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SPECIES AND BIOTYPE DISTRIBUTION OF THERMOTOLERANT CAMPYLOBACTERS IN ANIMAL RESERVOIRS IN SOUTHERN CHILE[1]

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SUMMARY

The prevalence of thermotolerant campylobacters in mammals and birds from Southern Chile was determined. Campylobacters were isolated from 46.3% of the animals studied being **C. jejuni** biotipe 1 the most frequent (25.7%) followed by **C. coli** (17.4%) and **C. jejuni** biotipe 2 (3.2%).

KEY WORDS: Campylobacter; Campylobacteriosis; Reservoirs; Epidemiology.

INTRODUCTION

Thermotolerant campylobacters - **Campylobacter jejuni** and **C. coli** - are recognized as important agents of acute diarrheal illness in humans[14,31]. These microorganisms are widely distributed all over the world having as natural reservoirs a great variety of animal species including companion, domestic and wild animals[1, 8, 13, 24].

Human illness considered as zoonosis[30] can be acquired by contact with animals[9, 20, 30], by drinking contaminated water with animal faeces[2, 27, 36] or by ingestion of contaminated food of animal origin[2, 20].

In view of the lack of information about the natural distribution of thermotolerant campylobacters in this region of the world, a study was undertaken to determine the prevalence of **C. jejuni** and **C. coli** in different mammals and birds from southern Chile (province of Valdivia), southern latitude 39°15' - 40°15'.

MATERIALS AND METHODS

A total of 631 fecal samples obtained by rectal swabs from 150 dogs, 80 pigs, 80 cows, 40 cats, 100 hens, 75 sparrows, 60 geese and 46 pigeons were studied. None of the animals subjeted to this study showed clinical symptoms of diarrhea. Rectal samples were obtained using commercial sterile swabs (Linsan Laboratories, Chile) supplied with a 1 ml ampule of Cary Blair transport medium. After collection of the sample the ampules were crushed to moisten the swabs. Each sample was identified with an appropriate code number, and within 2 to 6 hours after collection were seeded on modified Skirrow's medium[26] comprising Brucella agar (Difco), 5% horse blood, Skirrow's Antibiotic Supplement (Oxoid) plus 10 mg/l of cephalotin (Lilly) and FBP Supplement (Oxoid). Inoculated plates were incubated at 43°C for 48 hours under microaerobic conditions utilizing the Gas generating box **Campylobacter** system (bio Mérieux).

After the incubation period, the plates were examined for the presence of colonies presumed to be **Campylobacter** sp. The identification was made first presumptively[17] using catalase and oxidase reactions (both positive) and the morphological features observed in Gram stain (curved S-shaped rods). Later, the **Campylobacter** species and biotypes were identified using the criteria propose by SKIRROW & BENJAMIN[33].

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RESULTS

Campylobacters were isolated from 292 (46.3%) of the animals studied. The highest isolation rate was found in pigs (70%), followed by hens (60%) and dogs (51.4%). The lowest rate was found in pigeons (17.4%).

C. jejuni biotype 1 was the most frequently isolated (25.7%) and was found in all the animals studied. **C. coli** was isolated from all the animals but not from cats and pigeons, with an average frequency of 17.4%. **C. jejuni** biotype 2 was not found in hens, sparrows and pigeons and presented the lowest isolation rate (3.2%). Table 1 shows the distribution of these campylobacters among the animal reservoirs studied.

TABLE 1

Distribution of thermotolerant campylobacters among various mammals and birds from southern Chile.

Animals studied	(n)	C. jejuni biotype 1 Nº	%	C. jejuni biotype 2 Nº	%	C. coli Nº	%	Total Nº	%
Dogs	(150)	46	30.7	6	4.0	25	16.7	77	51.4
Pigs	(80)	8	10.0	4	5.0	44	55.0	56	70.0
Cows	(80)	12	15.0	6	7.5	6	7.5	24	30.0
Cats	(40)	6	15.0	3	7.5	0	0.0	9	22.5
Hens	(100)	45	45.0	0	0.0	15	15.0	60	60.0
Sparrows	(75)	21	28.0	0	0.0	9	12.0	30	40.0
Geese	(60)	16	26.7	1	1.7	11	18.3	28	46.7
Pigeons	(46)	8	17.4	0	0.0	0	0.0	8	17.4
Total	(631)	162	25.7	20	3.2	110	17.4	292	46.3

DISCUSSION

Birds and mammals constitute important links in the epidemiological chain of **Campylobacter** infections[2, 20]. We studied several animal reservoirs that, according to their epidemiological relations with humans, can be divided in four groups:
1. companion animals (dogs and cats); 2. food animals (cows and pigs); 3. poultry (hens and geese); 4. wild birds (sparrows and pigeons). Our results show that thermotolerant species of **Campylobacter** were present in high frequency in all of them.

