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Detection of Pathogenic *Campylobacter* Species in Blood Culture Systems

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Because differences in recognition of *Campylobacter fetus* and *Campylobacter jejuni* in systemic infections may be due partially to differences in the ability to cultivate these organisms, we studied their growth characteristics in two widely used blood culture systems. In the Roche Septi-Chek system (Hoffman-La Roche, Inc., Nutley, N.J.), over a broad range of inocula all strains were detected in broth within 2 days and on paddles within 3 days. In the BACTEC 6B aerobic bottles (Johnston Laboratories, Inc., Towson, Md.), *C. jejuni* and *C. fetus* took a median of 5 and 3 days, respectively, to reach the growth index threshold. However, in the BACTEC 7D anaerobic bottles, *C. fetus* required a median of 2 days to reach the growth index threshold, whereas for *C. jejuni* the median was greater than 10 days. The poor performance of *C. jejuni* in both BACTEC systems may have been due to unfavorable incubation atmospheres and may partially explain why *C. jejuni* bacteremia is so infrequently detected. Overall, the Roche Septi-Chek system was excellent for detecting *Campylobacter* strains in blood cultures.

Campylobacter fetus subsp. *fetus* has been recognized primarily as a cause of systemic illnesses (2, 4), whereas *Campylobacter jejuni* and *Campylobacter coli* are predominently recognized as causes of diarrheal illnesses. Compared with the thousands of isolates of fecal origin, these organisms are recognized in bloodstream infections only occasionally (1a, 4–6, 9, 10–13, 17, 19; W. LeBar, Clin. Microbiol. Newsl. 4:5–6, 1982). Possible reasons for low rates of bloodstream *C. jejuni* isolations are that blood cultures usually are not taken in patients with enteritis and that procedures used to culture blood may not be optimum for the growth of *C. jejuni* (1a). Furthermore, at present not all clinical laboratories use optimal subculture methods for isolation of campylobacters nor are they familiar with the microscopic morphology of campylobacters, and thus may overlook or misidentify their presence. Because the efficiency of blood culture systems for isolating these microaerophilic organisms have not been thoroughly investigated, we studied the ability of the BACTEC radiometric ^{14}C anaerobic and aerobic and the Roche broth-paddle blood culture systems to support their growth. The utility of both the BACTEC and Roche systems for isolating a wide variety of pathogens have been investigated by other workers, but *Campylobacter* was not mentioned in their studies (14, 23, 24).

The BACTEC systems are based on the detection of microbial uptake of radioactive carbon from the broth, whereas the Roche system is partially dependent on the detection of growth on agar surfaces of the paddle. *Campylobacter* species are known to be relatively inert, including the inability to metabolize glucose (8, 18), and most *C. jejuni* strains form a thin-spreading growth on agar surfaces which is not easy to visualize. Because each of the blood culture systems described above involves one of these two features, the potential exists for some systemic *Campylobacter* infections not to be diagnosed. Further, because it is possible that differences in growth characteristics of these pathogenic *Campylobacter* strains could affect recognition of the relative frequency with which they cause infections, we studied their growth in these two widely used commercially available blood culture systems. Our studies involved the use of laboratory strains of clinical origin although not in a clinical setting.

MATERIALS AND METHODS

Bacterial strains. We used six *C. jejuni* strains, two *C. coli* strains, and six *C. fetus* strains in this study (Table 1). All but one isolate was of human origin. The organisms had been frozen at −70°C after five or fewer passages on laboratory media (1). After removal from the freezer, strains were cultured on brucella (BBL Microbiology Systems, Cockeysville, Md.) or tryptic soy agar with 5% sheep blood (blood agar plate; PASCO, Wheat Ridge, Colo.) in an atmosphere with 5% oxygen, 10% carbon dioxide, and 85% nitrogen for 48 h at 37°C for *C. fetus* or 42°C for *C. jejuni* and *C. coli*. After reisolation, pure cultures were stored in Wang transport medium (22) prior to study. For each experiment, the strains to be used were subcultured onto the brucella or tryptic soy blood agar to check purity, and an isolated colony was inoculated into brucella broth. All plates and broths were incubated overnight in the microaerobic atmosphere as described above. These subcultures of *Campylobacter* in brucella broth were diluted in Trypticase soy broth (BBL) to yield concentrations of approximately 10^5 to 10^0 CFU/ml for inoculations of blood culture media.

