Eur J Clin Microbiol Infect Dis (2006) 25: 242–246
DOI 10.1007/s10096-006-0119-x

ARTICLE

A. Bordes-Benítez · M. Sánchez-Oñoro · P. Suárez-Bordón · A. J. García-Rojas · J. A. Saéz-Nieto · A. González-García · I. Álamo-Antúnez · A. Sánchez-Maroto · M. Bolaños-Rivero

Outbreak of *Streptococcus equi* subsp. *zooepidemicus* infections on the island of Gran Canaria associated with the consumption of inadequately pasteurized cheese

Published online: 21 March 2006

Abstract *Streptococcus equi* subsp. *zooepidemicus* infections are infrequent in humans. A clinical and epidemiological study of a milk-borne outbreak caused by this organism is described. Fifteen patients (5 females, 10 males) with a median age of 70 years (range 47–86) were infected. Twelve (80%) had underlying diseases. Infection with *S. equi* subsp. *zooepidemicus* presented as primary bacteremia in six cases, as bacteremia associated with aortic aneurism in four cases, as septic arthritis in two cases, as pneumonia in two cases, and as meningitis in one case. Five (33.3%) patients died. A case-control study proved that consumption of inadequately pasteurized cheese of a specific brand was associated with *S. equi* subsp. *zooepidemicus* disease (OR=4.5; 95% CI 1.57–19.27; $p<0.001$). This outbreak serves as a reminder that *S. equi* subsp. *zooepidemicus* causes serious infections that are usually zoonoses. Identification of beta-hemolytic streptococci to the species level to detect contaminated foods of animal origin is important for preventing new food-borne outbreaks. For a precise characterization of the isolates, the application of molecular markers is recommended.

Keywords *S. equi* subsp. *zooepidemicus* · Group C streptococci · Food-borne outbreak · Zoonoses · Bacteremia · Aortic aneurism

A. Bordes-Benítez (✉) · M. Sánchez-Oñoro · P. Suárez-Bordón · A. González-García · I. Álamo-Antúnez
Laboratorio de Microbiología, Hospital Universitario de Gran Canaria Dr. Negrín,
Bco. de la Ballena s/n,
35020 Las Palmas de Gran Canaria, Canary Islands, Spain
e-mail: aborben@gobiernodecanarias.org
Tel.: +34-28-449522
Fax: +34-28-449292

A. J. García-Rojas
Public Health Department (Epidemiology Section),
Alfonso XIII 5,
35003 Las Palmas de Gran Canaria, Canary Islands, Spain

J. A. Saéz-Nieto · A. Sánchez-Maroto
National Reference Laboratory of Streptococcus (Servicio de Bacteriología), Centro Nacional de Microbiología, Instituto de Salud Carlos III,
Carretera Majadahonda-Pozuelo km 2,
28220 Majadahonda, Madrid, Spain

M. Bolaños-Rivero
Laboratorio de Microbiología, Hospital Universitario Insular de Gran Canaria,
35016 Las Palmas de Gran Canaria, Canary Islands, Spain

Introduction

Large-colony Lancefield group C streptococci are grouped into two species, each with two subspecies: *Streptococcus dysgalactiae* subsp. *equisimilis*, *S. dysgalactiae* subsp. *dysgalactiae*, *Streptococcus equi* subsp. *equi*, and *S. equi* subsp. *zooepidemicus* [1]. *S. dysgalactiae* subsp. *equisimilis* is the subspecies most frequently detected in humans, either as a commensal organism or as a pathogen. The other subspecies are more commonly isolated in animals.

Of all group C streptococci subspecies, *S. equi* subsp. *zooepidemicus* is probably the most aggressive human pathogen, and several outbreaks and sporadic cases of severe infection due to this microorganism have been reported, including nephritis, arthritis, sepsis, meningitis, and pneumonia [2–8]. Human infections are linked to close contact with domestic animals [2, 9], with consumption of unpasteurized milk [9–14], and even with consumption of pork [15]. This report describes a clinical and epidemiological study of an outbreak of *S. equi* subsp. *zooepidemicus* infections on the island of Gran Canaria (Spain) associated with the consumption of inadequately pasteurized cheese between February and April 2003.

