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## SURVIVAL AND GROWTH OF CAMPYLOBACTER JEJUNI IN MEAT

(With 1 table)

By

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### نمو ميكروب الكامبيلوباكتر ومدى حيويته أثناء حفظ اللحوم في درجات الحرارة المختلفة

رمضان رفايي ، باهي الجمال

اختيرت عترة من ميكروب الكامبيلوباكتر وتم حقنها في اللحوم البقرى بعد تقطيعها الى مكعبات متساوية (حجم كل منها ٢,٢ x ٢,٢ x ٢,٢ سم). وبالكشف الدوري - لعد ذلك الميكروب - على مكعبات اللحم البقرى عند درجة حرارة ٢٠ - ٢٥ درجة مئوية بعد صفر ٢٤، ٤٨، ٧٢ ساعة وكذلك مكعبات اللحم المحفوظ عند درجة ٤ درجة مئوية بعد الصفر ٢، ٤، ٦، ٨، ١٠، ١٢، ١٤ يوم والمحافظة عند ٢٠ درجة مئوية بعد الصفر ١٠، ٢٠، ٣٠، ٤٠، ٥٠، ٦٠ اسبوع. تبين ان هذا الميكروب ينقص في العدد في درجات الحرارة المختلفة. وعند تسخين المكعبات عند درجات الحرارة ٥٠، ٥٥، ٦٠، ٧٠ درجة مئوية. وجد ان ميكروب الكامبيلوباكتر لا يستطيع مقاومة هذه الحرارة ويختفي نهائيا وقد تم مناقشة الامة الصحية لوجود الميكروب على الصحة العامة.

### SUMMARY

Experiments were carried out to assess the ability of *C. jejuni* to survive in fresh meat during storage at different holding temperature. Fresh meat were experimentally inoculated by *C. jejuni* strain after cutting aseptically into cubes (2.2x2.2x2.2 cm) each weighing approximately 10g. and then wrapped in aluminum foil. The samples were stored at different holding temperatures: 20-25 C for 72 h; 4 C for 14 days and -20 C for 6 weeks respectively. Decrease in *C. jejuni* counts occurred. Thermal inactivation occurred when the samples were heated at 50 C, 55 C, 60 C and 70 C. Public health importance of the obtained results were discussed.



## INTRODUCTION

There is increasing evidence that *Campylobacter fetus* subsp. *jejuni* can cause gastroenteritis in consumers if they consumed water or food (CHRISTOPHER et al., 1982). Both food and water have been implicated as vehicles responsible for transmitting *Campylobacters* to susceptible individuals. Foods derived from animals are of particular concern. Many species of animals, both wild and domestic, harbor *C. fetus* subsp. *jejuni* as part of their normal intestinal flora. Examples include poultry, swine, cattle and sheep. Since *C. fetus* subsp. *jejuni* may be present on meat or in milk obtained from such animals, it is important to know how these organisms will respond to conditions that may be present when foods are prepared and/or stored (DOYLE, 1981 and DOYLE and ROMAN, 1982).

More recently, contamination of meat by *C. fetus* subsp. *jejuni* is of concern for public health depends in part upon the survival of the organisms during storage and their ability to grow on raw meats or in any prepared dishes to which they be inadvertently transferred (GILL and HARRIS, 1981).

DOYLE and ROMAN (1981) reported that *C. Fetus* subsp. *jejuni* does not grow at temperatures of 30 C and below or at 47 C and above. This suggests that foods maintained at room temperature or below would not allow growth of *C. fetus* subsp. *jejuni*.

Little information is available regarding the survival of *C. fetus* in frozen or refrigerated raw animal foods and about the effect on *C. fetus* of commercial heat processes to which these foods are subjected in food processing plants or at home (CHRISTOPHER et al, 1982).

## MATERIAL AND METHODS

### Cultures:

*C. jejuni* strain used in this study was obtained from cultures made from carcasses of chickens collected from Assiut area. Isolated strain was purified and identified following the techniques of Bates (1981).

### Preparation of samples:

Biceps femoris muscles of beef was examined for presence of *C. jejuni* as follows: The surfaces of muscles were sterilized by means of hot spatula. A clean deep incision was made with a sterile scalpel and a sterile swabs was then rubbed along the incision and placed in screw capped bottles containing 5 ml of brucella broth (Difco).



The inoculated brucella broth tubes were incubated at 37 C in a Gaspak jar containing one injected envelope of *Campylobacter* microaerophilic system (Difco) for generating hydrogen and carbon dioxide inside the jar. Incubation was carried out for 72 hours, after which a loopful was taken from each tube and spread onto clean dry slide, covered and then examined under dark ground microscope for detection of motility. Tubes containing motile *M. Os.* having the characteristic cork-screw motility of *Campylobacter*, were subcultured onto *Campylobacter* selective media plates (SKIRROW, 1977) and incubated at 37 C in a Gas-pak jar at microaerophilic atmosphere for 72 hours. The plates were examined for growth and characters of *Campylobacter* colonies. Further identification was carried out according to the techniques of BATES (1981).

