

Campylobacter upsaliensis isolated from young dogs with and without diarrhea

Campylobacter upsaliensis isolado de cães jovens com e sem diarréia

Dear Editor,

Campylobacter upsaliensis is considered a human enteropathogen associated with diarrhea and bacteremia^{3,7,8,12,14,15}. This is an atypical species of *Campylobacter* genus as it is thermophilic, weakly catalase-positive, and generally sensitive to cephalosporins. Immunosuppressive factors seem to be of major importance in triggering the disease^{5, 11, 16}. Cats and dogs are considered the major carriers for humans^{4, 7, 17}, more frequently in under 12-month-old dogs⁴. In Brazil, studies on dogs as potential *C. upsaliensis* carriers for humans are scarce. We studied the isolation frequency in 100 dogs with diarrhea and 100 dogs without diarrhea; all animals were less than 12 months old.

Dog feces were submitted to two parallel procedures: 1) filtration technique - one gram of feces was suspended in a test tube with 9ml saline solution, vigorously homogenized for 1 min, centrifuged at 2,500 rpm for 5 min, and filtered using 0.65mM cellulose acetate membrane filter. Three drops from this filtrate were grown on *Petri* dishes in sodium thioglycolate agar supplemented with 20% bovine blood and incubated at 37°C, 2) Direct growth - one aliquot of feces was grown in smears in the same agar with *Butzler* selective supplementation (bacitracin, novobiocin, cycloheximide, colistin and cefazoline) and incubated at 43°C. In both procedures, the plates were examined with a phase-contrast microscope (1000 X) for morphological evaluation of vibriion characteristics and typical spirillum movement. After presumptive diagnosis, these colonies were replicated in *Tarozzi* medium and incubated at 37°C for 72 hours to obtain the inoculum, with density adjusted to 1 MacFarland standard turbidity (3x10⁸ CFU/mL). Definitive diagnosis was made using biochemical characteristics from following tests¹⁰: catalase (-) and hippuricase production (-); growth at 43°C (+), 25°C (-), in 1% glycine (-), and in 3.5% NaCl (-); resistance to nalidixic acid (-) and cephalothin (-); production of H₂S with (-) or without cysteine (-); and tolerance to 2'3'5'-triphenyltetrazoline chloride (-). The animals with diarrhea showed 3 (3%) *C. upsaliensis* strains and the diarrhea-free 2 (2%). Our results were relatively

lower than other data in literature, suggesting heterogeneity of this bacterium isolation frequency^{2,4,9}. To increase the possibility of *C. upsaliensis* detection, a filtration technique should be used on a routine basis; as has already been reported¹³. *C. upsaliensis* isolation from dogs highlights the risk of zoonosis, especially in Brazil, although no reports were found in literature.

José Rafael Modolo¹ and Rogerio Giuffrida¹

ACKNOWLEDGEMENTS

The authors wish to thank Fundação de Amparo à Pesquisa de São Paulo for financial support and Ms. Tânia Maria Martins and Adriana Cristina Pavan Vieira for their valuable technical assistance.

REFERENCES

1. Albert, MJ, Tee W, Leach A, Asche V, Penner JL. Comparison of blood-free medium and a filtration technique for isolation of *Campylobacter* spp. from diarrhoeal stools of hospitalised patients in central Australia. *Journal of Medicine Microbiology* 37:176-179, 1992.
2. Baker J, Barton MD, Lanser J. *Campylobacter* species in cats and dogs in South Australia. *Australian Veterinary Journal* 77:662-666, 1999.
3. Bourke B, Chan VL, Sherman P. *Campylobacter upsaliensis*: waiting in the wings. *Clinical Microbiology Reviews* 11:440-449, 1998.
4. Burnens AP, Ageloz-Wick B, Nicolet J. Comparison of *Campylobacter* carriage rates in diarrheic and healthy pet animals. *Zentralblatt fur Veterinarmedizin [B]* 39:175-180, 1992.
5. Carnahan AM, Beadling J, Watsky D, Ford N. Detection of *Campylobacter upsaliensis* from a blood culture by using the BacT/Alert system. *Journal of Clinical Microbiology* 32:2598-2599, 1994.
6. Goossens H, Vlaes L, Butzler JP, Adnet A, Hanicq P, N'Jufom S, Massart D, De Schrijver G, Blomme W. *Campylobacter upsaliensis* enteritis associated with canine infections. *Lancet* 337:1486-1487, 1991.

