Campylobacter Infection in Man and Animals

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INTRODUCTION

Frequent in animals, particularly in bovines and ovines, campylobacter has been known for more than 40 years exclusively as a veterinary disease. In 1909, two veterinary surgeons, McFadyean and Stockman, discovered the animal disease during a survey on epizootic abortion in ewes. They observed an unknown bacterium, frequently present in abortion, isolated from aborted fetuses and resembling a vibron.

In 1913, the same authors demonstrated that their organism could be observed in infectious abortions, but World War I prevented diffusion of their work.

In 1919, Theobald Smith, while investigating infectious abortions of bovines in the U.S., isolated (apart from the "Bacillus of Bang") another bacterium which he described as a spirillum. Finishing his study, he became acquainted with McFadyean and Stockman's work and assumed they had been studying the same bacteria. He confirmed this, together with Taylor, and proposed "Vibrio fetus" as a name.

In 1931, Jones, Orcutt, and Little attributed winter dysentery in calves to infection with a "vibrio" they called Vibrio jejuni and in 1944 Doyle described a similar organism associated with swine dysentery which in man was a milkborne outbreak of acute diarrheal illness reported by Levy in 1946; organisms resembling Vibrio jejuni were seen in blood cultures from several of the victims; but they could not be isolated on solid media and thus positively identified. In 1947 Vinzent isolated Vibrio fetus from the blood of three pregnant women, admitted because of fever of unknown origin. The illness lasted about 4 weeks, and two of the three women aborted. On examination, the placenta revealed large necrotic and inflammatory areas.

In 1949, Stegenga and Terpstra demonstrated the pathogenic role of C. fetus venerealis in enzootic sterility in cows. In 1959, Florent was able to distinguish two types of C. fetus by their biochemical characteristics and pathogenic powers: C. fetus venerealis and C. fetus intestinalis.

In 1957, E. King described a Vibrio that presented several features in common with the agents described by Vinzent, but had different biochemical and antigenic characteristics. She called it related Vibrio. This condition was for a long time unrecognized. Indeed, until 1972 only 12 cases of related Vibrio infections were known: 7 infants, 2 children, and 3 adults. The reason for the paucity of reports was that the selective culture techniques necessary for the isolation of the Vibrio, later renamed by Sebald and Veron as Campylobacter, from feces were not known at that time. Consequently, the infection could be diagnosed only from the blood of bacteremic patients. However, King believed that the infection was not so rare as these few reports suggested and she emphasized the need to devise a method for culturing the organisms from feces. It is sad that such a method was not developed in her lifetime.

It was not until 1972 in Brussels that the application of veterinary techniques to the culture of human material provided the necessary breakthrough. The Belgian team isolated Campylobacter jejuni from five percent of children with diarrhea and later Skirrow confirmed and extended this observation. Since then other workers have reported similar findings and in some laboratories Campylobacter isolations outnumbered those of Salmonella and Shigella together.

In human pathology we distinguish C. fetus intestinalis, now called C. fetus subsp. fetus and C. jejuni. C. fetus subsp. fetus is an opportunistic, chiefly attacking debilitating patients with impaired defences against infections. C. jejuni is one of the most common etiologic agents of bacterial diarrhea.

Veterinary medicine has to investigate the etiologic role of different new subspecies of Campylobacter. There is a lot of evidence to consider human campylobacteriosis as a zoonosis. This book reflects the different efforts made by veterinary and medical doctors.
for a better knowledge of the disease. It shows how much we depend upon each other to understand better the clinical features, pathogenesis, and epidemiology of campylobacter infections and other diseases.

I wish to thank all my colleagues and friends for their contributions.

J. P. Butzler

REFERENCES


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Among other awards, he is a recipient of the Award of the Belgian Royal Academy of Medicine for his work in infectious diseases and of the Van Beneden and Brohée awards for his research in gastroenterology. Dr. Butzler is author of over 150 articles. He has presented his research findings at more than 70 international meetings. His current major research interests include rapid diagnosis and pathogenesis of infectious diseases and the control of diarrhoeal disease in relation to child morbidity and mortality in developing countries.
To Miss Elisabeth King (1912—1966)
whose vision and diligence
paved the way
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Chapter 1

TAXONOMY OF THE GENUS CAMPYLOBACTER

Mohamed A. Karmali and Martin B. Skirrow

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I. INTRODUCTION

Even though campylobacters were first recognized about 70 years ago, their remarkable association with disease is only now becoming widely appreciated. Not only are these organisms among the most frequent causes of bacterial diarrhea in man, but they are also leading causes of enzootic sterility in cattle and sporadic abortion in various domestic animals.

The taxonomy of the genus Campylobacter has a confusing past. Renewed interest in this genus during the past decade has led to significant improvements in the identification, nomenclature, and classification of its members. The major impetus to these improvements has undoubtedly been the practical need to improve understanding of the epidemiology of campylobacter infections in man. The extent of current progress is such that descriptions of new and unnamed species are already appearing in the literature. Continuing modifications to the classification of campylobacters are thus to be anticipated. The purpose of this chapter is to provide a historical perspective to the taxonomy of the genus Campylobacter, and to outline practical approaches to the identification and classification of its species and strains. For a more detailed description of the taxonomy of this genus, the reader is referred to publications by Véron and Chatelain and Smibert.

II. GENERAL DESCRIPTION

Campylobacters were originally referred to as “micro-aerophilic vibrios”. The new generic term Campylobacter (“curved rod” in Greek) was proposed by Sebald and Véron in 1963 on the grounds that the microaerophilic vibrios differed significantly from Vibrio cholerae and certain other vibrios and vibrio-like organisms with respect to their biochemical and physiological properties, and their DNA base-pair ratios.

Campylobacters are small, nonsporeforming, Gram-negative bacteria that have a characteristic curved, S-shaped, or spiral morphology. The cells may vary from 0.5 to 8 μm in length, and 0.2 to 0.5 μm in width. Virtually all members of the genus are oxygen sensitive and can grow only under conditions of reduced oxygen tension that vary from almost anaerobic to microaerobic for the different species; an aerotolerant Campylobacter group has recently been described. The cells are highly motile with a characteristic rapid, darting, corkscrew-like motility; they have a single polar flagellum at one or both ends of the cell.

Campylobacters use amino acids and tricarboxylic acid cycle intermediates as their principal energy sources. They are oxidase positive and reduce nitrates; some species are catalase positive, others are catalase negative. Their metabolism is strictly respiratory; carbohydrates are neither fermented nor oxidized. Campylobacters are rather inert when tested in conventional biochemical media and this has made it difficult to classify them on biochemical grounds.