Inoculation of bottles. Outdated human banked blood was used for inoculating the bottles. The blood had been kept refrigerated and was tested for sterility under aerobic, anaerobic, and microaerobic conditions prior to use. Based on the recommendations of the manufacturers for the amount of blood to culture, prior to the addition of the diluted *Campylobacter* culture, 5.0 ml of sterile blood was added to the 30 ml of broth in each of the BACTEC bottles, and 10 ml was added to the 70 ml of broth in the Roche

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TABLE 1. Sources of *Campylobacter* strains used for blood culture study

Campylobacter species	DVAMC strain no.[a]	Source[b]
C. jejuni	84–37	Blood
	84–47	Blood
	84–185	Blood
	79–10	Feces
	79–263	Blood
	85–6	Blood
C. coli	84–187	Feces
	84–157	Blood
C. fetus	84–192	Feces
	82–40	Blood
	84–48	Feces
	78–10	Blood
	78–11	Feces
	78–15	Blood

[a] DVAMC, Denver Veterans Administration Medical Center *Campylobacter* laboratory.
[b] Strain 79–10 was isolated from a duck; all other strains were of human origin.

bottles. All bottles were inoculated with dilutions of each of the *Campylobacter* strains, yielding final concentrations ranging from 0.005 to 2,100 CFU/ml, and each dilution was done in duplicate. In each experiment, dilutions of the *Campylobacter* cultures were inoculated into the Roche and BACTEC bottles so that each bottle had approximately the same bacterial concentration per milliliter of broth.

Processing of blood culture bottles. After inoculation, the duplicate BACTEC 6B (30 ml of tryptic soy broth with an aerobic atmosphere) and 7D (30 ml of tryptic soy broth with an anaerobic atmosphere) bottles (Johnston Laboratories, Inc., Nutley, N.J.) were incubated at 35°C and assessed for elevation in the growth index twice daily for the first 2 days and daily for an additional 8 days if negative according to the instructions of the manufacturer. A growth index reading of $\geq$20 or $\geq$31 was considered the threshold for positive cultures in the anaerobic or aerobic bottles, respectively, and these were confirmed by Gram stain examination of broth and subculturing to blood agar plates which were incubated as described above to permit the growth of campylobacters. On some occasions, bottles showed turbidity prior to a rise in the growth index. We also performed Gram stains and subcultured these bottles. To determine whether shaking was beneficial, two early experiments were set up in duplicate with *C. jejuni* strains in BACTEC 6B bottles. One set was placed on a shaker, and the other set was not. Growth was only slightly more rapid in the bottles which had been shaken, and therefore shaking was not continued in other experiments.

After inoculation, Roche Septi-Chek blood culture bottles (Hoffman La Roche, Inc., Nutley, N.J.) consisting of 70 ml of Trypticase soy broth and paddles with chocolate, MacConkey, and malt agar sections were incubated at 35°C according to the instructions of the manufacturer and observed for turbidity of the broth and growth on paddles according to the same schedule as for the BACTEC bottles. Bottles showing turbidity of broth or growth on paddles or both were examined by Gram stain and subcultured onto blood agar plates incubated as described above to allow for growth of campylobacters. The growth on these plates was examined macroscopically and microscopically, and when necessary, oxidase and biochemical tests were made for confirmation. As most *C. jejuni* strains form spreading growth in a thin film on the paddles, it took several experiments before we recognized that growth was occurring.

RESULTS

Detection in the BACTEC system. Since we usually did not take any further readings once a bottle became positive by exceeding the threshold, the exact height of the mean growth index after that point was lowered due to our calculation method. To avoid the artifactual appearance of the growth index curve falling with time, we included in all subsequent days the value of the last reading (Fig. 1 and 2). This type of calculation dampens the actual heights that the mean growth index may have reached, but these values are well above the range that is clinically important.