Patients and methods

Clinical and microbiological methods

A confirmed case of infection by *S. equi* subsp. *zooepidemicus* was defined as the isolation of *S. equi* subsp. *zooepidemicus* from a clinical sample. Samples

obtained from normally sterile sites and from the respiratory tract were processed according to conventional microbiological methods. Blood cultures were processed using the Bactec 9000 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA). Dairy product samples were processed according to conventional microbiological methods [16].

Characterization of isolates was carried out by means of the following conventional tests: Gram stain, catalase activity, beta-hemolysis on blood agar plates, colony size, ability to ferment sorbitol and trehalose, and detection of Lancefield group C antigen (Phadebact Streptococcus Test; Boule Diagnostics, Huddinge, Sweden). Identification to species level was performed using the API Rapid 32 Strep System (bioMérieux, Marcy l'Toile, France) and the BBL-Crystal GP ID (Becton Dickinson Microbiology Systems, Sparks, MD, USA). The relatedness of isolates was determined by *Sma*I restriction enzyme digests of chromosomal DNA separated by pulsed-field gel electrophoresis according to a method described previously [17, 18].

The E test (AB Biodisk, Solna, Sweden) was used to test susceptibility to the following antibiotics: penicillin, vancomycin, tetracycline, rifampin, erythromycin, and clindamycin. Susceptibility to levofloxacin and high-level resistance to gentamicin were determined using the agar dilution method following guidelines published by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) [19].

Medical records of patients were reviewed for demographic and clinical data. All patients with confirmed infection as well as individuals who lived with them were subjected to an epidemiological survey to look for evidence of epidemiological linkage: animal contact, contact with laboratory products, and consumption of milk, cheese and other raw dairy products.

Table 1 Clinical features of the patients

Case number	Age/sex	Date of positive sample	Underlying disease	Fever	Focal symptoms	Peripheral leukocyte count	Source of isolate	Diagnosis	Outcome
1	72/F	25 Feb	None	Yes	Joint pain	12,210	Joint	Septic arthritis	Limited mobility
2	86/F	27 Feb	AH, COPD, HD	No	Neurologic, respiratory	19,300	Blood	Bacteremia and pulmonary thromboembolism	Died
3	70/M	01 Mar	DMII, AH, CRF	Yes	GI	22,600	Blood	Bacteremia	Recovered
4	76/M	01 Mar	DMII, AH	Yes	Neurologic	21,800	Blood	Bacteremia	No follow-up
5	83/F	02 Mar	AH	Yes	Neurologic	29,300	CSF, bronchial aspirate	Meningitis	Died
6	47/M	02 Mar	None	Yes	Respiratory, GI	17,200	Blood	Pneumonia and bacteremia	Recovered
7	79/M	02 Mar	DMII, AH, HD	Yes	None	10,930	Blood	Bacteremia and septic arthritis	Recovered
		07 Mar		No	Joint pain	ND	Joint		
8	70/F	06 Mar	None	Yes	GI	20,430	Blood, bronchial aspirate	Pneumonia, endocarditis, and bacteremia	Disorientation to time and place
9	63/F	10 Mar	DMII, AH, HD	No	Cardiologic, respiratory	25,800	Blood	Bacteremia, tracheobronchitis, MI	Recovered
10	59/M	24 Mar	DMII, AH, DLP	Yes	Lumbar pain	14,000	Blood	Bacteremia and AA	Recovered
11	66/M	27 Mar	Pulmonary carcinoma	Yes	None	6,160	Blood	Bacteremia	Recovered
12	70/M	03 Apr	DMII, AH, HD	Yes	GI	11,340	Blood	Bacteremia	Recovered
13	68/M	12 Apr	DMII, AH, COPD	Yes	GI	16,880	Blood	Bacteremia and AA	Died
14	69/M	19 Apr	DMII, AH, DLP	Yes	Lumbar pain	8,780	Blood	Bacteremia and AA	Died
15	83/M	21 Apr	AH, HD	Yes	Lumbar pain, GI	15,500	Blood	Bacteremia and AA	Died

AH arterial hypertension, *COPD* chronic obstructive pulmonary disease, *CRF* chronic renal failure, *DLP* dyslipemia, *DMII* diabetes mellitus type II, *GI* gastrointestinal, *HD* heart disease, *ND* not done, *MI* myocardial infarction, *AA* aortic aneurism

Following the descriptive analysis, a hypothesis was established regarding the origin of the outbreak. Then, a case-control study was performed in order to confirm the hypothesis. Two controls per case were selected from among patients admitted with any other disease; one had to have been admitted immediately before and one immediately after the admission of the confirmed case. Both controls matched the confirmed case in terms of age and sex.

