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Deibel, Kurt Eugene

A STUDY OF CAMPYLOBACTER JEJUNI

The Ohio State University

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A STUDY OF CAMPYLOBACTER JEJUNI

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By

Kurt Eugene Deibel, B.S., M.S.

* * * * *

The Ohio State University
1985

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I would like to dedicate this dissertation to my wife, Debbie, her love, support and encouragement were instrumental in the preparation of this work.

I would also like to dedicate this dissertation to my parents and my sister for their persistent interests and support throughout my academic career.
ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. George J. Banwart for his assistance, encouragement and close friendship throughout my years in graduate school. Without his input, this work would not have been possible.

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I would also like to express my appreciation to Elsie Pykonen and Sharon Sanborn for their help in the preparation of this dissertation.
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Ting, W.T., Deibel, K.E. and Banwart, G.J. Induced fluid accumulation in rat ileal loops by Bacillus cereus. Ohio Branch, American Society of Microbiology, Athens, Ohio, October 16, 1982.


May, R.N., Deibel, K.E. and Banwart, G.J. Effect of Orange-All on the growth of various microorganisms. The 44th Annual National Institute of Food Technologists Meeting, Anaheim, California, June 10-13, 1984.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>VITA</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>A. The Organism</td>
<td>3</td>
</tr>
<tr>
<td>1. Taxonomy</td>
<td>3</td>
</tr>
<tr>
<td>2. Morphology</td>
<td>6</td>
</tr>
<tr>
<td>3. Growth Requirements</td>
<td>6</td>
</tr>
<tr>
<td>4. Sources</td>
<td>7</td>
</tr>
<tr>
<td>5. Survival</td>
<td>11</td>
</tr>
<tr>
<td>B. Human Illness</td>
<td>14</td>
</tr>
<tr>
<td>1. Incidence</td>
<td>14</td>
</tr>
<tr>
<td>2. Age and Sex</td>
<td>20</td>
</tr>
<tr>
<td>3. Seasonal Frequency</td>
<td>21</td>
</tr>
<tr>
<td>4. Incubation Period</td>
<td>21</td>
</tr>
<tr>
<td>5. Symptoms</td>
<td>23</td>
</tr>
<tr>
<td>6. Duration</td>
<td>24</td>
</tr>
<tr>
<td>7. Complications</td>
<td>24</td>
</tr>
<tr>
<td>8. Treatment</td>
<td>25</td>
</tr>
<tr>
<td>C. Virulence</td>
<td>26</td>
</tr>
<tr>
<td>1. Toxin</td>
<td>26</td>
</tr>
<tr>
<td>2. Animal Models</td>
<td>26</td>
</tr>
<tr>
<td>D. Transmission</td>
<td>30</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (Continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Methodology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Presumptive Identification in Human Stool</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>2. Presumptive Identification in Foods</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>3. Biochemicals</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>4. Serology</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>5. Storage of Cultures</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>F. Control</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>1. Effect of Heat</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>a) Temperature Effect</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>b) Microwave Heating</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>2. Effect of Chemicals</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>a) pH</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>b) Ascorbic Acid</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>c) Spices</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>3. Gamma Radiation</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Experiment 1 - Prevalence of <em>Campylobacter jejuni</em> in Retail Meats</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Experiment 2 - Identification of Non-Campylobacter Colonies from Selective Agars</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Experiment 3 - Comparison of Blending Method, Time and Diluent for the Enumeration of <em>Campylobacter jejuni</em></td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Experiment 4 - Survival of <em>Campylobacter jejuni</em> in Bovine Feces</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Experiment 5 - Effect of an Iodine Udder Wash on <em>Campylobacter jejuni</em></td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Experiment 6 - Effect of Hydrogen Peroxide on <em>Campylobacter jejuni</em> in Milk</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Experiment 7 - Effect of Sodium Diacetate on <em>Campylobacter jejuni</em></td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Experiment 8 - Effect of Spices on <em>Campylobacter jejuni</em></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Experiment 9 - <em>Campylobacter jejuni</em> Survival in Meat Cubes and Milk Heated in a Microwave Oven</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Experiment 10 - Iron Dextran Enhanced Colonization of <em>Campylobacter jejuni</em> in Adult Mice</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (Continued)

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prevalence of Campylobacter jejuni in Retail Meats</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>Identification of Non-Campylobacter Colonies on Selective Agars</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>Comparison of Blending Method, Time and Diluent for the Enumeration of Campylobacter jejuni</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>Survival of Campylobacter jejuni in Bovine Feces</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>Effect of Iodine Udder Wash on Campylobacter jejuni</td>
<td>102</td>
</tr>
<tr>
<td>6</td>
<td>Effect of Hydrogen Peroxide on Campylobacter jejuni in Milk</td>
<td>105</td>
</tr>
<tr>
<td>7</td>
<td>Effect of Sodium Diacetate on Campylobacter jejuni</td>
<td>116</td>
</tr>
<tr>
<td>8</td>
<td>Effect of Spices on Campylobacter jejuni</td>
<td>129</td>
</tr>
<tr>
<td>9</td>
<td>Campylobacter jejuni survival in Meat Cubes and Milk Heated in a Microwave Oven</td>
<td>144</td>
</tr>
<tr>
<td>10</td>
<td>Iron Dextran Enhanced Colonization of Campylobacter jejuni in Adult Mice</td>
<td>156</td>
</tr>
</tbody>
</table>

SUMMARY                                                                                      163

BIBLIOGRAPHY                                                                              166
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Time of Survival of Campylobacter jejuni in Biological Milieus</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Isolation rates of enteropathogens from diarrheic individuals from different areas</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>Composition of selective media for detection of Campylobacter jejuni</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>List of biochemical tests used in the confirmation of Campylobacter jejuni</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>The recovery of Campylobacter jejuni from blind inoculated meat samples</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>Isolation of Campylobacter jejuni from retail meats during June 1983</td>
<td>78</td>
</tr>
<tr>
<td>7</td>
<td>Isolation of Campylobacter jejuni from retail meats during September 1983</td>
<td>79</td>
</tr>
<tr>
<td>8</td>
<td>Isolation of Campylobacter jejuni from retail meats during December 1983</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>Isolation of Campylobacter jejuni from retail meats during March 1984</td>
<td>81</td>
</tr>
<tr>
<td>10</td>
<td>The incidence of Campylobacter jejuni in several types of meat</td>
<td>82</td>
</tr>
<tr>
<td>11</td>
<td>The incidence of Campylobacter jejuni during four periods of analyses of animal products</td>
<td>84</td>
</tr>
<tr>
<td>12</td>
<td>The identification of non-Campylobacter jejuni colonies isolated from Campy-BAP agar during analyses of animal products</td>
<td>86</td>
</tr>
<tr>
<td>13</td>
<td>Detection of Campylobacter jejuni in the intestinal tract of BALB/C adult mice fed this organism suspended in iron dextran</td>
<td>159</td>
</tr>
</tbody>
</table>
LIST OF TABLES (continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Detection of <em>Campylobacter jejuni</em> in the intestinal tract of BALB/C adult mice fed this organism suspended in phosphate buffer</td>
<td>161</td>
</tr>
<tr>
<td>15</td>
<td>Presence of <em>Campylobacter jejuni</em> in the intestinal tract of adult BALB/C mice fed <em>Campylobacter jejuni</em> suspended in raw milk</td>
<td>162</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Schematic Classification scheme of Campylobacter (update from Skerman et al. 1980)</td>
<td>4-5</td>
</tr>
<tr>
<td>2</td>
<td>Season Frequency of Campylobacter jejuni isolated from Fecal specimens (Figure taken with permission from Blaser, et al. 1982)</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>The effect of blending method, time and diluent on the recovery of Campylobacter jejuni from milk</td>
<td>92-93</td>
</tr>
<tr>
<td>4</td>
<td>Survival of Campylobacter jejuni in and the aerobic plate count of bovine feces stored at 25°C</td>
<td>97-98</td>
</tr>
<tr>
<td>5</td>
<td>Survival of Campylobacter jejuni and the aerobic plate count in bovine feces at 4°C</td>
<td>99-100</td>
</tr>
<tr>
<td>6</td>
<td>The effect of an iodine solution on Campylobacter jejuni</td>
<td>103-104</td>
</tr>
<tr>
<td>7</td>
<td>The effect of various concentrations of H₂O₂ in Campylobacter jejuni strain 29428 inoculated into sterile skim milk</td>
<td>106-107</td>
</tr>
<tr>
<td>8</td>
<td>The effect of various concentrations of H₂O₂ on Campylobacter jejuni strain 5-4 inoculated into sterile skim milk</td>
<td>108-109</td>
</tr>
<tr>
<td>9</td>
<td>The effect of various concentrations of H₂O₂ inoculated into sterile skim milk</td>
<td>110-111</td>
</tr>
<tr>
<td>10</td>
<td>The effect of 0.05% H₂O₂ on Campylobacter jejuni strain 29429 inoculated into raw milk</td>
<td>113-114</td>
</tr>
<tr>
<td>11</td>
<td>The effect of 0.45% sodium diacetate aseptically added or sterilized with ethylene oxide on Campylobacter jejuni at 2 pH levels at 42°C</td>
<td>117-118</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES (continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Response of <em>Campylobacter jejuni</em> to autoclaved sodium diacetate-</td>
<td>121-122</td>
</tr>
<tr>
<td></td>
<td>brucella broth and brucella broth at pH 4.9 and 7.0 incubated at</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42°C</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>The effect of 0.45% sodium diacetate sterilized with ethylene oxide</td>
<td>123-124</td>
</tr>
<tr>
<td></td>
<td>on <em>Campylobacter jejuni</em> incubated at 25°C and 4°C</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Response of <em>Campylobacter jejuni</em> to autoclaved sodium diacetate</td>
<td>126-127</td>
</tr>
<tr>
<td></td>
<td>at 25°C and 4°C</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Response of <em>Campylobacter jejuni</em> to various concentrations of</td>
<td>130-131</td>
</tr>
<tr>
<td></td>
<td>oregano at 42°C</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Response of <em>Campylobacter jejuni</em> to various concentrations of</td>
<td>132-133</td>
</tr>
<tr>
<td></td>
<td>sage at 42°C</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Response of <em>Campylobacter jejuni</em> to various concentrations of</td>
<td>134-135</td>
</tr>
<tr>
<td></td>
<td>ground cloves at 42°C</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Response of <em>Campylobacter jejuni</em> to 0.5% of oregano, sage and</td>
<td>137-138</td>
</tr>
<tr>
<td></td>
<td>ground cloves at 42°C</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Response of <em>Campylobacter jejuni</em> to 0.5% of oregano, sage and</td>
<td>139-140</td>
</tr>
<tr>
<td></td>
<td>ground cloves at 25°C</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Response of <em>Campylobacter jejuni</em> to 0.5% of oregano, sage and</td>
<td>141-142</td>
</tr>
<tr>
<td></td>
<td>ground cloves at 4°C</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>The effect of the microwave treatment on <em>Campylobacter jejuni</em></td>
<td>145-146</td>
</tr>
<tr>
<td></td>
<td>heated in 39.6 ml of sterile skim milk</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>The effect of the microwave treatment on <em>Campylobacter jejuni</em></td>
<td>148-149</td>
</tr>
<tr>
<td></td>
<td>in 200 ml of skim milk</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>The effect of the microwave treatment on <em>Campylobacter jejuni</em></td>
<td>150-151</td>
</tr>
<tr>
<td></td>
<td>in 200 ml of raw milk</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Survival of <em>Campylobacter jejuni</em> inoculated into beef cubes and</td>
<td>153-154</td>
</tr>
<tr>
<td></td>
<td>heated in the microwave oven</td>
<td></td>
</tr>
</tbody>
</table>
INTRODUCTION

At the present time, *Campylobacter jejuni* is considered to be a major cause of gastroenteritis in humans. The isolation rate of this organism from diarrheic patients is similar to that reported for *Salmonella* (Blaser et al. 1982). Higher isolation rates of this organism are reported in developing countries (Bokkenheuser et al. 1979), than in industrialized countries (Blaser et al. 1983b). This may be attributed to the poorer sanitation in developing countries.

The primary source of this organism is animals. It has been isolated from cattle (Munroe et al. 1983), sheep (Rosef et al. 1983), swine (Sticht-Groh 1982), poultry (Smitherman et al. 1984), wild birds (Kapperud and Rosef 1983), a variety of zoo animals (Luechtfeld et al. 1981a), rodents (Fernie and Parks 1977) house pets (Blaser et al. 1980c) as well as other animals.

Both water (Vogt et al. 1982) and food have been implicated as vehicles for the transmission of this organism. The consumption of raw or improperly pasteurized milk is the most important vehicle of *C. jejuni*, both in terms of numbers of outbreaks and numbers of individuals becoming ill. Other foods such as chicken (Brouwers et al. 1979), pork (Doyle 1981), beef (Oosterom et al. 1982) and clams (Blaser et al. 1982) have been implicated in the transmission of this organism.
One common denominator that all food vehicles for the transmission of *C. jejuni* have is that the food was eaten either raw or undercooked. This organism is usually heat sensitive and will not withstand the acceptable pasteurization time and temperatures for milk.

*C. jejuni* reportedly produces a toxin which is immunologically similar to cholera toxin (Ruiz-Palacios et al. 1983). Attempts to develop a suitable animal model to demonstrate and study *C. jejuni* gastroenteritis have not been successful to date.

The objective of this study was to evaluate and determine the sources, survival, control, methodology and an animal model for pathogenicity of *Campylobacter jejuni*. 
I. Campylobacter jejuni

A. The Organism

1. Taxonomy

In 1919 Smith and Taylor described a vibrioid microaerophilic microorganism isolated from an aborted bovine fetus. Subsequently they named this organism *Vibrio fetus*. Sebald and Veron (1963) found that the G+C content of *V. fetus* was quite different from that of the other *Vibrio* species that they studied. They also noted that *V. fetus* differed from other vibrios phenotypically. Due to these differences, they reclassified this organism as *Campylobacter*. Currently *Campylobacter* is found in section 2, the "Aerobic/Microaerophilic, Motile, Helical/Vibrioid Gram Negative Bacteria" is Bergey's Manual of Systematic Bacteriology Volume 1 (Krieg and Holt 1984). The classification scheme for the *Campylobacter* is diagrammed in Figure 1 (Krieg and Holt 1984; Skerman et al. 1980).

Two species of *Campylobacter*; *C. jejuni* and *C. coli* are important pathogens that can be transmitted through food.
Figure 1: Schematic Classification Scheme of the *Campylobacter* (updated from Skerman et al. 1980).
Campylobacter jejuni is isolated primarily from sheep and cattle. Usually, Campylobacter coli is isolated from pigs. These organisms have very few phenotypical differences and for the extent of this dissertation both of them will be referred to as Campylobacter jejuni.

2. Morphology

Campylobacter is a gram negative curved or spiral rod. It ranges from 0.2-0.5μm in width and from 0.5-5.0μm in length. These nonsporeforming rods appear as single cells, but two cells may form a short chain and appear S shaped or gull-winged shaped. In old cultures, the cells become coccoid (Buck et al. 1983). These microorganisms are motile by virtue of a single polar flagellum and move with a corkscrew-like or tumbling motion.

3. Growth Requirements

Campylobacter jejuni requires a microaerophilic environment in which the optimal gas mixture is 5% O₂, 10% CO₂ and 85% N₂ (Doyle 1981). The oxygen range is 3-15% and the carbon dioxide range is 3-5% (Krieg and Holt 1984). When a combination of 0.025% of each of FeSO₄·7H₂O, sodium metabisulfite and sodium pyruvate is added to the growth medium, the aerotolerance of C. jejuni is increased up to 17% O₂ (George et al. 1978). These
compounds are scavengers of superoxide anions and hydrogen peroxide which may build up in a culture medium and become toxic to C. jejuni.

Organisms of the genus Campylobacter are unable to ferment or oxidize carbohydrates. Their energy is obtained by utilizing amino acids or tricarboxylic acid cycle intermediates (Krieg and Holt 1984).

The optimal growth temperature is 42°C. The temperature range for C. jejuni is 30.5-46.0°C (Doyle and Roman 1981; Krieg and Holt 1984).

This organism will grow over a pH range of 5.5-8.0, with an optimal pH range of 6.5-7.5 (Doyle and Roman 1981).

4. Sources

The primary source of this organism is animals. In 1931, winter scours in cattle was associated with the organism we now know as C. jejuni (Jones and Little 1931). The organism was isolated from 40% of 127 diarrheic calves (Firehammer and Myers 1981). Abortions in cows have been caused by Campylobacter (Maclaren and Wright 1977). This organism has been isolated from the feces of healthy cows (Hanninen 1981a; Munroe et al. 1983;
Skirrow and Benjamin 1980). It was found in the cecal content of 11 of 200 cows (Oosterom et al. 1982). A survey was conducted to determine the distribution of _C. jejuni_ in healthy cattle (Rosef et al. 1983). The feces were sampled from 254 cows from 36 herds, and _C. jejuni_ was isolated from 0.8% of the samples. The consumption of raw or improperly pasteurized milk and undercooked beef has been implicated in campylobacteriosis of humans.

When feces of 197 sheep were tested for _C. jejuni_, 8.1% were positive (Rosef et al. 1983). The organism also has been isolated from asymptomatic sheep (Hanninen 1982a; Stern 1981) and from aborted lambs (Firehammer and Myers 1981; Smibert 1978).

When feces of swine were tested for this organism, 70% were positive for _C. coli_ (Sticht-Groh 1982). Also, _C. coli_ has been isolated from diarrheic pigs (Munroe et al. 1983). It is considered that swine are a common source of _C. coli_.

Smibert (1978) reported that certain strains of _C. jejuni_ cause infectious hepatitis in chickens. When chickens at various chicken ranches were tested for the presence of _C. jejuni_, 0-100% of chicken fecal droppings per ranch were determined to be positive for this organism (Smitherman et al. 1984). It was determined that for chickens excreting _C. jejuni_, the intestinal
content contained as high as $10^7$ \textit{C. jejuni} per gram (Oosterom et al. 1983a). The consumption of undercooked chicken was implicated in \textit{C. jejuni} gastroenteritis in humans (Goodman et al. 1983). Twenty-two percent of frozen broiler chickens analyzed were positive for \textit{C. jejuni} (Norberg 1981).

