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Short communication

Salmonella Cerro isolated over the past twenty years from various sources in the US represent a single predominant pulsed-field gel electrophoresis type

K. Hoelzer [a,*], K.J. Cummings [b], E.M. Wright [a], L.D. Rodriguez-Rivera [a], S.E. Roof [a], A.I. Moreno Switt [a], N. Dumas [c], T. Root [c], D.J. Schoonmaker-Bopp [c], Y.T. Grohn [b], J.D. Siler [b], L.D. Warnick [b], D.D. Hancock [d], M.A. Davis [e], M. Wiedmann [a]

[a] Department of Food Science, Cornell University, Ithaca, NY, USA
[b] Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, USA
[c] Wadsworth Center, New York State Department of Health, Albany, NY, USA
[d] Veterinary Clinical Sciences Department, Washington State University, Pullman, WA, USA
[e] Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, USA

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ABSTRACT

Salmonella Cerro prevalence in US dairy cattle has increased significantly during the past decade. Comparison of 237 *Salmonella* isolates collected from various human and animal sources between 1986 and 2009 using pulsed-field gel electrophoresis, antimicrobial resistance typing, and *spvA* screening, showed very limited genetic diversity, indicating clonality of this serotype. Improved subtyping methods are clearly needed to analyze the potential emergence of this serotype. Our results thus emphasize the critical importance of population-based pathogen surveillance for the detection and characterization of potentially emerging pathogens, and caution to critically evaluate the adequacy of diagnostic tests for a given study population and diagnostic application.

1. Introduction

In 2007, *Salmonella* Cerro was one of the most commonly isolated serotypes from healthy lactating dairy cattle in the US, representing a marked increase in prevalence relative to estimates from 1996 and 2002 (Aphis, 2008). In a recent study of *Salmonella* from dairy cattle in New York serotype Cerro was also the most prevalent serotype and significantly associated with gastrointestinal disease (Cummings et al., 2010). Persistence of *Salmonella* Cerro in a dairy herd for more than 18 months without clinical disease has also been reported (van Kessel et al., 2007). Furthermore, *Salmonella* Cerro has been occasionally isolated from healthy humans, clinical human cases and outbreaks among humans such as the 1985 'Carne Seca'-associated outbreak in New Mexico (CDC, 1985; Mammina et al., 2000). Between 1996 and 2006, serotype Cerro represented 0.12% (447/360,948 isolates) of serotyped isolates from human sources in the US (CDC, 2008).

The potential animal and human health concern merits further characterization of this possibly emerging serotype (CDC, 1985). We, thus, selected 237 *Salmonella* Cerro isolates from sick and healthy cattle, farm environments, humans, and other domestic animals collected over a 20-year period for PFGE analysis, antimicrobial resistance typing and *spvA* screening.

* Corresponding author at: Department of Food Science, Cornell University, 410 Stocking Hall, Ithaca, NY 14850, USA.
Tel.: +1 607 255 1266.
E-mail address: kh294@cornell.edu (K. Hoelzer).

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Table 1
Overview of study isolates.

Isolate category	Isolate subcategory			Isolation period	Simpson's Index of Diversity[a]	No. isolates tested[b]	PFGE pattern numbers	No. isolates with PFGE pattern (%)
	Region	Host	Attributes					
Recent isolates	Northeast	Cattle	Clinical	2007–2009	0.26 (0.12–0.40)[c]	64	CU-213	55 (86)
							CU-843	1 (2)
							CU-846	1 (2)
							CU-848	3 (5)
							CU-849	1 (2)
							CU-973	3 (5)
	Northeast	Cattle	Non-clinical	2007–2009	0.06 (0.00–0.18)[c]	32	CU-213	31 (97)
							CU-843	1 (3)
	Northeast	Farm environment		2007–2009	0.25 (0.08–0.42)[c]	45	CU-213	39 (87)
							CU-839	1 (2)
							CU-840	2 (4)
							CU-843	2 (4)
							CU-874	1 (2)
Human isolates	Northeast	Human	Clinical	2007–2009	n/a	3	CU-213	3 (100)
Comparison isolates	Northwest	Animals	Clinical and non-clinical	1986–2007	0.43 (0.30–0.55)[c]	93	CU-213	70 (75)
							CU-967	5 (5)
							CU-968	6 (6)
							CU-969	8 (9)
							CU-970	1 (1)
							CU-971	1 (1)
							CU-972	1 (1)
							CU-975	1 (1)

[a] Simpson's Index of Diversity value for all recent isolates combined: 0.21 (0.12–0.31).
[b] None of the isolates were resistant to any of the antimicrobial drugs tested, and none of the tested isolates were positive for *spvA*.
[c] 95% confidence interval.

2. Materials and methods

2.1. Isolate characterization

A total of 141 previously described (Cummings et al., 2010) *Salmonella* Cerro isolates, collected from cattle and farm environments between 2007 and 2009 (i.e., "recent isolates", Table 1), including 115 isolates for which *XbaI* PFGE patterns have been reported previously and 26 isolates that appeared resistant to one or more antimicrobial drugs on initial testing or that were isolated from clinically sick cattle (Cummings et al., 2010) but for which no PFGE patterns have been reported previously, were compared to a convenience sample of serotype Cerro isolates isolated from clinical human cases ($n = 3$, "human isolates") and from domestic animals ($n = 93$, "comparison isolates") collected in the Pacific Northwest between 1986 and 2007. Historical isolates from the Northeastern US were not available for comparison. Comparison isolates originated from cattle ($n = 87$), cats ($n = 2$), dogs ($n = 1$), birds ($n = 2$), and unspecified sources ($n = 1$), and they were collected in the states of Washington ($n = 91$), Utah ($n = 1$), and Nebraska ($n = 1$).

2.2. Pulsed field gel electrophoresis (PFGE) pattern analysis

PFGE analysis with restriction enzyme *XbaI* (Roche Molecular Diagnostics, Pleasanton, CA) was performed according to the CDC PulseNet protocol (Hunter et al., 2005; Ribot et al., 2006). PFGE patterns were analyzed using BioNumerics version 5.1 (Applied Maths, Austin, TX). Similarity analyses were based on Dice coefficients with a maximum space tolerance of 1.5%. Exact 95% binominal confidence intervals (CI) were calculated using SAS version 9.2 (SAS, Cary, NJ). To compare subtype diversity between isolate categories, we calculated Simpson's Index of Diversity (D) and 95% confidence intervals (Grundmann et al., 2001; Simpson, 1949). A D value of 0 signifies no diversity and a value of 1 signifies complete diversity.

2.3. Antimicrobial resistance typing

Antimicrobial susceptibility testing of all 237 isolates was performed according to the National Antimicrobial Resistance Monitoring System (NARMS) protocol, and results for 141 of the recent isolates have been described previously (Cummings et al., 2010).

2.4. Screening for the presence of spvA

To test for differences in the presence of virulence gene *spvA*, 41 isolates representing each combination of sample subcategory, PFGE pattern, host species, and initial resistance type, were screened for *spvA*, using a previously described PCR (Gebreyes et al., 2009). Due to the clearly high level of clonality not all isolates were selected. For each subcategory, a representative isolate was selected randomly (using www.random.org).