The genus Campylobacter is classified together with the genus Spirillum in the family Spirillaceae. The distinction between these genera is based on the number of polar flagella, the ability of cells to accumulate intracellular granules of polyhydroxybutyric acid (PHB), and the DNA base composition. Campylobacters usually have a single polar flagellum and are unable to accumulate PHB, whereas the spirilla have tufts of flagella at their poles and readily accumulate intracellular granules of PHB.

The guanine-plus-cytosine (G + C) content of the genus Campylobacter ranges from 29 to 38 mol %, which, as pointed out by Neill et al., is among the lowest known for bacteria; the G + C ratio of the genus Spirillum ranges from 38 to 65 mol %. There are now about a dozen species and subspecies recognized in the genus Campylobacter. These may be conveniently separated into two broad groups: the catalase-positive campylobacters, and the catalase-negative campylobacters. A list of species and subspecies...
Table 1

CAMPYLOBACTER SPECIES AND SUBSPECIES

<table>
<thead>
<tr>
<th>Catalase positive</th>
<th>Catalase negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fetus subsp. fetus&lt;sup&gt;15&lt;/sup&gt;</td>
<td>C. sputorum subsp. sputorum&lt;sup&gt;15&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. fetus subsp. venerealis&lt;sup&gt;35&lt;/sup&gt;</td>
<td>C. sputorum subsp. babulus&lt;sup&gt;35&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. jejuni&lt;sup&gt;35&lt;/sup&gt;</td>
<td>C. sputorum subsp. mucosalis&lt;sup&gt;35&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. coli&lt;sup&gt;35&lt;/sup&gt;</td>
<td>C. concisus&lt;sup&gt;36&lt;/sup&gt;</td>
</tr>
<tr>
<td>NARTC group&lt;sup&gt;38,67&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>C. fecalis&lt;sup&gt;39&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Aerotolerant Campylobacter sp.&lt;sup&gt;9&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Nitrogen-fixing Campylobacter sp.&lt;sup&gt;75&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Free-living Campylobacter sp.&lt;sup&gt;76&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 belonging to each of these groups is shown in Table 1. The use of the catalase test, which has proved to be a very useful taxonomic tool in classifying campylobacters, was first reported in this context in 1955 by Bryner and Frank.<sup>13</sup>

III. CATALASE-POSITIVE CAMPYLOBACTERS

A. Historical Background

Microaerophilic vibrios, now considered to be campylobacters, were first described in 1913 in England by McFadyean and Stockman,<sup>1</sup> who implicated these organisms as causal agents of abortion in sheep. A few years later, in 1918, Smith<sup>44</sup> in the U.S. reported on the association of similar organisms with bovine abortion. The latter organisms, which were isolated from aborted bovine fetuses, were named Vibrio fetus by Smith and Taylor<sup>15</sup> and the disease became known as vibriotic abortion. In 1927, Smith and Orcutt<sup>46</sup> described the isolation of microaerophilic vibrios from cultures of livers and spleens obtained from calves with diarrhea. They noted that the calf diarrhea strains differed serologically from Vibrio fetus and speculated that the former might be causally linked to bovine enteritis.

Investigations into the role of microaerophilic vibrios in bovine enteritis were subsequently conducted by Jones and colleagues<sup>17-19</sup> who, in 1931, published evidence showing a causal relationship between these organisms and winter dysentery in cattle. They noted<sup>19</sup> that the calf enteritis strains "while presenting certain slight morphological differences such as length, the number of coils, and to some extent the depth of coils," resembled Vibrio fetus sufficiently to be regarded as a closely related group. Jones et al. named the calf enteritis strains Vibrio jejuni.<sup>19</sup>

Microaerophilic vibrios became linked with yet another disease when Doyle<sup>20</sup> in 1944, suggested that these organisms caused swine dysentery. Doyle named the swine organism Vibrio coli.<sup>21</sup>

A significant development during the 1940s was the association of microaerophilic vibrios with human disease. In 1946, Levy<sup>22</sup> described a large institutional outbreak of gastroenteritis that affected about 350 people. Microaerophilic vibrios were isolated from blood cultures of 13 of 39 patients and were seen in fecal smears from about 20% of the cases. Levy suggested that these organisms were identical to V. jejuni described earlier by Jones et al.<sup>19</sup> in association with winter dysentery. Vinzent et al.,<sup>23</sup> in 1947, reported the isolation of a microaerophilic vibrio that they considered to be V. fetus from the blood culture of a pregnant woman who aborted during the course of a febrile illness. Vinzent<sup>24</sup> went on to describe two further cases of a similar nature.

V. fetus, V. jejuni, and V. coli owe their names more to their association with specific diseases in animals rather than to any observed taxonomic differences between them. The position was somewhat clarified in 1957 when King<sup>25</sup> showed that catalase-positive microaerophilic vibrios could be distinguished by their ability to grow at different temperatures.
Table 2
NOMENCLATURES USED FOR CAMPYLOBACTER FETUS

<table>
<thead>
<tr>
<th>Original description</th>
<th>Vibrio fetus (Smith and Taylor, 1919)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florent (1959)27</td>
<td>Vibrio fetus var. intestinalis</td>
</tr>
<tr>
<td>Véron and Chatelain (1973)6*</td>
<td>Campylobacter fetus subsp. fetus</td>
</tr>
<tr>
<td>Smiter (1974)6</td>
<td>Campylobacter fetus subsp. intestinalis</td>
</tr>
<tr>
<td></td>
<td>Vibrio fetus var. venerealis</td>
</tr>
<tr>
<td></td>
<td>Campylobacter fetus subsp. venerealis</td>
</tr>
</tbody>
</table>

* Officially recognized nomenclature in the “Approved Lists” of bacterial names.35

She noted that strains of V. fetus were able to grow at 25 and 37°C, but not at 42°C; by contrast, a closely related group of organisms which she termed “related vibrios” failed to grow at 25°C, but grew at 37°C, and even better at 42°C. Notably all four human isolates of “related vibrios” that she studied were obtained from blood cultures of infants or young children with diarrhea. In a subsequent report,26 King suggested that her “related vibrios” were identical to the V. jejuni of Jones et al.19 and V. coli of Doyle.21 King26 also hinted at possible morphological differences between V. fetus and the “related vibrios” when she observed that in cells of the latter the undulations tended to be closer together than in V. fetus.

