

## PREVALENCE OF THERMOTOLERANT SPECIES OF *CAMPYLOBACTER* AND THEIR BIOTYPES IN CHILDREN AND DOMESTIC BIRDS AND DOGS IN SOUTHERN CHILE

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### SUMMARY

The prevalence of thermotolerant *Campylobacter* in diarrhoeic and healthy children as well as in dogs, hens, ducks and pigeons was determined in Southern Chile. *Campylobacter* were found in 34.5% of the faecal samples examined. The isolation rate of *Campylobacter* in diarrhoeic and healthy children was 16.3% and 6.4% respectively. Despite *C. jejuni* was always more frequent than *C. coli*, the latter was isolated with a high frequency (29%) from patients with diarrhoea. *C. jejuni* and *C. coli* biotypes I and II were found in healthy and diarrhoeic children and were predominant in all the animals species studied. This may point out towards the possible origin of strains infecting children.

**KEYWORDS:** *Campylobacter*; Diarrhea; Epidemiology; Ecological distribution.

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### INTRODUCTION

In the last two decades, the thermotolerant species of *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari*) have acquired great importance in public health, especially as agents of human diarrhoeal illness<sup>1, 13, 21</sup>.

The epidemiological aspects involved in the spread of these bacteria are complex and not well understood<sup>1</sup>, particularly in developing countries<sup>4</sup>.

The present study was undertaken to determine the prevalence of thermotolerant species of *Campylobacter* and their biotypes in diarrhoeic, non diarrhoeic children and in domestic animals in Southern Chile.

### MATERIAL AND METHODS

#### *Faecal samples*

Nine hundred and fifteen faecal samples were studied. The samples were collected by means of sterile rectal swabs from 190 diarrhoeic and 150 non diarrhoeic children, aged between 5 days and 6 years, attending the

county hospital (Valdivia city, 39° 46' Southern latitude), and from 150 hens, 100 ducks, 104 pigeons and 214 dogs. All the animals studied were apparently healthy at the time the sample collection.

#### *Isolation and identification methods*

Immediately after their collection, each sample was introduced into a semisolid transport and enrichment medium (TEC), consisting of brucella broth 28 g/l, agar 1.5 g/l, sodium metabisulfite 0.5 g/l, sodium pyruvate 0.5 g/l, ferrous sulphate 0.5 g/l, trimethoprim 10 mg/l, rifampicin 15 mg/l, colistin 10,000 U.I./l, amphotericine 10 mg/l and defibrinate horse blood 30 ml/l<sup>5</sup>.

In the laboratory, the samples were seeded on modified Skirrow medium<sup>6, 7</sup> consisting of Brucella agar 43 g/l, vancomycin 10 mg/l, trimethoprim 5 mg/l, polymyxine B 2,500 U.I./l, cephalotine 10 mg/l, sodium metabisulfite 0.5 g/l, sodium pyruvate 0.5 g/l, ferrous sulphate 0.5 g/l and sterile horse blood 60 ml/l.

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TEC medium was incubated microaerobically (GasPak system without a catalyst) for enrichment during 24h at 42°C. Following incubation, subcultures were made on modified Skirrow medium and the plates incubated for 48h under the conditions described above.

*Campylobacter* strains were identified morphologically (Gram stain) and biochemically, according to Lior's biotyping scheme <sup>19</sup>.

## RESULTS

The isolation rates of *Campylobacter* species and their respective biotypes in diarrhoeic and healthy children, and in domestic animals are shown in Tables 1 and 2.

*Campylobacter* were found in 34.5% of the faecal samples examined, being *C. jejuni* more frequently isolated (27.4%) than *C. coli* (7.1%), whereas *C. lari* was not found.

The isolation rates of *Campylobacter* in diarrhoeic and healthy children were 16.3% and 6.4%, respectively. This difference was statistically significant.

The four biotypes described for *C. jejuni* were found only in dogs and hens faecal samples, being biotypes I and II the most frequently isolated. Both were found amongst faecal samples collected from children, ducks and pigeons, being biotype II the most frequent.

The two biotypes described for *C. coli* were isolated from all the groups under study, except from pigeons where only biotype II was found.

## DISCUSSION

The isolation rate from faecal specimens obtained from patients with diarrhoea is comparable to that reported in most studies carried out on various developing countries <sup>1,4</sup>. However, it is higher than that reported by PRADO <sup>21</sup> and FIGUEROA et al. <sup>10</sup> in Central Chile (Santiago city, 33° 43' Southern latitude). This difference could be associated to the strong relationship commonly observed in Southern Chile between man and animals, where dogs and fowls could be natural reservoirs of *Campylobacter* <sup>4,6,7</sup>. GRADOS et al. <sup>13</sup> and MARQUIS et al. <sup>16</sup> have demonstrated that a close contact with animals, particularly with poultry, is a high risk factor in the transmission of *Campylobacter* to human beings.

Asymptomatic excretion of *Campylobacter* is a common epidemiological fact observed in developing countries <sup>1,7,21</sup>. In this study, the carrier rate (6.4%) obtained from healthy children was statistically significant ( $p < 0.05$ ) when compared with that obtained from diarrhoeic children. On the other hand, it was higher than the carrier rates reported previously by us (4%) in Valdivia <sup>3</sup> and by PRADO (3.2%) in Santiago <sup>21</sup>, but lower than that observed in Brazil <sup>9</sup>, North India <sup>17</sup>, Egypt <sup>19</sup> and in other developing countries <sup>1</sup>. Perhaps the high frequency of asymptomatic carriers could be strongly related to deficient environmental sanitary conditions which promote much higher transmission opportunities, with repeated infections with *Campylobacter* from their natural sources to children, than it occurs in developed countries <sup>1,5,7,13</sup>.