As expected, *C. jejuni* growth was poor in the anaerobic incubation condition (Fig. 1A). For the lowest inocula, no growth was detected at all, whereas with increasing inoculum size, late growth was detected although at low levels. At the highest inoculum, which represented a mean of about 300 CFU/ml, or more than 9,000 CFU per bottle, the mean exceeded the threshold pre-established by the manufacturer of 20 only on day 9. In contrast, under aerobic conditions (Fig. 1B), growth was detected much more rapidly and easily. In general, the highest inocula produced the most rapid detection. An examination of the results by the length of time required to reach the threshold of 20 makes it clear that growth in the anaerobic system was very poor (Table 2). Of 46 trials, only 13 showed an index rise, and this generally was with inocula of greater than 1 CFU/ml. The earliest index rise was at 3 days, but regardless of inoculum size, the median time for each rise was greater than 10 days. This reflects the fact that at every inoculum at least 70% of the strains studied did not show an index rise. In contrast, in the aerobic system there was an index rise in each of the studies done. Nevertheless, in 30 (65%) of the 46 trials, incubation of 5 or more days was necessary for an index rise to 31. In some experiments involving *C. jejuni*, turbidity was noted in the bottles although the growth index was considerably below the usual threshold of 31. Gram stains of these turbid cultures always yielded organisms with morphology typical for *Campylocacter* spp., and subcultures of these bottles showed campylobacters.

In limited studies of two *C. coli* strains, the growth rates in both the aerobic and anaerobic systems were slightly but not significantly faster than for similar inocula of the *C. jejuni* strains tested.

C. fetus was more rapidly and more consistently detected in both the aerobic and anaerobic systems than *C. jejuni* or *C. coli* (Table 2). In the anaerobic system, the median index rise was 2 days regardless of the inoculum size. For the highest inoculum range, there was little or no lag phase since the mean growth index rose very early. Only in two trials were organisms not detected by 10 days and that occurred with calculated inocula of 0.005 and 0.018 CFU/ml. However, there was some variability in inoculum size and resulting CO_2 release (Table 2 and Fig. 2A). Growth in the aerobic system (Fig. 2B) was nearly as rapid, with 39 of 40 trials positive by 3 days regardless of inoculum size. The mean growth index for all inocula was above the threshold for positivity by day 3. Nevertheless, even at the highest inoculum, the mean did not approach the threshold until more than 48 h, suggesting that there was a brief lag period.

Detection in the Roche system. For *C. jejuni*, turbidity was detected in the broth by 24 h in every experiment (Table 2). In each case, Gram stain and subculture confirmed identity with the initial inoculum. Growth occurred on the chocolate