During the late 1940s and early 1950s the clinical spectrum of abortion in cattle associated with V. fetus was becoming better understood. It became clear that there were two distinct clinical entities of bovine vibrionic abortion. The first was the syndrome of sporadic bovine abortion occurring among pregnant cows that were members of an otherwise fertile herd. The infection that resulted in abortion was acquired not venereally, but probably following a bacteremia by V. fetus that had established itself in the intestinal tract of the cow,27,28 i.e., in a manner similar to that proposed for ovine vibrionic abortion.29 The second clinical entity, of much greater economic importance, was that of sporadic abortion occurring in a herd with strikingly reduced conception rates.30 In this syndrome of enzootic sterility or infectious infertility,31 V. fetus was transmitted from the bull to the cow during coitus. The bull was a symptomless carrier of the organism on the prepuce, and transmitted the organism venereally to an entire herd. Infertility was the major consequence, although conception and subsequent abortion also sometimes occurred.

The reasons for the involvement of V. fetus in two apparently different abortion syndromes was clarified when Florent27 showed in 1959 that there were in fact two different varieties of V. fetus. One variety, which he termed V. fetus var. intestinalis, originated in the intestine and caused sporadic abortion among members of a fertile herd; the second, which he called V. fetus var. venerealis, was the one transmitted venereally and implicated in the infertility syndrome.

Florent27 distinguished V. fetus var. intestinalis from V. fetus var. venerealis on the basis of two tests: the ability of the strains to produce hydrogen sulfide (H₂S) in cysteine-containing media (using a lead acetate strip as indicator), and their ability to grow in the presence of 1% glycine, a test originally described by Lecce32 for distinguishing ovine from bovine strains of V. fetus. V. fetus var. intestinalis grew in the presence of 1% glycine, and produced H₂S in a cysteine-containing medium, whereas V. fetus var. venerealis gave the opposite reactions.

Following the work of Sebald and Véron6 in 1963, in which microaerophilic vibrios were assigned to the new genus Campylobacter, Véron and Chatelain,6 in 1973, published the
Table 3

NOMENCLATURES USED FOR C. JEJUNI AND C. COLI

<table>
<thead>
<tr>
<th>Original description</th>
<th>Vibrio jejuni (Jones et al., 1931)</th>
<th>Vibrio coli (Doyle, 1948)</th>
</tr>
</thead>
<tbody>
<tr>
<td>King (1957)</td>
<td>&quot;Related vibrios&quot;</td>
<td></td>
</tr>
<tr>
<td>Véron and Chatelain (1973)</td>
<td>Campylobacter jejuni</td>
<td>Campylobacter coli</td>
</tr>
<tr>
<td>Smibert (1974)</td>
<td></td>
<td>Campylobacter fetus subsp. jejuni</td>
</tr>
</tbody>
</table>

- Officially recognized nomenclature in the "Approved Lists" of bacterial names.

first comprehensive account of the taxonomy of this genus. In accordance with the International Code of Nomenclature of Bacteria, Véron and Chatelain had to establish one of the species, preferably that which conformed most closely to the original description of V. fetus by Smith and Taylor. Since Smith and Taylor’s original strains were no longer available for study, Véron and Chatelain argued for selecting V. fetus var. intestinalis (Florent) as the type species and, according to the International Code, renamed that species C. fetus subsp. fetus (Table 2). The choice of V. fetus var. intestinalis (Florent) rather than V. fetus var. venerealis for the type species was subsequently supported by Karmali et al. who showed that the measurements of the wavelength and amplitude of cells corresponding to V. fetus var. intestinalis (Florent) were similar to those documented for V. fetus by Smith.

Another major taxonomic study of the genus Campylobacter was published by Smibert in 1974 in the 8th edition of Bergey’s Manual of Determinative Bacteriology. In contrast to Véron and Chatelain’s nomenclature, Smibert speculated that the original strains of V. fetus were more likely to have been V. fetus var. venerealis (Florent). He therefore designated the latter variety as the type species, and renamed it C. fetus subsp. fetus (Table 2). Smibert also gave the name C. fetus subsp. jejuni to strains that Véron and Chatelain called C. jejuni and C. coli (Table 3).

Because the nomenclatures of both Smibert and Véron and Chatelain have been widely used in the literature, confusion has arisen over the use of names for species and subspecies. The different nomenclatures used for catalase-positive campylobacters are listed in Tables 2 and 3. The officially recognized nomenclature for campylobacters published in the “Approved List” of bacterial names is that of Véron and Chatelain. It should be noted that the nomenclature to be used in the 9th edition of Bergey’s Manual of Determinative Bacteriology (in press) is in accordance with the List of Approved Names.

A dilemma until recently was whether C. jejuni and C. coli were different species or merely biotypes or variants within the same species; laboratory tests for distinguishing between the two were not available. A major breakthrough occurred in this area in 1980 when Harvey showed that strains in the C. jejuni-C. coli group could be separated on the basis of their ability to hydrolyze hippurate. Skirrow and Benjamin separated the C. jejuni-C. coli group by cultural characteristics. Using Harvey’s hippurate test, they went on to show that hippurate positivity was a feature of strains they considered to be C. jejuni, while hippurate negativity was linked with characteristics of strains they recognized as C. coli. It has now become established on the basis of DNA hybridization studies that the hippurate-positive strains (C. jejuni), and hippurate-negative strains (C. coli) represent two different species. There is reason to question the validity of the term C. coli because whereas strains now considered to be C. coli by Véron and Chatelain and Skirrow and Benjamin are nitrate positive, the strains originally described by Doyle as C. coli were reported by him to be nitrate negative. This controversy is now of academic interest only, because the listing of "C. coli (Doyle 1948) Véron and Chatelain 1973” in the Approved
Lists of Bacterial names\(^{35}\) gives official recognition to this species and assumes that the organisms studied by Doyle and Véron and Chatelain were the same. The type strain of \textit{C. coli} (CIP 7080)\(^{15}\) is nitrate positive. There are no reports in the recent literature of nitrate-negative strains in the \textit{C. jejuni-C. coli} group. Nevertheless, in support of Doyle’s nitrate-negative strains\(^{21}\) there are reports in the 1950s by Kuzdas and Morse\(^{41}\) and Di Liello et al.\(^{52}\) which describe nitrate-negative strains of “\textit{Vibrio jejuni}”. It is thus conceivable that there may indeed exist within the \textit{C. jejuni-C. coli} group a subgroup of nitrate-negative strains.

Apart from \textit{C. fetus} subsp. \textit{fetus}, \textit{C. fetus} subsp. \textit{venerealis}, \textit{C. jejuni} and \textit{C. coli}, a number of other species (Table 1) are now included in the catalase-positive \textit{Campylobacter} group. These species are described below.