Statistical analysis

Cheese consumption and disease were compared by chi-square test with Fisher's exact test. $p<0.05$ was considered statistically significant. Relative risk was calculated from odds ratios and 95% confidence intervals. Data from the case-control study were validated using EpiInfo 2000 (CDC, Atlanta, GA, USA).

Results

Patients

The island of Gran Canaria in Spain has a population of 790,360, and all inhabitants have access to the National Health Service. From February to April 2003, 15 patients infected with *S. equi* subsp. *zooepidemicus* were detected in the two reference hospitals on the island: 12 in the Hospital Universitario de Gran Canaria Doctor Negrin and 3 in the Hospital Universitario Insular de Gran Canaria.

Clinical and microbiological data

Patients from whom *S. equi* subsp. *zooepidemicus* was isolated had a median age of 70 years (range 47–86). The clinical features of the patients are summarized in Table 1. All patients except cases 2 and 4 received a beta-lactam agent as antibiotic treatment. Case 2 died immediately after admission, and case 4 was discharged from the emergency unit and was lost to follow-up, with no follow-up data recorded in the clinical chart.

All the isolates were confirmed as *S. equi* subsp. *zooepidemicus* on the basis of biochemical tests. The API Rapid 32 Strep System showed a profile number of 05012061101, and with Crystal GP panels, three profile numbers were found: 1667351540 (6 isolates), 1767351561 (4 isolates), and 1667351563 (7 isolates).

All isolates were sensitive to penicillin, erythromycin, vancomycin, rifampin, and levofloxacin but were resistant to clindamycin and tetracycline. No isolate presented high-level gentamicin resistance.

Epidemiological study

When as an increasing number of cases of infection by *S. equi* subsp. *zooepidemicus* was being detected in the microbiology laboratory of Hospital Universitario de Gran Canaria Doctor Negrin, a possible outbreak was suggested and the situation was communicated to the Public Health Department (Epidemiology Section). That agency alerted all hospitals and health centres on the island about the possibility of an epidemic, and other cases were sought out, but, as previously described, only confirmed cases were assessed.

The epidemiological inquiry suggested that consumption of fresh cheese ("queso fresco") (present in 9 of 15 patients) was the possible origin of the outbreak. The case-control study proved an association between consumption of fresh cheese of a specific brand and *S. equi* subsp. *zooepidemicus* (OR=4.5, 95% CI 1.57–19.27; $p<0.001$). The cheese was made from cow's milk produced at two dairy farms located