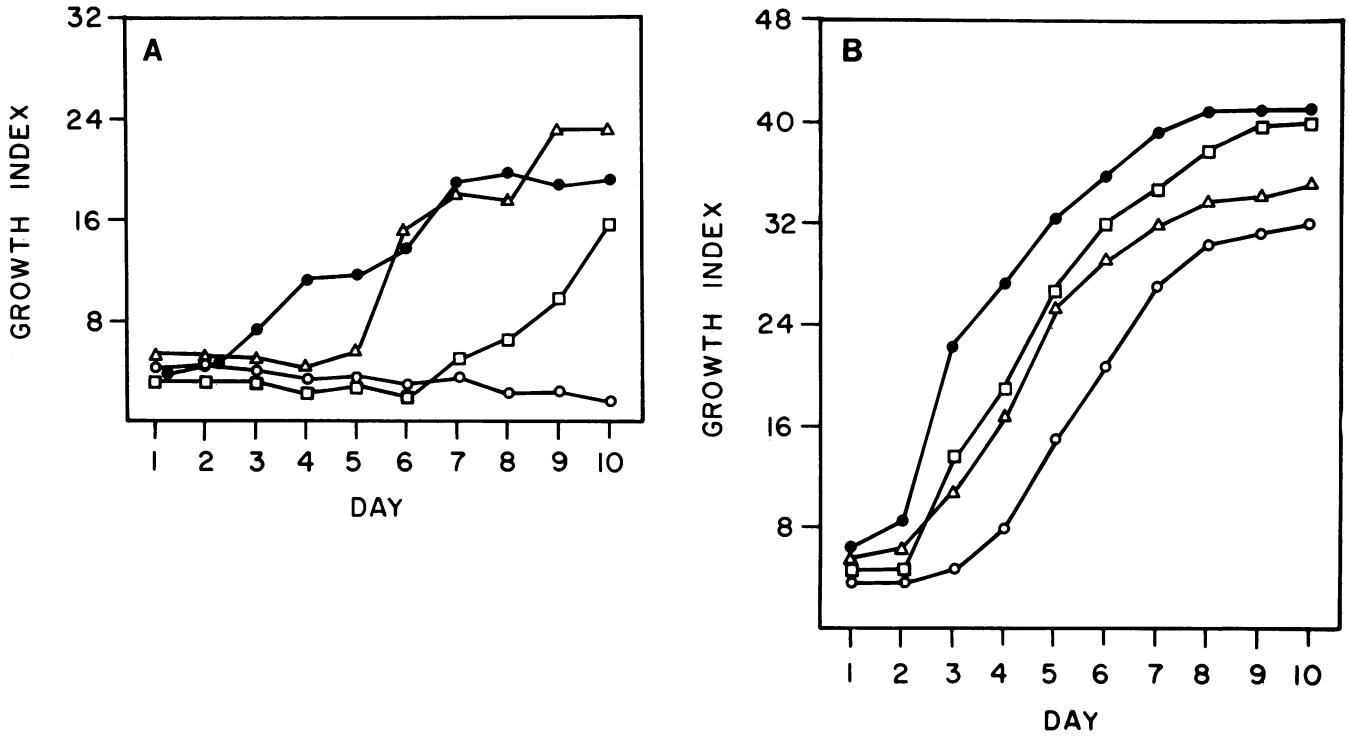

FIG. 1. Detection of *C. jejuni* and *C. coli* in BACTEC blood culture system. Dilutions (10 -fold) from 48-h cultures were added to banked human blood, and 5 ml was inoculated into each of duplicate bottles. The points represent inoculum sized (in CFU per milliliter) of <1.0 (○), 1.0 to 9.9 (□), 10 to 99 (△), and ≥100 (●). In general, when the index was greater than 30, no further readings were done. So as not to falsely show a decline in the mean index, the last positive reading was extended throughout the period of the experiment. (A) Detection in BACTEC 7D (anaerobic) bottles. The means and standard error of the mean of the actural inoculum (in CFU per milliliter) were 0.18 ± 0.06 (○), 2.50 ± 0.59 (□), 25.2 ± 4.7 (△), and 182.0 ± 25.9 (●). From 7 to 10 separate studies were done for each inoculum range. (B) Detection in BACTEC 6B (aerobic) bottles. The means and standard error of the mean of the actual inoculum (in CFU per milliliter) were 0.28 ± 0.08 (○), 4.33 ± 0.86 (□), 33.7 ± 6.4 (△), and 296.8 ± 116.9 (●). From 8 to 15 separate studies were done for each inoculum range.

agar portion of the paddles but as expected not on the malt or MacConkey agar. Detectable growth on the paddles was much slower, requiring at least 42 h, although all were positive by 72 h. No effect of inoculum size was noted. Similar to results for *C. jejuni, C. coli* inocula were detected in the broth overnight, whereas growth time until detection on the paddles was under 72 h in all cases.

For *C. fetus*, turbidity was detected in the broth within 48 h in every case, although there appeared to be an unexpected direct relationship between inoculum size and time until growth was detected (Table 2). Detectable growth on the paddles took 48 to 72 h but showed the expected inverse relationshop between inoculum size and time until growth was detected.

DISCUSSION

Isolation of *C. jejuni* from stools of persons with enteritis has been frequent, but bloodstream isolations are relatively uncommon, In contrast, *C. fetus* subsp. *fetus* has been cultured from blood of debilitated patients but less often from stools (2, 4). Butzler (4) stated that of 315 *Campylobacter* isolates from 11,000 stools, 314 were *C. jejuni* and only one was *C. fetus*, but of 8 positive blood cultures, 5 were *C. fetus* and 3 were *C. jejuni*. Data from our laboratory over 3 years showed 154 *C. jejuni* and 2 *C. fetus* isolates from stools (unpublished data). National surveillance of *Campylobacter* infections by the Centers for Disease Control shows that the numbers of *C. fetus* and *C. jejuni* bacteremias reported are similar despite a 490-fold difference in reported stool isolations (16).