C. jejuni biotype 1 was isolated from all the animals under study with an average frequency of 25.7%. **C. jejuni** biotype 2, with a frequency of 3.2%, was found in all the animals but not in hens, sparrows and pigeons. With the exception of the cats and pigeons fecal samples, **C. coli** was isolated from the other animal feces with a frequency of 17.4%. SKIRROW[30-33] and SHANKER et al.[28] reported that **C. jejuni** biotype 1 was the most prevalent in fecal samples from hens and dogs. Our results support these findings. However they found that **C. jejuni** biotype 2 was also frequent in these two animal species. **C. coli** was not isolated by SHANKER et al.[28] from cloacal samples of hens. In our study **C. jejuni** biotype 2 was found in low frequency (4%) in dogs but not in hens. On the other hand, the prevalence of **C. coli** was 16.7% in dogs and 15% in hens. This results are very simmilar to that obtained by FERNÁNDEZ[3] in fecal samples from dogs and hens in São Paulo - Brazil.

Some differences in the epidemiology of campylobacteriosis have been established among developed and developing countries such as the high percentage or normal carriers and a greater frequency of cases in the first two years of life observed in the latter countries [4,5].

The fact that **C. jejuni** biotype 2 is less and **C. coli** more frequent in hens and dogs in Brazil [3] and southern Chile, could be considered a peculiar epidemiological situation observed in developing countries. However, more data must be made available from third world countries to establish definitively this statement.

The prevalence of **Campylobacter** in dogs (51.4%) was higher than that obtained by

McORIST & BROWNING[19] in Australia (9.5%) and JORGENSEN[12] in Denmark (11.1%). Nevertheless, it was simmilar to that reported by SIMPSON et al.[29] in stray dogs (52.6%) in England. Remarkable differences exist in the incidence of intestinal carriage of **Campylobacter** among stray and pet dogs[2, 29, 30]. Although all the dogs studied had an individualized owner, many of them presented loiterer habits reflecting a poor standard of canine hygiene that predisposes to intestinal colonization with **Campylobacter**[30].

The prevalence of **Campylobacter** in cats (22.5%) was higher than that reported by McORIST & BROWNING[19] in Australia (3%) and by PATTON et al.[22] in U.S.A. (14%). Because the cats studied presented the same habits observed in the dogs (owners references) we can use the same considerations as SKIRROW[30] to explain the high isolation rates of **Campylobacter** obtained in both animal species. These results constitute another evidence of the importance of dogs and cats as natural reservoirs of **Campylobacter** as potencial sources for human infections[2, 9, 20, 24, 30].

The fecal carriage of **Campylobacter** in pigs (70%) was very simmilar to that reported by STICHT-GROH[34] in Germany (77%) and LUECHTEFELD & WANG[16] in U.S.A. (71%), but lower to that published by ROSEF et al.[25] in Norway (100%). The fact that **C. coli** forms part of the normal intestinal flora of pigs[3] explains the high isolation frequency of this bacteria (55%) in the pigs subjected to our study.

Campylobacter was found in 30% of the cows under study. This result is lower than reported by LUECHTEFELD & WANG[16] in U.S.A. (43%) but higher than the results obtained by SWEDHEM & KAIJSER[35] in Sweden (19%).

The isolation of thermotolerant species of **Campylobacter** in high frequency from these two animals indicate their importance as natural reservoirs and sources of these bacteria for man. Humans can acquire the infection by contact with these animals[9, 20] or by consumption of contaminated meats and milk[2, 10, 20, 23].

In hens and geese the **Campylobacter** isolation rate was 60% and 46.7% respectively. High isolation rates of **Campylobacter** from both, slaughtered and alive hens, is a common fact in many countries[2,6]. Wild geese harbour these microorganisms frequently in their intestinal tract[15].

Poultry constitutes an important vehicle of **Campylobacter** and the role of poultry meat in the illness transmission is well established[2, 10, 11].

The isolation rate of Campylobacter in sparrows was 40%. The corresponding values found by MATSUSAKI et al.[18] in Japan was only 10% whereas, KAPPERUD & ROSEF[13] did not isolated any **Campylobacter** from sparrows in Norway.

In pigeons we isolated only **C. jejuni** biotype 1 with a frequency of 17.4% MATSUSAKI et al.[18] and KAPPERUD & ROSEF[13] reported 7% and 4.2% of intestinal carriage of **Campylobacter** in pigeons respectively. WEBER et al.[37] in Germany, demonstrated that **Campylobacter** can be isolated with high frequency (54.9%) from carrier pigeons. Sparrows and pigeons are wild birds that can live near man and may be sources of contamination for water supplies and the environment as it has been previously suggested [7, 21].

Except for studies on pigs and hens[6, 7], this work is the first study in Chile to ascertain the prevalence of thermotolerant campylobacters in animals. We think that more studies are necessary to understand the epidemiology of campylobacteriosis that seems to be a very complex phenomenon in developing countries.

RESUMO

Espécies e biotipos de Campylobacter **termotolerantes em reservatórios animais do sul do Chile.**

Foi determinada a prevalência de **Campylobacter** termotolerantes em mamíferos e aves do sul do Chile.

Campylobacter foi isolado em 46,3% dos animais estudados sendo **C. jejuni** biotipo 1 o mais freqüente (25,7%) seguido de **C. coli** (17,4%) e **C. jejuni** biotipo 2 (3,2%).

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