Turkeys also are a source of \textit{C. jejuni} (Luechtefeld and Wang 1981; Rosef and Kapperud 1982). A range of 16-76\% of the fecal swabs from turkeys sampled at various brooder facilities were positive for \textit{C. jejuni}.

A study in Colorado showed that 35\% of 445 wild ducks shed \textit{C. jejuni} in their feces (Luechtefeld et al. 1980). Pigeons, blackbirds, starlings, sparrows, puffins, common terns, ural owls and reed buntings were found to excrete \textit{C. jejuni} (Kapperud and Rosef 1983; Smibert 1969). A high percent of crows and seagulls were found to excrete \textit{C. jejuni} in their feces (Fricker et al. 1983; Kapperud and Rosef 1983). This high percentage was attributed to these birds scavenging and scrounging through garbage.

In zoo animals, primates showed the highest incidence of \textit{C. jejuni} (9.3\%). Also, this organism was isolated from the feces of birds (8.7\%), hooved animals (6.9\%), felids (4.5\%) and
reptiles (3.8%) (Luechtefeld et al. 1981a). Bauwens and DeMeurichy (1981) concluded that *C. jejuni* was commonly found in warm-blooded zoo animals.

The organism has been isolated from various rodents such as bank voles, wild hares, ferrets, hamsters and laboratory rats (Fernie and Park 1977; Fox et al. 1983a).

Dogs, especially puppies, were diarrheic and asymptomatic excretors of this organism (Bruce and Fleming 1983; Firehammer and Myers 1981; Fox et al. 1983c; Holt 1980). There is clear evidence of dog to human transfer of *Campylobacter* from diarrheic dogs (Blaser et al. 1978b; Skirrow 1977). Cats can be a source of *C. jejuni* (Blaser et al. 1980c). These animals also have been implicated as vectors in a human infection of *C. jejuni* (Svedhem and Norkrans 1980).

The importance of food animals as well as other animals as potential reservoirs of *C. jejuni* for human infections should not be overlooked. There is not sufficient information in the literature to demonstrate a definite link of human outbreaks of *C. jejuni* being transmitted from animal products that are consumed by humans.
Campylobacter jejuni is considered to be a very important enteropathogen. Since various foods may be sources of this organism, the ability of this organism to survive during food preservation processes is important. This survival is dependent on the temperature, pH, water activity and the environment in which the organism is found.

Heat is very effective in killing or inactivating microorganisms. Factors that affect the destructive capability of heat are the type, number and age of microorganisms present. The environment in which the microorganisms are heated is also an important factor (Banwart 1981). Pasteurization is a means of destroying pathogens, thus reducing the risk factor of a given food. C. jejuni was inoculated into skim milk at a concentration of 10⁷ organisms per ml. After the milk was heated for 1 minute at 60°C, no C. jejuni was detected by the spread plate method (Christopher et al. 1982a). Gill et al. (1981) heated raw milk seeded with 10⁶ C. jejuni for 1 minute at 60°C. They found no survivors after heating. Pasteurized milk was inoculated with 10⁷ C. jejuni. No C. jejuni was present after the pasteurized milk was heated for 1 minute at 60°C (Watterman 1982). When 10⁶
C. jejuni was inoculated with minced pork and beef, no C. jejuni was noted after heating at 60°C for 15 minutes. C. jejuni showed a D value of less than 1 minute when heated at 60°C in peptone-yeast extract broth (Gill and Harris 1982b). When the organism was heated in lamb meat cubes, the D value at 60°C was 15.8 seconds and in ground beef the D value at 58°C was 21 seconds (Koidis and Doyle 1983b). It is very apparent that pasteurization times and temperature will kill any C. jejuni that may be present in food.

Refrigeration at 0-5°C will slow or inhibit microbial growth. Over 90% of the C. jejuni inoculated into ground beef survived storage for 2 weeks at 4°C (Koidis and Doyle 1983b). The holding of inoculated ground beef liver at 4°C for 6 days resulted in very little reduction in the number of C. jejuni. By this time, the meat was organoleptically unacceptable (Hanninen 1981a). A 1-2 log decrease in inoculated C. jejuni was determined when either egg yolk or liquid whole egg was stored at 4°C for 3 weeks. However, when it was inoculated into egg white and stored at 4°C, no C. jejuni were detected at 48 hr (Hanninen et al. 1984). This could be attributed to the antimicrobial properties found in egg white (Mayes and Takeballi 1983). C. jejuni showed virtually no change in numbers when inoculated into minced pork and minced beef which was held for 1 week at 4°C (Svedhem et al. 1981). A reduction of 2 log units of C. jejuni
In ground chicken meat was noted after 17 days storage at 4°C (Blankenship and Cravens 1982).

When frozen (-20°C), \textit{C. jejuni} survived for 3 months in minced pork and minced beef (Svedhem et al. 1981), and 84 days in chicken livers (Oosterom et al. 1983a). There was a reduction of 3 logs by 14 days in ground beef (Stern and Kotula 1982), 2-3 log units during 12 weeks in ground beef liver (Hanninen 1981a) and 0.5-2.0 logs during 12 weeks storage on frozen broiler carcasses (Hanninen 1981a). Gill and Harris (1982b) showed that \textit{C. jejuni} survived better when frozen in a high pH environment than at neutral pH. Cell death of \textit{C. jejuni} occurs at pH 4.7 and 9.0 (Christopher et al. 1982a; Doyle and Roman 1981).

The effect of salt on \textit{C. jejuni} is temperature dependent. At 42°C, 2.0% NaCl inhibits the growth of \textit{C. jejuni}. The rate of death of \textit{C. jejuni} is enhanced with 1.5% NaCl at 25°C and with 1.0% NaCl at 4°C. It was determined that 0.5% NaCl was required for growth of this organism (Doyle and Roman 1982b; Hanninen 1981b).

Survival of the organism in chemicals or foods is also temperature dependent. When in a non-growth environment, the death rate is greatest at 42°C, slower at 25°C and the slowest at
4°C. This trend has been observed in egg yolk and liquid whole egg (Hanninen et al. 1984), salt (Doyle and Roman 1982b), hydrochloric acid at pH 3 (Doyle and Roman 1981), sensitivity to drying (Doyle and Roman 1982a) and survival in inoculated meat (Gill and Harris 1982b).

The survival of *C. jejuni* in several biological milieus is shown in Table 1. Generally, the organism survives longer as the temperature decreases from the optimal growth temperature.

B. Human Illness

1. Incidence

*Campylobacter jejuni* is well known as an animal pathogen. However, only more recently was the bacterium isolated from the stools of two patients in Brussels (Dekeyser et al. 1972). After that, microbiologists became aware that this microorganism is a potential human pathogen. The reason that *C. jejuni* was not recognized as a human enteric pathogen prior to this isolation, is due to the stringent growth requirements. Dekeyser et al. (1972) filtered stool samples with a 0.65μm membrane filter and seeded the filtrate on agar plates. *C. jejuni* is significantly small enough to pass through the 0.65μm filter. To follow up on
Table 1. Time of Survival of *Campylobacter jejuni* in Biological Milieus

<table>
<thead>
<tr>
<th>Biological Milieus</th>
<th>4°C</th>
<th>25°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl pH 2.3²</td>
<td></td>
<td>ND³</td>
<td>ND</td>
</tr>
<tr>
<td>Human Bile</td>
<td>2 mo</td>
<td>1-3 w</td>
<td>ND</td>
</tr>
<tr>
<td>Human Feces</td>
<td>3 mo</td>
<td>&lt;1 w</td>
<td>ND</td>
</tr>
<tr>
<td>Pasteurized Milk</td>
<td>3 w</td>
<td>&lt;3 d</td>
<td>ND</td>
</tr>
<tr>
<td>Human Urine</td>
<td>5 w</td>
<td>ND</td>
<td>&lt;48 hr</td>
</tr>
</tbody>
</table>

1 Taken from Blaser et al. 1980b
2 pH resembles gastric acid
3 ND = Not Done
their findings, this group of researchers from Brussels conducted a survey on the prevalence of \textit{C. jejuni} in diarrheic patients (Butzler et al. 1973). They reported that 5.2% of 800 children and 4.0% of 100 adults were excreting this organism. They also recovered the organism from 1.3% of 1000 non-diarrheic controls. Four years later, Skirrow (1977) used a selective agar system containing several antibiotics to recover the organism from human stools. He reported that 7.1% of the individuals tested who had diarrhea were culture positive for this organism. He found no \textit{C. jejuni} in stools of 194 control patients.

Since Skirrow (1977) substantiated the findings of the Brussels group, world-wide recognition of this enteropathogen has been reported. The incidence of \textit{C. jejuni} in developing countries differs quite considerably from industrialized countries. In Bangladesh, it has been recovered from 14.0-38.8% of the individuals tested (Blaser et al. 1980a; Glass et al. 1983; Speelman et al. 1983). The variability in recovery rates may be attributed to the number and type of individuals tested. In Gambia, an isolation rate of \textit{C. jejuni} from diarrheic patients ranged from 10.2-14.3% (Billingham 1981; Lloyd-Evans et al. 1983). Nigeria (12%) (Olusanya et al. 1983), India (9.6%) (Nair et al. 1983), Indonesia (10%) (Ringertz et al. 1980), South India (14.8%) (Rajan and Mathan 1982) and South Africa (38%)
(Bokkenheuser et al. 1979) are developing countries which show a similar prevalence of C. jejuni. In industrialized areas, the incidence of C. jejuni is lower than that in developing countries. This may be attributed to better sanitation in these areas. This organism was isolated from patients with diarrhea from New Zealand (4.9%) (Watson et al. 1979), Sweden (10.9%) (Svedhem and Kaijser 1980), Finland (5.8%) (Pitkanen 1982) and Canada (4.3%) (Pai et al. 1979). In the United States several surveys on the frequency of C. jejuni in the feces of patients with diarrhea were conducted. In New Orleans, a 13% rate of isolation was reported (Gordon et al. 1983). In Eastern Tennessee (Lambe et al. 1981) and Honolulu, Hawaii (Pien et al. 1983), it was detected at the 8.0% and the 8.7% levels, respectively. In Denver, Colorado (Blaser et al. 1982) and in a nationwide survey (Blaser et al. 1983b) the organism was isolated in 4.6% of the patients with diarrhea.

To better understand the importance of this pathogen, surveys were conducted on patients with diarrhea for the detection of this organism as well as other common gastrointestinal pathogens. This information is listed in Table 2. C. jejuni was isolated at a higher frequency in developing areas than in industrial areas. This trend can also be observed for Salmonella.
<table>
<thead>
<tr>
<th>Region</th>
<th>Campylobacter jejuni</th>
<th>Salmonella</th>
<th>Shigella</th>
<th>Yersinia enterocolitica</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Gambia&lt;sup&gt;2&lt;/sup&gt;</td>
<td>14.3%</td>
<td>7.0%</td>
<td>6.3%</td>
<td>ND&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Billingham (1981a)</td>
</tr>
<tr>
<td>Nigeria&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12.0%</td>
<td>6.0%</td>
<td>10.0%</td>
<td>ND</td>
<td>Olusanya et al. (1983)</td>
</tr>
<tr>
<td>Canada&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.3%</td>
<td>5.1%</td>
<td>1.4%</td>
<td>2.8%</td>
<td>Pai et al. (1979)</td>
</tr>
<tr>
<td>Honolulu, HI&lt;sup&gt;3&lt;/sup&gt;</td>
<td>8.7%</td>
<td>4.2%</td>
<td>3.8%</td>
<td>ND</td>
<td>Pien et al. (1983)</td>
</tr>
<tr>
<td>Denver, CO&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.6%</td>
<td>3.4%</td>
<td>2.9%</td>
<td>ND</td>
<td>Blaser et al. (1982)</td>
</tr>
<tr>
<td>U.S.A. Survey&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.6%</td>
<td>2.3%</td>
<td>1.0%</td>
<td>ND</td>
<td>Blaser et al. (1983b)</td>
</tr>
</tbody>
</table>

<sup>1</sup> ND = Not determined
<sup>2</sup> Developing countries
<sup>3</sup> Developed areas
and Shigella. In most cases, C. jejuni is detected more frequently or at least as often as any of the other important gastrointestinal disease causing agents. The data listed in Table 2 are important findings, however, interpretation of these data should be made cautiously. The enteric response to C. jejuni in many cases may be more severe than that of the other enteropathogens. Blaser et al. (1982) explained that before a specimen was obtained for testing of pathogens, a physician must have considered the patient ill enough to have the specimen analyzed. This type of testing is obviously biased for the more severe diseases.

The detection of asymptomatic carriers of C. jejuni in developed countries are extremely low and in many instances nonexistent. This is not the case in developing countries. In Bangladesh 17.7% of non-diarrheic children carried this organism (Blaser et al. 1980a). In Nigeria (2%) (Olusanya et al. 1983) and South Africa, (5-40%) (Bokkenheuser et al. 1979) symptomless carriers of C. jejuni were present in their population, with children showing the highest frequency of carriage. These data may indicate that not all strains of C. jejuni can cause diarrhea or possibly that the organism can be carried for an extensive time after an individual recovers from the illness. Other possibilities may be that high levels of certain strains are
required to achieve the infective dose, or that some individuals are more resistant than others.

2. Age and Sex

In most cases there is no sex preference in the rate of illness caused by _C. jejuni_. A few surveys showed a slight predominance of male patients becoming infected. However, this could possibly be due to a dominance of exposure of males (Blaser et al. 1982; Butzler and Skirrow 1979.)

Although, more isolates of _C. jejuni_ are recovered from children, more stool samples from children are usually analyzed. It is a more common practice for children than adults to be taken to the doctor and have stool cultures collected and analyzed when they have diarrhea. To determine the age incidence of _C. jejuni_ gastroenteritis, the number of positive stools per total stools analyzed in a given age group must be determined. With this assumption taken into consideration, the age range for contacting _C. jejuni_ enteritis is 10-29 years (Blaser et al. 1983b). Similar results were found by Butzler and Skirrow (1979).
3. Seasonal Frequency

The seasonal frequency of *C. jejuni* enteritis is similar to that of Salmonella. Peaks are observed in the warmer months and a decrease in incidence in the colder months (Figure 2). These trends have been observed in England and Belgium (Butzler and Skirrow 1979) and in the United States (Blaser et al. 1983b).

4. Incubation Period

When determining the epidemiology of an infection (especially foodborne), it is usually difficult to determine what agent caused the infection and when the infection occurred. Therefore, the incubation period is an estimated value. Things that may affect the incubation time include the dose and strain of the organism as well as the susceptibility of the host. A 2-11 day incubation period was suggested by Skirrow (1977). Other researchers have stated that a 2-5 day incubation period is a more reasonable assumption (Blaser et al. 1979a; Karmali and Fleming 1979). In an outbreak in which *C. jejuni* was transmitted through milk in which 205 individuals contacted the organism, the incubation period was 2-11 days with a mean of 5 days (Porter and Reid 1980.) In an outbreak of *C. jejuni* enteritis, 57 people became ill in a range of 1-8 days after consumption of raw milk (McNaughton et al. 1982).
Figure 2. Season Frequency of *Campylobacter jejuni* Isolated from Fecal Specimens (Figure taken, with permission, from Blaser et al. 1982)
5. Symptoms

The consumption of the organism may result in asymptomatic carriers to mild diarrhea to severe, bloody diarrhea. *C. jejuni* can cause a colitis which resembles ulcerative colitis or Crohn's disease (Colgan et al. 1980; Doberneck 1983). The most frequent symptoms are diarrhea, abdominal pain and fever. In more severe diseases, blood is usually present in the stool. The abdominal pains may mimic those of appendicitis. The organism was isolated from the appendix of a child (Chan et al. 1983). Infants are usually afebrile (Andersen et al. 1981). Symptoms seem to be less severe in children than adults (Butzler and Skirrow 1979). Other symptoms which may or may not be present are: malaise, headache, dizziness, backache, myalgia, rigor, nausea, mucus in stool, bile-stained stool, anorexia, musculoskeletal pains and delerium. Vomiting is not common (Blaser et al. 1982; Blaser and Reller 1981; Butzler and Skirrow 1979; Doyle 1981; Snydman 1982). In severe cases, patients may have more than 8 bowel movements on the worst day of illness, which could be approximately 8-9 liters of stool volume (Blaser et al. 1982; Miner and Voth 1983). Due to the severity of the more critical cases, Lambe et al. (1981) proposed that this stage of the illness be reclassified as an "acute dysentery syndrome."
6. **Duration**

In untreated patients, the symptoms usually persist for 7-10 days (Gordon et al. 1983). According to Butzler and Skirrow (1979) children are subject to a more persistent or recurring illness of campylobacteriosis. Even though symptoms subside, *C. jejuni* can persist in the stool for several months (Blaser and Reller 1981). Blaser et al. (1979a) reported that 20% of the infected patients may have a relapse, prolonged or severe illness.

7. **Complications**

A very common feature of the illness is that it resembles inflammatory bowel disease (Gordon et al. 1983). When a barium enema examination was performed, the colitis induced by *C. jejuni* appeared as colonic cancer (Doberneck 1983). The organism can cause a nonspecific colitis that appears similar to ulcerative colitis (Colgan et al. 1980; Lambert et al. 1979). Mucosal ulcers have been found in the terminal ileum and at the ileocecal valve (Michalak et al. 1980). At least in one case, this illness was misdiagnosed as Crohn's disease (Coffin et al. 1982). Patients with *C. jejuni* gastroenteritis have developed arthritis that lasted up to one week (Johnsen et al. 1983; Pitkanen et al. 1980).
1983). Other complications have been proctitis (Lambert et al. 1979), pancreatitis (Pitkanen et al. 1983), bacteremia (Guerrant et al. 1978), convulsion in young adults with a high fever (Blaser and Reller 1981) and C. jejuni has been isolated from an inflamed appendix (Megraud et al. 1982). Death has occurred in debilitated or elderly patients, however, this is considered to be very rare (Blaser and Reller 1981).

