

CHICKEN AS POTENTIAL CONTAMINATION SOURCE OF *CAMPYLOBACTER LARI* IN IQUITOS, PERU.

Alvaro TRESIERRA-AYALA (1), Maria Elena BENDAYAN (1), Alfonso BERNUY (1), Gustavo PEREYRA (1) & Heriberto FERNANDEZ (2)

SUMMARY

In order to know the importance of chicken as natural reservoir of *Campylobacter lari* in Iquitos, Peru; samples were obtained by cloacal swabs from 200 chickens and immediately placed into a semisolid enrichment medium; these were streaked on modified Skirrow Agar. The organism was isolated from 21 (10.5%) samples, corresponding 58.8% to biovar I and 41.2% to biovar II (Lior scheme). The results provide evidence that chicken appear to be prominent reservoirs of *Campylobacter lari* in Iquitos.

KEYWORDS: *Campylobacter lari*; Chicken; Reservoir.

INTRODUCTION

Diarrhoeal disease is an important factor in the infection-malnutrition cycle of infants and young children in Peru^{2,13}. The classical thermotolerant species of *Campylobacter* have become recognized as an important cause of acute diarrhoeal disease, but *C. jejuni ssp. jejuni* and *C. coli* are considered the most important because human illness associated with *C. lari* (formerly *C. laridis*)²⁵ is infrequently recognized. However, even when this bacteria has been recovered from humans with *C. lari*-associated enteritis, its role as an enteric pathogen and their virulence factors have not been clearly established^{4, 8, 10, 20, 22, 24}.

C. lari was described as a nalidixic acid-resistant thermophilic *Campylobacter* (NARTC) by SKIRROW and BENJAMIN²³. The first human isolates were reported to be from the faeces of four asymptomatic individuals, but posteriorly, in sporadic cases, faecal isolation of the organism was associated with an enteric illness⁶.

The epidemiology of human illness associated with this agent remains uncertain. *C. lari* was chiefly recovered from the cloacal contents of wild herring gulls (*Larus argentatus*). Some isolates were subsequently obtained from a variety of birds and other animals^{1, 10, 16, 17}. However, unlike *C. jejuni ssp. jejuni* and *C. coli*, *C. lari* appear to become uncommon isolate from domestic animals⁹.

Chickens may be important reservoirs of *C. lari* in Peru; consequently, the purpose of this study was to determine the presence of *C. lari* in these birds.

MATERIALS AND METHODS

Samples were obtained by cloacal swabs from 200 free-ranging domestic chickens living in different peri-urban zones of Iquitos city (in the Peruvian jungle region). All samples were immediately placed into the

Financial support: Grant of Investig. Office - U.N.A.P. and CONCYTEC. Grant S-92-05 DID-UACH.

(1) Department of Microbiology. Universidad Nacional de la Amazonia Peruana. P.O. Box 751. Iquitos-Peru.

(2) Institute of Clinical Microbiology. Universidad Austral de Chile. P.O. Box 567. Valdivia - Chile.

Correspondence to: Prof. Alvaro Tresierra Ayala, Casilla Postal 751, Iquitos - Peru

semisolid enrichment medium proposed by FERNANDEZ⁸ and streaked on modified Skirrow plates⁷. These were incubated at 42°C for 48h, in microaerophilic conditions.

Suspected colonies were identified morphologically (Gram stain) and biochemical characterization of the isolates was done using the differential tests proposed by LIOR¹⁸ and GOOSSENS & BUTZLER¹¹. Antimicrobial susceptibility to nalidixic acid (30 µg) and cephalotin (30 µg) was determined by disc diffusion assay on FBP-blood agar incubated at the same conditions mentioned above.

RESULTS

After 48 h of incubation, some cultures showed some smooth, translucent, spreading colonies on modified Skirrow medium. Gram stain revealed curved Gram-negative bacilli with typical appearance of *Campylobacter spp.* Colonies were screened for campylobacters using the oxidase and catalase tests, being both positive.

From the 200 chickens studied, 21 (10.5%) harbored *C. lari* in their intestinal tract (Table 1). Only 17 strains were analyzed for biovars (Lior scheme) because 4 isolates were lost during the freezing process used to preserve them in freezing medium¹⁵, before the tests were done. Then (58.8%) of the strains corresponded to biovar I and 7 (41.2%) to biovar II (Table 1).

Table 1

Isolation rate of *Campylobacter lari* and their biovars (Lior scheme) from 200 free-ranging domestic chickens.

