Lactococcus lactis subsp. lactis Infection in Waterfowl: First Confirmation in Animals

Joaquín Goyache, Ana I. Vela, Alicia Gibello, María M. Blanco, Víctor Briones, Sergio González, Sonia Téllez, Cristina Ballesteros, Lucas Domínguez, and José F. Fernández-Garayzábal
Facultad de Veterinaria, Universidad Complutense, Madrid, Spain

We report the first description, confirmed by bacteriologic and molecular (polymerase chain reaction and pulsed-field gel electrophoresis) analysis, of an infection in animals caused by Lactococcus lactis subsp. lactis, affecting waterfowl.

Until recently, members of the genus Lactococcus were considered opportunistic pathogens (1,2). They are often misidentified as enterococci or streptococci (3,4), and the difficulties in correctly identifying them have probably hindered elucidation of their clinical significance. However, the number of clinical cases associated with infections by these microorganisms has increased in the last decade in both humans and animals (5-7). Lactococcus lactis subsp. lactis, L. piscium, and L. garvieae are recognized as the species with clinical significance for human and veterinary medicine (2,8). In humans, L. garvieae and L. lactis subsp. lactis have been associated with endocarditis (9,10) and have also been isolated from clinical samples of blood, skin lesions, and urine (5,7). In veterinary medicine, L. garvieae and L. piscium are pathogenic for various fish species (8,11,12), and L. garvieae causes mastitis in ruminants (13,14). However, infection by L. lactis subsp. lactis in animals has not previously been reported. We present the first microbiologic and molecular evidence for infection produced by L. lactis subsp. lactis in waterfowl.

The Study

From September to November 1998, a mass die-off was detected among waterfowl in southwestern Spain, affecting >3,000 birds (0.6% of the total waterfowl population in the area). The species most affected were coots (Fulica atra) (26.9%), shovellers (Anas clypeata) (25.1%), and mallards (Anas platyrhynchos) (13.8%). Overall, 20% of the birds died. Affected birds showed general weakness, evidenced by drooping wings and sluggishness; approximately 50% had respiratory distress. At necropsy, most animals had mild lung congestion; no other lesions were found at postmortem examination.

Samples from the lungs, liver, and spleen of five diseased birds (one mallard, S-15; three shovellers, S-16, S-18, and S-19; and one coot, S-17) were submitted to the Animal Health Department at the School of Veterinary Medicine of Madrid for microbiologic analysis. After 48 hours of incubation at 37°C, pure cultures of weakly α-hemolytic catalase-negative cocci were obtained on blood agar plates from samples of lung (S-15, S-16, and S-17) and liver and spleen (S-15, S-16, S-17, and S-18). All 11 clinical isolates had an identical biochemical profile, which was identified as L. lactis subsp. lactis by the Rapid ID 32 Strep system (bioMérieux España, S.A., Madrid).

L. lactis subsp. lactis and L. garvieae are the two species more frequently found in human and animal infections (2). Routine clinical microbiologic diagnosis requires accurate discrimination of the two species, as their similar biochemical reaction patterns may lead to misidentification (2,4). Although physiologic tests, differences in antimicrobial susceptibility, whole-cell protein, and DNA or RNA analysis (4,7,13) have been proposed to distinguish them, some of these techniques are not reliable or may be too time-consuming, limiting their use for routine identification. For these reasons, the clinical isolates were also identified by a polymerase chain reaction (PCR) assay, which has been successfully used to identify many other pathogens (15,16).