8. Treatment

Usually C. jejuni enteritis is self-limiting and no treatment is needed. If treatment is required, the drug of choice is erythromycin (Blaser 1982). A dose of 50 mg per kg of body weight per day in four divided doses was recommended by Snydman (1982). Erythromycin has a narrow spectrum of activity and a low toxicity (Butzler and Skirrow 1979). There appears to be a low level of erythromycin-resistant strains (Blaser and Reller 1981). Tetracycline, doxycycline, furazolidone and chloramphenicol are alternative drug choices (Blaser 1982; Blaser and Reller 1981; Butzler and Skirrow 1979). Clindamycin is suggested only in severe infections (Snydman 1982). Treatment of symptoms, such as replacement of fluids and electrolytes is also recommended (Blaser and Reller 1981).
C. Virulence

1. Toxin

Since fever is a major symptom, one would assume that this illness is an infection, not an intoxication. However, a cholera-like toxin has been demonstrated by Ruiz-Palacios et al. (1983). Supernatants of cultures of *C. jejuni* isolated from humans, produced fluid accumulation in rat ileal loops. The supernatants also caused elongation and increased intracellular cyclic AMP levels in Chinese hamster ovary cells. When cholera antitoxin was added simultaneously with the culture supernatant, activity of the toxin was not detected. The toxin has an approximate molecular weight of $10^4$-$10^5$ daltons. Studies still need to be performed to determine the ability of isolates from animal or food origin to produce a toxin.

Culture filtrates of *C. jejuni* can also cause rounding of Y-1 mouse adrenal cells (McCardell et al. 1984).

2. Animal Models

Rhesus monkeys were administered $10^6$-$10^7$ cells per os of human isolates of *C. jejuni* (Butzler and Skirrow 1979;
Fitzgeorge et al. 1981). Diarrhea was demonstrated in some animals, however very inconsistent results were obtained using this model.

Young calves were fed $10^8$ cells of \textit{C. jejuni} and colonization was observed by 24-48 hr post-inoculation (Firehammer and Myers 1981). Presence of \textit{C. jejuni} in the feces was determined by these researchers to be evidence of colonization. No data were given to indicate how long the organism was excreted in the feces of these young calves.

Chickens are considered to be a source of \textit{C. jejuni} (Oosterom et al. 1983b). Due to their relationship to this organism and their susceptibility to other enteric pathogens, these animals were studied as models of campylobacteriosis. Three day old chicks infected orally with $10^7$ \textit{C. jejuni} showed diarrhea by 45 hr (Ruiz-Palacios et al. 1981). As many as 88% of the infected chicks produced diarrhea. The organism was found to be localized in the jejunum and ileum. Other researchers showed that when chicks of similar age were used, a comparable dose produced diarrhea in these chicks, although it took 5 days for 81% of the chicks to become diarrheic (Sanyal et al. 1984). Manninen et al. (1982) could not induce diarrhea in 3 day old chicks by inoculation of \textit{C. jejuni}. Welkos (1984) demonstrated
that 12 hr chicks produced bloody, mucousy and watery stools after being inoculated orally with *C. jejuni*. The incubation period was related to the inoculum size. Differences in the results of these studies may be due to the different strains tested. Monoxenic and holoxenic chicks fed $10^4-10^5$ *C. jejuni* produced watery diarrhea during the first week post challenge. The organism was found to be colonized in the cecum (Soerjadi et al. 1982). The problem with using chicks as experimental models is that these animals have uncolonized or sparsely colonized intestines, therefore, they are not representative of most human cases. The holoxenic and monoxenic chicks require special housing which some laboratories do not have.

Prescott and Barker (1980) produced a mild diarrhea in gnotobiotic puppies. The organism was limited to the cecum and colon. These animals require special housing and have a lack of bacterial competition from intestinal flora, thus making colonization easier.

A mouse model was investigated due to the ease of care, lack of expense and availability of these animals. Adult Swiss mice appear to be quite resistant to this microorganism when orally challenged (Fields et al. 1981). When adult female Swiss mice were pretreated with tobramycin and fed $10^8-10^9$ *C. jejuni*,
colonization but no diarrhea was achieved (Field et al. 1984). The organism was colonized in the cecum and colon and remained in these tissues for 2 weeks before the normal flora overgrew C. jejuni. When tobramycin-treated mice were fed a bacterial suspension made from a normal fecal pellet 24 hr before challenge, C. jejuni colonization was prevented. Blaser et al. (1983) demonstrated chronic asymptomatic excretors of C. jejuni when adult HA-ICR Caesarian-section derived and barrier reared mice were fed C. jejuni. These mice excreted the organism up to 2 months. The organism was colonized in the stomach and the upper part of the small intestine. Problems with this animal model are that these animals require special rearing and they are devoid of normal intestinal flora. Neonatal Swiss mice infected with a high dose (10^8-10^9) of C. jejuni became colonized for 2 weeks (Fields et al. 1981). The organism was colonized in the lower small intestine, cecum and colon. The colon was the most populated area.

BALB/C mice are more susceptible to bacterial infection than are other strains of mice (Cheers et al. 1978). For example, when Listeria monocytogenes was injected intravenously into BALB/C mice and C57BL/10 mice, the C57BL/10 strain was 100 times more resistant to L. monocytogenes than was the BALB/C strain. BALB/C mice have a low macrophage inflammatory response and are
less responsive to complement-derived chemotactic factors than other strains of mice (Stevenson et al. 1981). These mice are more sensitive to Mycobacterium bovis BCG Pasteur strain than are other mice (Forget et al. 1981; Ormie and Collins 1984).

When *C. jejuni* was passed through weanling BALB/C mice pretreated with iron dextran 3 hr before bacterial challenge, the LD₅₀ was reduced from 10¹⁰ to 10⁵. Neonatal BALB/C mice that were inoculated with *C. jejuni* in a 1:1 concentration with iron dextran showed loose mucus stools, sometimes with blood (Kazmi et al. 1984).

D. Transmission

Various foods have been incriminated as vehicles for transmission of *Campylobacter jejuni*. Epidemiological and serological verification of this transmission is usually unavailable. One reason for this lack of verification is the lengthy incubation period of 2-5 days. After a long incubation time and the time it takes for a proper diagnosis of this illness, the incriminated food is: a) unknown, b) unavailable for analysis, or c) spoiled, thus making detection of this organism very difficult. For some foods, the methodology of culturing this organism is still unacceptable (Taylor et al. 1979).
Waterborne outbreaks of *C. jejuni* enteritis have been documented in the literature. The first reported and the largest outbreak occurred in Bennington, Vermont during the month of June, 1978 (Vogt et al. 1982). Approximately 3,000 residents of this town became ill during a 2-week period. *C. jejuni* transmitted through the town's water supply system was implicated in the outbreak, however no *C. jejuni* was recovered from samples of water. Since this outbreak, a most probable number method (Bolton et al. 1982) and a filtration method (Mathewson et al. 1983) have been devised for the detection of *C. jejuni* in water. Outbreaks of waterborne transmitted *C. jejuni* enteritis occurred during the summers of 1980 and 1981 in the Rocky Mountains in Wyoming (Taylor et al. 1983). Young adults who drank untreated surface mountain water became ill. *C. jejuni* was isolated from a suspected stream.

Other waterborne outbreaks of *C. jejuni* enteritis in Northern Illinois, Wales and British Columbia were attributed to the consumption of unchlorinated or inappropriately chlorinated water (Taylor et al. 1982). Low levels of chlorination will inactivate this microorganism (Wyatt and Timm 1982).

Milk has been the most important foodborne vehicle of *C. jejuni*, both in terms of numbers of outbreaks and numbers of individuals becoming ill.
Raw milk has been implicated as a vehicle in *C. jejuni* gastroenteritis in Kansas (Tosh et al. 1981), Oregon (Terhune et al. 1981) Arizona (Taylor et al. 1982), Colorado (Blaser et al. 1983), Georgia (Potter et al. 1983), Illinois (Bryan 1983), Minnesota (Blaser et al. 1983a) and Pennsylvania (Blessing et al. 1983). The consumption of Grade A raw chocolate milk was implicated in New Mexico (Bryan 1983). Outbreaks associated with the consumption of certified raw milk were reported in California (Anon 1981) and Georgia (Bryan 1983). In all cases, *C. jejuni* could not be isolated from the suspect milk samples.

An outbreak was reported in Canada in which raw milk was the contributing factor for campylobacteriosis (McNaughton et al. 1982).

The milkborne transmission of *C. jejuni* is a major problem in England where approximately 3% of the milk is consumed unpasteurized (Blaser 1982). In England, a large number of milkborne outbreaks have been cited in the literature (Robinson et al. 1979; Porter and Reid 1980; Jones et al. 1981a; Jones et al. 1981b; Wright et al. 1983). These outbreaks were associated with the consumption of either raw or improperly pasteurized milk. Jones et al. (1981b) estimated that over 2,500 individuals contacted *C. jejuni* during a 3 week period in March, 1979. Free milk that was given to school children was considered to be the vehicle for transmission. Upon
inspection of the supplying dairy, it was determined that the dairy's pasteurization processes were unacceptable. In another case, _C. jejuni_ was isolated from the milk filter in a dairy (Robinson et al. 1979). When this isolate was compared to clinical isolates from an outbreak of _C. jejuni_ gastroenteritis in which this dairy was implicated, the 2 strains were "indistinguishable from one another." This report appears to be the only proven link of _C. jejuni_ transmission through milk.

A major problem in implicating _C. jejuni_ milkborne outbreaks is the culturing techniques used to recover this organism from raw milk. Since the organism is usually present only in low numbers, enrichment techniques are employed. Lovett et al. (1983) used a selective procedure in the detection of _C. jejuni_ from raw milk and concluded that _C. jejuni_ was present in 1-2% of farm bulk milk tanks. Koidis and Doyle (1984) found that if raw milk were treated with 0.01% sodium bisulfite in an atmosphere of 100% nitrogen, _C. jejuni_ survived 15 or more days longer than did this organism in untreated milk.

Raw chicken (Brouwers et al. 1979), undercooked chicken livers (Barot et al. 1983; Goodman et al. 1983), undercooked chicken (Hayek and Cruickshank 1977) and undercooked barbecued chicken (Doyle 1981) have been implicated in _C. jejuni_ transmission. In chicken associated campylobacteriosis, it was determined that the infected individuals used shorter cooking times that did non-infected controls.
(Severin 1982). This insinuates that the infected individuals consumed undercooked chicken.

The consumption of luncheon meat made from pork, and vinegared pork was incriminated in an outbreak of \textit{C. jejuni} enteritis (Doyle 1981).

Other foods that have been suspected as vehicles for transmission of this organism are: cake, primarily the icing (Blaser et al. 1982); raw hamburger (Oosterman et al. 1982); a salad, cross contamination from chicken livers (Goodman et al. 1983) and raw clams (Blaser et al. 1982).

One thing that all these food vehicles have in common is that the food that was implicated was eaten raw or undercooked. Normal cooking time and temperature will inactivate this organism (Christopher et al. 1982a; Stern and Kotula 1982).

Person to person transmission has been documented (Blaser et al. 1981). A human stillborn fetus was delivered to a mother who previously had \textit{C. jejuni} enteritis (Gribble et al. 1981). The organism was isolated from the blood and spleen of the fetus.

Various animals such as puppies (Blaser et al. 1978b) and kittens (Svedhem and Norkrans 1980) have transmitted \textit{C. jejuni} to humans.
E. Methodology

1. Presumptive Identification in Human Stool

The resistance of *Campylobacter jejuni* to several antibiotics has been employed in selective media for its isolation. This organism is resistant to vancomycin, polymyxin B, trimethoprim lactate, cephalosporin, amphotericin B and cycloheximide. Vancomycin is active against gram positive cocci. Polymyxin B and colistin are inhibitory toward Enterobacteriaceae and *Pseudomonas* sp. Trimethoprim is inhibitory against *Proteus* sp. The cephalosporin and cephalothin are active against *Streptococcus faecalis*, *Enterobacter* sp., *Serratia* sp., *Pseudomonas aeruginosa*, some *Proteus* sp., *Yersinia enterocolitica* and *Bacteroides fragilis*. The amphotericin B and cycloheximide are inhibitory to yeasts and molds.

Combinations of some of these antibiotics are used in most of the media for the isolation of *C. jejuni*. These media were developed for isolation of *C. jejuni* from feces and since have been used for the isolation of *C. jejuni* from food. A composite comparison of the four selective media used for the identification of *C. jejuni* is shown in Table 3.
<table>
<thead>
<tr>
<th>Medium Composition [1]</th>
<th>Butzler's Medium</th>
<th>Modified Butzler's Medium</th>
<th>Skirrow's Medium</th>
<th>Campy-BAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal Medium</td>
<td>Fluid Thioglycollate + Agar</td>
<td>Fluid Thioglycollate + Agar</td>
<td>Brucella agar or Blood agar base no. 2</td>
<td>Brucella agar</td>
</tr>
<tr>
<td>Blood</td>
<td>15% defibrinated sheep</td>
<td>10% defibrinated sheep</td>
<td>5-7% lysed horse</td>
<td>5-7% defibrinated horse</td>
</tr>
<tr>
<td>Actidione</td>
<td>50 mg</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>50 mg</td>
<td>2 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>25,000 IU</td>
<td>25,000 IU</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>-</td>
<td>15 mg</td>
<td>-</td>
<td>15 mg</td>
</tr>
<tr>
<td>Colostin</td>
<td>-</td>
<td>40,000 IU</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>5 mg</td>
<td>5 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>10,000 IU</td>
<td>-</td>
<td>2,500 IU</td>
<td>2,500 IU</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>-</td>
<td>-</td>
<td>5 mg</td>
<td>5 mg</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>-</td>
<td>-</td>
<td>10 mg</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

\[1\] All ingredients added to 1 liter of distilled H₂O
Billingham (1981b) compared Skirrow's and Butzler's media for isolation of \textit{C. jejuni} from human feces. Butzler's medium allowed easier identification than did Skirrow's medium. Patton et al. (1981) compared Skirrow, Butzler and modified Butzler media for the isolation of \textit{C. jejuni} from rectal swabs from cats and dogs. Results showed that a significantly higher rate of recovery was obtained using modified Butzler's medium.

Rapid diagnosis of \textit{C. jejuni} from stool specimens is helpful. Due to its characteristic shape, a modified gram stain has been suggested as a rapid presumptive identification of this organism in stool samples (Saize and Titus 1982; Park et al. 1983). The use of a darkfield microscope to detect the characteristic darting, corkscrew motion that is quite distinctive from other enteric pathogens has been suggested as a presumptive method of detecting this organism in stool specimens (Paisley et al. 1982).

The use of selective enrichment media has been suggested for isolation and detection of \textit{C. jejuni} from human stools. Combinations of antibiotics are added to a basal medium. Martin et al. (1983) reported that using their enrichment broth allowed the recovery of 46.3% more \textit{Campylobacter} than did direct plating. Similar results were shown by other scientists (Hodge and Terro
Hutchinson and Bolton (1983) stated that a selective enrichment of stools is needed only when *C. jejuni* is found in low numbers.

2. **Presumptive Identification in Foods**

Stern (1982a) compared Campy-BAP, Butzler's medium and a modified Skirrow (containing 15 μg cephalothin per ml) for the recovery of inoculated *C. jejuni* from ground beef. The number of *C. jejuni* recovered from the ground beef was consistently lower than the number originally inoculated in all three media. Campy-BAP recovered the highest number of *C. jejuni* as compared to the other two agars. However, Butzler's medium showed the best selectivity for *C. jejuni* with the fewest contaminating microorganisms. In view of this, Stern (1982a) suggested a combination of Campy-BAP and Butzler's medium for the recovery of *C. jejuni* from food.

Due to the difficulty of isolation and the selectivity of the selective media, enrichment techniques were applied for *C. jejuni* detection from food. Most of the enrichment media contain a lower concentration of antibiotics than are used in the selective agars.
Acuff et al. (1982a) devised an enrichment procedure in which the food sample is suspended in brucella broth supplemented with 0.15% agar, 0.05% FeSO₄·7H₂O, 0.05% sodium metabisulfite, 0.05% sodium pyruvate, vancomycin (10mg/l), trimethoprim (5mg/l), polymyxin B sulfate (2,500 IU/l), amphotericin B (2mg/l) and cephalothin (15mg/l). After inoculation, the enrichment broth is incubated at 4°C for 12 hr and then transferred to 42°C for 48 hr in an atmosphere containing 5% O₂, 10% CO₂ and 85% N₂. After incubation, a loopful of the enrichment broth is streaked onto a Campylobacter selective agar plate. With this system they could detect as few as 0.3 to 3.3 cells per g of food.

Park et al. (1981) devised an enrichment procedure for whole chickens. The whole chickens were washed in 250 ml of nutrient broth. The washing was then filtered through 2 layers of sterile cheesecloth. The filtrate was centrifuged and the resultant pellet was resuspended into 5 ml of brucella broth. A part of this suspension was streaked onto a selective agar and the remainder was transferred into 100 ml of enrichment broth. The enrichment broth consisted of 8 mg of vancomycin, 4 mg of trimethoprim, 3 mg of cephalothin, 3 mg of colistin and 30 ml of calf serum per liter of brucella broth. The inoculated broth was incubated at 42°C microaerophilically for 24-48 hr. After incubation, 5 ml of the broth was filtered through a 0.65 μm
membrane filter and a portion of the filtrate plated onto a selective agar. Reportedly, this enrichment technique is able to recover 1 cell of *C. jejuni* in 10 g of chicken in the presence of $10^5$-$10^7$ cells of indigenous flora. Park et al. (1981) recovered 32% of *C. jejuni* inoculated onto chicken when analyzed by direct plating and 62% by using the enrichment method.