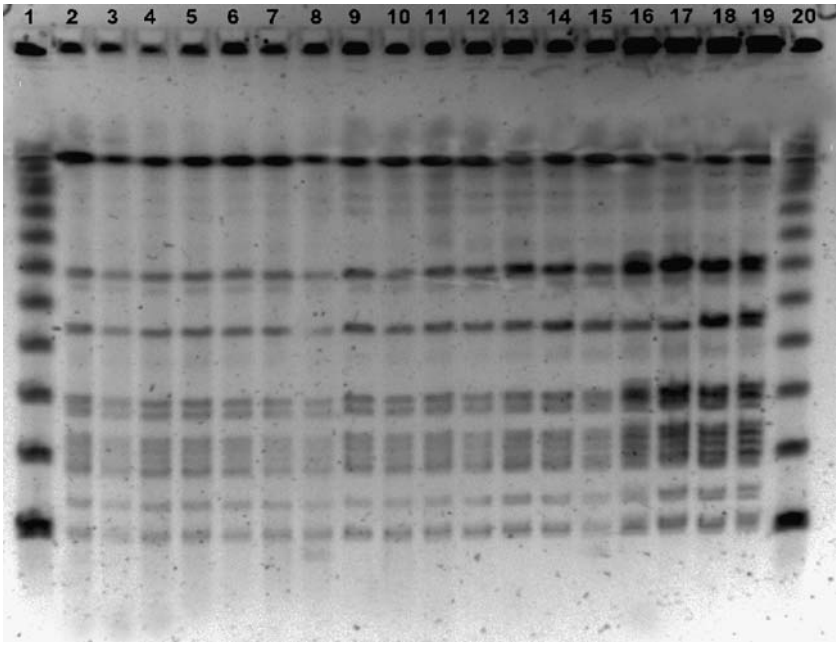

Fig. 1 Restriction patterns obtained after digestion of *Sma*I by pulsed-field gel electrophoresis. *Lanes 1 and 20* molecular size marker. *Lanes 2–16* patient isolates. *Lanes 17–19* milk isolates

in the north of the island. It was distributed to two supermarkets and grocery shops located in the same area. After this link was proved, a public health inspection of the cheese factory was performed. Inspectors established that the equipment used for pasteurization did not meet the necessary requirements, and, as a result, cheese production at the factory was stopped. At the same time, samples of raw milk and fresh cheese were obtained, and *S. equi* subsp. *zooepidemicus* was isolated from raw milk samples. Subsequently, the local Public Health Department undertook an investigation of the herd of cows at the two dairy farms, but *S. equi* subsp. *zooepidemicus* was not isolated from the eight samples of unpasteurized milk obtained from those cows. The animals on the farm did not show any evidence of pathology at that time.

All isolates of *S. equi* subsp. *zooepidemicus* recovered from patients and milk samples were available for molecular typing. All of them displayed the same macrorestriction pattern by pulsed-field gel electrophoresis, with the *Sma*I restriction pattern (Fig. 1) indicating a common link.

Since July 2003, only one more case of *S. equi* subsp. *zooepidemicus* infection has been detected. The microorganism was isolated from blood in a patient with diabetes mellitus and chronic alcoholic hepatopathy. The patient had had an episode of diarrhea, but he did not eat fresh cheese. The strain had the same macrorestriction pattern as the outbreak strain.

Discussion

A wide spectrum of illnesses due to *S. equi* subsp. *zooepidemicus* in animals has been described [20–23], but human infections associated with *S. equi* subsp. *zooepidemicus* are not frequent and can usually be traced back to animal sources [12, 24]. This group C streptococci subspecies appears to be more virulent than the other subspecies, and it is also less sensitive to antibiotics. Consequently, it causes more aggressive infections [2, 24], sometimes complicated by acute poststreptococcal glomerulonephritis [10, 13, 14, 25, 26]. Bacteremia is frequently detected in patients infected by group C streptococci [27], and our findings were in agreement: 90.9% of the patients whose blood was cultured were bacteremic.

Food-borne outbreaks by *S. equi* subsp. *zooepidemicus* are already well documented. Table 2 summarizes these outbreaks and their epidemiological and clinical characteristics. In most cases, cow's milk is the source of infection. The outbreaks differ with respect to the severity of the clinical disease: some present as noninvasive infections (upper respiratory tract infections) and others as invasive disease (meningitis, pneumonia, aortic aneurisms) [8–15]. All patients in our outbreak had invasive disease, possibly because our strain was more virulent than those of other outbreaks or because patients with noninvasive infections had not gone to the hospital or been etiologically diagnosed. Pinto et al. [25] reported a high rate of hypertension and frequent abnormalities of renal function after the follow-up of patients from a large outbreak of acute glomerulonephritis. Serious infections with *S. equi* subsp. *zooepidemicus* have mainly been reported in patients over the age of 70 or in neonates [9], and they seem to be rare in healthy young adults [4]. In the present report, 60% of the patients were 70 or older, and no neonates or pregnant women were documented.