Blaser et al. (3) reported that *C. jejuni* strains are usually susceptible to the complement-mediated bactericidal activity present in normal human serum, whereas *C. fetus* strains usually are resistant. This phenomenon may help explain why systemic infection due to *C. fetus* is much more commonly recognized than that due to *C. jejuni*. In our current studies, we used outdated human blood that had been refrigerated and then mixed with the broth present in all of the blood culture systems. Since either of these steps would remove all complement activity, the differences in growth characteristics that we observed could not have been due to complement-mediated injury or death of the inoculated cells. Of eight *C. jejuni* and *C. coli* laboratory isolates chosen for study, six originally had been isolated from blood cultures; selection of such strains might bias our results toward better growth characteristics when reintroduced to blood culture systems. Nevertheless, growth of these organisms in the aerobic BACTEC system was marginal and, at best, very poor under the anaerobic condition. Because this study was performed under laboratory conditions, the results may not apply to a clinical setting.

C. fetus and *C. jejuni* are microaerophilic; the optimum concentrations of O_2 and CO_2 are 5% and 10%, respectively (18, 20, 21). None of the present commercial blood culture systems have this atmosphere. Thus, we studied the widely used aerobic (6B) and anaerobic (7D) BACTEC blood culture systems and the aerobic Roche Septi-Chek blood cul-

