Isolation of *Campylobacter* spp. from Slaughtered Cattle and Swine on Blood-Free Selective Medium

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ABSTRACT. The charcoal-cefazolin-sodium deoxycholate agar (CCDA) was compared with Butzler agar for selectivity of campylobacters from fecal samples. A total of 279 samples of cecal contents from 176 cattle and 103 pigs slaughtered in Saitama were examined. Fifty-five (31.3%) cattle were found to contain campylobacters by the direct plating culture on CCDA plates, compared with 29 (16.5%) on Butzler agar plates. The positive rates of pig samples on CCDA and Butzler agar were very high, being 96 (93.2%) and 86 (83.5%), respectively. After enrichment culture of 107 cattle specimens in Preston, CEM or Bu 10 broth, the isolation rate on CCDA was also higher ($p<0.01$) than that on Butzler agar after enrichment in any medium. These results indicate that CCDA medium is easy to handle without blood and, as to this study shows, is superior to Butzler agar for isolation of *Campylobacter* species from fecal samples. — **KEY WORDS:** blood-free medium, *Campylobacter*.

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Campylobacters have been recognized as a cause of diarrhea or enteritis in humans [2, 6]. Many studies [7, 17, 20] have been conducted to find the reservoir of *C. jejuni* in many mammals and birds. In general, Skirrow [18] and Butzler [14] agar media are used for isolation of *Campylobacter* species, but for its poor selectivity, Skirrow agar is not considered to be suitable for isolation of the microbes from fecal samples [3, 16]. Bolton *et al.* [4, 5] found that charcoal could effectively replace blood in the medium for growing campylobacters. The ability of charcoal-based media for isolation of *Campylobacter* spp. has been discussed only in a few reports [9, 13]. We made a study to compare CCDA with Butzler agar in the isolation rate of campylobacters from fecal samples.

In the present study, the cecal contents of cattle and pigs were used as test materials. Samples were obtained from apparently healthy cattle and pigs carried into the O slaughterhouse in Saitama prefecture during the period from July to November, 1993. Ages and numbers of the cattle examined in this study were: Holstein, 4 to 8 years ($n=151$); Japanese black cows, 2 to 4 years ($n=25$); and Landrace, about 6 months ($n=103$).

Samples of animals were taken by wiping out with sterilized swabs and transported immediately to our laboratory under cool condition. All samples were directly inoculated onto CCDA (Oxoid) and Butzler agar (Oxoid) plates, which were incubated at 37°C for 48 hr in a microaerophilic atmosphere of 85% N₂, 10% CO₂, and 5% O₂.

By the direct culture method, *Campylobacter* spp. were isolated from 55 (31.3%) cattle samples on CCDA compared with 29 (16.5%) on Butzler agar. The isolation rate of the former was significantly ($p<0.01$) higher than that of the latter. The species were isolated from 96 (93.2%) pigs on CCDA and 86 (83.5%) on Butzler agar. The rate of isolation from pigs was basically high, so there was no significant

difference in the isolation rate between the two media.

In this study, some samples taken from cattle were also enriched in Preston broth [3], CEM broth [21] and Bu 10 broth [11] for 24 hr before inoculation onto the two kinds of selective agar plates. Table 1 illustrates the detection rate of *Campylobacter* on the two selective media by the direct and enrichment methods. The highest isolation rate (32.7%) was obtained by direct plating on CCDA. Statistical analysis by the Wilcoxon's rank-sum test revealed that the isolation rate on CCDA was significantly ($p<0.01$) higher than that on Butzler agar after enrichment culture in any medium. Butzler agar is considered to have a relatively high selectivity; however, this medium often allowed contaminants to grow, including those swarming in the present study. Few contaminants were observed on CCDA plates even in direct plating of fecal samples, and there was no overgrowing of contaminants such as swarming *Proteus* spp. These results indicate that CCDA is more selective and less vulnerable to contamination than is Butzler agar.

In this study, three kinds of broth were used for enrichment. Statistical analyses (Kruskal-Wallis procedure) of the isolation rates of the enrichment methods were made. When CCDA was used after enrichment, there was no significant difference ($p<0.01$) in the isolation rate among the methods. On the contrary, when Butzler agar was used after enrichment, CEM broth and Butzler agar were not a good combination for isolation of *Campylobacter* species.

