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OCCRRENCE AND SURVIVAL OF CAMPYLOBACTER JEJUNI IN RAW MILK

(With 3 Tables)

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(Received at 17/5/1994)

مدى تواجد وقدرة ميكروب الكامبيلوباكتر جيوجيناى على المعيشه فى اللبن الخام

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نظرا لخطورة ميكروب الكامبيلو باكتر جيوجيناى على صحة الانسان وتزايد حالات الاصابه بالاسهال الناتجه عن تلوث الالبان بهذا الميكروب اجريت هذه الدراسه لمعرفة مدى تواجد وقدرة الميكروب على المعيشه فى اللبن الخام وكذلك تواجده فى براز تلك الحيوانات وقد تم فحص عدد ٢٠٠ و١٠٠ عينه لبن من أبقار سلبيه وايجابيه لاختبار التهاب الضرع السرى على التوالى وكذلك عدد ٣٠٠ عينه من براز تلك الحيوانات التى تم جمعها من عدة مزارع بمدينة الاسماعيليه وقد تبين ان الميكروب متواجده بنسبه ٥ ر٢ ، صفر ، ٣٣ ر٥ ٪ على التوالى . بالنسبه لتأثير اللاكتوبيروكسيديز على عزل الميكروب من اللبن الخام تبين ان عند عدم تنشيطه كانت نسبة العزل ٨٥ ٪ بينما عند تنشيطه كانت نسبة العزل ٢٢ ٪ فقط . وقد وجد ان الميكروب يستمر حيا لمدة ٧ ، ٢ يوم فى اللبن الخام عند حفظه عند درجة حرارة الثلاجه (٤ م) ودرجة الغرفه (٢٢ م)) . وقد نوقشت خطورة الميكروب على الصحه العامه وكذلك الاشتراطات الصحيه الواجب توافرها عند انتاج وتداول الالبان .

SUMMARY

Different samples of normal raw milk (200), mastitic milk (100) and faeces (300) from individual cows collected from different farms in Ismailia City were examined for the presence of C. jejuni. The organism was isolated from 2.5, 0.0 and 5.33% of samples, respectively. Results of laboratory experiment showed that when LPS was inactivated, the organism was isolated from 85% of the samples, whereas 22% of the samples were positive when LPS was not inactivated. The organism could survive in raw milk kept at refrigerated temperature for 7 days, while at 22°C it survived up to 2 days.

Keywords: Campylobacter Jejuni, milk.

INTRODUCTION

In the recent years, C. jejuni has been recognized as an important cause of acute enteritis in humans *(SKIRROW, 1977 and BECKERS, 1987)*. Several outbreaks have been associated with the consumption of raw or insufficiently heat treated milk *(ROBINSON et al., 1979 and PORTER and REID, 1980)*. It has been indicated that the organism failed to survive heating in skim milk at 60°C for 1 min *(CHRISTOPHER et al., 1982)*.

C. jejuni could contaminate the milk from cattle faeces because the organism appeared to be a commensal in the intestinal tract of numerous animal species *(OoSTEROM et al., 1982 and ROBINSON, 1982)*. However, faecal matters do not constitute the only source of contamination of milk, it has been shown that C. jejuni can cause mastitis in cattle *(LANDER and GILL, 1980 and NEUMANN, 1986)*, and this might be a more important factor in the epidemiology of milk-borne outbreaks, since large numbers of C. jejuni can be excreted by udder.

C. jejune has seldom been isolated from raw milk. This discrepancy has to be attributed mainly to the inability of the organism to multiply in milk, and to its rapid reduction in raw or insufficiently heat treated milk. Some authors have suggested that the presence of natural bactericidal compounds or systems in milk such as lactoperoxidase in the presence of adequate amounts of thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2) affect the viability of the organism in milk *(BJöRCK et al., 1975)*. Lp is not inactivated by low temperature

pasteurization (72°C for 15 s or 63°C for 30 min, but more intensive heating (85°C for 10s) destroys the enzyme. The enzyme also becomes inactivated at pH values above 7, and its maximum activity is at pH 5 or lower *(REITER et al., 1976)*.