B. Differentiation of Catalase-Positive \textit{Campylobacter} from Catalase-Negative \textit{Campylobacter}

Catalase-negative \textit{campylobacters}\(^{7}\) tend to be more oxygen sensitive than catalase-positive \textit{campylobacters} and thus require a lower level of oxygen tension for optimal growth than the latter. Virtually all \textit{Campylobacter} species are able to reduce nitrates. However the reduction of nitrites is a feature only of the catalase-negative group. As a general rule, the production of hydrogen sulfide in triple sugar iron (TSI), Kliger’s iron (KI), or SIM media is confined to the catalase-negative \textit{campylobacters}; \textit{C. fecalis} is the only catalase-positive \textit{Campylobacter} species that produces hydrogen sulfide in these media.

C. Differentiation of Catalase-Positive \textit{Campylobacter} Species and Subspecies

Practical tests for differentiating catalase-positive \textit{campylobacters} are outlined in Table 4. Other differential characteristics are discussed below under the heading of individual species.

D. Description and Pathogenic Significance of Species and Subspecies

1. \textit{C. jejuni} and \textit{C. coli}

The \textit{C. jejuni-C. coli} group of organisms has recently emerged from obscurity to rank among the leading causes of bacterial diarrhea in man.\(^{2,3}\) This discovery was the result of pioneering work done by Butzler and colleagues\(^{43}\) in Belgium and Skirrow\(^{44}\) in England during the past decade.

The majority of human \textit{Campylobacter} isolates from cases of gastroenteritis are \textit{C. jejuni}\(^{38,45,46}\) (Table 5). \textit{C. jejuni} is widely distributed in the animal kingdom\(^{4,38,45,47}\) and has been isolated from poultry and wild birds, domestic animals such as dogs, cats, cattle, sheep and pigs, primates, and also from various exotic species in zoos. \textit{C. jejuni} is an established cause of sporadic abortion in sheep\(^{4}\) and has, in the past, also been associated with avian infectious hepatitis,\(^{48}\) bluecomb disease of turkeys,\(^{49}\) and enteritis in cattle.\(^{17-19}\)

\textit{C. coli} accounts for only about 3 to 5\% of the \textit{Campylobacter} isolates from cases of human gastroenteritis\(^{38,45,46}\) (Table 5); it appears to cause an illness in man indistinguishable from that caused by \textit{C. jejuni}\.\(^{38,46}\) Although the pig is its major reservoir,\(^{38,45}\) \textit{C. coli} is also sometimes found in other domestic animals, but with a frequency considerably less than that for \textit{C. jejuni}\(^{45}\) (Table 5).

The principal features and differential characters of \textit{C. jejuni} and \textit{C. coli} are shown in Table 4. Both these species have a higher than usual optimal growth temperature, about 42°C.\(^{25,26}\) It should be noted that the mere presence of growth at 42°C is not in itself sufficient for assigning an organism to the \textit{C. jejuni-C. coli} group; as originally pointed out by King,\(^{25}\) it must be shown that the organism grows better at 42°C than at 37°C to qualify for \textit{C. jejuni-C. coli} status. This factor should help to resolve the identity of those strains of \textit{C. fetus} subsp. \textit{fetus} that reportedly also grow at 42°C.\(^{50}\) Skirrow and Benjamin\(^{38}\) observed that
Table 4
KEY DIFFERENTIAL CHARACTERISTICS OF CATALASE-POSITIVE CAMPYLOBACTERSa,b

<table>
<thead>
<tr>
<th></th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>C. fetus subsp. fetus</th>
<th>C. fetus subsp. venerealis</th>
<th>NARTC</th>
<th>C. falcis</th>
<th>Aerotolerant Campylobacter spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average wavelength A (μm)</td>
<td>1.12</td>
<td>1.80</td>
<td>2.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average amplitude α (μm)</td>
<td>0.48</td>
<td>0.55</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative dimensions of λ and α</td>
<td>‘‘Short’’</td>
<td>‘‘Short’’</td>
<td>‘‘Medium’’</td>
<td>‘‘Long’’</td>
<td>‘‘Short’’</td>
<td>‘‘Medium’’</td>
<td>‘‘Long’’</td>
</tr>
<tr>
<td>Rapid coccal transformation</td>
<td>+</td>
<td>w/−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Swarming on moist media</td>
<td>+</td>
<td>w/−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth at</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.0°C</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>30.5°C</td>
<td>−</td>
<td>d(78%)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>37.0°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>43.0°C</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>45.5°C</td>
<td>d</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Susceptibility to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid §</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Cephalothin §</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth in 1% glycine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>H₂S production:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSI or SIM medium</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Iron/metabisulfite (FBP) medium</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+/w</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cysteine medium with PbAc strip</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Growth anaerobically in presence of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumarate</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>d(70%)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Trimethylamine N-oxide</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. jejuni</td>
<td>C. coli</td>
<td>C. fetus subsp. fetus</td>
<td>C. fetus subsp. venerealis</td>
<td>NARTC</td>
<td>C. fecalis</td>
<td>Aerotolerant Campylobacter spp.</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------</td>
<td>---------</td>
<td>-----------------------</td>
<td>---------------------------</td>
<td>-------</td>
<td>-----------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Growth on triphenyl tetrazolium chloride agar (0.4 g/l)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Presence of C-19 fatty acid in cellular lipids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Note:  
+ = 90—100% strains positive  
++ = optimal reaction  
- = 90—100% strains negative  
d = 10—90% strains positive  
w = weak positive or intermediate reaction  
S = sensitive  
R = resistant  
† = 30 μg disc or 40 mg/l  
§ = 30 μg disc or 64 mg/l

* Excluding the nitrogen-fixing Campylobacter species of McClung and Patriquin and the free-living Campylobacter species of Laanbroek et al.