Specific primers LLF 5'-GCAATTGCATCACTCAAAGA and LLR 5'-ACAGAGAACTTATAGCTCCC were designed from diagnostic regions of the L. lactis subsp. lactis 16S rRNA gene sequence (accession number M58837). PCR amplifications were performed in a 100-µL reaction volume containing 150 ng each of the two primers, 1 mM each of deoxynucleoside triphosphate, 1 U of Taq DNA polymerase (Promega, Inc., Madison, WI), under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturation for 1 minute at 92°C, primer annealing for 1.5 minutes at 50°C, primer extension for 2 minutes at 72°C, and a final extension of 5 minutes at 72°C. The following bacterial strains were used to test the specificity of the PCR assay: L. lactis subsp. lactis, ATCC 19435 and ATTC 11007; L. garvieae, NCFB 2155; four clinical isolates of Lactococcus garvieae (1336, 1458, 1982, and 4294, isolated from lactococcosis in trout); L. piscium, NCFB 2778; Streptococcus iniae, ATCC 29187; Vagococcus fluvialis, NCFB 2497; and Enterococcus faecalis, CECT 481. All the L. lactis subsp. lactis clinical isolates generated an expected PCR amplification product of 650 bp. No amplification was observed with any other Lactococcus species tested, indicating the specificity of the PCR assay (Figure 1). These results confirmed those of the biochemical identification, as well as the utility of this PCR assay for spe-
cate that infection was produced by a single strain of
sotypes of
able restriction patterns were obtained from all clinical
isolates, as described by Vela et al. (18). This technique has
been successfully applied for strain identification and epide-
miologic investigations of lactococci (19,20). Indistinguish-
been applied for strain identification and epide-
ic, rapid, and accurate identification of this microorgan-
ism. In addition, the fact that no PCR amplification was
observed from
L. lactis
subsp. lactis ATCC 19435 (lane C), L. lactis subsp. lactis ATCC 11007 (lane D), and the clinical isolates (lanes J, K, and L) generated
a PCR amplification product of 650 bp. No amplification was
specific, rapid, and accurate identification of this microorgan-
ism. In addition, the fact that no PCR amplification was
observed with clinical isolates when tested with a PCR specific
for L. garvieae (17) corroborates the identification (data not shown).

Pulsed-field gel electrophoresis (PFGE) with the enzyme
SmaI digests of Smal digests of genomic DNA of Lactococcus lactis subsp. lactis clinical isolates. Lane A, molecular weight marker: lanes B, D, F, and H, liver isolates from samples S-15, S-16, S-17, and S-18; lanes C, E, and G, lung isolates of samples S-15, S-16, and S-17; and lane I, spleen isolate from sample S-18.

As L. lactis subsp. lactis is considered nonpathogenic for
animals (1,2) and no additional histopathologic or toxicologic
studies could be carried out in the diseased animals, we
not rule out other possible causes for the mass deaths. Therefore, although the PFGE results, together with the
recovery of L. lactis subsp. lactis in pure culture from the
clinical samples, may suggest clinical significance, no direct
link between the L. lactis subsp. lactis infection and this epi-

wild animals, including waterfowl, are known reservoirs
for various pathogens (21). We can only speculate about the
possibility that waterfowl may be a reservoir for this bacte-
rium. However, wild animal reservoirs for other species of
lactococci have been described (22).

Dr. Goyache is associate professor, Facultad de Veterinaria, Universidad Complutense de Madrid. His responsibilities include
research and teaching related to clinical microbiology of wildlife and
exotic animals.