Doyle and Roman (1982c) reported the isolation of 0.1-1.0 cell of *C. jejuni* per gram of food in the presence of $10^6$ to $10^9$ indigenous bacteria. In their enrichment procedure, 10 or 25 grams of food are added to 100 ml of enrichment broth. The enrichment broth consists of 7% lysed horse blood, 3 g of sodium succinate, 0.1 g of cysteine HCl, 15 mg of vancomycin, 5 mg of trimethoprim, 20,000 IU of polymyxin B and 50 mg of cycloheximide per liter of enrichment broth. The broth is incubated microaerophilically for 24 hr while being shaken at 100 gyrations per minute at 42°C. After incubation, the enrichment broth is plated onto selective agar in plates.

Hanninen (1982c) compared four enrichment broths containing various concentrations of antibiotics with and without the presence of bile salts. The enrichment broth with the best recovery contained brucella broth supplemented with 0.05% sodium pyruvate, FeSO$_4$·$7H_2$O, Na$_2$S$_2$O$_5$ (FBP) and 5 IU polymyxin B, 10 μg
vancomycin, 5 μg trimethoprim, 2 μg amphotericin B, 2 mg of sodium deoxycholate (or 2 mg of sodium cholate) per ml.

Three enrichment broths used in the recovery of *C. jejuni* from foods were compared by Rothenberg et al. (1984). The enrichment broths devised by Doyle and Roman (1982c), Park et al. (1981) and a modified Doyle-Roman medium were studied. The modified Doyle-Roman medium contained the same ingredients as that of Doyle and Roman (1982c), however, filtered sterilized FBP, 0.1% sodium lauryl sulfate and 0.0075% agar were added. Isolation rates of these broths were: Doyle and Roman 61%, Park et al. 60% and modified Doyle-Roman 50%. Direct plating of the same samples recovered 40% of spiked *C. jejuni*.

An enrichment broth described by Wesley et al. (1983) consisted of 20 g tryptone, 2.5 g yeast extract, 5 g sodium chloride, FBP, 10 g bicine and 1 g agar per liter of distilled water. The broth was then autoclaved at 121°C. Hematin solution (6.25 ml), rifampin (25 mg), cefsulodin (6.25 mg) and polymyxin B sulfate (20,000 IU) were filter sterilized and added aseptically to the broth. The pH of this solution was adjusted to 8.0 and the broth was incubated microaerophilically at 42°C for 48 hr. They compared this enrichment broth to that described by Park et al. (1981). They found that their enrichment broth was superior
for recovery of \textit{C. jejuni} from poultry products. As few as 0.07 \textit{C. jejuni} per gram of chicken meat was detected by this method.

3. Biochemicals

Following enrichment, a sample is spread plated onto a blood based selective agar. After incubation of the selective plates, \textit{C. jejuni} colonies appear as flat or slightly raised, usually round, gray to pink in color and non-hemolytic. Colonies tend to spread, especially on moist surfaces. Adding an additional 0.5\% agar to the selective agar, reduces the amount of spreading. Adding glycerol soaked filter disks to the anaerobe jar also helps reduce spreading (Stern 1981). Typical colonies are further tested to obtain a presumptive identification. The testing for motility using a dark-field or phase contrast microscope should be done. This organism is both catalase and oxidase positive. Also, the use of a modified gram stain (using 1\% carbol fuschin as a counterstain) is suggested as a good presumptive diagnostic test (Luechtefeld et al. 1981b). Bergey's Manual of Systemic Bacteriology, Volume 1 (Krieg and Holt 1984) lists additional biochemical tests that can be used to confirm presumptive \textit{C. jejuni} isolates. This information is listed in Table 4.
TABLE 4. LIST OF BIOCHEMICAL TESTS USED IN THE CONFIRMATION OF CAMPYLOBACTER JEJUNI

<table>
<thead>
<tr>
<th>Test</th>
<th>C. jejuni Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate broth</td>
<td>No reduction</td>
</tr>
<tr>
<td>1% glycine</td>
<td>No growth</td>
</tr>
<tr>
<td>42°C microaerophilically</td>
<td>Growth</td>
</tr>
<tr>
<td>25°C microaerophilically</td>
<td>No growth</td>
</tr>
<tr>
<td>3.5% NaCl</td>
<td>No growth</td>
</tr>
<tr>
<td>SIM agar with Lead acetate strips</td>
<td>Blackening of strip indicating H₂S production</td>
</tr>
<tr>
<td>1% bile</td>
<td>Growth</td>
</tr>
<tr>
<td>Carbohydrate fermentation</td>
<td>No growth</td>
</tr>
<tr>
<td>30 µg naladixic acid/disc²</td>
<td>Growth inhibition</td>
</tr>
</tbody>
</table>

¹All biochemicals except 30 µg NAD/disc were performed in brucella broth + 0.7% agar + test chemical.

²Disc containing NAD was placed on lawn surface of C. jejuni and incubated.
4. **Serology**

The use of serotyping can be an aid for epidemiologists when attempting to prove that an isolate from a human with gastroenteritis is the same strain as isolated from a given food. *Campylobacter* contains O, H and K antigens (Ullmann 1979). Procedures for serotyping *C. jejuni* are described by Abbott et al. (1980). Munroe et al. (1983) typed 316 animal isolates of *C. jejuni* on the basis of the thermostable antigen with 20 antisera produced from serotypes of *Campylobacter enteritis* outbreaks. They determined that 96% of the chicken isolates and 67% of the cattle isolates belonged to 11 of the human *C. jejuni* serotypes. These data may link isolates of animal origin to human gastroenteritis.

5. **Storage of Cultures**

Due to the susceptibility of this organism to various environmental conditions, storage of this microorganism has been difficult. Wang et al. (1980) devised a storage-transport medium in which 0.5% agar and 10% difibrinated sheep blood was added to brucella broth. A lawn of *C. jejuni* was removed from an agar surface via a cotton swab and transferred to 4-6 ml of this medium in a screw capped tube. After incubation overnight, it
was then stored at 4°C. The isolate should survive for at least one month in this medium. When an egg based medium was used for storage of *C. jejuni*, the cells were viable for over 3 months when stored at 4°C (Nair et al. 1984). The addition of 10-15% glycerol to a 24 hr broth culture assists in the resistance of this organism to freezing. When *C. jejuni* was stored at -20°C and -70°C in this cryoprotective agent, it survived for one year and three years, respectively (Luechtefeld et al. 1981; Rollins et al. 1983). The organism can be stored for many years when lyophilized and stored in liquid nitrogen (Krieg and Holt 1984).

F. Control

1. Effect of Heat

a) Temperature Effect

The heat used in cooking foods destroys many microorganisms, toxins and enzymes. When ground beef inoculated with $10^7$ *C. jejuni* per gram was heated at 190°C for 10 minutes (resulting in an internal meat temperature of 70°C), no *C. jejuni* was detected (Stern and Kotula 1982). Beef roasts spiked with *C. jejuni* and heated to a final internal temperature of 50-53°C contained no viable *C. jejuni*. 
Christopher et al. 1982a). The normal cooking of meat dishes should destroy C. jejuni. No foodborne outbreaks have been attributed to the consumption of properly cooked food.

b) Microwave Heating

The use of microwave ovens has become popular in homes, restaurants, hospitals and in the food industry. It has been estimated that approximately 20% of the households in the United States contain microwaves (Baldwin 1983). Microwave ovens are used as a method of cooking, reheating and thawing foods.

Microwaves by definition are a form of non-ionizing electromagnetic energy produced by means of a magnetron tube. Normally, microwaves have a frequency of 2450 MHz. Polar molecules (such as water) absorb the microwaves. These molecules attempt to align with oppositely charged molecules. A microwave frequency of 2450 MHz will alternate the current at a rate of 2450 million times per second. As the polar molecules try to align with the alternating current, they create molecular friction and as a result heat the food (Banwart 1981). Microwave heating is an extremely rapid process.
The use of microwave heating has generated a lot of interest in the effect of this cooking process on microorganisms. There has been some controversy in the literature as to whether the lethal effect of microwaves on microorganisms is associated with heat alone, or heat and microwave energy. One major disadvantage in microwave cooking is that the food is unevenly heated. There are hot and cold points found in the food. Ohlsson and Risman (1978) suggested that the unevenness of heating is associated with "reflection and refraction of microwaves at boundaries of dissimilar materials." Culkin and Fung (1975) heated soup in a microwave and found that the middle was the hottest point with the bottom and then the top being the coolest. They reported that the fewest cells were recovered on the top of the soup, which was the coldest point of the microwaved soup. They suggested that the cause of death must not only be associated with a lethal heat effect, but a microwave energy effect.

A modified microwave was used to test if there was a microwave radiation effect. Bacterial cultures were kept cool during extensive microwaving. No reduction in colony forming units was observed when the cells were microwaved and kept cool (Lechowich et al. 1969).
When cooking meat in a microwave and in a convection oven, it was determined that the inactivation of *E. coli* and *B. subtilis* was nearly identical for each method (Goldblith and Wang 1967). Crespo and Ockerman (1977) found that the microwave was less effective in destroying microorganisms than the conventional cooking oven.

2. **Effect of Chemical**

a) **pH**

Foods can be categorized according to their pH values. A food that has a pH of 3.7 or lower is considered to be a high acid food. One that has a pH range of 3.7-4.6 is an acid food. A medium acid food has a pH range of 4.6-5.3 and any food with a pH over 5.3 is a low or non-acid food. *C. jejuni* will not grow at a pH of 4.7 or lower. When the pH of brucella broth was lowered to 4.5 with hydrochloric acid, cell death of *C. jejuni* was observed (Doyle and Roman 1981). When lactic acid was used to adjust the pH of the culture medium, a pH of 5.7 was the minimal pH for growth of *C. jejuni* (Gill and Harris 1982b). This organism poses no problem in either acid or high acid foods.
b) **Ascorbic Acid**

*C. jejuni* was inhibited by 0.5 mg of L-ascorbic acid per ml. When L-ascorbic acid was oxidized, it was more inhibitory than in the reduced state (Fletcher et al. 1983).

c) **Spices**

Spices are added to food for flavor, aroma or aesthetic qualities. Spices are usually dried plant parts that may or may not be ground. According to Shelef (1983) the use of spices has generated much interest, recently. The reasons for this are: (1) an increase in usage of natural foods and (2) spices can be blended and used as a salt substitute.

The inhibitory activities of certain spices toward a broad spectrum of bacteria has been reported. Beuchat (1976) studied the effects of 13 different spices on the growth of *Vibrio parahaemolyticus*. He found that oregano and thyme possessed the highest degree of inhibition at the 0.5% level. The spices sage, rosemary and allspice were tested with a broad spectrum of foodborne bacteria by Shelef et al. (1980). At a concentration of 2% spice, they observed that gram positive bacteria were more sensitive than gram negative bacteria. It was revealed that 0.3% of sage or rosemary was
needed for growth inhibition. A bactericidal effect was evident at the 0.5% level of these spices. Julseth and Deibel (1974) reported the inhibition of *Salmonella* by allspice, cassia, onion and oregano. Garlic inhibited the growth of *Escherichia coli* and *Salmonella typhimurium* (Johnson and Vaughn 1969). Cinnamon and its extracts were found to inhibit the growth of *Aspergillus parasiticus* as well as aflatoxin production (Bullerman 1974; Bullerman et al. 1977). The antimicrobial properties of spices are usually located in the essential oils of the spice. The work described above was performed in culture media containing spices. When these spices are put into foods, their antimicrobial properties are reduced (Farbood et al. 1976; Shelef 1983).

3. **Gamma Radiation**

Lambert and Maxcy (1984) showed a $D_{10}$ reduction in *C. jejuni* spiked in various foods that were subjected to gamma radiation at 32K-rad. The resistance of this organism was both temperature and environment dependent. It survived radiation the longest in brucella broth stored at $-30^\circ C$. 
MATERIALS AND METHODS

Experiment 1 - Prevalence of Campylobacter jejuni in Retail Meats

Samples of flank steak, ground beef, pork chops, ground pork, broiler chicken (whole or parts) and lamb stew meat, each weighing approximately 1-2 pounds were obtained. Five samples of each type of meat were purchased from each of two local retail stores (the Kroger Co. and Big Bear Stores) during each of the months of June, September and December, 1983 as well as March, 1984. The total number of samples analyzed was 240 samples. After each sample was purchased, the meats were transported immediately to the laboratory and stored at 4°C until tested. Two sampling techniques were employed, depending on if the meat were ground or whole.

For whole cuts, each meat was aseptically transferred into a 10 9/16 in. x 11 in. x 2.7 mil. Ziploc heavy duty freezer bag (The Dow Chemical Co.). Then 250 ml of brucella broth (Oxoid) was added, and the meat was massaged through the plastic bag and/or shaken for 2 minutes. Then the broth was filtered through 2 layers of sterile cheesecloth into a sterile centrifuge bottle. After centrifuging (RC2-B Centrifuge; GSA Rotor; Sorvall) at 16,300 x g at 4°C for 20 minutes, the supernatant was discarded and the pellet was resuspended into 100 ml of enrichment broth (Doyle and Roman 1982c) in a 250 ml side-arm flask.
Each ground meat sample was analyzed by aseptically weighing 25 g of ground meat into a 250 ml side-arm flask containing 100 ml of enrichment broth. Dialysis tubing was attached to the side-arm of the flask and the top was covered with a no. 7 or 7 1/2 rubber stopper. Each flask was attached through the dialysis tubing to a vacuum pump. The flask was evacuated three times. With each evacuation, the air was replaced with a gaseous mixture of 5% O₂, 10% CO₂ and 85% N₂. The atmosphere was secured within the flask with a screw clamp collapsing the dialysis tubing. The prepared flasks were incubated in a shaker water bath (Precision Instrument model G-76; New Brunswick Scientific Co., New Brunswick, NJ) and shaken at 100 gyrations per minute for 16-18 hr at a water temperature of 42°C.

After this enrichment of cells, 0.1 ml of both the enrichment broth and of a 10⁻¹ dilution was spread plated onto Campy-BAP (Oxoid) (Blaser et al. 1979a) prepoured into petri dishes. The inoculated plates were incubated microaerophilically at 42°C, and were observed for typical _C. jejuni_ colonies at 24, 48 and 72 hr. The microaerophilic environment was replaced after each observation. The selected colonies were presumptively identified as _C. jejuni_ by testing for motility, catalase and oxidase production. Further confirmatory tests were performed on these presumptive, positive isolates. These tests were the inability to reduce nitrate, growth in 1% glycerine, no growth in 3.5% NaCl and growth in 1% bile. All of these biochemical tests were accomplished in brucella broth (Oxoid), 0.3% agar (Oxoid) and the appropriate test chemical. Other
tests were the ability of *C. jejuni* to grow at 42°C microaerophilically but not at 25°C, and inhibition of growth by naladixic acid (Neg Gram; Winthrop Lab., New York NY). The nalidixic acid was added to sterile antibacterial assay filter discs (No. 740-E; 1/2 in. dia.; Schleicher and Schuell, Inc.; Keene, NH) at a concentration of 30 µg per disc and dried at room temperature. A dried disc was placed on a lawn of *C. jejuni* and incubated as previously described. Each isolate also was verified by the United States Department of Agriculture (USDA) Meat Research Laboratory, Beltsville, Maryland. Isolates were transported in Wang's transport medium (Wang et al. 1980).

A study was conducted to determine the reproducibility of recovering *C. jejuni* in inoculated meat samples before and after the survey. Twenty cubes of beef were analyzed during May, 1983 and May, 1984. Each cube weighed approximately 25 g and was stored in a coded Ziploc bag (The Dow Chemical Co.). The cubes were seeded with varying concentrations of *C. jejuni* ranging from 0.07–192 cells per gram of meat, by the USDA Meat Research Laboratory for shipment to the Food Microbiology Laboratory, The Ohio State University, Columbus, Ohio. Some meat samples were not spiked with *C. jejuni*. The coded-spiked samples were then put inside an additional plastic bag and into an insulated styrofoam box containing ice and shipped by United Parcel Service. Upon arrival at the laboratory, the samples were refrigerated (< 48 hr) until analyzed. The method for analysis of the spiked beef was the same as that described for whole meat cuts.
Experiment 2 - Identification of Non-Campylobacter Colonies from Selective Agars

Several non-Campylobacter colonies were observed on the Campy-BAP plates during the meat survey (Experiment 1). Representative colonies were isolated and identified to genus level. These isolates were determined to be non-Campylobacter because they yielded negative reactions in the biochemical tests performed for the identification of C. jejuni. The isolates were streaked onto brucella agar (Oxoid) plates containing 5% defibrinated horse blood (BAB) incubated at 42°C in a modified atmosphere of 5% O₂, 10% CO₂ and 85% N₂. Upon verification of a pure culture through colonial morphology and simple staining, the pure cultures were restreaked onto another BAB surface and plate count agar (Difco) and incubated at 42°C microaerophilically and 35°C aerobically, respectively. All identified isolates grew at 35°C on plate count agar. Duplicate isolates were stored on plate count agar slants at 4°C. Growth-temperature studies were determined by inoculating each isolate into brucella broth (Oxoid) and incubating at 4, 25, 35, 42 and 55°C. Each isolate was tested for the gram reaction, cellular morphology, colonial morphology and oxidase and catalase production. Using these basic tests, the genus of each isolate was determined with biochemical tests using the aid of several published sources (Stanier et al. 1976; Buchanan and Gibbons 1974; Banwart 1981; Burns et al. 1982; Krieg and Holt 1984).
Experiment 3 - Comparison of Blending Method, Time and Diluent for the Enumeration of Campylobacter jejuni

A strain of Campylobacter jejuni was obtained from The Ohio State University Hospital. The culture was stored according to Luechtefeld et al. (1981). When needed, the culture was streaked onto brucella agar (Difco) supplemented with 5% defibrinated horse blood (BAB) to test for purity. The inoculated plates were incubated at 42°C in an atmosphere of 5% O₂, 10% CO₂ and 85% N₂ for 24-48 hr. The inoculum for testing was prepared by transferring a loopful of C. jejuni into brucella broth (Difco) and incubating it for 24 hr as stated above. The culture was centrifuged (RC2-B Centrifuge; GSA Rotor; Sorvall) for 20 minutes at 4°C at 16,300 x g. The resultant pellet was resuspended in sterile 0.85% saline. Using a spectrophotometer (Spectronic 20), the optical density of the inoculum was adjusted to 0.05 with saline at wavelength 540 nm. This prepared inoculum of C. jejuni was added to 250 ml of sterile skim milk (Carnation Co., Los Angeles, CA).

