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Survival rate of c*ampylobacter coli* strains in sterile buffalo and bovine milk [*]

Tasas de sobrevida de *Campylobacter coli* en leche de búfalo y de bovino

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SUMMARY

The survival rate of five *Campylobacter coli* strains, isolated from bovine faeces (3) and from buffalo faeces (2), was studied in sterile buffalo and bovine milk kept at 4°C under aerobic conditions. All strains lost their viability substantially more rapidly in buffalo milk than in bovine milk.

Palabras claves: *Campylobacter coli*, sobrevida, leche.

Key words: *Campylobacter coli*, survival, milk.

INTRODUCTION

diarrhoea in both developing and industrialized countries. Many animal species harbour these agents in their intestinal tract; cows, pigs and poultry being the most important reservoirs (Skirrow & Blaser, 1992; Tresierra-Ayala et al., 1995; Fernández & Pisón, 1996).

Campylobacter enteritis is mainly a foodborne disease and human beings become infected by consumption of contaminated water or food of animal origin (Butzler & Oosterom, 1991). Some researchers (Barrell, 1981; Doyle & Roman, 1982; Koidis & Doyle, 1984; Tresierra-Ayala et al., 1999) have studied the survival rate of Campylobacter species in milk, but most of these studies have been done in bovine milk, considering only C. jejuni, although C. coli has a similar importance in public health (Tresierra-Ayala et al., 1996). In Iquitos (Eastern Peru), unpasteurized buffalo and bovine milk are frequently consumed and they are usually stored at 4°C for several days. For these reasons, it was decided to determine the survival rate of C. coli strains both in buffalo and bovine milk kept at 4°C under aerobic conditions.

MATERIAL AND METHODS

Five C. coli strains, 3 isolated from bovine and 2 from buffalo faeces were studied. Strains were cultured on blood agar plates according to Hoffman et al. (1979) consisting of (wt/vol): Brucella agar 4,3 g%; ferrous sulphate 0,05 g%, sodium metabisulfite 0,05 g%; sodium piruvate 0,05 g% and defibrinated horse blood 5 ml%. Plates were incubated at 42°C for 48 h under microaerophilic conditions. For survival experiments, bacterial suspensions of each strain were prepared in distilled water (10^9 CFU/ml) and 1ml aliquot added to 99 ml of sterile buffalo and bovine milk (pre-heated at 121°C for 15 min), for eliminating any influence of the accompanying flora or indigenous systems present in normal milk, and kept at 4°C, throughout the experiments, under aerobic conditions, without agitation. The pH of milk was monitored at the beginning and at the end of each experiment, ranging between 6.7 to 6.9 throughout the study.

Using a modified Miles and Misra method (Miles & Misra, 1938), viable counts were carried out at six-hour intervals up to 30 h or until no viable bacteria were detected. To achieve this, a 1-ml sample of the content of each assay was obtained, serially diluted in 0.1% peptone and 20 µl from each dilution plated out (in quintuplicate) onto blood agar plates and incubated at 42°C for 36 h under microaerophilic conditions. Finally, the results were corrected using the lineal regression method (Colton, 1974).

RESULTS AND DISCUSSION

A relative rapid decrease in viable counts of all strains was observed, especially in buffalo milk, in which viable bacteria were not detected after 30 h of incubation at 4°C. Strains 1, 4 and 5 did not survive after 24 h and viable cells of strains 2 and 3 were not detected after 30 h (table 1).

Table 1. Viable counts of Campylobacter coli strains in sterile buffalo and bovine milk stored at 4°C
Recuento de células viables de Campylobacter coli en leche bovina y de búfalo mantenida