Information compiled from References 5-7, 34, 38, 45, 51, 53-59.
Table 5
DISTRIBUTION OF C. JEJUNI, C. COLI, AND NARTC IN MAN AND DOMESTIC ANIMALS

<table>
<thead>
<tr>
<th></th>
<th>C. jejuni biotype 1</th>
<th>C. jejuni biotype 2</th>
<th>C. coli</th>
<th>NARTC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>157 (74)</td>
<td>42 (20)</td>
<td>12 (5.5)</td>
<td>1 (0.5)</td>
<td>212 (100)</td>
</tr>
<tr>
<td>Cattle</td>
<td>104 (83)</td>
<td>10 (8)</td>
<td>10 (8)</td>
<td>1 (1)</td>
<td>125 (100)</td>
</tr>
<tr>
<td>Sheep</td>
<td>55 (78)</td>
<td>8 (12)</td>
<td>7 (10)</td>
<td>0</td>
<td>70 (100)</td>
</tr>
<tr>
<td>Dogs</td>
<td>55 (68)</td>
<td>18 (22)</td>
<td>4 (5)</td>
<td>4 (5)</td>
<td>81 (100)</td>
</tr>
<tr>
<td>Chickens</td>
<td>29 (43)</td>
<td>21 (32)</td>
<td>15 (22)</td>
<td>2 (3)</td>
<td>67 (100)</td>
</tr>
<tr>
<td>Pigs</td>
<td>5 (5)</td>
<td>0</td>
<td>90 (95)</td>
<td>0</td>
<td>95 (100)</td>
</tr>
</tbody>
</table>

* Indigenous infections diagnosed at Worcester Royal Infirmary, U.K., over a period of 2 years.

a temperature of 43°C is more reliable for distinguishing the C. jejuni-C. coli group from the C. fetus group, but they placed greater relevance on growth at 25°C as a differential test.

It has been demonstrated by Karmali et al.\textsuperscript{34} that the spiral forms of C. jejuni, C. fetus subsp. fetus, and C. fetus subsp. venerealis differ substantially with respect to wavelength and amplitude when observed by phase-contrast microscopy (Table 4); these differences can be distinguished by a trained observer on the basis of size alone, without actual measurement (Figure 1). C. jejuni rapidly undergoes coccal degeneration within 24 to 48 hr upon exposure to normal atmospheric air,\textsuperscript{34} whereas the two C. fetus subspecies undergo coccal degeneration much less readily. C. jejuni has a pronounced tendency to swarm on moist agar plates, a feature that is absent in C. fetus subsp. fetus and C. fetus subsp. venerealis.

C. jejuni and C. coli are sensitive to nalidixic acid (40 mg/\text{ml} or a 30-\text{\mu g nalidixic acid disc}) and resistant to cephalothin (64 mg/\text{ml} or a 30-\text{\mu g cephalothin disc}) in contrast to the two C. fetus subspecies which give the opposite susceptibility pattern.\textsuperscript{51,52} However, we have now recognized a few strains of C. coli (=2% of strains in our collections) that are sensitive to cephalothin; zone sizes of between 20 to 40 mm around a 30-\text{\mu g cephalothin disc}\textsuperscript{53} have been observed. A few strains of nalidixic-acid-resistant C. jejuni have also been recognized;\textsuperscript{54} these are distinct from the NARTC group described below. C. jejuni and C. coli are unable to grow anaerobically in the presence of fumarate, aspartate, or nitrate, whereas C. fetus subsp. fetus and C. fetus subsp. venerealis are able to grow anaerobically in the presence of fumarate and also usually in the presence of aspartate and nitrate.\textsuperscript{55,56} The ability of campylobacters to grow anaerobically in the presence of the latter substrates can be assessed readily by inoculation into semisolid agar and looking for growth throughout the medium as opposed to growth restricted to near the surface.\textsuperscript{56}

Studies of the cellular fatty acid composition of campylobacters by gas-liquid chromatography\textsuperscript{57-59} have also shown differences between the C. jejuni-C. coli group and the C. fetus group. Most strains of the former group contain a 19-carbon cyclopropane acid which is absent in C. fetus subsp. fetus and C. fetus subsp. venerealis; some strains in the C. jejuni — C. coli group have only trace amounts or undetectable levels of the 19-carbon acid.\textsuperscript{60} Differences between the C. jejuni—C. coli and the C. fetus have also been evident in studies examining the total protein profiles of these organisms using polyacrylamide gel electrophoresis.\textsuperscript{61} The C. jejuni-C. coli group are serologically distinct from the C. fetus group.\textsuperscript{62}

C. jejuni and C. coli are differentiated on the basis of the hippurate hydrolysis test.\textsuperscript{36,37,39}
C. jejuni is hippurate positive, and C. coli hippurate negative.59 In addition, C. coli may be distinguished by its ability to grow at 30.5°C (78% of strains), whereas most strains (94%) of C. jejuni do not grow at this temperature.38 There are other qualitative and in some cases subtle differences between the two species. C. coli grows somewhat more rapidly than C. jejuni. The tendency of C. coli to undergo rapid coccal transformation is less pronounced than that for C. jejuni, and C. coli shows only a limited tendency to swarm on moist agar plates. This latter difference is of practical significance because isolates of C. coli are likely to be missed on moist primary plates if only swarming or effuse colonies are looked for.

There have not been any studies published so far with respect to possible differences in cell dimensions between C. jejuni and C. coli. Our own observations suggest that subtle differences in cellular morphology probably do exist between the species. For example, the spiral forms of C. coli tend to be more “stretched out” than those of C. jejuni, and occasionally appear even rod-like.

Other tests that have been suggested for differentiating C. jejuni from C. coli include tolerance to brilliant green (1/100,000), triphenyl tetrazolium chloride (TTC), and 8% glucose.5 Tolerance to brilliant green and 8% glucose have not proved to be useful tests.38,51 Skirrow and Benjamin59 found a wide range of tolerance to TTC which facilitated strain identification, but did not specifically separate C. jejuni from C. coli.

Strains of C. jejuni may be biotyped according to the H₂S test (in an iron/metabisulfite medium) described by Skirrow and Benjamin.59 Biotype 1 strains are H₂S negative and biotype 2 strains are H₂S positive. Biotyping of C. jejuni is discussed in more detail later on in this chapter.

Estimates for the G+C content (mol %) of the DNA of C. jejuni have ranged from 29 to 34.5,7,11,12
2. C. fetus subsp. fetus and C. fetus subsp. venerealis

C. fetus subsp. fetus is the type species of the genus Campylobacter.\(^5,35\) It is a recognized cause of sporadic abortion in cattle and sheep;\(^7\) its distribution in other animal species is not known. In man, C. fetus subsp. fetus is an occasional cause of septicemic illness, particularly in patients who are immunocompromised or have other serious underlying disease.\(^63,64\)

C. fetus subsp. venerealis is a major cause of enzootic sterility and abortion in cattle.\(^31\) The isolation of this organism from other animal species has not been reported.

The differentiation of the C. fetus group from the C. jejuni-C. coli group has been discussed in the previous section and its differentiation from catalase-positive campylobacters in general is outlined in Table 4.