Two blending methods were used. A 25 g sample of the inoculated milk was weighed into a stomacher bag or Waring blender jar. Two hundred and twenty-five ml of diluent was added to each bag or jar. Four diluents were tested: brucella broth (Difco), 0.1% peptone water (Difco), modified cary blair diluent (cary blair transport medium minus the agar) and FBP-peptone diluent (0.025% FeSO₄·7H₂O,
sodium metabisulfite, sodium pyruvate and 0.1% peptone). Each sample was stomached or blended for 1, 2 and 4 minutes. At the end of each time interval, appropriate dilutions were spread plated on prepoured BAB. The plates were incubated at 42°C for 48 hr microaerophilically. After incubation, the colony forming units were enumerated with the aid of a Quebec colony counter. Each experiment was repeated four times. The statistical analysis was performed with Duncan's multiple range test for variable counts. This test can determine the significant difference of data between methods, diluents and blending times.
Experiment 4 - Survival of *Campylobacter jejuni* in Bovine Feces

Three strains of *Campylobacter jejuni* (29428, L-7 and 5-4) were obtained from the USDA Research Laboratory, Beltsville, MD. The cultures were stored according to the method by Luechtefeld et al. (1981b). A 24 hr viable culture was centrifuged (RC2-B Centrifuge, GSA Rotor; Sorvall) at 4°C and 16,300 x g for 20 minutes. The resultant pellet was resuspended in sterile 0.85% saline. Using a spectrophotometer (Spectronic 20), the optical density of the inoculum was adjusted to 0.05 with saline and wavelength 540 nm. An inoculum of approximately $10^6$-$10^7$ *C. jejuni* was used per sample of bovine feces.

Bovine feces were collected with aseptic instruments from The Ohio State University cattle herd. Representative samples of the feces were weighed into sterile Whirlpack bags (Model no. B1065; 8 oz.; Nasco) in 10 g quantities. A 0.1 ml volume of each culture was added separately to each fecal sample. The culture was distributed throughout the sample by massaging through the bag. Thirty bags of each strain-spiked feces were stored at either 4°C or 25°C. At various intervals, three bags, one with each strain, were removed from storage and analyzed. Ninety ml of 0.1% peptone water (Difco) was added to each bag and the sample was stomached with a Colworth Stomacher 400 for 2 minutes. Appropriate dilutions were prepared in 0.1% peptone water. The aerobic plate counts were made
using the pour plate technique with plate count agar (Difco). The
plates were incubated at 35°C for 48 hr. *C. jejuni* were counted by
spread plating appropriate dilutions onto Campy-BAP (Blaser et al.
1979a). The plates were incubated at 42°C in an atmosphere of 5% O₂,
10% CO₂ and 85% N₂ for 48 hr. All colony forming units on both agars
were counted with the aid of a Quebec colony counter. The plating
was replicated three times.
Experiment 5 - Effect of an Iodine Udder Wash on Campylobacter jejuni

Bioguard Iodine Udder Wash L.A. (low acid; Biolab, Inc., Decatur, GA) was obtained from The Ohio State University Dairy Barn. The iodine solution was diluted to 25 ppm in sterile water according to the manufacturer's directions. Campylobacter jejuni 29428 was obtained from the USDA Beltsville Agriculture Research Laboratory. A 24 hr viable broth culture was centrifuged (RC2-B centrifuge; GSA Rotor; Sorvall) for 20 minutes at 4°C at 16,300 x g. The resultant pellet was resuspended in sterile 0.85% saline. The optical density of the inoculum was adjusted to 0.05 at a wavelength of 540 nm via a spectrophotometer (Spectronic 20). Two levels of C. jejuni were tested ($10^3$-10$^4$/ml and 10$^7$-10$^8$/ml). The organism was suspended in luke-warm (45°C) iodine solution and then immediately transferred to a waterbath (Magni Whirl Constant Temperature bath; Blue M Electric Co.; Blue Island, IL) at 45°C. Plate counts for C. jejuni were determined at 0, 1, 2, 4, 8, 15, 30 and 60 minutes of organism-iodine interaction. The plate count was performed by spread plating the sample on brucella broth (BBL) solidified with 2% bacto agar (Difco) and supplemented with 5% defibrinated horse blood. The prepared plates were incubated microaerophilically at 42°C for 48 hr. The colony forming units were determined with the aid of a Quebec colony counter. The plating was repeated three times.
Experiment 6 - Effect of Hydrogen Peroxide on Campylobacter jejuni in Milk

Three Campylobacter jejuni strains (29428, L-7 and 5-4) were obtained from the USDA Beltsville Agriculture Research Laboratory. Each culture was stored on Wang's transport medium and, when needed, streaked onto brucella agar (BBL) which was supplemented with 5% defibrinated horse blood (BAB) and incubated microaerophilically at 42°C.

The inoculum was prepared by transferring one loopful of the organism into brucella broth (Oxoid) and incubating microaerophilically at 42°C for 24 hr. Using a spectrophotometer (Spectronic 20), the optical density of the inoculum was adjusted to 0.05 at wavelength 540 nm.

Sterile reconstituted skim milk (Carnation Co.; Los Angeles, CA) (50 ml) in screw cap milk dilution bottles was inoculated with 1 ml of the inoculum and incubated at 4°C. After a 10 minute holding period, an appropriate amount of hydrogen peroxide (H_2O_2) was added to each milk dilution bottle to the final concentrations of 0.005%, 0.01% and 0.05%. An inoculated skim milk sample containing no H_2O_2 was considered to be the control. The colony forming units of C. jejuni were determined at 0, 10, 30 and 60 minutes after the addition of H_2O_2. Decimal dilutions were made in 0.1% peptone water.
(Difco), and the appropriate dilutions were spread plated on prepoured BAB. The plates were incubated at 42°C for 48 hr as previously described. This experiment was repeated three times with each strain.

Raw milk, which was obtained from The Ohio State University Dairy Herd, was aseptically transferred into sterile screw cap milk dilution bottles. Each bottle containing 50 ml of the raw milk sample was inoculated with 1 ml of C. jejuni strain 29428 and incubated at 4°C. After a 10 minute incubation period, H₂O₂ was added to a final concentration of 0.05%. An inoculated raw milk sample without added H₂O₂ was included as a control. At 0, 10, 30, 60 and 120 minutes after the H₂O₂ treatment, the aerobic plate count, psychrotrophic count and C. jejuni counts in the raw milk sample were determined. C. jejuni was enumerated as previously described except that Campy-BAP (Blaser et al. 1979a) was used. The aerobic plate count and the psychrotrophic counts were determined by plating appropriate dilutions of the raw milk samples in plate count agar (Difco) using the pour plate method. The aerobic plate count plates were incubated at 35°C for 48 hr. The psychrotrophic plates were incubated at 4°C for 10 days. This experiment was repeated three times.
Experiment 7 - Effect of Sodium Diacetate on Campylobacter jejuni

A culture of Campylobacter jejuni was obtained from The Ohio State University Hospital. A 0.45% (w/v) of sodium diacetate obtained from Food Technology Products (Chicago, IL) was chosen as the experimental concentration due to its suggested usage. Sodium diacetate was added to sterile brucella broth (Difco) aseptically without sterilization, sterilized with ethylene oxide (Anpro model no. A N74, H.W. Anderson Prod.) and added aseptically, or added before the broth was sterilized and autoclaved for 20 minutes at 121°C. A Pyrex bottle containing brucella broth with no sodium diacetate was considered to be the control.

The pH of the autoclaved brucella broth was 7.0. The pH was determined with the aid of a Corning model 10 pH meter. The pH of the autoclaved brucella broth - sodium diacetate solution was 4.9. The pH of the aseptically added as well as the ethylene oxide treated sodium diacetate-brucella broth solution was 4.7. In order to determine if the pH caused the entire inhibitory effect, if any, on C. jejuni or if the sodium diacetate had a chemical effect on the organism, regardless of pH, the pH of some brucella broth was adjusted to 4.9 and the pH of some sodium diacetate-brucella broth solutions were adjusted to pH 7.0.
A 1 ml portion of *C. jejuni* which had been incubated microaerophilically at 42°C for 24 hr in brucella broth, was added to each test bottle.

The prepared test bottles were incubated at either 4°C, 25°C or 42°C in the microaerophilic environment. A sample from each test bottle was removed at 0, 3, 6, 9, 12, 24, 36 and 48 hr and analyzed for colony forming units of *C. jejuni*. The sample was diluted in 0.1% peptone water (Difco), when needed, and spread plated onto prepoured brucella agar (Difco) surfaces containing 5% defibrinated sheep blood. The plates were incubated at 42°C for 48 hr in the microaerophilic environment. After incubation, the colony forming units were enumerated. Each experiment was repeated three times.
Experiment 8 - Effect of Spices on *Campylobacter jejuni*

**Culture and Conditions**

A *Campylobacter jejuni* A7455 culture was obtained from the Centers for Disease Control (Atlanta, Georgia). The culture was stored according to Leuchtefeld e t al. (1981b). When needed, the culture was taken out of storage and inoculated on brucella agar (Difco) supplemented with 5% defibrinated sheep blood (BAB). The culture was then incubated microaerophilically at 42°C for 24 hr.

Modified gas-pak jars used for incubation, were evacuated to 25% atmospheric pressure and a mixture of gases containing 10% CO₂ and 90% N₂ was added. This provided an approximate atmosphere of 5% O₂, 10% CO₂ and 85% N₂.

**Test Criteria**

The spices used in this study were oregano, sage and ground cloves (McCormick-Stange Flavor Co., Inc., Hunts Valley, MD). Four concentrations of each spice (0, 0.1, 0.5 and 1% (w/v)) were added individually to 100 ml of brucella broth (Difco) in 250 ml cotton-plugged Erylenmeyer flasks before sterilization at 121°C for 20 minutes. A 1 ml aliquot of the 24 hr culture of *C. jejuni* was added to each test flask.
The prepared flasks were incubated at 4°, 25°C, or 42°C in the microaerobic atmosphere. A 1 ml aliquot from each incubated flask was tested for _C. jejuni_ at 0, 2, 4, 8, 16, 24 and 48 hr. Decimal dilutions were prepared in 0.1% peptone water (Difco) and spread plated onto prepoured BAB surfaces. The plates were incubated microaerobically for 48 hr at 42°C. After incubation, the colony forming units were enumerated with the aid of a Quebec colony counter. Each experiment was repeated three times.
Experiment 9 - Campylobacter jejuni Survival in Meat Cubes and Milk
Heated in a Microwave Oven

a) Heat Penetration of Distilled Water and Milk

Three volumes (9.9 ml, 39.6 ml or 69.3 ml) of distilled water or rehydrated skim milk (Carnation Co.; Los Angeles, CA) were added to Pyrex beakers (50 ml, 100 ml, 250 ml or 400 ml). The size of beaker depended upon the amount of water or milk. Some beakers containing milk or water were covered with Saran wrap (Reynolds 914 Film) and heated in a microwave oven (Amana Radar Range model no. RR4) for various time intervals (0, 10, 20, 30, 40, 50, 60 and 120 seconds). Other beakers containing milk or water were heated without a Saran wrap covering. After each microwave exposure, the temperature of the solution was determined with a YSI Model 42SC-Tele-Thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). This experiment was performed in triplicate.

A similar experiment was performed to resemble the effect of microwave heating on milk in a small glass. A volume of 200 ml of either sterile skim milk or raw milk (obtained from The Ohio State University dairy herd) was added to a sterile 250 ml Pyrex beaker and heated in the microwave oven for 0, 1, 2, 3 and 4 minutes. The temperatures at the top, middle, bottom and side of the milk were determined with the YSI Model 42SC Tele-Thermometer.
b) Aseptic Meat Preparation

The procedure described by Christopher et al. (1982) was used to obtain aseptic meat. A 10 lb. beef roast was purchased from a local retail store. The outer surface of the roast was alcohol flamed and then charred to destroy surface contamination. The exterior portion of the meat was aseptically removed with sterile instruments. The interior portion of the meat was cut into cubes of approximately 2.2 x 2.2 x 2.2 cm. The cubes were weighed and trimmed to provide a weight of 10 g per cube. Each cube was transferred individually to a Whirlpak bag (model no. B1065; 8 oz.; Nasco) and frozen at -20°C.

Several meat cubes were removed from frozen storage, thawed and tested for sterility. Each cube was transferred to a stomacher bag. The Whirlpak bag was rinsed with a portion of 90 ml of 0.1% peptone water (Difco). The rinse water and the remainder of the 90 ml of peptone water were transferred to the stomacher bag and stomached (Colworth Stomacher 400) for two minutes. Aerobic plate counts were performed by using the pour plate technique with plate count agar (Difco). These plates were incubated at 35°C for 48 hr. C. jejuni counts were determined by spread plating the sample onto prepoured Campy-BAP (Blaser et al. 1979a). These plates were incubated microaerophilically at 42°C for 48 hr. All plates were enumerated with the aid of a Quebec colony counter.
c) **Heat Penetration of Meat**

The cubes of meat that were aseptically collected and frozen were thawed at 4°C. The metal tie was removed from each of the Whirlpak bags with a sterile scissors, and the meat samples were microwaved for 0, 10, 20, 30, 40, 50 and 60 seconds. The temperature of the top, center, bottom and side of each sample was determined by using the YSI model 42SC Tele-Thermometer. This experiment was repeated three times.

d) **Culture Preparation**

Two strains of *C. jejuni*, 29428 and L-7, were obtained from the USDA Beltsville Agriculture Research Laboratory. The cultures were stored according to Luechtefeld et al. (1981b). A 24 hr viable culture was centrifuged (RC2-B Centrifuge; GSA Rotor; Sorvall) for 20 minutes at 16,300 x g at 4°C. The resultant pellet was resuspended in sterile 0.85% saline. The optical density was adjusted to 0.05 at wavelength 540 nm with a spectrophotometer (Spectronic 20).

e) **Microwave Heating of Inoculated Distilled Water and Milk**

A 39.6 ml volume of either sterile skim milk or distilled water in a Pyrex beaker (250 ml) was inoculated with 0.4 ml of culture. Each beaker was covered with Saran wrap and heated for 0, 10, 20, 30,
40, 50, 60 and 120 seconds. After each time interval of microwave treatment, the beaker was immersed in an ice-water bath and, after cooling, the sample was analyzed for \textit{C. jejuni}. The procedure for enumerating \textit{C. jejuni} was the same as already described, except that brucella agar (Difco) supplemented with 5% defibrinated horse blood (BAB) was used instead of Campy-BAP agar. This experiment was repeated three times for each strain.

A 200 ml volume of either raw or sterile skim milk was aseptically transferred to a sterile 250 ml Pyrex beaker. \textit{C. jejuni} was inoculated at a level of $10^5$-$10^6$ per ml of milk. The milk was heated in the microwave oven for 0, 1, 2, 3 and 4 minutes. After each heating period, the contents in the beaker were analyzed for \textit{C. jejuni}. The BAB plates were used when enumerating the sterile skim milk and the Campy-BAP plates were used when analyzing the raw milk. This experiment was repeated three times for each strain.

\textbf{f) Microwave Heating of Inoculated Meats}

When needed, an appropriate number of bags containing aseptically obtained frozen meat were stored at 4°C to thaw the meat. A 0.1 ml suspension of standardized culture was inoculated into the center of each thawed meat cube with a tuberculin syringe. The metal tie was removed from the Whirlpak bags and the inoculated cubes were
heated for 0, 10, 20, 30, 40, 50 and 60 seconds in the microwave oven. Each of the heated cubes was transferred to a separate stomacher bag. The Whirlpak bag was rinsed with a portion of 90 ml of 0.1% peptone water. All of the diluent was transferred to the stomacher bag and the sample was stomached for two minutes. *Campylobacter* counts were performed as previously described using BAB agar.
Experiment 10 - Iron Dextran Enhanced Colonization of Campylobacter jejuni in Adult Mice

BALB/C mice were obtained from the Charles Rivers Animal Co. The mice were fed antibiotic free animal feed (Ralston Purina Mouse Chow) (Model no. 5051; St. Louis, MO) and water ad libitum.

Campylobacter jejuni 29428 was obtained from the USDA Beltsville Agriculture Research Laboratory and strain HIMONT was obtained from the Children's Hospital (Columbus, OH). All cultures were stored according to Luechtefeld et al. (1981b). When needed, a culture was streaked onto brucella agar (Oxoid) supplemented with 5% defibrinated horse blood. The plates were incubated microaerophilically at 42°C for 48 hr. The inoculum was prepared by inoculating a loopful of this viable pure culture into brucella broth (Oxoid) and incubating it for 24 hr as stated above. The culture was centrifuged (RC2-B Centrifuge; GSA Rotor; Sorvall) for 20 minutes at 4°C at 16,300 x g. The pellet was resuspended in 0.85% saline and the turbidity was adjusted with a spectrophotometer (Spectronic 20) to 0.10 at wavelength 540 nm.