<i>C. lari</i>	Number (%) of		
	Chicken	Biovar I*	Biovar II*
Present	21 (10.5)	10 (58.8)	7 (41.2)
Absent	179 (89.5)	-	-

* Calculated with 17 strains.

DISCUSSION

Previous reports^{1, 8, 9, 10, 15}, have indicated that thermotolerant species of *Campylobacter* are commensals in the intestinal tract of poultry, being chickens frequently associated as infection sources for human beings^{5, 21}.

The isolation rate of *C. lari* found in this study (10.5%) is higher than that reported by KWIA TEK et al.¹⁷ in Poland (6.1%). In Latin America⁹, this microorganism was isolated from cattle and sewage in Brazil, from aquatic birds and from one documented diarrhea episode affecting a chicken eviscerator in Chile, and from chicken meat in Costa Rica.

The key feature for primary differentiating *C. lari* from the other classical thermotolerant species of the genus is the resistance to a 30 µg disk of nalidixic acid. However, some investigators¹ have identified rare strains of *C. jejuni* resistant to this antibiotic. Indoxil acetate hydrolysis¹⁴ and H₂S production on iron metabisulfite medium¹⁸ are complementary tests that, showing a good level of discrimination, are useful for distinguishing *C. lari* from *C. jejuni* and *C. coli*.

Because large number of *Campylobacter spp.* are released from intestinal content during the defeathering and eviscerating operations, they could be present, also in large numbers, contaminating poultry carcasses¹⁷. Transmission of *Campylobacter spp.* to humans via these food products is well established³. Moreover, GRADOS et al.¹² showed that chickens maintained in close association with humans represent a potential source of *Campylobacter* infection.

Most of the chicken faecal samples submitted to our study come from the peri-urban area, where the risk of acquiring viable *Campylobacter spp.* is increased because of the close association observed between humans and birds in similar places¹². Besides, the house floors are usually made of dirt or unfinished concrete, so the complete elimination of chicken faeces is difficult to achieve, contributing to increase the infection risks¹².

Despite further clinical and epidemiological studies are required to better define the role of *C. lari* in human disease, our data provide evidence that chicken appear to be prominent reservoir of *C. lari* in Iquitos. That should remind us to search for this microorganism in gastroenteritis cases, specially when the patient has close contact with chickens.

RESUMO

Frangos como fonte potencial de contaminação por *Campylobacter lari* em Iquitos, Peru.

Com o objetivo de conhecer a importância dos frangos como reservatório natural de *Campylobacter*

lari na cidade de Iquitos, Perú, foram estudadas amostras cloacais obtidas de 200 aves. Cada amostra foi semeada em meio de enriquecimento semi-sólido e no ágar de Skirrow modificado. *C. lari* foi isolado em 21 (10,5%) amostras. Destes, 58,8% corresponderam ao biotipo I e 41,2% ao biotipo II do esquema de Lior. Os resultados obtidos sugerem que os frangos podem ser um importante reservatório de *C. lari* em Iquitos, Perú.