1. Disciplina de Panejamento de Saúde Animal e Veterinária Preventiva do Departamento de Higiene Veterinária e Saúde Pública da Faculdade de Medicina Veterinária e Zootecnia da Universidade do Estado de São Paulo, Botucatu, SP.

Supported by grants from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo)

Address to: Dr José Rafael Modolo. Caixa Postal 524, 18618-000 Botucatu, SP.

Tel: 14 6802-6270, Fax: 14 6802-6075

e-mail: jrmodolo@fmvz.unesp.br

Recebido para publicação em 14/8/2002.

Aceito em 13/11/2003

7. Goossens H, Vlaes L, De Boeck M, Pot B, Kerstrs K, Levy J, De Mol P, Butzler JP, Vandamme P. Is "*Campylobacter upsaliensis*" an unrecognized cause of human disease? *Lancet* 335:584-586, 1990.
8. Grossens H, Giesendorf BA, Vandamme P, Vlaes L, Van Den Borre C, Koeken A, Quint WG, Blomme W, Hanicq P, Koster DS. Investigation of an outbreak of *Campylobacter upsaliensis* in day care centers in Brussels: analysis of relationships among isolates by phenotypic and genotyping methods. *Journal of Infectious Diseases* 172:1298-1305, 1995.
9. Hald B, Madsen M. Healthy puppies and kittens as carriers of *Campylobacter* spp, with special reference to *Campylobacter upsaliensis*. *Journal of Clinical Microbiology* 35:3351-3352, 1997.
10. Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. *Bergey's manual of determinative bacteriology*. 9th edition, Philadelphia: Williams & Wilkins, p. 799, 1994.
11. Jerkin GA, Tee W. *Campylobacter upsaliensis*-associated diarrhea in human immunodeficiency virus-infected patients. *Clinical Infectious Diseases* 27:816-821, 1998.
12. Jimenez SG, Heine RG, Ward PB, Robins-Browne RM. *Campylobacter upsaliensis* gastroenteritis in childhood. *Pediatric Infectious Diseases Journal* 18: 988-992, 1999.
13. Lopez L, Castillo FJ, Clavel A Rubio MC. Use of a selective medium and a membrane filter method for isolation of *Campylobacter* species from Spanish paediatric patients. *European Journal of Clinical Microbiology Infectious Diseases* 17: 489-492, 1998.
14. Mooney A, Clyne M, Curran T, Doherty D, Kilmartin B, Bourke B. *Campylobacter upsaliensis* exerts a cytolethal distending toxin effect on HeLa cells and lymphocytes. *Microbiology* 147:735-743, 2001.
15. Musmanno RA, Russi M, Figura N, Guglielmetti P, Zanchi A, Signori R, Rossolini A. Unusual species of campylobacters isolated in the Siena Tuscany area, Italy. *New Microbiology* 21:15-22, 1998.
16. Snijders, F, Kuijper EJ, De Wever B, Van Der Hoek L, Danner SA, Dankert J. Prevalence of *Campylobacter*-associated among patients infected with immunodeficiency virus. *Clinical Infectious Diseases* 24:1107-1113, 1997.
17. Steinhauserova I, Fojtikova K, Klimes J. The incidence and PCR detection of *Campylobacter upsaliensis* in dogs and cats. *Letters in Applied Microbiology* 31: 209-212, 2000.