Strain Origin	Incubation Time (h)	Buffalo milk CFU/ml	Buffalo milk Corrected values*	Bovine milk CFU/ml	Bovine milk Corrected values*
1 bovine	0	$3,9x10^6$	$6,8x10^6$	$3,9x10^6$	$2,3x10^6$
	6	$8,1x10^5$	$3,7x10^5$	$9,0x10^5$	$9,4x10^5$
	12	$6,1x10^3$	$2,0x10^4$	$3,0x10^5$	$3,8x10^5$
	18	$1,7x10^3$	$1,1x10^3$	$9,4x10^4$	$1,5x10^5$
	24	Not detected	---	$5,0x10^4$	$6,1x10^4$
	30			$1,2x10^4$	$2,4x10^4$
	48			$5,0x10^3$	$1,6x10^3$
	96			Not detected	---
2 buffalo	0	$1,0x10^6$	$1,0x10^6$	$1,0x10^6$	$2,2x10^5$
	6	$2,7x10^5$	$1,9x10^5$	$9,4x10^5$	$1,7x10^5$
	12	$3,9x10^4$	$3,5x10^4$	$2,0x10^5$	$1,3x10^5$
	18	$8,7x10^3$	$6,8x10^3$	$8,0x10^4$	$1,0x10^5$
	24	$9,2x10^2$	$1,3x10^3$	$3,0x10^4$	$8,2x10^4$
	30	Not detected	---	$1,1x10^4$	$6,4x10^4$
	48			$8,0x10^3$	$3,1x10^4$
	96			$3,0x10^3$	$4,3x10^3$
	144			$2,0x10^3$	$6,x10^2$
	192			Not detected	---
3 buffalo	0	$4,5x10^6$	$4,5x10^6$	$4,5x10^6$	$2,1x10^6$
	6	$1,4x10^6$	$5,7x10^5$	$8,0x10^5$	$6,0x10^5$
	12	$3,4x10^4$	$7,2x10^4$	$4,0x10^4$	$1,7x10^5$
	18	$4,2x10^3$	$9,1x10^3$	$3,8x10^4$	$4,7x10^4$
	24	$1,7x10^3$	$1,2x10^3$	$2,0x10^4$	$1,3x10^4$
	30	Not detected	---	$3,5x10^3$	$3,7x10^3$
	48			$1,0x10^2$	$8,1x10^1$
	96			Not detected	---
4 bovine	0	$2,5x10^6$	$4,1x10^6$	$2,5x10^6$	$8,6x10^5$
	6	$7,3x10^5$	$4,1x10^5$	$7,0x10^5$	$3,9x10^5$
	12	$4,2x10^4$	$4,2x10^4$	$6,0x10^4$	$1,8x10^5$
	18	$4,3x10^3$	$4,2x10^3$	$3,9x10^4$	$8,1x10^4$
	24	Not detected	---	$3,0x10^4$	$3,7x10^4$
	30			$9,5x10^3$	$1,7x10^4$
	48			$4,0x10^3$	$1,6x10^3$
	96			Not detected	---
5 bovine	0	$1,4x10^6$	$2,6x10^6$	$1,4x10^6$	$2,6x10^5$
	6	$5,3x10^5$	$2,0x10^5$	$6,0x10^5$	$1,9x10^5$
	12	$5,8x10^3$	$1,5x10^4$	$3,0x10^5$	$1,3x10^5$
	18	$1,4x10^3$	$1,1x10^3$	$4,2x10^4$	$9,5x10^4$
	24	Not detected	---	$2,0x10^4$	$6,7x10^4$
	30			$8,2x10^3$	$4,8x10^4$
	48			$4,0x10^3$	$1,7x10^4$
	96			$5,0x10^3$	$1,1x10^3$
	144			Not detected	---

* lineal regression method (Colton, 1974)

On the other hand, the survival time of C. coli strains in bovine milk was more prolonged; strains 1, 3 and 4 did not survive after 96 h of incubation, whereas viable cells of strains 2 and 5 were not

The results of the present study have shown that, apparently, C. coli strains do not survive for extended times in sterile milk kept at 4°C under aerobic conditions, loosing their viability substantially more rapidly in buffalo milk than in bovine milk. Under the experimental conditions of this study, none of the 5 C. coli strains were recovered after 30 h of incubation in buffalo milk. This product is well known for its "heaviness" due to higher proportions of milk constituents such as fat, protein, lactose and minerals compared to bovine milk (Ganguli, 1980). We believe that chemical composition of buffalo milk may play an important role in the survival rate of Campylobacter strains in milk.

On the other hand, all C. coli strains were recovered after storage at 4ºC for 48 h or more in bovine milk. Interestingly, one strain (C. coli N°2) was still recoverable at 144 h after inoculation (table 1). Barrell (1981) and Koidis & Doyle (1984) determined that some Campylobacter strains were still recovered from bovine milk after storage at 4°C for more than 120 h.

The greatest survival time of C. coli strains in milk, specially in bovine milk, indicates that these organisms remain viable for most of the time the milk is stored in the consumer's house. Being C. coli closely related to C. jejuni and also associated with enteric infections (Skirrow and Blaser, 1992; Fernández, 1992) and considering that the infective dose of C. jejuni for humans appears to be low (ca. 500 cells) as it was stated by Robinson (1981), the latter could be extrapolated to C. coli. Therefore, the presence of small numbers of Campylobacter cells in milk during storage in the consumer's house as well as the survival times of these bacteria, such as that found in this study, are other aspects to be considered for under-standing campylobacteriosis as a foodborne disease.

RESUMEN

Se determinó, en leche de bovino y de búfalo autoclavadas y mantenidas a 4ºC bajo condiciones de aerobiosis, la sobrevida de cinco cepas de Campylobacter coli aisladas de fecas de bovino (3) y de búfalo (2). Todas las cepas perdieron su viabilidad más rápidamente en leche de búfalo que en leche bovina.

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