REFERENCES

- BENJAMIN, J.; LEAPER, S.; OWEN, R.J. & SKIRROW, M.B. - Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic *Campylobacter* (NARTC) group. **Curr. Microbiol.**, 8: 231-238, 1983.
- BLACK, R.; LOPEZ DE ROMAÑA, G.; BROWN, K. et al. - Incidence and etiology of infantile diarrhea and major routes of transmission in Huascar, Peru. **Amer. J. Epidem.**, 129: 785-799, 1989.
- BLASER, M.J.; TAYLOR, D.N. & FELDMAN, R.A. - Epidemiology of *Campylobacter jejuni* infections. **Epidem. Rev.**, 5: 157-176, 1983.
- BORCZYK, A.; THOMPSON, S.; SMITH, D. & LIOR, H. - Waterborne outbreak of *Campylobacter laridis*-associated gastroenteritis. **Lancet**, 1: 164-165, 1987.
- BUTZLER, J.P. & OOSTEROM, J. - *Campylobacter*: pathogenicity and significance in foods. **Int. J. Food Microbiol.**, 12: 1-8, 1991.
- CARLSON, J. - 162 cases of *Campylobacter* and *Salmonella* as a result of a waterborne outbreak in a nuclear reactor station. **Ontario Dis. Surveill. Rep.**, 6: 544-545, 1985.
- FERNANDEZ, H. - Thermophilic species of *Campylobacter*: bacteriological, epidemiological and pathogenical aspects. São Paulo, 1983. (Doctoral Thesis - School of Medicine of São Paulo - Escola Paulista de Medicina).
- FERNANDEZ, H. - Increase of *Campylobacter* isolation rates using an enrichment medium. **Rev. Microbiol. (S. Paulo)**, 23: 5-7, 1992.
- FERNANDEZ, H. - Thermotolerant *Campylobacter* species associated with human diarrhea in Latin America. **Cienc. e Cult. (J. Braz. Ass. Adv. Sci.)**, 44: 39-43, 1992.
- FERNANDEZ, H.; LANDSKRON, E.; FIGUEROA, G.; GESCHE, W. & MONTEFUSCO, A. - *Campylobacter laridis*: first clinical isolation and identification of reservoir in Chile. **Rev. méd. Chile.**, 118: 699-701, 1990.
- GOOSSENS, H. & BUTZLER, J.P. - Isolation and identification of *Campylobacter* spp. In: NACHAMKIN, I.; BLASER, J.M. & TOMPKINS, L.S., ed. - *Campylobacter jejuni*. Current status and future trends. Washington, American Society for Microbiology, 1992.
- GRADOS, O.; BRAVO, N.; BLACK, R.E. & BUTZLER, J.P. - Paediatric *Campylobacter* diarrhoea from household exposure to live chickens in Lima, Peru. **Bull. Wild. Hlth. Org.**, 66: 369-374, 1988.
- GREENBERG, B.L.; SACK, R.B.; SALAZAR-LINDO, R.E. et al. - Measles-associated diarrhea in hospitalized children in Lima, Peru: pathogenic agents and impact on growth. **J. infect. Dis.**, 163: 495-502, 1991.
- HODGE, D.S.; BORCZYK, A. & WAT, L.L. - Evaluation of the indoxil acetate hydrolysis test for the differentiation of *campylobacters*. **J. clin. Microbiol.**, 28: 1482-1483, 1990.
- HUTCHINSON, D.N. & BOLTON, F.J. - An improved blood-free medium for isolation of *Campylobacter jejuni* from faecal specimens. **J. clin. Path.**, 37: 956-957, 1984.
- KASRAZADEH, M. & GENIGEORGIS, C. - Origin and prevalence of *Campylobacter jejuni* in ducks and duck meat at the farm and processing plant level. **J. Food protect.**, 50: 321-326, 1987.
- KWIATEK, K.; WOJTON, B. & STERN, N.J. - Prevalence and distribution of *Campylobacter* spp. on poultry and selected meat carcasses in Poland. **J. Food protect.**, 53: 321-326, 1990.
- LIOR, H. - New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli* and "*Campylobacter laridis*". **J. clin. Microbiol.**, 20: 636-640, 1984.
- LUECHTEFELD, N.; CAMBRE, R. & WANG, W. - Isolation of *Campylobacter fetus* subsp. *jejuni* from zoo animals. **J. Amer. Med. vet. Ass.**, 179: 1119-1122, 1981.
- MEGRAUD, F.; CHEVRIER, D.; DESPLACES, N.; SEDALLIAN, A. & GUESDON, J.L. - Urease-positive thermophilic *Campylobacter* (*C. laridis* variant) isolated from an appendix and from human feces. **J. clin. Microbiol.**, 26: 1050-1051, 1988.
- SHANE, S.M. - The significance of *Campylobacter jejuni* in poultry: a review. **Avian Path.**, 21: 189-213, 1992.
- SIMOR, A. & WILCOX, L. - Enteritis associated with *Campylobacter laridis*. **J. clin. Microbiol.**, 25: 10-12, 1987.
- SKIRROW, M.B. & BENJAMIN, J. - "1001" campylobacters: cultural characteristics of intestinal campylobacters from man and animals. **J. Hyg.**, 85: 427-442, 1980.
- TAUXE, R.V.; PATTON, C.M.; EDMONDS, P. et al. - Illness associated with *Campylobacter laridis*, a new recognized *Campylobacter* species. **J. clin. Microbiol.**, 21: 222-225, 1985.
- VON GRAEVENITZ, A. - Revised nomenclature of *Campylobacter laridis*, Enterobacter intermedium and "*Flavobacterium brachiophila*". **Int. J. system. Bact.**, 40: 211, 1990.

Recebido para publicação em 03/01/1994.

Aceito para publicação em 25/05/1994.