All mice were examined for two days prior to each experiment to determine if they were excretors of C. jejuni. No C. jejuni was recovered from the fecal pellets of the mice prior to the start of an experiment.
a) A group of ten mice were used for each experiment. Five mice were fed intragastrically (with a blunted 18 ga. needle) 0.1 ml of C. jejuni 29428 suspended in saline. This inoculum provided an approximate number of $10^8$ C. jejuni. Five mice were fed 0.1 ml of sterile saline intragastrically. Each mouse was tested daily for 12 days. They were weighed to determine any weight change that might occur. Blood smears were obtained via the tail vein. The smear was methanol fixed, giemsa stained and observed for the presence of C. jejuni in the blood. Fecal pellets were collected from each mouse and suspended in 10 ml of cary blair diluent (Luechtefeld et al. 1981a). The consistency of each pellet was observed (hard, soft mucusy) and recorded. The pellet was transferred to a Whirlpak bag (model no. B1065; 18 oz.; Nasco) and stomached (Colworth Stomacher 400) for two minutes. Two slides were prepared from this suspension. One slide was air dried, methanol fixed and giemsa stained. It was observed microscopically for blood cells. The other slide was air dried, heat fixed and gram stained (1% carbol fuschin counterstain). It was observed microscopically for C. jejuni. A 0.1 ml suspension of the stomached feces was spread plated onto prepoured Campy-BAP (Blaser et al. 1979) agar and incubated microaerophilically at $42^\circ$C for 48 hr. The typical C. jejuni colonies were presumptively identified by motility as well as catalase and oxidase production. Motility was determined with the aid of a
darkfield microscope. This experiment was repeated three times for strain 29428. The same experiment was repeated two times for strain HIMONT.

A similar set of experiments was conducted in which the organism was suspended in a 1% solution of iron dextran (no. D-8517; Sigma Chemical Co.; St. Louis, MO). This suspension was fed intragastrically (0.1 ml) to five mice. Five additional mice were fed 0.1 ml of 1% iron dextran in saline solution intragastrically. Each mouse was tested for 12 d as stated earlier. This experiment with iron dextran was repeated two times for strain 29428 and two times for strain HIMONT.

b) A group of 24 adult BALB/C mice was fed C. jejuni 29428 suspended in 1% iron dextran, raw milk or phosphate buffered saline. Each day, 2 mice were cervically dislocated. The mice were aseptically opened and the stomach, intestines, cecum and colon were removed aseptically. The intestine was separated into three sections of equal length. All tissues were suspended into 10 ml of 0.1% peptone water (Difco) in a Whirlpak bag. The contents inside the tissue were extruded into the 0.1% peptone water. Then, the contents in the bag were stomached for 2 minutes. The mixed sample was spread plated onto Campy-BAP agar (Blaser et al. 1979) and incubated microaerophilically at 42°C.
for 48 hr. Typical colonies were presumptively identified by observing for motility, and testing for catalase and oxidase production.

An additional experiment was performed to determine if the organism was removed totally from the tissue by stomaching. Any tissue fragments that remained in the bag after stomaching were washed two times in 10 ml of peptone water and then transferred to 10 ml of Doyle and Roman (1982c) enrichment broth in a screw-capped tube. The tube was incubated for 24 hr as stated above. After incubation, its contents were transferred to a Whirlpak bag, stomached and plated for C. jejuni as previously described.
Experiment 1 - Prevalence of Campylobacter jejuni in Retail Meats

A survey was conducted to determine the prevalence of Campylobacter jejuni in retail meats. Beef cubes were spiked with C. jejuni in a blind study to predict the recovery potential of the procedure chosen to detect this organism. This blind study also tested the accuracy of the experimenter. This study was performed before and after the survey. Results from these tests are observed in Table 5.

The recovery percentage is greater after the survey than before. This can be expected because experience with a given procedure, increases the competency of the experimenter. However, these results may be misleading since different levels of inoculum were used in the two testing periods. According to Doyle and Roman (1982c), whose procedure was employed in this survey, the level of detection is 0.1 cell per gram of food. In the first spiked testing, C. jejuni was inoculated at levels of 1.8 and 180 C. jejuni cells per 25 g of beef cube. This is only 0.072 and 7.200 cells of C. jejuni per gram of beef. The former level is below the limits of detection reported for this system. With this level of C. jejuni, only 20% recovery was noted. However, when the results of the first samples inoculated with 180 cells per sample (80% recovered) and the results of the
TABLE 5. THE RECOVERY OF *CAMPYLOBACTER JEJUNI* FROM BLIND INOCULATED MEAT SAMPLES

<table>
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<th>Sampling Time</th>
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<th>May, 1984</th>
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<tbody>
<tr>
<td>Inoculum per Sample $^1$</td>
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<td>No. Pos/ No. Sample</td>
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<td>1/5</td>
</tr>
<tr>
<td>% Recovery/Inoculated Sample</td>
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<td>20</td>
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$^1$Inoculum data obtained from the Beltsville Research Laboratory
second samples with 48 cells per sample (100% recovered) are compared, it is obvious that the researcher was also not as proficient in the detection of C. jejuni during the first analysis. It should be noted that no false positive results were reported during either sampling time.

Results from the survey of retail meats are listed in Tables 6-9. The detection rate of meat naturally contaminated with C. jejuni ranged from 0% (ground beef) to 35% (chicken) (Table 10). Park et al. (1981) reported the recovery of C. jejuni from 54% of 50 fresh eviscerated whole chickens purchased in Ohio. One possible explanation to account for this difference in the recovery of C. jejuni from chicken is that different detection systems were used. Both systems recover a similar level of C. jejuni from food, however it could be possible that the system employed by Park et al. (1981) is better suited for the detection of C. jejuni from chickens. Another explanation is that this variation is due to the different chickens that were sampled in the two experiments. When chickens at four chicken ranches were sampled for the presence of C. jejuni, a range of 0-86% of the chickens contained C. jejuni (Smitherman et al. 1984). It appears that brooder facilities can be maintained to give a C. jejuni free environment. The presence or absence of this organism at a given chicken ranch could influence the recovery of this organism from the chickens purchased at the retail store.
<table>
<thead>
<tr>
<th>Product</th>
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<tbody>
<tr>
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<td>Kroger</td>
<td>Total</td>
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<tr>
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<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Lamb Stew Meat</td>
<td>2/5</td>
<td>0/5</td>
<td>2/10</td>
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</table>

\(^{a}4/5\  \ 4 = \text{number positive for } C. \text{jejuni}; 5 = \text{number analyzed.}\)
### TABLE 7. ISOLATION OF CAMPYLOBACTER JEJUNI FROM RETAIL MEATS DURING SEPTEMBER, 1983

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<sup>a</sup>1/5  1 = number positive for C. jejuni; 5 = number analyzed.
TABLE 8. ISOLATION OF CAMPYLOBACTER JEJUNI FROM RETAIL MEATS DURING DECEMBER, 1983

<table>
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<tr>
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<tr>
<td>Pork Sausage</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
<td>0/10</td>
</tr>
<tr>
<td>Ground Beef</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
<td>0/10</td>
</tr>
<tr>
<td>Flank Steak</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
<td>0/10</td>
</tr>
<tr>
<td>Lamb Stew Meat</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
<td>0/10</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 0/5 0 = number positive for \textit{C. jejuni}, 5 = number analyzed
<table>
<thead>
<tr>
<th>Product</th>
<th>Retail Store</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Big Bear</td>
<td>Kroger</td>
<td>Total</td>
</tr>
<tr>
<td>Chicken</td>
<td>2/5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/5</td>
<td>6/10</td>
</tr>
<tr>
<td>Pork Chops</td>
<td>0/5</td>
<td>0/5</td>
<td>0/10</td>
</tr>
<tr>
<td>Pork Sausage</td>
<td>0/5</td>
<td>0/5</td>
<td>0/10</td>
</tr>
<tr>
<td>Ground Beef</td>
<td>0/5</td>
<td>0/5</td>
<td>0/10</td>
</tr>
<tr>
<td>Flank Steak</td>
<td>0/5</td>
<td>0/5</td>
<td>0/10</td>
</tr>
<tr>
<td>Lamb Stew Meat</td>
<td>0/5</td>
<td>1/5</td>
<td>1/10</td>
</tr>
</tbody>
</table>

<sup>a</sup>2/5, 2 = number positive for *C. jejuni*, 5 = number analyzed
TABLE 10. THE INCIDENCE OF CAMPYLOBACTER JEJUNI IN SEVERAL TYPES OF MEAT

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. Positive/No. Tested</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>14/40</td>
<td>35</td>
</tr>
<tr>
<td>Pork Chops</td>
<td>1/40</td>
<td>2.5</td>
</tr>
<tr>
<td>Pork Sausage</td>
<td>1/40</td>
<td>2.5</td>
</tr>
<tr>
<td>Ground Beef</td>
<td>0/40</td>
<td>0</td>
</tr>
<tr>
<td>Flank Steak</td>
<td>1/40</td>
<td>2.5</td>
</tr>
<tr>
<td>Lamb Stew Meat</td>
<td>3/40</td>
<td>7.5</td>
</tr>
</tbody>
</table>
The total incidence of *C. jejuni* isolated from red meat was 3% with 7.5% of the lamb stew meat containing *C. jejuni*. This is higher than the incidence of *C. jejuni* in red meat reported in the United Kingdom (Turnbull and Rose 1982). Again, a different detection system was employed in the United Kingdom survey.

The highest levels of *C. jejuni* detection were during June, 1983 (16.7%) and March, 1984 (11.4%) (Table 11). The lowest levels of *C. jejuni* detection were during December, 1983 (0.0%) and September, 1983 (5.0%). These data show a seasonal trend in the detection of *C. jejuni* in retail meats. The highest percentage of detection of this organism occurred during the warmest month (June) and the lowest percentage of recovery was observed during the coldest month (December).

This study shows that *C. jejuni* is present in some fresh meats and that chickens are a prime source of this organism.
<table>
<thead>
<tr>
<th>Month</th>
<th>No. Positive/No. Analyzed</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>June, 1983</td>
<td>10/60</td>
<td>16.7</td>
</tr>
<tr>
<td>September, 1983</td>
<td>3/60</td>
<td>5.0</td>
</tr>
<tr>
<td>December, 1983</td>
<td>0/60</td>
<td>0.0</td>
</tr>
<tr>
<td>March, 1984</td>
<td>7/60</td>
<td>11.7</td>
</tr>
</tbody>
</table>
Experiment 2 - Identification of Non-Campylobacter Colonies on Selective Agars

During the survey of retail meats for *Campylobacter jejuni*, it was noticed that many non-Campylobacter colonies grew on the selective agars. The overall procedure entails a selective enrichment and a selective plating step. Both systems employ antibiotics, a microaerophilic atmosphere and an elevated temperature as selective characteristics. It would seem that very few microorganisms would grow with these conditions. However, at times it was difficult to detect *C. jejuni* due to overgrowth of other microorganisms. It was decided that the genera of these microorganisms should be identified. Once the contaminating microorganisms are known, possibly selective agents could be added to the selective systems in order to improve the recovery of *C. jejuni*.

A total of 217 isolates were selected randomly from all types of meat samples for identification. The data of the isolates recovered from each meat are shown in Table 12.

The genus *Bacillus* was the most frequent randomly chosen microbe obtained from the selective plates. It made up 24.8% of the genera identified. The next most frequent genus was *Citrobacter* which made up 9.7% of the randomly chosen isolates. A total of 18 different genera were identified.
<table>
<thead>
<tr>
<th>Genera</th>
<th>Chicken</th>
<th>Lamb Stew</th>
<th>Flank Steak</th>
<th>Ground Beef</th>
<th>Pork Chop</th>
<th>Pork Sausage</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter</td>
<td>2¹</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>20</td>
<td>9.2</td>
</tr>
<tr>
<td>Bacillus</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>15</td>
<td>8</td>
<td>15</td>
<td>54</td>
<td>24.9</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>21</td>
<td>9.7</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>4</td>
<td>2</td>
<td>13</td>
<td>6.0</td>
</tr>
<tr>
<td>Escherichia</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>4.6</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Hafnia</td>
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<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>Klebsiella</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>4.1</td>
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<tr>
<td>Leuconostoc</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>-</td>
<td>4</td>
<td>20</td>
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<tr>
<td>Proteus</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
<td>14</td>
<td>6.5</td>
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<tr>
<td>Pseudomonas</td>
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<td>3</td>
<td>5</td>
<td>-</td>
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<td>Serratia</td>
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<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>Shigella</td>
<td>-</td>
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<td>-</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>Streptococcus</td>
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<td>1</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>5</td>
<td>20</td>
<td>9.2</td>
</tr>
<tr>
<td>Vibrio</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>Zymomonas</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>0.9</td>
</tr>
</tbody>
</table>

¹Number of times detected
Eight of the genera identified resulted in 81% of the total number of microbes randomly isolated. These are in descending order: **Bacillus, Citrobacter, Acinetobacter, Leuconostoc, Streptococcus, Proteus, Enterobacter and Pseudomonas**. Making the assumption that these 8 genera were isolated from the selective plates more frequently because they made up the bulk of the contaminants on these plates, the inhibition of these genera would greatly improve this selective system for the detection of *C. jejuni* in meats. For this reason, the remainder of this discussion will be directed toward these genera. The species of each genus was not determined.

**Bacillus** was recovered from all of the meats. It was the most detected genus in all meats except chicken from which **Proteus** was the most prominent genus. The genus **Bacillus** consists of gram positive or gram variable sporeforming rods. These organisms are very ubiquitous, and are common isolates from various foods. Some species of this genus are able to grow microaerobically and at elevated temperatures. The spores of this organism are very resistant to adverse conditions.

The genus **Citrobacter** consists of gram negative non-sporeforming rods. This genus is a member of the coliform group. **Citrobacter** is found in food, water, feces and urine. It is normal flora of the intestinal tract of many humans (Buchanan and Gibbons 1974). It was
recovered from every meat except beef. Approximately 10% of the isolates were in this genus.

*Citrobacter* can utilize citrate as a sole carbon source. It will ferment glucose as well as other carbohydrates. *Campylobacter* neither ferments nor oxidizes carbohydrates. If the dextrose was removed from the brucella broth/agar, the growth rate of *Citrobacter* might be impeded.

Polymyxin B is supposed to inhibit the growth of *Citrobacter*. Possibly if the concentration of polymyxin B was increased, the growth of this organism might be controlled. There is a limit as to how much antibiotic can be added, since *C. jejuni* is sensitive to approximately 6.25 μg/ml of polymyxin B (Vanhoof et al. 1978).

*Acinetobacter* is a gram negative coccobacillus, and is commonly isolated from fresh and processed meat and poultry. Although labeled as a strict aerobe, this organism has been shown to grow in CO₂ enriched vacuum packaged meat. Polymyxin B is active against *Acinetobacter*.

*Leuconostoc* consists of gram positive cocci. This facultative anaerobe ferments various carbohydrates. It frequently requires amino acids for growth (Buchanan and Gibbons 1974). This organism is resistant to acidic conditions. *Leuconostoc* is an important
organism in the fermentation of fruit, vegetables and milk. Also, it has been a common spoilage organism of some foods. Again, the removal of dextrose from the media might hinder the growth of this organism. Vancomycin is bactericidal to Leuconostoc.

Streptococcus is a gram positive coccus. It is a facultative anaerobe and is important in both the fermentation and spoilage of food. It is found in the intestinal tract of man and animals. The antibiotic vancomycin is bactericidal to Streptococcus.

Proteus, a gram negative rod, is usually distinguished on culture media (agar surfaces) due to its characteristic swarming. It is found in the intestinal tract of man and animals. Proteus is important in the spoilage of eggs and meats. Trimethoprim and cephalothin are inhibitory toward Proteus.

Enterobacter is a short gram negative rod. These organisms are included in the coliform group. They are ubiquitous. They ferment several carbohydrates with the production of acid and gas. Cephalothin inhibits the growth of these organisms.

Pseudomonas is a gram negative rod. These bacteria are commonly associated with the spoilage of refrigerated foods, especially meats. These organisms are extensively distributed throughout nature. Pseudomonas is sensitive to polymyxin B and cephalothin.
Many of these contaminating organisms theoretically should be inhibited by the antibiotics incorporated in these selective systems. Possibly, the antibiotic concentrations are not sufficiently high or resistant strains were isolated. Another possibility is that there were too many contaminating microorganisms for the antibiotics to interact with every single cell.
Experiment 3 - Comparison of Blending Method, Time and Diluent for the Enumeration of Campylobacter jejuni

A study was conducted to determine the best mixing method, time and diluent combination for enumerating Campylobacter jejuni from skim milk.

The results of this experiment are diagrammed in Figure 3. The statistical analysis test of Duncan's multiple range test for variable counts showed that there were no significant differences between blending methods, except when FBP-peptone diluent was used. With this diluent, the Stomacher resulted in significantly higher counts than did the Waring blender at the 5% level of confidence.

When comparing the different blending times, there were no significant differences between blending a spiked skim milk sample for either 1, 2 or 4 minutes.

When the diluents were compared, there were no significant differences between the results obtained using either peptone water, cary blair diluent or brucella broth. All three diluents yielded similar counts. However, the use of any of these three diluents resulted in significantly higher counts than those obtained from using the FBP-peptone diluent.
Figure 3. The effect of blending method, time and diluent on the recovery of *Campylobacter jejuni* from milk [□ Waring blender (WB) - peptone (P), ○ WB-brucella broth (BB), △ WB-Cary blair (CB), ◇ WB-FBP, ■ Stomacher (S) - P, ● S-BB, ▲ S-CB, ◆ S-FBP].
C. jejuni is an aerotolerant bacterium. It was suspected that the type of blending might influence counts of C. jejuni. The Waring blender produces excessive foam when certain foods are mixed. Less foam is produced in the Stomacher. Foam production is an indication of air being beaten into a sample. During this experiment, foam production was observed when the Waring blender was used, but not when the Stomacher was the mixer. No significant differences in isolations of the organism were obtained when either blender was used.

A blending time of one minute is suggested when enumerating Clostridium perfringens in foods (FDA 1978). It is thought that a longer blending time may decrease the chances of recovering this aerotolerant microbe in foods. When the blending time was extended to four minutes in the enumeration of C. jejuni, no significant reduction of counts of C. jejuni was noted.

