long way to explain the failure to isolate C. jejuni, C. coli and C. laridis from canals. It may be caused by the effect of temperature. C. jejuni may be survive in water at 4°C for a year and the isolation from environmental sources is markedly decreased when the water temperature rise above 15°C, and at 25°C they became non-viable within 4 days and could not multiply in water

(Knill et al. 1982, Blaser et al. 1980-a). The temperature of canals were unfavorable for their growth. The optimal temperature for C. jejuni growth is 36°C to 42°C. In addition, single specimen was taken from each sample site of canal in each duration of time. However, repeated isolation should be made from the same sample site of canals in the same duration of time in order to recover the failure of primary isolation.

The identification and differentiation of Campylobacter isolates are based on the careful limited number of morphology and biochemical criteria because misidentification may result from atypical reaction. In this study, the result of identification of Campylobacter isolates are C. cryaerophilis and C. cryaerophila-like organisms. They were differentiated by urease activity only. Therefore C. cryaerophila-like organism might belonged to unique species or was variant of C.cryaerophila. For further studies, to solve this problem, the alternative approaches to strain differentiation including DNA-DNA hybridization dot blotting or DNA base composition assay (Tee et al. 1987, Steele et al. 1985, Ursing et al. 1983), gas-liquid chromatographic analysis of cellular fatty acid composition (Edmonds et al. 1987), whole cell protein electrophoretic profile studies (Ferguson et al. 1984) can be very useful tools for differentiation among Campylobacter spp. However, DNA-DNA hybridization is undoubtedly an extremely useful tool to differentiate organisms to species level when the

conventional method fails.

In this study Campylobacter organisms were obtained from water samples taken from community area. No isolation were obtained from water samples which far from community area. The presence of C. cryaerophila and C. cryaerophila-like organism in canals can not be implicated whether they are associated with human infection, because clinical significance and pathogenesis of these organisms are unknown now. However, C. cryaerophila was reported to be associated with porcine and ovine abortions and was reported to be associated with diarrhea in man (Neill et al. 1979, Tee et al. 1988). The study show that canals can be an important source of these two Campylobacter species that might be considered as cause of diarrhea in the future.

Conclusion

1. The 0.2-um membrane filter was better to concentrate Campylobacter species from canal water samples and enriched in Doyle's medium with the filter further. The 0.45-um membrane filter was used to concentrate the other enteric pathogens and enriched in proper enrichment selective media.

2. Isolation rates of Campylobacter species from canals were 44.23%, 51.19%, and 46.15% in duration of July to September 1988, November 1988 to January 1989, and

February to April 1989 respectively which were not statistical significantly difference.

3. The isolated Campylobacter species were 74 C. cryaerophila strains and 42 C. cryaerophila - like organism strains. They were differentiated by urease activity only, therefore C. cryaerophila-like organism might belonged to unique or was variant of C. cryaerophila.

4. The temperature of canals (26°C-32°C) were unfavorable for the thermophilic Campylobacter (C. jejuni, C. coli and C. laridis) growth (36°C-42°C).

Suggestion

1. In this study, C. cryaerophilas-like organism was differentiated from C. cryaerophila by positive urease test only. C. cryaerophila-like organism may be unique species or variant of C. cryaerophila. To solve this problem, DNA-DNA hybridization dot blotting or DNA hybridization assays can further determine whether these C. cryaerophila-like organisms belong to unique species or are variant of C. cryaerophila.

2. Repeated isolation should be made from the same site samples of canals in the same duration of time.

3. Antibiotic susceptibility test and serotyping of these isolated organisms would be studied for the further studies of epidemiology of these organisms.

4. Further studies emphasizing Microbiology, Seroepidemiology and Clinical patterns are required to determined firmly establish the etiological role of these organisms in human diseases.