References
1. Aguirre M, Collins MD. Lactic acid bacteria and human clinical
infection. J Appl Bacteriol 1993;75:95-107.
2. Facklam RR, Elliot JA. Identification, classification, and clinical
relevance of catalase-negative, gram-positive cocci, excluding
streptococci and enterococci. Clin Microbiol Rev 1995;8:479-95.
3. Schleifer KH. Recent changes in the taxonomy of lactic acid
bacteria. FEMS Microbiol Rev 1987;46:201-3.
4. Elliot JA, Facklam RR. Antimicrobial susceptibilities of Lacto-
coccus lactis and Lactococcus garvieae and a proposed method
to discriminate between them. J Clin Microbiol 1996;34:1296-8.
5. Facklam RR, Pigott NE, Collins MD. Identification of Lactococ-
cus species from human sources. Proceedings of the XI Lance-
field International Symposium on Streptococci and Streptococcal Diseases, Siena, Italy. Stuttgart: Gustav Fischer
Verlag; 1990:127.
6. Mannion PT, Rothburn MM. Diagnosis of bacterial endocarditis
caused by Streptococcus lactis and assisted by immunoblotting
of serum antibodies. J Infect 1990;21:317-8.
7. Elliot JA, Collins MD, Pigott NE, Facklam RR. Differentiation of
Lactococcus lactis and Lactococcus garvieae from humans by
comparison of whole-cell protein patterns. J Clin Microbiol 1991;29:2731-4.
8. Doménech A, Prieta J, Fernández-Garyzábal JF, Collins MD, Jones D, Domínguez L. Phenotypic and phylogenetic evidence
for a close relationship between Lactococcus garvieae and Enterococcus seriolicida. Microbiologia SEM 1993;9:63-8.
9. Furutan NP, Breiman RF, Fischer MA, Facklam RR. Lactococ-
cus garvieae infection in humans: a cause of prosthetic valve
endocarditis [C297]. Proceedings of the 91st America Society of
Microbiology Conference. Dallas: American Society of Microbi-
ology; 1991. p. 109.
10. Fefer JJ, Ratzan KR, Sharp SE, Saiz E. Lactococcus garvieae
endocarditis: report of a case and review of the literature. Diagn Microbiol Infect Dis 1998;32:127-30.
11. Eldar A, Ghittino C, Asanta L, Bozzeta E, Goria M, Prearo M,
et al. Enterococcus seriolicida is a junior synonym of Lactococ-
cus garvieae a causative agent of septicemia and meningoen-
cephalitis in fish. Curr Microbiol 1996;32:85-8.
12. Eldar A, Goria M, Ghittino C, Zlotkin A, Bercovier H. Biodiversity of Lactococcus garvieae strains isolated from fish in Europe, Asia, and Australia. Appl Environ Microbiol 1999;65:1005-8.
13. Collins MD, Farrow JAE, Phillips BA, Kandler O. Streptococcus garvieae sp. nov. and Streptococcus plantarum sp. nov. J Gen Microbiol 1983;129:3427-31.
14. Teixeira LM, Merquior VLC, Vianni MCE, Carvalho MGS, Fracalanza SEL, Steigerwalt AG, et al. Phenotypic and genotypic characterization of atypical Lactococcus garvieae strains isolated from water buffalos with subclinical mastitis and confirmation of L. garvieae as a senior subjective synonym of Enterococcus seriolicida. Int J Syst Bacteriol 1996;46:664-8.
15. Amann RI, Ludwig W, Schleifer K. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 1995;59:143-69.
16. Gibello A, Blanco MM, Moreno M, Cutuli MT, Doménech A, Domínguez L, et al. Development of a PCR assay for detection of Yersinia ruckeri in tissues of inoculated and naturally infected trout. Appl Environ Microbiol 1999;65:346-50.
17. Zlotkin A, Eldar A, Ghittino C, Bercovier H. Identification of Lactococcus garvieae by PCR. J Clin Microbiol 1998;36:983-5.
18. Vela AI, Vázquez J, Gibello A, Blanco MM, Moreno MA, Liébana P, et al. Phenotypic and genetic characterization of Lactococcus garvieae isolated in Spain from lactococcosis outbreaks and comparison with isolates of other countries and sources. J Clin Microbiol 2000;38:3791-6.
19. Tanskanen EI, Tulloch DL, Hillier AJ, Davidson BE. Pulsed-field gel electrophoresis of SmaI digests of lactococcal genomic DNA, a novel method of strain identification. Appl Environ Microbiol 1990;56:3105-11.
20. Carvalho MG, Vianni MCE, Elliot JA, Reeves M, Facklam RR, Teixeira LM. Molecular analysis of Lactococcus garvieae and Enterococcus gallinarum isolated from water buffalos with subclinical mastitis. Adv Exp Med Biol 1997;418:401-4.
21. Hannam DAR. Zoonoses and health implications including COSHH. In: Beynon PH, Forbes NA, Harcourt-Brown NH, editors. Manual of raptors, pigeons and waterfowl. Ames: Iowa State University Press; 1996. p. 113-5.
22. Kusuda R, Salati F. Enterococcus seriolicida and Streptococcus iniae. In: Woo PTK, Bruno DW, editors. Fish diseases and disorders. Vol 3. Viral, bacterial and fungal infections. Wallingford: CABI Publishing; 1999. p. 303-17.