The number of tests for differentiating C. fetus subsp. fetus from C. fetus subsp. venerealis is limited. C. fetus subsp. fetus grows in the presence of 1% glycine and produces H\(_2\)S in cysteine-containing media (Table 4), whereas C. fetus subsp. venerealis does neither. A biotype of C. fetus subsp. venerealis termed biotype intermedius is recognized;\(^3\) this produces an “intermediate” result in the latter tests in that it fails to grow in the presence of 1% glycine, but does produce H\(_2\)S in cysteine-containing media. Chang and Ogg\(^65\) have questioned the reliability of the glycine test for separating C. fetus subsp. fetus from C. fetus subsp. venerealis because the ability to grow in the presence of 1% glycine is a transducible characteristic. The use of only the H\(_2\)S and glycine tests for separating the two C. fetus subspecies is clearly unsatisfactory. Karmali et al.\(^34\) have shown that they can be distinguished on the basis of cell size with respect to the wavelength and amplitude of the spiral forms (Table 4 and Figure 1).

No distinct serological differences have been reported between C. fetus subsp. fetus (Group 3, serotype A in the classification of Berg et al.)\(^62\) and C. fetus subsp. venerealis (Groups 1 and 2, both also serotype A).\(^62\) The DNA relatedness between the two subspecies is close.\(^12\) Most strains of C. fetus subsp. venerealis grow less well than C. fetus subsp. fetus.\(^66\)

Because of the paucity of differential characters between C. fetus subsp. fetus and C. fetus subsp. venerealis, Véron and Chatelain\(^3\) consider the latter to be a “defective mutant” of C. fetus subsp. fetus that has adapted itself to a very restricted ecological niche, the genital tract of cattle.

Estimates for the G + C content (mol %) of the DNA of C. fetus subsp. fetus and C. fetus subsp. venerealis have ranged from 33 to 36.\(^5,7,11,12\)

3. Nalidixic Acid-Resistant Thermophilic Campylobacters (NARTC)

NARTC strains were first isolated by Skirrow and Benjamin\(^38\) from the cloacal contents of wild seagulls of the genus Larus. They have been isolated from about 25% of apparently healthy seagulls, but only infrequently from other animal species.\(^38,45\) (Table 5). They have been isolated from the feces of four children, two of whom were symptomless and two who had mild recurrent diarrhea.\(^54\) The pathogenicity of NARTC strains is not known.

The main differential characters of NARTC strains are outlined in Table 4. NARTC strains resemble the C. jejuni-C. coli group in being thermotolerant and cephalothin-resistant. They resemble C. jejuni in having a short cellular wavelength and amplitude and in undergoing rapid coccal transformation, but differ from the latter in their decreased tendency to swarm on moist agar plates. They resemble C. jejuni biotype 2 strains in their ability to produce H\(_2\)S in the iron/metasulfite medium used by Skirrow and Benjamin.\(^39\) They resemble C. coli in being hippurate negative, being able to grow at 30.5°C, and showing little or no swelling on moist media. NARTC strains differ from the C. jejuni-C. coli group in being resistant to nalidixic acid and in having the ability to grow anaerobically in the presence of aspartate or trimethylamine N-oxide HCl.\(^56,67,68\)

Recent DNA hybridization studies\(^40\) have indicated that NARTC strains probably constitute a distinct species; the DNA relatedness between a reference NARTC strain (NCTC 11352)
and representative strains of *C. jejuni* biotypes 1 and 2, and *C. coli* was found to be 17, 26, and 13%, respectively. The mean value for the G+C content (mol %) of the DNA of NARTC was found to be 32.1 ± 0.5.\(^{11}\)

The new species name *C. laridis* (λαρός, Laros — a sea bird) has been proposed for the NARTC strains.\(^{67}\)

4. *C. fecalis*

During the course of an investigation into the occurrence of *C. fetus* subsp. *fetus* in ovine feces, Firehammer\(^{60}\) in 1965 isolated a hitherto unrecognized microaerophilic vibrio that was quite distinct from *C. fetus* subsp. *fetus*. This organism, which he named *Vibrio fecalis*, is now referred to as *Campylobacter fecalis*. *C. fecalis* has also been isolated from bovine semen and vagina.\(^{7}\) Its pathogenic significance is not known.

*C. fecalis* can easily be differentiated from other catalase-positive campylobacters since it is the only member of the group that produces hydrogen sulfide in peptone iron media such as triple sugar iron, Kligler’s iron, or SIM media (Table 4); this feature is more characteristic of the catalase-negative campylobacters.\(^{7}\)

*C. fecalis* strains grow optimally at 37°C but also grow at 42°C, and poorly at 25°C; they are resistant to nalidixic acid and sensitive to cephalothin.\(^{53}\)

Owen and Leaper\(^{11}\) have reported the G+C content (mol %) of a single strain of *C. fecalis* to be 36.6.

5. Aerotolerant Campylobacter Species

Ellis et al.\(^{70}\) in 1977 reported on the isolation of *Spirillum*-like organisms from the internal organs of aborted bovine fetuses. Primary isolation of these organisms was achieved only in semisolid *Leptospira* isolation media incubated at 30°C; growth was detectable as a cloudy zone slightly below the surface of the media after about 2 to 3 days of incubation. However, after one or more passes in semisolid media, visible growth could be obtained after 24 hr on blood agar under aerobic, microaerobic, and anaerobic conditions; growth occurred at 37°C, but more readily at 30°C. Ellis et al.\(^{71}\) went on to isolate similar organisms from aborted porcine fetuses. Subsequent characterization of these aerotolerant organisms led to their inclusion in the genus *Campylobacter*.\(^{9}\) Neill et al.\(^{9,72}\) showed that these organisms (which they termed “group 2” strains) were, like the *C. jejuni-C. coli* group, susceptible to 40 mg/l of nalidixic acid, but unlike the latter, grew at 25°C but not at 42°C. The isolation of aerobic vibrios conforming to the description of campylobacter from bovine genital sources has also been reported previously by Florent.\(^{22}\) In addition to their proposed etiological role in bovine and porcine abortion, aerotolerant campylobacters have recently also become implicated as causes of mastitis in cows.\(^{73}\)

In limited studies\(^{53}\) performed on five strains of aerotolerant *Campylobacter* species supplied by Dr. S. D. Neill, we found all strains to be hippurate-negative, cephalothin-resistant (30-μg cephalothin disc), and negative for hydrogen sulfide in triple sugar iron medium; the strains did not undergo rapid coccoc transformation nor did they swarm on moist agar plates. An unexpected finding was that all strains that we tested were negative for nitrate reduction using the plate nitrate method\(^{74}\) (with *C. fetus* subsp. *fetus* as a positive control). Confirmation of our results would make aerotolerant *Campylobacter* species the only campylobacters with available strains known to be nitrate-negative.