Several investigators [10, 15] have shown the advantage of combination of the plating method and enrichment culture. In our study, however, the direct plating method on CCDA was superior to any other enrichment procedure for isolation of *Campylobacter* spp. Generally, fecal samples contain many kinds of bacteria, so during enrichment, not only *Campylobacter* spp. but also other organisms also increase. Therefore, it may be difficult to separate the target organisms on a selective medium after

Table 1. Detection rate (%) of *Campylobacter* from cecal contents of cattle on CCDA and Butzler agar by using both direct and enrichment methods

Enrichment broth	Agar plate	Number of samples showing the result on the agar plate (%) (n=107)			
		+	±	–	HC
–	Butzler	16 (15.0)	0 (0.0)	84 (78.5)	7 (6.5)
	CCDA	29 (27.1)	6 (5.6)	68 (63.6)	4 (3.7)
Preston	Butzler	13 (12.1)	4 (3.7)	74 (69.2)	16 (15.0)
	CCDA	24 (22.4)	3 (2.8)	76 (71.1)	4 (3.7)
CEM	Butzler	10 (9.4)	4 (3.7)	52 (48.6)	41 (38.3)
	CCDA	20 (18.7)	11 (10.2)	60 (56.1)	16 (15.0)
Bu 10	Butzler	13 (12.1)	5 (4.7)	58 (54.2)	31 (29.0)
	CCDA	18 (16.8)	6 (5.6)	73 (68.2)	10 (9.4)

Symbols: +, *Campylobacter* colonies were detected in almost pure culture; ±, *Campylobacter* colonies were separated despite of some contaminants; –, no colony on the inoculated area; HC, no *Campylobacter* colonies were detected because of heavy contamination.

Table 2. *Campylobacter* species isolated from cecal contents of 176 cattle and 103 pigs by the direct plating method on CCDA and Butzler agar

<i>Campylobacter</i> species	Number (%) of positive samples			
	Cattle		Pigs	
	CCDA	Butzler	CCDA	Butzler
<i>C. jejuni</i>	36 (20.5)	23 (13.1)	7 (6.8)	5 (4.9)
<i>C. coli</i>	2 (1.1)	1 (0.6)	75 (72.8)	74 (71.8)
<i>C. fetus</i> subsp. <i>fetus</i>	18 (10.2)	6 (3.4)	2 (1.9)	2 (1.9)
<i>C. lari</i>			11 (10.7)	4 (3.9)
<i>C. hyointestinalis</i>			1 (1.0)	1 (1.0)
Total	56 (31.8)	30 (17.1)	96 (93.2)	86 (83.5)

enrichment.

Among 107 samples from cattle, *Campylobacter* spp. were detected from 28 samples by enrichment culture followed by plating on CCDA, nine samples by the direct plating method only and 12 samples by the enrichment procedure only. So, if time permits, the enrichment followed by plating procedure will be recommended for the maximum isolation of campylobacters.

Table 2 shows the species and the numbers of isolates from cecal contents of cattle and pigs, identified after plating on CCDA and Butzler agar. The colonies developed on both media were identified by the tests as described by Skirrow & Benjamin [19] and Itoh *et al.* [12]. From cattle samples, *C. jejuni* was isolated most frequently, and *C. fetus* subsp. *fetus* was also a dominant species. It seemed that CCDA had a much greater ability to support the growth of these two species than did Butzler agar. In one instance, both *C. jejuni* and *C. fetus* subsp. *fetus* were detected on the same plate of both CCDA and Butzler agar. Giacoboni *et al.* [8] reported that six *Campylobacter* species were detected in the feces of healthy calves and cattle by the Preston-

Skirrow combination method. In our study, *C. hyointestinalis*, *C. fetus* subsp. *venerialis* or *C. fecalis* was not detected from cattle. The difference may have been caused by the different selectivities of the media, as Skirrow agar they used has a lower inhibitory effect on bacteria [1]. In addition, Bolton *et al.* [5] reported that they had developed CCDA primarily for isolation of *C. jejuni* and *C. coli* from human stools. So, the selective agents in CCDA may be inhibitory to other species than these. In pigs, however, *C. coli* was the most dominant species, followed by *C. lari*, *C. jejuni* and *C. fetus* subsp. *fetus*. *C. hyointestinalis* was isolated only once, possibly for the reason described above. As compared with CCDA, Butzler agar gave a lower the isolation rate of *C. lari*.

For epidemiological studies of campylobacters, CCDA, requiring no blood, is easy to use and direct plating of fecal samples on CCDA is recommended for rapid and sample tests.

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