The percentage of patients with aortic aneurysm, 26.7%, is elevated. Yuen et al. [15] found a particular association between *S. equi* subsp. *zooepidemicus* infection and cardiovascular disease. They described 12 patients with *S. equi* subsp. *zooepidemicus* septicemia and reviewed 34 patients described elsewhere, and they found that 27% of them had cardiovascular disease (7 with endocarditis, 3 with abdominal aortic aneurysms, and 2 with deep venous thromboses). Albarracin et al. [28] also described another case of *S. equi* subsp. *zooepidemicus* bacteremia associated with an abdominal aortic aneurism.

The high percentage (33.3%) of deaths in the present report, exceeded only by that in the series reported by

Table 2 Outbreaks caused by *S. equi* subsp. *zooepidemicus*

Country	Study period	Number of cases	Source of infection	Main clinical features	Number of deaths (%)	Reference
Romania	May–July 1968	85	Cow's milk	Sore throat	0	[10]
England (N. Yorkshire)	Feb.–Apr. 1982	3	Cow's milk?	URTI, nephritis	0	[13]
New Mexico	July–Sept. 1983	16	Cow's milk (homemade cheese)	Bacteremia	2 (12.5)	[11]
England (W. Yorkshire)	Mar.–June 1984	12	Cow's milk	Septicemia	8 (66.6)	[9]
Hong Kong	Jan. 1982–Dec. 1986	12	Pigs?	Septicemia	2 (18)	[15]
Australia	No data	1+2 ACs	Cow's milk	Septicemia	0	[26]
Brazil (Nova Serrana)	Dec. 1997–July 1998	253	Cow's milk	Acute nephritis	3 (1.18)	[14]
Spain (Gran Canaria)	Feb.–Apr. 2003	15	Fresh cheese	Bacteremia, aortic aneurism	5 (33.3)	PR

URTI upper respiratory tract infection, *ACs* asymptomatic carriers, *PR* present report

Edwards et al. (66.6%) [9], is noteworthy. Although 60% of our patients were 70 or older and 80% had an underlying disease, we believe that the high death rate may be related to the severity of the clinical manifestations, i.e. bacteremia associated with aortic aneurisms or meningitis may have resulted in higher mortality [2, 12].

This study is limited because only confirmed cases were evaluated, and therefore the full extent of the outbreak is unknown. It is likely that more people were infected, but the infection probably presented as a noninvasive disease. People who remained undiagnosed could not be followed-up to monitor blood pressure and renal function, as would have been desirable.

In conclusion, *S. equi* subsp. *zooepidemicus* causes serious infections that are usually zoonoses. It is important to identify beta-hemolytic streptococci to the species level in order to detect contaminated foods of animal origin to prevent new food-borne outbreaks. Moreover, we recommend the application of molecular markers for precise characterization of the isolates.

Acknowledgements We wish to thank Maria Jose Pena López for her critical review and for assistance in writing the manuscript, Pilar García Castellano for her assistance in the epidemiologic survey, and Montserrat Riba Armenter and M[a] Angeles Capon García-Caro for processing the samples of dairy products.