Substantial pleomorphism in cellular morphology is seen when 48-hr blood agar cultures (grown at 30°C in an airtight polyethylene bag containing a culture of *S. aureus* to reduce the oxygen tension) are examined by phase-contrast microscopy;\(^{53}\) single cells appear slightly curved or rod-like, while the morphology of chains of cells varies from spiral to rod-like with intermediate forms showing variable degrees of flattening of the wave forms. The size range of the wavelength and amplitude of the spiral forms resembles that of *C. fetus* subsp. *venerealis*.
Using the buoyant density method, Neill et al. found the G+C content (mol%) of the aerotolerant "group 2" Campylobacter strains to range from 29 to 34.

6. Nitrogen-Fixing Campylobacter Species CI

McClung and Patriquin in 1980 reported the isolation of a microaerophilic nitrogen-fixing bacterium from the roots (which had been surface sterilized) of the plant Spartina alterniflora Loisel growing in a Nova Scotian salt marsh. Further study of this organism, referred to as CI, supported its inclusion in the genus Campylobacter. It is the only plant-associated Campylobacter species that has been described. It is distinguished from other campylobacters by various biochemical and physiological characteristics, but particularly by its unique ability to fix nitrogen, hydrolyze urea, produce pigment from tryptophan, and grow in the presence of up to 7% sodium chloride. Its association with plant roots further distinguishes it from the other campylobacters which usually inhabit animals. The G+C content (mol%) of CI was reported to be 32.1 ± 1.0.

7. "Free-Living" Aspartate-Fermenting Campylobacter Species

While studying the microflora of an anaerobic digester continuously fed with waste water from a potato-flour factory, Laanbroek et al. isolated a free-living aspartate-fermenting microaerophilic vibrio. This was subsequently identified as a Campylobacter species. It could be differentiated from other campylobacters on biochemical and physiological grounds; it was found to have an unusually high (for the genus Campylobacter) mean G+C content (mol%) of 41.6.

E. Methods of Strain Discrimination in the C. jejuni-C. coli Group

The need for studying the epidemiology of C. jejuni-C. coli infections has led to significant progress in the development of methods for discriminating among strains of these two species by means of serotyping and biotyping. Phages have been described in campylobacters, and phage-typing methods for the C. jejuni-C. coli group are being developed. The occurrence of bacteriocins in campylobacters has not been reported. However, there have been reports of the inhibitory action of bacteriocins from Pseudomonas aeruginosa on some Campylobacter strains.

1. Serotyping

Penner and Hennessy in Canada and Lauwers in Belgium have developed a serotyping system based on thermostable soluble antigens. The Penner and Hennessy system now recognizes over 50 different serogroups. Lior et al. also in Canada, have developed a typing system based on thermolabile antigens containing 21 different serogroups. Serotyping systems are discussed in separate chapters in this volume.

2. Biotyping

Skirrow and Benjamin made the observation that some strains of C. jejuni caused blackening in a medium supplemented with the FBP (ferrous sulfate, sodium metabisulfite, sodium pyruvate) supplement described by Hoffman et al. A positive reaction was evident within 4 hr of inoculation and clearly indicates the presence of a preformed enzyme, presumably a sulfatase, leading to the production of hydrogen sulfide. Strains that were H2S negative in the latter test were referred to as C. jejuni biotype 1, and those that were H2S positive, as biotype 2 by Skirrow and Benjamin. The relative distribution of the two biotypes among human and nonhuman isolates is shown in Table 5. The precise nature of the enzyme(s) responsible for H2S production in FBP-supplemented media as well as the optimal conditions for performing the test are currently under investigation. It should be emphasized that performance in the H2S test using FBP media bears no relationship to H2S production using conventional methods (cysteine, lead acetate, or T.S.I. tests).
Table 6
KEY DIFFERENTIAL CHARACTERISTICS OF CATALASE-NEGATIVE CAMPYLOBACTERS

<table>
<thead>
<tr>
<th></th>
<th>Growth in the presence of</th>
<th>Dirty yellow colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1% Glycine</td>
<td>1% Bile</td>
</tr>
<tr>
<td><em>C. spitorum</em> subsp. <em>spitorum</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>C. spitorum</em> subsp. <em>bubulus</em></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>C. spitorum</em> subsp. <em>mucosalis</em></td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><em>C. concisus</em></td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

* Information compiled from References 5—7, 89, 90, 94—97.

Skirrow and Benjamin58 have also shown that the differential susceptibility of strains of *C. jejuni*-*C. coli* to triphenyl tetrazolium chloride and metronidazole may be a valuable tool in the investigation of outbreaks of infections due to these organisms.

Hébert et al.84 have recently described the use of three tests (hippurate hydrolysis, DNA hydrolysis, and growth on charcoal-yeast extract agar) for separating the members of the *C. jejuni-* *C. coli* group into eight biotypes. The latter scheme remains to be tested under field conditions.

IV. CATALASE-NEGATIVE CAMPYLOBACTERS

The four main taxa in the catalase-negative *Campylobacter* group are listed in Tables 1 and 6.

A. Historical Background

Tunnicliff55 in 1914 reported the isolation of an anaerobic vibrio that "was found in fair numbers in the sputum of a patient suffering with acute bronchitis". It was described as being a strictly anaerobic, Gram-negative organism showing one or two curves but sometimes more. When stained by the Zettnow method, the vibrio showed "one long fine wavy flagellum attached to its extremity." Tunnicliff's albeit limited description served to differentiate her anaerobic vibrio from a number of other anaerobic vibrio-like organisms often present in the human mouth. In 1940, Prevot84 named the Tunnicliff anaerobic vibrio *Vibrio spitorum*. MacDonald77 in 1953 isolated 12 strains of *V. spitorum* from the oral cavity and characterized them as being straight or curved rods possessing usually monotrichous polar flagella; all strains were strictly anaerobic and produced hydrogen sulfide in peptone-iron medium.

Florent88 in 1953 isolated what he referred to as a saprophytic vibrio from the bovine vagina and semen. This vibrio differed from *Vibrio fetus* in that it was more anaerobic than the latter, and also produced abundant hydrogen sulfide in contrast to the latter. A detailed study on this organism, termed *Vibrio bubulus* by Florent,88 was subsequently reported by Thouvenot and Florent.89

The isolation of anaerobic vibrios from bovine reproductive tracts similar to those described as *Vibrio bubulus* by Florent was also reported by Bryner and Frank13 in 1955. They made the important observation that the anaerobic vibrios could be distinguished from *Vibrio fetus* on the basis of the catalase test; the former were catalase negative and the latter catalase positive. In 1963, Sebald and Véron8 transferred *V. bubulus* to the genus *Campylobacter*.