Because of the involvement of milk in human campylobacter enteritis, this work was planned to study the occurrence of C. jejuni in normal raw milk, mastitic milk, and faecal matter, as well as the effect of LPS on the isolation rate and survival of the organism in raw milk.

MATERIAL AND METHODS

Collection of samples

A total of 200 freshly drawn normal milk samples, and 100 samples of subclinical mastitic milk (by application of Schalm test) from individual cows were collected from different farms in Ismailia City in sterile test tubes. Faecal samples (300) were obtained from all examined individual animals with sterile swabs.

Isolation technique (Park et al., 1984)

Directly after sampling, 2 ml of each milk sample and the faecal swabs were transferred into tubes each containing 10 ml of thioglycolate broth (Merck Art No. 8190) with antibiotic supplement (Oxoid code Sr 69), and FBP (Oxoid code SR 84) to raise the oxygen tension of campylobacter. After raising the pH to 7.5 with sterile 1 mol/L NaOH to inactivate LPS, the tubes were incubated at 42°C for 48 h in microaerobic incubator, then subcultured on Skirrow agar (Oxoid code CM 271) with antibiotic supplement (Oxoid code SR 69), and incubated for 48 h at 42°C in microaerobic incubator.

Typical colories which are non haemolytic, catalase and oxidase positive and do not grow in aerobic condition were picked up, purified and then identified according to *PARK et al. (1984)*.

Experimental study

1- Effect of Lps on the isolation rate of C. jejuni

C. jejuni, freshly isolated from milk and identified was grown in thioglycolate broth under microaerobic conditions at 42°C for 48 h, then centrifuged at 1500 rpm for 5 min and the sediment was diluted with sterile solution to give a concentration of 10^3 cells/ml. A volume of 2 ml of the dilution was added to 2 litre of freshly drawn raw cow's milk resulting in a concentration of one cell of C. jejuni/ml. The milk was

distributed into two flasks (1 litre capacity). To one flask, 1 mol/L NaOH was added until the normal pH of milk (6.7) has risen to 7.5. Then flasks were cooled rapidly to 4°C in refrigerator for 8 h and then examined for the presence of C. jejuni in one ml samples (100 samples from each flask) as described before.

2- Viability of C. jejuni in raw milk

Two freshly drawn cow's milk samples (500 ml each) were placed in sterile screw capped bottles of one litre capacity, then the test milk samples were inoculated with the prepared C. jejuni cultures to produce initial inocula of 10^6/ml and stored at 4°C and 22°C. The milk samples were examined after 8 h, and then daily for incidence and count of C. jejuni.

RESULTS

Results of incidence and survival of C. jejuni are recorded in Tables 1, 2 and 3.

DISCUSSION

The results presented in Table 1 indicate that C. jejuni could be isolated from 5 (2.5%) of 200 normal raw milk samples, and could not be isolated from all mastitic milk samples. It was recorded that several workers failed to detect C. jejuni in raw milk samples examined *(PORTER and REID, 1980, OoSTEROM et al., 1982 and STONE 1987)*. Lower incidence (0.2 to 1.5%) was reported by *LOVETT et al. (1983); DE BOER, et al. (1984) and ISMAIL et al. (1988)*. Whereas higher percentages (4.5 to 12.7%) were recorded by *HUMPHREY and BECKETT (1987); BEUMER et al. (1988); HUMPHREY and HART (1988) and MOUSTAFA (1989)*.

The low isolation rate of C. jejuni from raw milk samples could be attributed to the low contamination level, methods of isolation, and the competing microorganisms which might produce metabolites toxic to campylobacter *(DE BOER et al., 1984)*. Also, several workers have found that viable C. jejuni introduced into raw milk rapidly decreased *(BLASER et al., 1980; OoSTEROM et al., 1982 and DE BOER et al., 1984)*, and this may be due to the antibacterial action of LPS in milk *(BEUMER et al., 1985)*.

Contamination of the milk during or after milking is probably of faecal origin. However, improper washing and treatment of the udder with suitable disinfectant or contact of

the milking pails with the floor may result in high levels of contamination. Furthermore, naturally occurring campylobacter mastitis and contaminated water mostly by wild domestic animals may act as a source of contamination of milk by C. jejuni *(MENTZING, 1981)*. Also, the incidence of intestinal carriage of C. jejuni in dairy animals presented in Table 1 reveal that the organism was detected in 16 (5.33%) faecal samples. This finding explains that the occurrence of C. jejuni in bovine faeces constitutes a dangerous source of milk contamination through faulty method of production.