#### Survival of *C. jejuni* in meat:

When the muscle proved to be *C.* free were flamed thoroughly to destroy micro-organisms on the surface. The exterior part was removed with sterile scalpels and the interior portion was cut aseptically with sterile scalpel into cubes (2.2x2.2x2.2 cm) each weighing approximately 10g. One ml of a 72 hours culture of *C. jejuni* (the approximate count was 17 cells/ml) was injected into the center of each cube. Samples were wrapped in aluminum foil (CHRISTOPHER et al., 1982). The samples stored at different holding temperatures (20-25 C for 72 hours, at 4 C for 14 days and -20 C for 6 weeks.

#### Thermal inactivation:

The inoculated samples were heated in an electric oven at 50, 55, 60 and 70 C. Once the cubes had reached the desired temperature, survivors were enumerated using methods described below.

At time intervals, each sample was macerated in 90 ml of sterile 0.1% peptone and blended in a waring blender mixed for 2.5 minutes (2000 rpm) to provide a dilution of 10. Serial dilutions were prepared from the original dilution then streaked on *Campylobacter* selective media plates (SKIRROW, 1977) and incubated at 37 C in a Gas-pak jar at microaerophilic atmosphere for 72 hours. The survivors were enumerated for *C. jejuni*.

*Campylobacter* counts were determined at time of inoculation 24, 48 and 72 hours for meat held at 20-25 C; at time of inoculation, 2, 4, 6, 8, 10, 12, and 14 days for those held at 4 C and at time of inoculation 1, 2, 3, 4, 5 and 6 weeks for meat held at -20 C.



## RESULTS

The obtained results are recorded in table,1.

## DISCUSSION

The growth of *C. jejuni* strain in meat during storage as calculated from confirmed colonies on Campylobacter selective media plates are presented in table(1).

The counts of *C. jejuni* after inoculation held at 20-25 C were  $1 \times 10^8$ ,  $6 \times 10^7$ ,  $4 \times 10^7$ ,  $1 \times 10^7$  cells/gm in time of inoculation 24, 48 and 72 hours respectively. The results indicated that a decrease had occurred in counts which agreed with the findings recorded by (DOYLE and ROMAN, 1981; CHRISTOPHER et al., 1982 and GILL and HARRIS, 1982).

GILL and HARRIS (1982) reported that storage of meat at room or chilling temperature resulted in comparatively rapid and ultimately complete die-off, whereas freezing substantially reduced the total bacterial numbers.

The counts of *C. jejuni* organisms in meat held at 4 C were  $1 \times 10^8$ ,  $8 \times 10^7$ ,  $5 \times 10^7$ ,  $3 \times 10^7$ ,  $2 \times 10^7$ ,  $1 \times 10^7$ ,  $8 \times 10^6$  and  $6 \times 10^6$  cells/gm in time of inoculation, 2, 4, 6, 8, 10, 12 and 14 days respectively.

The results indicated that the counts of *C. jejuni* during this storage decrease in cells growth. Similar observations were recorded by (DOYLE and ROMAN, 1981; WYATT and TIMM, 1982 and DOYLE, 1983).

*C. jejuni* may represent a significant public health hazard in certain raw or improperly processed foods. The survival of *C. jejuni* at 4 C shows that refrigeration is not an adequate means of controlling *C. jejuni* (WYATT and TIMM, 1982).

The counts of *C. jejuni* organisms in meat held at -20 C were  $1 \times 10^8$ ,  $6 \times 10^7$ ,  $3 \times 10^7$ ,  $1 \times 10^7$ ,  $8 \times 10^6$ ,  $6 \times 10^6$  and  $5 \times 10^6$  cells/gm in time of inoculation 1, 2, 3, 4, 5 and 6 weeks respectively. These results were in accordance with the results obtained from (CHRISTOPHER et al., 1983).

OOSTEROM et al. (1983) found that during cooling and freezing in processing plants, Campylobacter contamination is sometimes reduced to below detectable levels.

The organisms of *C. jejuni* were rapidly inactivated when heated to 50 C or above (GILL and HARRIS, 1982).

Failure of *C. jejuni* organisms to survive in meat when heated at 50, 55, 60 & 70 C which were completely inactivated, these results were in a close agreement with those reported by DOYLE and ROMAN, 1981;



CHRISTOPHER et al., 1982; GILL and HARRIS (1982) and STERN and KOTULA, 1982.

KOIDIS and DOYLE (1983) reported that *C. jejuni* is more sensitive to heat than *Salmonellae*, hence ground beef heated to a temperature sufficient to inactivate *Salmonella* spp. should be free of viable *Campylobacters*.

Thermal sensitivity would not allow the *C. jejuni* organisms to survive even moderate cooking (GILL and HARRIS, 1982).

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Table (1): *C. jejuni* counts of stored meat at different holding temperatures.

Hours/20-25°C		Days/4°C							Weeks/-20°C									
0	24	48	72	0	2	4	6	8	10	12	14	0	1	2	3	4	5	6
1x10 <sup>7</sup>	6x10 <sup>6</sup>	4x10 <sup>6</sup>	1x10 <sup>6</sup>	1x10 <sup>7</sup>	8x10 <sup>6</sup>	5x10 <sup>6</sup>	3x10 <sup>6</sup>	2x10 <sup>6</sup>	1x10 <sup>6</sup>	8x10 <sup>5</sup>	6x10 <sup>5</sup>	1x10 <sup>7</sup>	6x10 <sup>6</sup>	3x10 <sup>6</sup>	1x10 <sup>6</sup>	8x10 <sup>5</sup>	6x10 <sup>5</sup>	5x10 <sup>5</sup>