References

1. Facklam R (2002) What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin Microbiol Rev 15:613–630
2. Bradley SF, Gordon JJ, Baumgartner DD, Marasco WA, Kauffman CA (1991) Group C streptococcal bacteremia: analysis of 88 cases. Rev Infect Dis 13:270–280
3. Rose HD, Allen JR, Witte G (1980) *Streptococcus zooepidemicus* (group C) pneumonia in a human. J Clin Microbiol 11:76–78
4. Barnham M, Ljunggren A, McIntyre M (1987) Human infection with *Streptococcus zooepidemicus* (Lancefield group C): three case reports. Epidem Infect 98:183–190
5. Collazos J, Echevarria MJ, Ayarza R, de Miguel J (1992) *Streptococcus zooepidemicus* septic arthritis: case report and review of group C streptococcal arthritis. Clin Infect Dis 15:744–746
6. Latorre M, Alvarez M, Fernandez JM, Berdonces P, Llanos A, Cisterna R (1993) A case of meningitis due to *Streptococcus zooepidemicus*. Clin Infect Dis 17:932–933
7. Ferrandiere M, Cattier B, Dequin PF, Hazonard E, Legras A, Perrotin D (1998) Septicemia and meningitis due to *Streptococcus zooepidemicus*. Eur J Clin Microbiol Infect Dis 17:290–291
8. Ural O, Tuncer I, Dikici N, Aridogan B (2003) *Streptococcus zooepidemicus* meningitis and bacteraemia. Scand J Infect Dis 35:206–207
9. Edwards AT, Roulson M, Ironside MJ (1988) A milk-borne outbreak of serious infection due to *Streptococcus zooepidemicus* (Lancefield group C). Epidem Infect 101:43–51
10. Duca E, Teodorovici G, Radu C, Vita A, Talasman-Nicolescu P, Bernescu E et al (1969) A new nephritogenic streptococcus. J Hyg 67:691–698
11. Espinosa FH, Ryan WM, Vigil PL, Gregory DF, Hilley RB, Roming DA et al (1983) Group C streptococcal infections associated with eating home-made cheese, New Mexico. MMWR Morb Mortal Wkly Rep 32:510–516
12. Shah SS, Matthews RP, Cohen C (2001) Group C streptococcal meningitis: case report and review of the literature. Pediatr Infect Dis J 20:445–448
13. Barnham M, Thornton TJ, Lange K (1983) Nephritis caused by *Streptococcus zooepidemicus* (Lancefield group C). Lancet i:945–948
14. Balter S, Benin A, Pinto SWL, Texeira L, Alvim GG, Luna E, et al (2000) Epidemic nephritis in Nova Serrana, Brazil. Lancet 355:1776–1780
15. Yuen KY, Seto WH, Choi CH, Ng W, Ho SW, Chau PY (1990) *Streptococcus zooepidemicus* (Lancefield group C) septicaemia in Hong Kong. J Infect 21:241–250
16. International Commission on Microbiological Specifications for Foods (1983) Microorganismos de los alimentos. Técnicas de análisis microbiológico, vol. 1. Editorial Acribia, Zaragoza, Spain pp 156–157
17. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233–2239
18. Mazon A, Gil-Setas, Sota de la Gandara LJ, Vindel A, Sáez-Nieto JA (2003) Transmission of *Streptococcus pyogenes* causing successive infections in a family. Clin Microbiol Infect 9:554–559
19. National Committee for Clinical Laboratory Standards (2003) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. NCCLS, Wayne, Pennsylvania
20. Carr EA, Carlson GP, Wilson WD, Read DH (1997) Acute hemorrhagic pulmonary infarction and necrotizing pneumonia in horses: 21 cases (1967–1993). J Am Vet Med Assoc 210:1774–1778
21. Las Heras A, Vela AI, Fernández E, Legaz E, Domínguez L, Fernández-Garayzábal JF (2002) Unusual outbreak of clinical mastitis in dairy sheep caused by *Streptococcus equi* subsp. *zooepidemicus*. J Clin Microbiol 40:1106–1108
22. Hewson J, Cebra CK (2001) Peritonitis in a llama caused by *Streptococcus equi* subsp. *zooepidemicus*. Can Vet J 42: 465–467
23. Chalker VJ, Brooks HW, Brownlie J (2003) The association of *Streptococcus equi* subsp. *zooepidemicus* with canine infectious respiratory disease. Vet Microbiol 95:149–156
24. Barnham M, Kerby J, Chandler RS, Millar MR (1989) Group C streptococci in human infection: a study of 308 isolates with clinical correlations. Epidem Infect 102:379–390
25. Pinto SW, Sesso R, Vasconcelos E, Watanabe YJ, Pansute AM (2001) Follow-up of patients with epidemic poststreptococcal glomerulonephritis. Am J Kidney Dis 38:249–255
26. Francis AJ, Nimmo GR, Efstratiou A, Galanis V, Nuttal N (1993) Investigation of milk-borne *Streptococcus zooepidemicus* infection associated with glomerulonephritis in Australia. J Infect 27:317–323
27. Berenguer J, Sampedro I, Cercenado E, Baraia J, Rodriguez-Créixems, Bouza E (1992) Group C β-hemolytic streptococcal bacteremia. Diagn Microbiol Infect Dis 15:151–155
28. Albarracin C, Rosencrance G, Boland J, Hernandez JE (1998) Bacteremia due to *Streptococcus zooepidemicus* associated with an abdominal aortic aneurysm. W V Med J 94:90–92