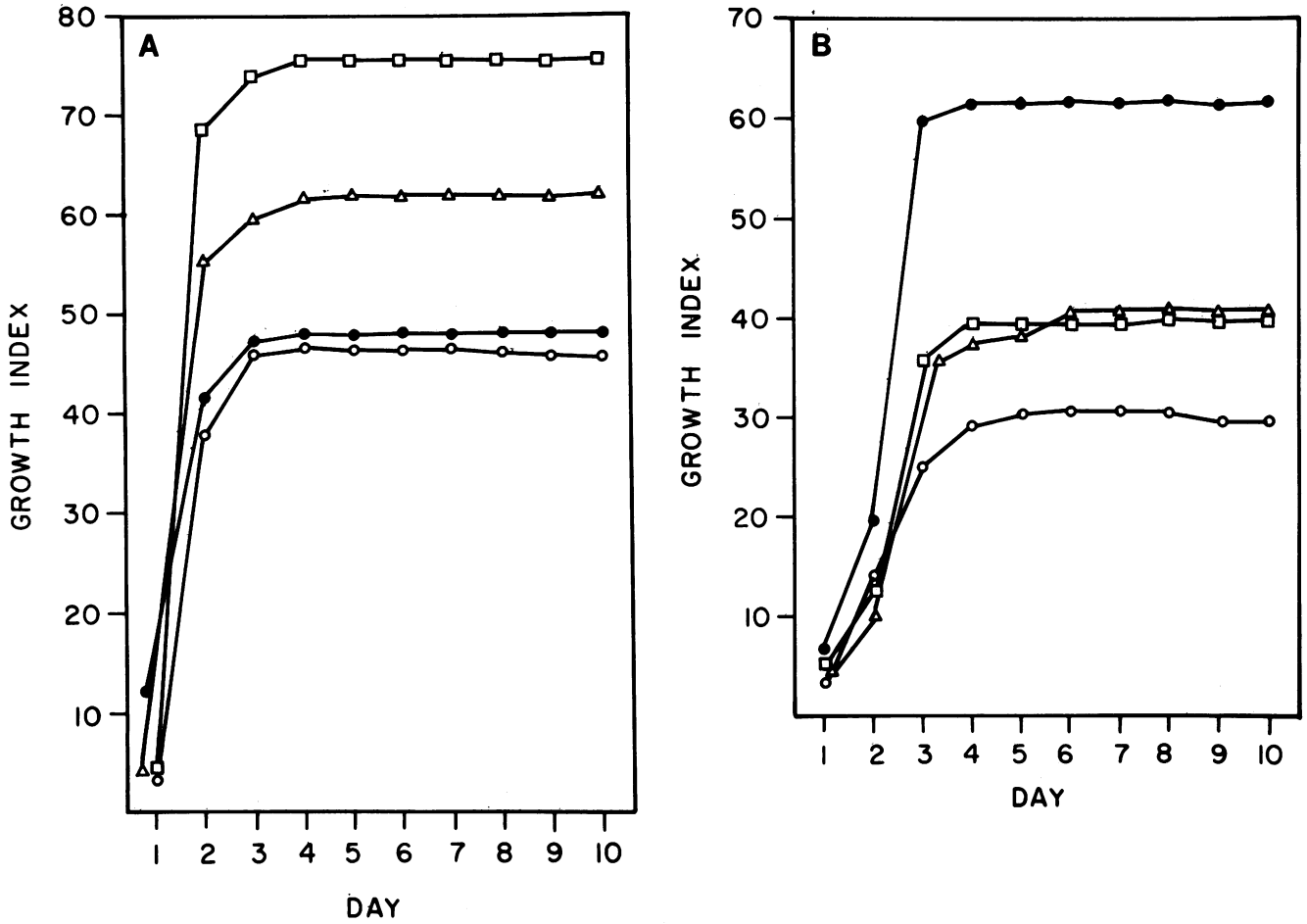

FIG. 2. Detection of *C. fetus* in BACTEC blood culture system. The methods and symbols are as described in the legend to Fig. 1. (A) Detection in BACTEC 7D (anaerobic) bottles. The means and standard error of the mean of the actual inoculum (in CFU per milliliter) were0.22 ± 0.06 (○), 3.66 ± 0.64 (□), 12.3 ± 2.6 (△), and 812.0 ± 341 (●). From 6 to 17 separate studies were done for each inoculum range. (B) Detection in BACTEC 6B (aerobic) bottles. The means standard error of the mean of the actual inoculum (in CFU per milliliter) were 0.20 ± 0.07 (○), 3.61 ± 0.58 (□), 29.8 ± 5.5 (△), and 812 ± 341 (●). From 6 to 17 separate studies were done for each inoculum range.

ture systems for the growth of these organisms. We found slow release of $^{14}CO_2$ by *C. jejuni* in the aerobic BACTEC bottles and practically none in the anaerobic bottles (7D), as indicated by changes in the growth index. It is possible that *C. jejuni* does not use the radioactively labeled substrates in this medium efficiently, as these organisms are relatively inert in conventional biochemical tests (8, 18); however, *C. jejuni* growth as shown by turbidity and subcultures also was slow. The BACTEC 6B and 7D bottles contain tryptic soy broth, vitamins, and other enrichments, and the 7D bottles also contain several reducing agents. *C. jejuni* should grow in either broth, thus differences in growth characteristics of *C. jejuni* in the anaerobic and aerobic systems most probably are due to differences in atmosphere. Previous in vitro studies have shown that *C. jejuni* does not tolerate anaerobiosis (20, 21). The few positive 7D bottles may have been due to introduction of air during the inoculation procedure or to differing tolerance of strains to anaerobiosis. Schwartz and Stamper (17) reported on *C. jejuni* isolated from the blood of a 79-year-old man and cultured in aerobic 6B bottles; after 5 days of incubation at 37°C, the growth index was 170. LeBar (LeBar, Clin. Microbiol. Newsl., 1982) reported *C. jejuni* isolated from blood cultured in the BACTEC system had a growth index of 60 at 48 h. The *C. jejuni* inocula from these patients may have been high, enabling detection in the aerobic bottles. An alternative explanation is that our laboratory studies may not mimic clinical conditions. Brucella agar and broth base have been widely used in *Campylobacter* studies, although ingredients used by different manufacturers vary. Our previous studies (unpublished data) showed that Trypticase soy base media do not support the growth of *C. jejuni* as well as Brucella base medium. The use of Brucella base medium (1) in blood culture bottles may permit *Campylobacter* species to grow better and faster.

Sodium polyanethol sulfonate, and anticoagulant added to blood culture media, inhibits the growth of a number of bacterial pathogens (15). That sodium polyanethol sulfonate was present at a higher concentration (0.05%) in the Roche broth than in the BACTEC broths (0.025%) suggests that it is not inhibitory to pathogenic campylobacters in the concentrations used.