KARMALI & SKIRROW <sup>14</sup> stated that *C. coli* accounts for only 3 to 5% of the *Campylobacter* strains

Table 1

Isolation rates of thermotolerant *Campylobacter* species isolated from children and domestic animals in Southern Chile.

Source	No. of Samples	<i>C. jejuni</i>		<i>C. coli</i>		Total	
		No.	%	No.	%	No.	%
Children w/diarrhoea	190	22	11.6	9	4.7	31	16.3*
Healthy children	157	5	3.2	5	3.2	10	6.4*
Dogs	214	63	29.4	28	13.1	91	42.5
Hens	150	88	58.7	12	8.0	100	66.7
Ducks	100	66	66.0	7	7.0	73	73.0
Pigeons	104	7	6.7	4	3.8	11	10.6
Total	915	251	27.4	65	7.1	316	34.5

\* $p < 0.05$

Table 2

Biotypes distribution amongst thermotolerant *Campylobacter* species isolated from children and domestic animals in Southern Chile.

Source	<i>Campylobacter jejuni</i>								<i>Campylobacter coli</i>					
	N	Biotypes								N	Biotypes			
		I	II	III	IV	I	II							
Children w/diarrhoea	22	2	9.1%	20	90.9%	0	0	0	0	9	1	11.1%	8	88.9%
Healthy children	5	1	20.0%	4	80.0%	0	0	0	0	5	4	80.0%	1	20.0%
Dogs	63	26	41.3%	20	31.7%	11	17.5%	6	9.5%	28	14	50.0%	14	50.0%
Hens	88	34	38.6%	37	42.0%	8	9.1%	9	10.2%	12	9	75.0%	3	25.0%
Ducks	66	11	16.7%	55	83.3%	0	0	0	0	7	6	85.7%	1	14.3%
Pigeons	7	1	14.3%	6	85.7%	0	0	0	0	4	0	0	4	100.0%
Total		75	29.9%	142	56.6%	19	7.6%	15	6.0%	65	34	52.3%	31	47.7%

isolated from cases of human enteritis. Contrasting with these authors, we found a high isolation rate of *C. coli* amongst children with diarrhoea. From the 31 diarrhoeic patients with *Campylobacter* positive stool culture, 22 (71%) corresponded to *C. jejuni* and 9 (29%) to *C. coli*. Similar results were observed previously in Chile <sup>11</sup>, Yugoslavia <sup>20</sup>, the Central African Republic <sup>12</sup> and Madagascar <sup>2</sup>. Perhaps, this unexpected finding is an epidemiological feature more frequently observed in developing than in industrialized countries, and probably linked to unadequate environmental sanitary conditions. Further field studies, correlating clinical trials with environmental conditions are needed to understand this epidemiological behavior.

In the animals studied, *C. jejuni* was always more frequent than *C. coli*. The isolation rates of *Campylobacter* from dogs and pigeons faecal samples (42.5 and 10.6% respectively) was slightly lower whereas in hens, the frequency of *Campylobacter* (66.7%) was similar to that reported in 1988 for the same animals in this geographical region <sup>4</sup>. The highest isolation rate of thermophilic campylobacters (73%) was found in ducks. This frequency is higher than that (27.3%) reported for a small group of ducks in Zaire <sup>22</sup> however, it is identical to that reported by PACHA et al. <sup>18</sup> in wild ducks. Apparently, this is the first report about intestinal carriage of *Campylobacter* species in domestic ducks in Chile.

Only biotypes I and II from *C. jejuni* were isolated from healthy and diarrhoeic children being biotype II the most frequent in both groups. Similar results were reported by FIGUEROA et al. <sup>11</sup> in Chile and by CASSELBERAUD et al. <sup>2</sup> in Madagascar. As regards to *C. coli*, biotype II was most frequent in children with diarrhoea,

whereas biotype I was the most frequent in healthy children. We are not aware whether these results have some epidemiological explanation, but we believe that more studies focusing *C. coli* will be necessary to establish its epidemiology and its role as an enteropathogen for human beings.

As reported in previous works carried out in Southern Chile <sup>5, 8</sup>, all the four biotypes described by LIOR <sup>15</sup> for *C. jejuni* and the two described for *C. coli* were isolated from dogs and hens, while biotypes I and II of *C. jejuni* and *C. coli* were isolated from ducks. This wide distribution of *C. jejuni* and *C. coli* biotypes amongst this kind of animals reflects contact with a highly contaminated environment <sup>5, 8</sup>.

*C. jejuni* and *C. coli* biotypes I and II were isolated from diarrhoeic and healthy children and were predominant in all the animal species studied. This may point out towards the possible origin of strain infecting children. However, it is necessary to conduct field prospective studies using more discriminatory typing methods which allow the assessment of the epidemiological relationships between human infections, animal reservoirs and environmental conditions in different geographical regions.

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## RESUMO

### Prevalência de espécies termotolerantes de *Campylobacter* e seus biotipos em crianças, aves domésticas e cães no sul do Chile.

Foi determinada, no sul do Chile, a prevalência de espécies termotolerantes de *Campylobacter* e seus

biotipos, em crianças normais e diarréicas, bem como em cães e aves domésticas.

*Campylobacter* foi isolado em 34,5% do total das amostras estudadas sendo sua frequência de isolamento de 16,3% e 6,4% nas crianças com diarréia e normais, respectivamente. *C. jejuni* foi a espécie mais freqüente. Porém, *C. coli* foi isolado em 29% das crianças com diarréia. Somente os biotipos I e II, tanto de *C. jejuni* como de *C. coli*, foram isolados das crianças, os quais foram também os mais frequentemente encontrados nos animais, sugerindo uma possível associação epidemiológica entre eles.

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