Diluents containing oxygen and oxygen free radical scavengers were tested to increase the aerotolerance of C. jejuni. C. jejuni has shown an increased resistance to oxygen under adverse conditions when incubated in brucella broth (Doyle and Roman 1982d). Sodium bisulfite (0.1%) was thought to be the agent that increased the aerotolerance of this organism. Cary blair diluent contains sodium thioglycollate which may increase the aerotolerance of C. jejuni (Luechtefeld et al. 1981a). The addition of 0.025-0.05% of each of
FeSO₄·7H₂O, sodium metabisulfite and sodium pyruvate (FBP) to brucella agar increases the aerotolerance of this organism (George et al. 1978). Peptone water was used as a control. The addition of oxygen quenchers did not increase the recovery of *C. jejuni* from skim milk. The use of the newly devised FBP-peptone diluent resulted in significantly lower counts that did the other three diluents. Possibly the concentration of FBP should be reduced in the broth diluent than what is suggested for use in an agar plate. Chemicals are more accessible to an organism in a broth than in an agar.

It appears that the blending method, time and diluent are not critical steps in the enumeration of *C. jejuni* from skim milk.
Experiment 4 - Survival of Campylobacter jejuni in Bovine Feces

A study was conducted to determine how long Campylobacter jejuni would survive in bovine feces at 25 and 4°C. The survival of three strains of this organism inoculated into bovine feces and stored at 25°C is shown in Figure 4. A slow decrease in C. jejuni occurred over the first 16 days of storage during which a 2 log decrease was noted. A more rapid reduction of C. jejuni was noted over the next 4 days of storage and by day 20, C. jejuni was not detected. The lower limit of detection was $10^2$ C. jejuni per gram of feces. This level was determined due to overgrowth of non-Campylobacter colonies on the Campy-BAP surfaces. The aerobic plate count increased approximately 0.5 of a log from $10^8$ per gram of feces during the first 4 days of storage. It remained relatively the same until day 24 when a decrease of 0.5 log was detected. All three of the inoculated C. jejuni strains demonstrated the same survival rates in bovine feces at 25°C. Due to this observation, only strain 29428 was tested for its survival in bovine feces at 4°C.

A gradual decrease in the number of C. jejuni was noted when it was stored in bovine feces at 4°C (Figure 5). No C. jejuni was detected by 8 weeks of incubation. The aerobic plate count remained consistently around $10^8$ colony forming units per gram of feces during the 8 week storage period.
Figure 4. Survival of *Campylobacter jejuni* in and the aerobic plate count of bovine feces stored at 25°C (○ APC, △ strain L-7, □ strain 5-4 ◇ strain 29428).
Figure 5. Survival of *Campylobacter jejuni* and the aerobic plate count in bovine feces at 4°C (○ aerobic plate count, □ strain 29428).
C. jejuni can remain viable for at least 20 days at 25°C and for 8 weeks at 4°C in bovine feces. Only a low dose of C. jejuni is believed to be needed to cause an enteric response (Robinson 1981). There is an ample number of C. jejuni present up to 20 days at 25°C, and up to 8 weeks at 4°C to cause illness. These data demonstrated that C. jejuni can survive for an extended time in bovine feces and may be an important vehicle by which C. jejuni can contaminate cows' milk. With the extended survival of this organism, especially during colder months, individuals coming into contact with cattle and cattle manure should disinfect clothing and wash their hands carefully before eating, drinking, smoking or performing any other function that would elicit hand to mouth contact.
Experiment 5 - Effect of Iodine Udder Wash on *Campylobacter jejuni*

The previous study showed that fecal contamination may be a potential source of *C. jejuni* for cows' milk. It was determined that a common sanitizing agent used on cows' teats should be evaluated on its effect on *C. jejuni*.

The iodine udder wash was tested at the manufacturer's recommended concentration of 25 ppm. Also, it was suggested by Campbell and Marshall (1975) that iodophors should be inhibitory toward most microorganisms at a concentration of 25 ppm and an exposure time of 2 minutes.

Two levels of *C. jejuni* (10⁷ and 10³) per ml of test solution were treated to determine the effect of the iodine udder wash. When 10⁷ *C. jejuni* was used, a rapid reduction in colony forming units occurred and by one minute of iodine exposure, a 3 log reduction of *C. jejuni* was determined (Figure 6). No *C. jejuni* (< 1 per ml) was noted by 8 minutes of exposure. When 10³ *C. jejuni* was tested, no colony forming units were detected on the 10⁹ dilution by 1 minute of iodine treatment.

It appears that if the iodine udder wash is used properly, according to the manufacturer's directions, the risk of fecal contamination of *C. jejuni* of cows' milk should be reduced.
Figure 6. The effect of an iodine solution on *Campylobacter jejuni* 
($\Delta 10^7$/ml control, ▲ $10^7$/ml iodine treated, ○ $10^3$/ml control, ● $10^3$/ml iodine treated).
Experiment 6 - Effect of Hydrogen Peroxide on Campylobacter jejuni in Milk

Various concentrations of hydrogen peroxide were tested for their effect on three strains of Campylobacter jejuni inoculated into sterile skim milk and raw milk. Figure 7 shows the effect of hydrogen peroxide on C. jejuni 29428 inoculated into sterile skim milk and stored at 4°C. When C. jejuni 29428 was interacted with 0.005% hydrogen peroxide a 1 log reduction of colony forming units was noted by 60 minutes. When 0.01% hydrogen peroxide was tested, a 4 log reduction was observed by 60 minutes. When 0.05% hydrogen peroxide was tested, no C. jejuni was observed by 30 minutes of incubation.

Figure 8 shows the effect of hydrogen peroxide on C. jejuni strain 5-4 in sterile skim milk. With a hydrogen peroxide concentration of 0.005%, a 2 log reduction of C. jejuni 5-4 was detected by 60 minutes. A similar reduction in C. jejuni 5-4 was observed with a concentration of 0.01% hydrogen peroxide. No C. jejuni 5-4 cells were observed by 30 minutes when 0.05% hydrogen peroxide was added.

Figure 9 shows the effect of hydrogen peroxide on Campylobacter jejuni L-7 inoculated into sterile skim milk and stored at 4°C. A 2 log reduction of C. jejuni L-7 was observed by 60 minutes when
Figure 7. The Effect of Various Concentrations of $H_2O_2$ on *Campylobacter jejuni* Strain 29428 Inoculated into Sterile Skim Milk.

( ● 0% $H_2O_2$, ○ 0.005% $H_2O_2$, ▲ 0.01% $H_2O_2$, △ 0.05% $H_2O_2$)
COLONY FORMING UNITS/ML (LOG$_{10}$)
FIGURE 8. The Effect of Various Concentrations of $H_2O_2$ on Campylobacter jejuni Strain 5-4 Inoculated into Sterile Skim Milk

(● 0% $H_2O_2$, ○ 0.005% $H_2O_2$, ▲ 0.01% $H_2O_2$, △ 0.05% $H_2O_2$)
FIGURE 9. The Effect of Various Concentrations of $H_2O_2$ on Campylobacter jejuni Strain L-7 Inoculated into Sterile Skim Milk

(● 0% $H_2O_2$, ○ 0.005% $H_2O_2$, ▲ 0.01% $H_2O_2$, △ 0.05% $H_2O_2$)
COLONY FORMING UNITS / ML (LOG_{10})

TIME (MIN)
0.005% hydrogen peroxide was interacted with this organism. Approximately a 4 log reduction of colony forming units was observed when C. jejuni L-7 was interacted with 0.01% hydrogen peroxide for 60 minutes. When 0.05% hydrogen peroxide was tested with C. jejuni L-7, only a 3 log reduction was noted by 30 minutes of incubation. This can be contrasted from a > 6 log reduction for the same time interval for strains 29428 and 5-4. However, when strain L-7 was incubated with 0.05% hydrogen peroxide for an additional 30 minutes, no C. jejuni was detected.

When C. jejuni strain 29428 was added to raw milk and the milk was then treated with 0.05% hydrogen peroxide, no C. jejuni colony forming units were detected by 30 minutes of incubation at 4°C (Figure 10). Using the same concentration of hydrogen peroxide the aerobic plate count from raw milk remained unchanged through the testing time, however, the psychrotrophic count was undetectable after 10 minutes of hydrogen peroxide treatment (Figure 10).

With the addition of hydrogen peroxide, the risk of C. jejuni gastroenteritis transmitted by raw milk may be reduced. It should be noted that different strains show differences in susceptibility towards hydrogen peroxide. The reduction in the risk of C. jejuni transmission can be attributed to the sensitivity of this organism to hydrogen peroxide. According to Hoffman et al. (1979a), C. jejuni is
FIGURE 10. The Effect of 0.05% $\text{H}_2\text{O}_2$ on *Campylobacter jejuni* Strain 29428 Inoculated into Raw Milk

(● *C. jejuni* 0% $\text{H}_2\text{O}_2$, ▲ *C. jejuni* 0.05% $\text{H}_2\text{O}_2$, ○ APC 0% $\text{H}_2\text{O}_2$, △ APC 0.05% $\text{H}_2\text{O}_2$, □ Psychrotrophs 0% $\text{H}_2\text{O}_2$, ■ Psychrotrophs 0.05% $\text{H}_2\text{O}_2$)
more sensitive to hydrogen peroxide and superoxide anions than are other aerotolerant bacteria. This observation might explain why _C. jejuni_ was eliminated from raw milk with the addition of hydrogen peroxide, but the number of aerobic mesophilic bacteria remained the same. This indicates that there are many hydrogen peroxide resistant microorganisms in this food. Some of these microorganisms might be pathogenic, therefore, the consumption of raw milk should not be recommended.
Experiment 7 - Effect of Sodium Diacetate on Campylobacter jejuni

Sodium diacetate is an effective inhibitor of rope formation and mold growth in bread. The use of sodium diacetate as an inhibitor of the growth of Campylobacter jejuni was evaluated.

The effect of 0.45% sodium diacetate on the growth of C. jejuni at 42°C is shown in Figure 11. When C. jejuni was incubated in two sodium diacetate-brucella broth solutions (ethylene oxide treated and added aseptically both at pH 4.6), a 5 log decrease was observed by 12 hr. A level of < 10 C. jejuni per ml was detected by 12 hr and 24 hr in the aseptically added and ethylene oxide sterilized sodium diacetate, respectively. Some sodium diacetate was sterilized with ethylene oxide. It was felt that ethylene oxide may remain in the treated sodium diacetate and alter its antimicrobial properties. To determine if any residual ethylene oxide remained in the treated sodium diacetate, another set of sodium diacetate-brucella broth solutions were tested in which the sodium diacetate was added aseptically without a presterilization treatment to sterile brucella broth. Similar counts were obtained in the ethylene oxide and aseptically added samples. This indicates that no ethylene oxide residue remained in the treated sample. Also few, if any, organisms were in the sodium diacetate even without sterilization.
Figure 11. The effect of 0.45% sodium diacetate aseptically added or sterilized with ethylene oxide on *Campylobacter jejuni* at 2 pH levels at 42°C (○ 0.0% sodium diacetate (SD) pH 7.0, ● 0.0% SD pH 4.6, ▽ 0.45% SD added aseptically pH 7.0, ▼ 0.45% SD added aseptically pH 4.6, □ 0.45% SD ethylene oxide sterilized pH 7.0, ■ 0.45% SD ethylene oxide sterilized pH 4.6)
The pH of both of these sodium diacetate-brucella broth solutions was 4.6. The brucella broth control had a pH of 7.0. Additionally, the brucella broth was adjusted with hydrochloric acid to pH 4.6 and the sodium diacetate-brucella broth solution was adjusted to pH 7.0 with sodium hydroxide to determine if the low pH was the sole inhibitory property of sodium diacetate. When brucella broth was adjusted to pH 4.6 and inoculated with C. jejuni, a 4 log decrease was observed by 12 hr. A level of < 10 colony forming units per ml was detected by 24 hr. At pH 7.0, the brucella broth control showed an increase in colony forming units. In the sodium diacetate-brucella broth solutions at pH 7.0, a gradual decrease in colony forming units was noted in both the ethylene oxide treated and the aseptically added samples. By 24 hr, the decrease had leveled off at a > 3 log decrease and remained at that level during the 48 hr incubation period.

An additional test was performed in which the sodium diacetate was added before the brucella broth was sterilized and then the solution was autoclaved for 20 minutes at 121°C. The pH of the autoclaved sodium diacetate-brucella broth solution was 4.9. The pH of the brucella broth and the sodium diacetate-brucella broth solutions were adjusted to pH 4.9 and 7.0, respectively. This again was to determine if the pH was the primary inhibitory agent of this chemical. Comparing the sodium diacetate at pH 4.9 to the control brucella broth at pH 4.9, it can be observed at 24 hr of
incubation, there was a 3 log greater decrease in colony forming units in the sodium diacetate-brucella broth solution as compared to brucella broth alone (Figure 12). At pH 7.0, the final concentration of cells enumerated from the sodium diacetate-brucella broth solution was lower than that of brucella broth at pH 7.0. Growth was observed in both broths at pH 7.0.

The inhibitory effect of autoclaved sodium diacetate at 42°C on *C. jejuni* was less than the aseptically added or ethylene oxide sterilized sodium diacetate. Sodium diacetate is a molecular compound of sodium acetate and acetic acid. The boiling point of acetic acid is 118°C. The difference in inhibition of *C. jejuni* in the autoclaved sodium diacetate compared to the aseptically added or ethylene oxide treated sodium diacetate may be attributed to the fact that during autoclaving some volatile acetic acid was driven off. This could also explain the difference in the pH's of the autoclaved sodium diacetate solution (pH 4.9) and the ethylene oxide treated or aseptically added sodium diacetate solutions (pH 4.6).

Figure 13 shows the effect of ethylene oxide sterilized sodium diacetate on *C. jejuni* at 25 and 4°C. This organism does not grow at either temperature. At 25°C, a gradual decrease in colony forming units was detected in the ethylene oxide sterilized sodium diacetate. By 48 hr of *C. jejuni* - sodium diacetate interaction, no colony
FIGURE 12. Response of Campylobacter jejuni to autoclaved sodium diacetate-brucella broth and brucella broth at pH 4.9 and 7.0 incubated at 42°C. ( O brucella broth a pH 7.0, • sodium diacetate-brucella broth pH 7.0, ▼ brucella broth pH 4.9, ▽ sodium diacetate-brucella broth pH 4.9)
FIGURE 13. The effect of 0.45% sodium diacetate sterilized with ethylene oxide on *Campylobacter jejuni* incubated at 25°C and 4°C
(〇 0% sodium diacetate (SD) - 4°C, ● 0.45% SD - 4°C,
▽ 0% SD-25°C, ▼ 0.45% SD-25°C).
forming units were detected. When the sodium diacetate was autoclaved (Figure 14), the inhibitory effect was reduced. Only a 1 log decrease was detected over a 48 hr incubation period at 25°C. Very little reduction in colony forming units was noted in the ethylene oxide sterilized sodium diacetate (Figure 13) or the autoclaved sodium diacetate (Figure 14) when they were stored at 4°C.

Sodium diacetate is considered to be a weak organic acid. Weak organic acids at low pH values tend to be undissociated. According to Eklund (1983) an undissociated acid may be from 10-600 times more inhibitory towards bacterial cells than a dissociated acid. Undissociated acids can enter the cell more readily than dissociated acids. Once inside the cell, the undissociated acid can ionize and lower the internal pH of the cell (Salmond et al. 1984). This low pH may interfere with intracellular enzymes or other intracellular components. Hydrochloric acid dissociates readily and does not pass through the cell membrane as easily as an undissociated acid; therefore, hydrochloric acid may be a less effective inhibitor of microbial cells.

In this experiment, it is apparent that the bacteriostatic or bactericidal effect of sodium diacetate on C. jejuni was decreased when the temperature was below the optimal growth temperature of 42°C. Similar results were observed in the inhibition of C. jejuni
FIGURE 14. Response of Campylobacter jejuni to autoclaved sodium diacetate at 25°C and 4°C (○ brucella broth at 25°C, ▼ sodium diacetate-brucella broth at 25°C, ● brucella broth at 4°C, ▼ sodium diacetate-brucella broth at 4°C)
with spices (Deibel and Banwart 1984), hydrochloric acid (Doyle and Roman 1981), sodium chloride (Doyle and Roman 1982b), sensitivity to drying (Doyle and Roman 1982a) and survival in inoculated meat (Gill and Harris 1982b).

In an outbreak of gastroenteritis in which cake icing was involved as the vehicle of C. jejuni, it was believed that cooked icing was contaminated by a food handler. Most bakery products are stored at room temperature (25°C). Figure 13 shows that 0.45% sodium diacetate can inactivate C. jejuni in a broth culture at 25°C. Autoclaving the sodium diacetate, reduces the bactericidal property of this chemical (Figure 14). Heating, such as by baking, might reduce the antimicrobial properties of sodium diacetate. If this occurs, post-cooking contamination of C. jejuni such as in the outbreak described by Blaser et al. (1982), the heat treated sodium diacetate may not be as effective in destroying this organism as the untreated chemical.
Experiment 8 - Effect of Spices on Campylobacter jejuni

Inhibitory (Beuchat 1976) and stimulatory (Kissinger and Zaika 1978; Zaika and Kissinger 1981) effects of oregano have been reported. When C. jejuni was subjected to oregano at 42°C for 16 hr, the 0.1% level showed a slight inhibition (Figure 15). However, when 0.5% oregano was added to the growth medium, a large decrease in colony forming units of C. jejuni was noted. When 1% oregano was tested, <30 colony forming units per ml were detected by 8 hr. Sage has been shown to have antibacterial activity against a wide spectrum of organisms (Shelef et al. 1980). When 0.1% sage was subjected to the culture at 42°C for 16 hr a slight inhibition was shown when compared to growth in the control broth (Figure 16). At the 0.5 and 1% levels of sage, a reduction of colony forming units was observed. By 16 hr of incubation of 1% sage and C. jejuni, <30 colony forming units per ml were detected. At the 0.1% level of ground cloves incubated at 42°C for 16 hr, a slight inhibition was observed when compared to the control (Figure 17). At the 0.5% level of ground cloves, the growth of C. jejuni was inhibited. Very little, if any, reduction in colony forming units was observed.