That all *C. fetus* strains grew more quickly in the anaerobic BACTEC 7D bottles than in the aerobic 6B bottles agrees with previous studies of two *C. fetus* strains; the best growth was in anaerobic bottles, followed by microaerobic bottles (5% oxygen), and growth was poorest in 17% oxygen (W.-L. L. Wang, unpublished data). The irregularity in the

TABLE 2. Time required for detection of *Campylobacter* species in blood culture systems

Species and inoculum (CFU/ml)	No. of BACTEC/ Roche trials	Time required for detection by: BACTEC[a] in conditions: Aerobic Median (day)	Aerobic Range (day)	Anaerobic Median (day)	Anaerobic Range (day)	Roche[b] with: Broth Median (h)	Broth Range (h)	Paddles Median (h)	Paddles Range (h)
C. jejuni									
<1.0	14/4	5.5	4–8	>10	5–>10	18	18	57	42–72
1–9.9	10/6	5.0	3–7	>10	8–>10	18	18–24	45	42–72
10–99	14/7	5.5	3–7	>10	6–>10	18	18–24	45	42–72
≥100	8/4	3.5	4–8	>10	3–>10	18	18–24	72	42–72
C. fetus									
<1.0	17/13	3	3–≥10	2	2–>10	18	18–24	72	48–72
1–9.9	11/7	3	3	2	2–3	18	18–24	72	48–72
10–99	6/5	3	3	2	2–3	24	18–48	48	48–72
≥100	6/5	3	1–3	2	2–3	48	18–48	48	48

[a] Detection in the BACTEC system was defined as having a growth index that exceeded 20 for anaerobic bottles and 31 for aerobic bottles according to the instructions of the manufacturer.

[b] Detection in the Roche system was defined as turbidity in the broth or growth on the paddles according to the instructions of the manufacturer.

data relating inoculum size and $^{14}CO_2$ release in the 7D bottles could reflect the accidental introduction of oxygen into the bottles during some of our experimental manipulations. Our present data suggest that if a patient has *C. fetus* bacteremia, growth can be detected in either the 6B or 7D bottles. Reimer and colleaques (14) have recommended and we currently use in our laboratory a threshold of 31 for aerobic bacteria and 20 for anaerobes. As all campylobacters are considered to be microaerophilic, in both BACTEC systems we monitored the growth indexes until 31 was reached. We found that the rise from 20 to 31 for *C. jejuni* cultures required a mean of 0.78 days in the aerobic bottles. For *C. fetus* this rise took 0.27 days in the anaerobic bottles and 0.28 days in the aerobic bottles. In those experiments in which the growth index reached 20, the Gram stain and subculture were always positive. Therefore we recommend a growth index of 20 as the threshold for detection of campylobacters in the BACTEC blood culture system. In clinical practice, for those inoculated bottles that show increases in growth index that do not reach the threshold but show turbidity, Gram stain and subculture under microaerobic conditions that will permit growth of campylobacters also should be considered. Clinical laboratories also need to determine whether establishing a policy of a threshold of 20 for screening aerobic blood cultures for campylobacters will be efficent and cost effective.

Despite an atmosphere of 17% O_2 and 3 to 5% CO_2, reflecting the incubator conditions, all *Campylobacter* species tested grew well in the Roche broth, in some cases nearly as rapidly as fast-growing organisms, such as members of the family *Enterobacteriaceae*. As we do not know the exact formulation of the Roche ingredients, we are not certain which compound permits faster campylobacter growth than that in the Trypticase soy base (BACTEC) alone. It is possible that some reducing agent as well as enrichment in the medium stimulated the growth of *Campylobacter*. Because of spreading, growth on the paddles was more difficult to detect. In our previous growth studies, we added extra agar to plates to obtain isolated *C. jejuni* colonies (21). It is possible that incorporating extra agar into the Roche paddles would produce more discrete colonies. Although primary isolation of *C. jejuni* from stool cultures can occur in 24 h, 48 h is usually required. As such, overnight growth of *C. jejuni* in the Roche bottles may be clinically useful; improvement of the paddle could make this system ideal. At least at the present, the Roche Septi-Chek bottle is more efficient for detection of campylobacters in blood cultures than either the aerobic or anaerobic BACTEC ^{14}C bottles.

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