In 1965 Loesche and colleagues90 compared the characters of *Vibrio spitorum* and *Vibrio bubulus* and concluded that the differences between the two were insufficient to justify their consideration as two species. They suggested that the two organisms be referred to as *V.
sputorum var. sputorum and V. sputorum var. bubulus, and showed that they could be distinguished on the basis of tolerance to 3.5% sodium chloride; var. bubulus was salt tolerant whereas var. sputorum was inhibited by 3.5% sodium chloride. Loesche et al.\textsuperscript{90} also pointed out that both varieties of V. sputorum were not, as they had been considered until then, strict anaerobes, but were in fact microaerophiles with a somewhat lower optimal oxygen tension for growth than V. fetus. The two varieties of V. sputorum are now referred to as C. sputorum subsp. sputorum, and C. sputorum subsp. bubulus.\textsuperscript{35}

In 1973, Rowland, Lawson, and Maxwell\textsuperscript{91} demonstrated the presence of an intracellular vibrio-like organism within the lesions of porcine intestinal adenomatosis. Further studies showed that this organism was probably a new catalase-negative Campylobacter species.\textsuperscript{92-94} The organism had many characters in common with C. sputorum, but it could be differentiated from C. sputorum subsp. sputorum and C. sputorum subsp. bubulus according to its tolerance to sodium chloride, glycine, and sodium deoxycholate.\textsuperscript{94} Lawson et al. named the organism C. sputorum subsp. mucosalis.\textsuperscript{94,95} The presence of a "dirty yellow" pigment in the colonies of C. sputorum subsp. mucosalis is another feature that distinguishes this subspecies from other campylobacters.\textsuperscript{94}

A completely new catalase-negative Campylobacter species termed C. concisus\textsuperscript{96} was described only recently, in 1981. This organism was isolated from gingival crevices of the human mouth, and when compared to C. sputorum subsp. sputorum and subsp. bubulus by DNA hybridization, showed less than 30% homology with each of the latter subspecies. The pathogenic significance of C. concisus is not known.

\section*{B. Description and Differentiation of the Catalase-Negative Campylobacter Group}

Catalase-negative campylobacters have all the general characteristics of the genus Campylobacter discussed earlier. Apart from the catalase reaction, catalase-negative campylobacters are distinguished from catalase-positive campylobacters as follows: catalase-negative campylobacters have a lower optimal oxygen tension for growth than do the catalase-positive campylobacter group; they reduce nitrite, and produce hydrogen sulfide in peptone-iron media. By contrast, catalase-positive campylobacters fail to reduce nitrite and fail to produce hydrogen sulfide in peptone-iron medium (except for C. faecalis).

The key differential characteristics of catalase-negative Campylobacter species and subspecies are listed in Table 6.

\section*{C. Principal Features and Pathogenic Significance of Catalase-Negative Campylobacter Species and Subspecies}

1. C. sputorum subsp. sputorum

This organism has been isolated only from the human mouth and intestine. Its pathogenic significance is not known. The frequent isolation of this organism from the healthy human oral cavity\textsuperscript{94,97} suggests that it is a commensal. Gibbons et al.\textsuperscript{97} found C. sputorum subsp. sputorum to account for about 5% of cultivable organisms isolated from gingival crevices, a proportion similar to that found for Bacteroides melaninogenicus. It has been isolated (filtration method) from 2% of fecal samples from healthy people.\textsuperscript{54}

C. sputorum subsp. sputorum grows in the presence of 1% glycine and 1% bile, but not 3.5% sodium chloride.\textsuperscript{5,7,90,94}

2. C sputorum subsp. bubulus

This organism has been isolated from the genital tracts of healthy male and female cattle and sheep.\textsuperscript{7,88,89} Its main distinguishing characteristic is its ability to grow in the presence of at least 3.5% sodium chloride.\textsuperscript{5,7,90,94} It has never been implicated in disease.

The mean G + C content (mol \%) of this organism was recorded as 32.3 ± 0.05.\textsuperscript{11}
3. C. sputorum subsp. mucosalis

This organism has been associated with lesions of porcine intestinal adenomatosis, hemorrhagic enteropathy, and regional ileitis. Its presence has not been recorded in man or animals other than the pig. It is distinguished from other catalase-negative campylobacters by virtue of the "dirty yellow" colonies that it produces on solid culture media. It shares with C. concisus the property of requiring hydrogen for microaerobic growth. Detailed accounts of this organism have been reported by Rowland and Lawson and colleagues. The G+C content (mol %) of a representative strain was found to be 33.9.

4. C. concisus

This newly described species was isolated from human gingival crevices. Its pathogenic significance is not known. It has been described in detail by Tanner et al. The G+C content (mol %) of this species was reported to range from 34 to 38. Like C. sputorum subsp. mucosalis, it requires hydrogen for microaerobic growth.

V. CONCLUDING REMARKS

Cowan and Steel have likened taxonomy to a cocktail made up of "three components (classification, nomenclature, and identification) that are skillfully blended so that the outsider relishes the whole and cannot discern the individual ingredients". In the case of Campylobacter, the cocktail has, without doubt, been indigestible for many years. Recent advances in the taxonomy of this genus have made available fresh ingredients that we have attempted to blend into what we hope is a more palatable concoction.

It will be apparent that the greater emphasis in this chapter has been placed on the catalase-positive as opposed to the catalase-negative campylobacters. This is because there is much more information available on catalase-positive campylobacters and they include most of the Campylobacter species pathogenic to man and animals. The only catalase-negative Campylobacter taxon of known pathogenic significance, C. sputorum subsp. mucosalis, is discussed in detail in a separate chapter in this volume.

Finally, for the sake of historical completion, it is tempting to speculate on the identity of strains originally described by McFadyean and Stockman in association with ovine abortion. The latter strains represent the very first time that organisms corresponding to the description of Campylobacter were described. The two groups now known to be associated with ovine abortion are C. jejuni and C. fetus subsp. fetus. McFadyean and Stockman’s organism failed to grow under anaerobic conditions, but was "capable of active growth in a rarefied atmosphere". Its cultural activity was greatest at incubation temperatures of 35 to 37°C, but it also grew well, although slowly, at room temperature. These features suggest that McFadyean and Stockman’s organism was most probably what is now referred to as C. fetus subsp. fetus.

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