Results of laboratory experiment given in Table 2 show that inactivation of LPS gave better recovery of C. jejuni. When LPS was inactivated, the organism was isolated from 85 of 100 samples, whereas only 22 of 100 samples were positive when the system was not inactivated. This difference in the isolation rate of C. jejuni reveals that the antibacterial action of the LPS may play an important role in the viability of C. jejuni in milk as recorded by *BEUMER* et al. *(1985) and BEUMER* et al. *(1988)*. The mode of action of LPS system is not yet known, but its antibacterial effect is due to an oxidation of the SH groups in various essential proteins in the bacterial cell wall, this causing the energy metabolism of the bacteria to become destroyed. However, more recently it was demonstrated that the SH-independent enzyme D-lactate dehydrogenase is also inhibited by LPS *(REITER and HARNULY, 1984)*.

It is clear from the data shown in Table 3 that C. jejuni inoculated into raw milk survived better at 4°C than at 22°C. At 4°C, C. jejuni count decreased from 2.6×10^6 to 1.1×10^1 on the 5th day, then the survivors remained viable up to 7th day. While at 22°C, C. jejuni count rapidly decreased to 1.2×10^1 by the end of first day, and no survivors could be detected after 2 days. It is appeared from these results that the survivability of C. jejuni is a temperature dependent, as the organisms survived better in refrigerated raw milk than in milk stored at 22°C. This finding run parallel to that observed by *BLASER* et at. *(1980), BARRELL (1981) and NEUMANN (1986)*. The decrease in viable numbers of C. jejuni in raw milk kept at 22°C may be caused by the antibacterial action of the LPS of milk *(BEUMER* et al., *1985)*, where the pH developed by the lactics in raw milk (5 or less) leading to activation of this system in milk, whereby the bacteria is destroyed *(BARRELL, 1981)*. Moreover, it has been recorded that lactic acid is an inhibitory to C. jejuni, as the pH 5 completely destroys the organism *(CHRISTOPHER* et al., *1982)*.

In Egypt, the fluid milk is usually sold as raw. In addition, some dairy products such as cream, butter and cheese

are often manufactured from raw milk and could be consumed fresh. *EHLERS* et al. *(1982)* have reported that several strains of C. jejuni inoculated into cheddar cheese milk survived in the curd for 30-60 days. Therefore, raw milk and some milk products may constitute public health hazards. It has been stated that as few as 2-3 cells/ml milk can infect an individual who consumed 240 ml of contaminated milk *(BLACK* et al., *1983)*. A considerable high isolation rates are obtained from raw milk, if the LPS of the milk is inactivated directly after drawing by raising the pH of the milk to 7.5 using NaOH. Also, the organism can survive in refrigerated raw milk for 7 days, thereby encompasses the whole period in which raw milk is normally consumed to cause the disease.

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Table 1: Incidence of C.jejuni in the examined samples of milk and faecal matter

Samples	No. of examined samples	Incidence	
		NO.	%
Normal raw milk	200	5	2.5
Mastitic milk	100	--	---
Faecal matter	300	16	5.33

Table 2: Isolation rate of C. jejuni from raw milk of LPS not inactivated and inactivated.

No. of examined samples (for each)	Isolation rate			
	LPS not inactivated (pH 6.7)		LPS inactivated (pH 7.5)	
	No.	%	No.	%
100	22	22	85	85

Table 3: Survival of C. jejuni in raw milk stored at 4°C and 22°C.

Time	Count and incidence	
	At 4°C	At 22°C
0.0	2.6×10^6	2.6×10^6
8 h	1.7×10^6	1.1×10^6
1 day	5.9×10^5	1.2×10^1
2 „	4.2×10^4	+
3 „	6.2×10^3	-
4 „	2.3×10^1	-
5 „	1.1×10^1	-
6 „	+	
7 „	+	
8 „	-	
9 „	-	