Beuchat (1976) reported that after an extended incubation period of Vibrio parahaemolyticus in media with added spice, cell growth was observed. Therefore, it was decided to determine the colony forming units of C. jejuni with an incubation time up to 48 hr at the three
FIGURE 15. Response of *Campylobacter jejuni* to Various Concentrations of Oregano at 42°C.

( □ = control, ■ = 0.1% ○ = 0.5% ● = 1.0%)
FIGURE 16. Response of *Campylobacter jejuni* to Various Concentrations of Sage at 42°C.

(□ = control, ■ = 0.1% ○ = 0.5% ● = 1.0%)
FIGURE 17. Response of *Campylobacter jejuni* to Various Concentrations of
Ground Cloves at 42°C.

( □ = control, ■ = 0.1% ○ = 0.5% ● = 1.0%)
COLONY FORMING UNITS (LOG$_{10}$)
temperatures. The lowest concentration of spice (0.5%) that was tested which showed inhibition or cell death was chosen.

Figure 18 shows the effects of the 0.5% level of the three spices at 42°C with an extended incubation time. Between 16 and 24 hr, an increase in colony forming units was observed in the presence of all three spices.

When 0.5% of each spice was individually tested at 25°C, a decrease in colony forming units was observed in the presence of either oregano or sage (Figure 19). The optimal temperature for \textit{C. jejuni} growth is 42°C and it will not grow at 25°C. Ground cloves showed results similar to the control with no spice added. At the end of a 48 hr interaction between spice and \textit{C. jejuni}, fewer viable cells were detected in the spice-broth mixtures held at 25°C than that at 42°C. When \textit{C. jejuni} was subjected to 0.5% of each spice at 4°C, very little, if any, reduction of colony forming units was evident at this temperature compared to the control (Figure 20). The concentrations of the three spices that were studied in this experiment may be used in the preparation of red meats and poultry. The effect of temperature on the inhibition of \textit{C. jejuni} by these spices shows that with a short incubation time (up to 16 hr), the greatest reduction in colony forming units was observed at 42°C with 25°C being less and 4°C showing the least death. After 16 hr of
FIGURE 18. Response of *Campylobacter jejuni* to 0.5% of Oregano, Sage and Ground Cloves at 42°C.

(□ = control, 〇 = ground cloves, ▲ = oregano, ● = sage)
FIGURE 19. Response of *Campylobacter jejuni* to 0.5% of Oregano, Sage and Ground Cloves at 25°C.

(□ = control, ○ = ground cloves, ▲ = oregano, ● = sage)
FIGURE 20. Response of *Campylobacter jejuni* to 0.5% of Oregano, Sage and Ground Cloves at 4°C.

(□ = control, ○ = ground cloves, ◇ = oregano, ● = sage)
incubation at 42°C, an increase in colony forming units was observed. This increase was not observed at 25°C or 4°C since _C. jejuni_ does not grow at either temperature. At 25°C, the 0.5% of sage or oregano lowered the number of _C. jejuni_ and this level was maintained during the course of this experiment. This study shows that these spices probably could not be effective in reducing the number of _C. jejuni_ in foods stored at refrigeration temperatures.
Experiment 9 - Campylobacter jejuni Survival in Meat Cubes and Milk
Heated in a Microwave Oven

Microwave cooking is a common system to thaw, heat or cook food. It is less expensive to operate and it heats foods faster than does the convection oven. With such a rapid rate of heating, much interest has been directed toward the effect of cooking food in a microwave oven on microorganisms. In this experiment, the effect of microwave heating on Campylobacter jejuni inoculated into milk and meat, was studied.

When C. jejuni was inoculated into 39.6 ml of sterile skim milk, very little reduction of colony forming units was observed after 10 seconds of microwave treatment (Figure 21). With continued treatment, a rapid reduction of C. jejuni was observed. By 50 seconds no cells were detected by plating the milk onto an agar surface. Similar results were obtained with strain 29428 and strain L-7. The temperature of this volume of skim milk after 50 seconds of treatment in the microwave oven was 93°C. A bubbling of the milk was observed at 45 seconds. When C. jejuni was heated at 60°C in skim milk, no survivors were detected after 1 minute of heating in a waterbath (Christopher et al. 1982a). When comparing the data shown in Figure 21 to that of Christopher et al. (1982a), a higher temperature was needed to inactivate C. jejuni in the microwave oven than in a waterbath.
Figure 21. The effect of microwave treatment on *Campylobacter jejuni* heated in 39.6 ml of sterile skim milk (○ strain L-7, □ strain 29428, ■ middle temp., ◆ side temp.)
A 200 ml quantity of skim or raw milk was put into a sterile 250 ml beaker, spiked with *C. jejuni* and subjected to a microwave treatment. This experiment was designed to determine how much microwave treatment is needed to inactivate *C. jejuni* in a small glass of milk. The results of the skim milk experiment are shown in Figure 22.

During the first minute of heating, the temperature of the milk rose to about 35°C and a small reduction in colony forming units was noted. When the milk was heated for 2 minutes in the microwave oven, a 4 log reduction was detected. After 3 minutes of microwave treatment, no *C. jejuni* cells were detected. The milk after treatment, had an average temperature of 80°C (Figure 22). When raw milk was spiked with *C. jejuni* strain 29428, the organism was undetectable by 3 minutes of treatment in the microwave oven (Figure 23). The sample boiled after treatment for 3.75 minutes. The temperature of the raw milk heated for 3 minutes in the microwave oven was 74°C. Figure 23 also demonstrates the uneven heating that occurs in a food that is being heated in a microwave oven. The center of the raw milk was the hottest point. When strain L-7 was added to raw milk, viable cells remained after 3 minutes of microwave treatment. However, no cells were detected after 4 minutes. The sample might have been heated beyond consumer acceptance because a skin was formed on the surface of the milk after it was heated for 4 minutes in the microwave oven. It should be noted that the heat resistance of *C. jejuni* in the microwave oven is strain and food dependent. Raw milk contains fat.
Figure 22. The effect of the microwave treatment on Campylobacter jejuni in 200 ml of skim milk ( O strain L-7, □ strain 29428, ● top temp., ▲ bottom temp., ■ center temp., ◆ side temp.).
Figure 23. The effect of the microwave treatment on *Campylobacter jejuni* heated in 200 ml of raw milk (○ strain L-7, □ strain 29428, ● top temp., ▲ bottom temp., ■ center temp., ◆ side temp.)
whereas skim milk is defatted milk. One possible reason that strain L-7 was still present in the raw milk and not in the skim milk after 3 minutes of microwave treatment is that the fat molecules are microenvironments that may protect the organism from heat.

It is recommended that milk that is suspected of contamination be brought to boiling in a microwave oven and allowed to remain for an additional 30-60 seconds. The best safety factor would be to discard the suspected milk.

Figure 24 shows the effect of treatment in the microwave oven on *C. jejuni* inoculated into beef cubes. Again, a very small reduction in colony forming units of *C. jejuni* was noted during the first 10 seconds of heating. By 20 seconds, a 2 log reduction was noted and by 30 seconds, a 3 log decrease was observed. No *C. jejuni* was detected after 40 seconds of heating in the microwave oven. The temperature of the center of the beef cube was 95.7°C. The meat cubes were determined to be completely cooked when no pink tissue was observed throughout the cube. This occurred after 40 seconds of microwave treatment. When beef roasts were spiked with *C. jejuni* and heated in a convection oven, no *C. jejuni* was observed when the internal meat temperature reached 57°C (Christopher et al. 1982b). A higher temperature is required to destroy *C. jejuni* in the microwave oven than in the convection oven.
Figure 24. Survival of *Campylobacter jejuni* inoculated into beef cubes and heated in the microwave oven ( ○ strain L-7, □ strain 29428, ● top temp., ▲ bottom temp., ■ center temp., ◆ side temp.)
These data demonstrate that heating in the microwave oven will destroy *C. jejuni* in skim milk, raw milk and beef cubes. They also show that some strains are more resistant to the microwave treatment and that some foods may protect the cells of *C. jejuni* from the action of microwaves.
Experiment 10 - Iron Dextran Enhanced Colonization of Campylobacter jejuni in Adult Mice

A study was conducted to attempt to infect adult BALB/C mice with Campylobacter jejuni. This strain of mouse reportedly is more susceptible to some bacterial infections than are other strains (Cheers et al. 1978).

In the first experiment, groups of 10 mice were used. Five mice were fed $10^8$ C. jejuni strain 29428 suspended in saline, the other five were fed saline. The mice were checked daily for 12 days for several signs of C. jejuni gastroenteritis. The stool was cultured for C. jejuni. No C. jejuni was detected from the fecal pellets of any mouse. Blood smears were negative for bacteremia. The stool was observed for signs of diarrhea. All mice had normal fecal pellets. The stool was analyzed via a giemsa stain for the presence of blood cells in the stool. This is a classical sign of C. jejuni gastroenteritis. No blood cells were observed on these slides. No typical Campylobacter cells were observed when the stools were gram stained. This experiment was repeated three times using three groups of mice with strain 29428. No C. jejuni, or signs of gastroenteritis were observed.

It was speculated that possibly the strain used was avirulent or unable to produce symptoms in this mouse model. Another strain of
C. jejuni (HIMONT) was obtained from the Children's Hospital (Columbus, Ohio) which was cultured from a diarrheic child. A similar experiment was repeated two times with this strain. Again, all results were negative. No C. jejuni was isolated from the stool, and the bloodsmear, gram stain and giemsa stain were all negative. The stool appeared to be normal for all mice.

After this study, a virulence enhancing agent (iron dextran) was used in conjunction with this mouse model. A similar experiment was designed in which groups of 10 mice were used and tested as before. The difference being that five mice were fed $10^8$ C. jejuni suspended in 1% iron dextran and five mice were fed a 1% iron dextran solution. This was repeated 2 times for strain 29428. C. jejuni was isolated from the stool of 2 of the 5 mice fed C. jejuni suspended in iron dextran. One was positive by day 3 and the other by day 4. All other tests were negative. These mice were asymptomatic excretors of the organism. All of the control mice were negative. When this experiment was repeated, 3 mice given the organism suspended in 1% iron dextran excreted C. jejuni in their feces. One mouse was positive after 1 day and the other 2 were positive by day 4. All other tests were negative. The same experiment was repeated twice with strain HIMONT. The first time this experiment was conducted using this strain, one mouse out of 5 fed C. jejuni in iron dextran gave a positive stool culture by day 2. All other tests were negative. The second experiment resulted in no mice excreting C. jejuni.
Iron dextran has been used to enhance the infective response of \textit{E. coli} and \textit{Salmonella} \cite{Suveges and Glavits 1976}. The role that iron dextran plays in increasing the virulence of enteric pathogens is unknown. It is thought that iron dextran may neutralize macrophages. Also iron is required by many microorganisms for growth. There are iron sequestering proteins (siderophores) that are found in the body that might bind up available iron \cite{Kochan et al. 1978}. When excess iron is added in the form of iron dextran, some may be available for growth of an organism.

These previous data indicated that \textit{C. jejuni} was being retained somewhere in the mouse. An experiment was designed to determine the location of \textit{C. jejuni} in the mouse. A group of 24 adult BALB/C mice were fed $10^8$ cells of strain 29428 suspended in 1\% iron dextran. Each day, 2 mice were cervically dislocated and the stomach, 3 sections of the small intestine, cecum and colon were removed and cultured for \textit{C. jejuni}. The results of this experiment are listed in Table 13. Initially, the lower third of the small intestine, cecum and colon were positive for \textit{C. jejuni}. The highest number of \textit{C. jejuni} was found in the cecum and the colon. By day 4, \textit{C. jejuni} was recovered only from the colon. By day 6, no \textit{C. jejuni} was recovered from any of these sections.

It was questioned if all of the \textit{C. jejuni} were removed from the tissue by the stomaching procedure. Tissue fragments that remained
TABLE 13. Detection of *Campylobacter jejuni* in the Intestinal Tract of BALB/C Adult Mice Fed This Organism Suspended in Iron Dextran

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<sup>a</sup> 0/2, 0 = no. mice with section containing *C. jejuni*, 2 = no. mice analyzed

<sup>b</sup> Small intestine was section in 3 equal lengths with Intestine 1 being the first third, etc.
In the bag after stomaching were transferred to an enrichment broth and incubated overnight. The tissue was then analyzed for the presence of *C. jejuni*. None of these tissue fragments contained any *C. jejuni*, as detected by the method used.

A further experiment was conducted to determine the effect of suspending *C. jejuni* in phosphate buffer or in raw milk instead of iron dextran. Groups of 24 mice were used in this experiment and 2 mice were sacrificed and sectioned daily as before. The results obtained by feeding phosphate buffer solution suspended *C. jejuni* are listed in Table 14. No *C. jejuni* was recovered from any tissue section over the 12 day testing period. The results of the raw milk suspended *C. jejuni* are shown in Table 15. No *C. jejuni* was recovered during this experiment.

It appears that the organism remains in the cecum and colon for several days in the presence of iron dextran. However, these animals were asymptomatic, and they did not appear to be acceptable models for reproducing campylobacteriosis.
TABLE 14. Detection of *Campylobacter jejuni* in the Intestinal Tract of BALB/C Adult Mice Fed This Organism Suspended in Phosphate Buffer

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<sup>a</sup> 0/2, 0 = no. mice with section containing *C. jejuni*, 2 = no. mice analyzed

<sup>b</sup> Small intestine was sectioned in 3 equal lengths with Intestine 1 being the first third, etc.
TABLE 15. Detection of *Campylobacter jejuni* in the Intestinal Tract of BALB/C Adult Mice Fed *C. jejuni* Suspended in Raw Milk

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Stomach</th>
<th>Intestine 1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Intestine 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Intestine 3&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Cecum</th>
<th>Colon</th>
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<td>1</td>
<td>0/2&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

<sup>a</sup> 0/2, 0 = no. mice with section containing *C. jejuni*, 2 = no. mice analyzed

<sup>b</sup> Small intestine was section in 3 equal lengths with Intestine 1 being the first third, etc.
An investigation to evaluate and determine the sources, methodology, survival, control and an animal model for pathogenicity of *Campylobacter jejuni* was conducted.

Six types of retail meats were analyzed for the presence of *C. jejuni* during four months, with each month representing a different season. The detection rate of meat contaminated with *C. jejuni* was: chicken (35%), lamb stew meat (7.5%), pork chops (2.5%), pork sausage (2.5%), flank steak (2.5%) and ground beef (0.0%). The highest level of *C. jejuni* was detected during June, 1983 (16.7%) and the lowest level was detected during December, 1983 (0.0%).

During the survey of retail meats for *C. jejuni*, it was noticed that many non-*Campylobacter* colonies grew on the selective agars. The genera of randomly selected colonies were determined. The most frequent randomly chosen microorganisms from the selective plates were in descending order: *Bacillus, Citrobacter, Acinetobacter, Leuconostoc, Streptococcus, Proteus, Enterobacter* and *Pseudomonas*. Possible measures to restrict the growth of these contaminating microorganisms on the selective agars are discussed.
A study was conducted to determine the best mixing method, time and diluent combination for enumerating *C. jejuni* from skim milk. It was suspected that blending this aerotolerant microorganism might reduce the count when enumerating *C. jejuni* in a food. The use of diluents that protect aerotolerant microorganisms and reduced blending times were also investigated. Results from this experiment show that the blending mechanism, time and diluent are not critical steps in the enumeration of *C. jejuni* from skim milk.

Raw milk is the most common food vehicle for the transmission of *C. jejuni*. It is theorized that milk can become contaminated with this organism through fecal contamination by cows that excrete *C. jejuni*. A study was conducted to determine how long this organism would survive in bovine feces at 25°C and 4°C. *C. jejuni* remained viable for at least 20 days at 25°C and 8 weeks at 4°C in bovine feces.

Since *C. jejuni* can survive in bovine feces for an extended period, an investigation was conducted to determine the effectiveness of a common sanitizing agent used on cows' teats to inactivate *C. jejuni* and therefore prevent fecal transmission of this organism into raw milk. The iodine udder wash, when used properly, can inactivate $10^6$ *C. jejuni* in less than 8 minutes.
Various concentrations of hydrogen peroxide were tested for their effect on *C. jejuni* inoculated into sterile skim milk and raw milk. When 0.05% hydrogen peroxide was tested in spiked skim milk, a greater than 6 log reduction in colony forming units of *C. jejuni* was noted with strains 29428 and 5-4. With the same concentration of hydrogen peroxide, it took 60 minutes of treatment before *C. jejuni* strain L-7 was undetectable. When *C. jejuni* strain 29428 was inoculated into raw milk and then treated with 0.05% hydrogen peroxide, no *C. jejuni* was detected by 30 minutes. The use of hydrogen peroxide will reduce the number of *C. jejuni* in milk.

The antimicrobial effect of sodium diacetate, oregano, sage and ground cloves on *C. jejuni* was temperature dependent. The greatest reduction of colony forming units of *C. jejuni* was observed at 42°C with 25°C being intermediate and 4°C showing the least death for all test chemicals.

The effect of the microwave oven treatment on *C. jejuni* inoculated into milk and meat was studied. It took 50 seconds of microwave treatment and a temperature of 92°C to inactivate $10^6$ *C. jejuni* spiked into sterile skim milk. When $10^6$ *C. jejuni* were inoculated into 10g beef cubes, a 40 second microwave treatment was required to destroy all of the spiked *C. jejuni*. The center temperature of the meat was 95.7°C. If these foods are suspected
to contain *C. jejuni*, they should be heated to >90°C in the microwave oven to be considered safe from transmission of this organism.

An attempt to reproduce campylobacteriosis in BALB/C mice was studied. When $10^8$ *C. jejuni* suspended in 1% iron dextran was fed to these mice, they became asymptomatic extretors of this organism for up to 5 days. An experiment was conducted to determine the location of *C. jejuni* in the digestive system of the inoculated BALB/C mice. It was recovered from the cecum and the colon. These mice and experimental parameters do not result in an acceptable model to reproduce campylobacteriosis.
BIBLIOGRAPHY


