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Salmonella Cerro isolated over the past twenty years from various sources in the US represent a single predominant Pulsed-Field Gel Electrophoresis type

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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.

Keywords

Salmonella Cerro; molecular epidemiology; PFGE; emerging clone

2. 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

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reported (VanKassel 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, 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.

3. MATERIALS AND METHODS

3.1. 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), 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).

3.2. 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 (Grundemann et al., 2001; Simpson, 1949). A D value of 0 signifies no diversity and a value of 1 signifies complete diversity.

3.3. 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).

3.4. 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). However, all human isolates were screened to maximize the probability of detecting differences between human and animal isolates. The final isolate set included 23 recent isolates from clinically sick

cattle (n=6), clinically healthy cattle (n=12), and environmental isolates (n=5), as well as human isolates (n=3), and 15 comparison isolates from cattle (n=10), birds (n =2), dogs (n=1), and cats (n=2).

4. RESULTS

4.1. PFGE pattern analysis

Of the 237 isolates, 198 (84%) shared pattern CU-213 (Table, Figure). The frequency of pattern CU-213 was somewhat higher among the recent isolates (89%, CI: 82 – 93) than among the comparison isolates (75%, CI: 64–83). Pattern CU-213 was detected among 86% (CI: 75 – 93) of isolates from clinically sick cattle, 97% (CI: 84–100) of isolates from clinically healthy cattle, and 87% (CI: 73–95) of environmental isolates (Table). Similarly, all human isolates shared pattern CU-213. Most other PFGE patterns were represented by 1 to 3 isolates, and only pattern CU-843 was detected in more than one isolate subcategory (Table). The comparison isolates with PFGE patterns other than CU-213 represented 7 PFGE patterns and originated from cattle (n=22) and cats (n=1). None of these 7 PFGE patterns were detected among the recent isolates, and the overwhelming majority of PFGE patterns differed from pattern CU-213 by only a single band (Figure).

Simpson's index of diversity values were considerably lower for recent than comparison isolates (Table). Intriguingly, values were similar for isolates from clinically sick cattle and farm environments, but they were considerably lower for isolates from healthy cattle and higher for comparison isolates.

4.2. Antimicrobial resistance typing results

All tested isolates eventually proved susceptible to all antimicrobial drugs. However, on initial analysis, 12 isolates (5%) were resistant to one or more antimicrobial drugs. Yet, PCR – based screening failed to detect resistance genes, and upon retesting using the same protocols and the same glycerol-frozen bacterial cultures, which were stored at −80°C, all 12 isolates were susceptible to all drugs, thus indicating previous false-positive results or, less likely, resistance loss.

4.3. Results of spvA screening

spvA was detected in none of the isolates, even though the gene was readily detected in the positive controls (i.e., Typhimurium isolates FSL S5-800 and FSL- S5-550, see www.pathogentracker.org).

5. DISCUSSION

Our data indicate that *Salmonella* Cerro strains circulating in the US represent a clonal subtype. The vast majority of cattle isolates, most isolates from other domestic animals, and all human isolates in our study shared a single *XbaI* PFGE pattern (CU-213), while nearly all other PFGE patterns differed by only one band from this predominant PFGE pattern. Moreover, all isolates were susceptible to all antimicrobial drugs tested, again supporting the hypothesis of one clonal subtype. While further studies are clearly needed, the absence of antimicrobial resistance genes among the 237 tested isolates might indicate that limited antimicrobial selection pressures have so far acted on this serotype, which might be congruent with a predominantly low virulence of this serotype. Our data might further suggest that host or environmental factors, rather than genetic changes, contributed to the recent increase in *Salmonella* Cerro prevalence. However, genetic changes not detectable by *XbaI* PFGE or a combination of host and pathogen factors might have also caused the increase in prevalence, and further studies are clearly needed.

Despite *Salmonella* Cerro being extremely common among dairy cattle in the US, human cases are rarely associated with this serotype. We found though that all three available human *Salmonella* Cerro isolates had the same *XbaI* PFGE pattern that was predominant among dairy cattle isolates. It is thus tempting to speculate that at least some *Salmonella* Cerro strains have some capacity to cause human disease. However, specific virulence characteristics remain to be elucidated. While it is tempting to speculate that the rare occurrence of human *Salmonella* Cerro cases may be due to reduced virulence of this subtype, scarcity of exposure cannot be excluded as one determining cause. All tested isolates lacked *spvA*, a generally plasmid-mediated virulence factor found among some strains of certain, predominantly host adapted, serotypes such as Abortusovis, Dublin, or Typhimurium (Chu and Chiu, 2006; Gassama et al., 2006; Guney et al., 1994). Still, absence of this virulence gene is unlikely to explain the rare association of serotype Cerro with human cases.

Importantly, our data also suggest that genetic diversity in contemporary US *Salmonella* Cerro isolates is low or cannot be adequately captured by standard *XbaI* PFGE. The development and validation of improved subtyping methods, an integral component of veterinary epidemiology, is clearly needed to allow further studies of this potentially emerging serotype (Belkum et al., 2007).

6. CONCLUSIONS

Overall, our study illustrates the importance of continuous integrated animal and human population-based pathogen surveillance, as this approach may be crucial for the reliable early detection and characterization of emerging pathogens. Moreover, our data provides strong evidence for the necessity to critically evaluate the adequacy of diagnostic tests with regard to the specific study populations and research aims at hand.

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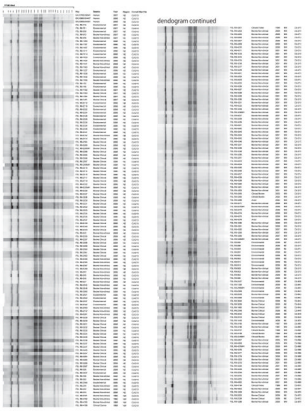

Figure.

XbaI pulsed-field gel electrophoresis (PFGE) patterns for 237 *Salmonella* Cerro isolates included in this study. The isolation year, isolate subcategory, PFGE pattern number and geographic area of isolation are indicating adjacent to individual isolates. ‘NE’= isolated in Pacific Northwest; ‘NW’ = isolated in Northeast.

Table

Overview of study isolates

Isolate category	Isolate subcategory			Isolation period	Simpson's Index of Diversity *	No. isolates tested †	PFGE pattern numbers	No. isolates with PFGE pattern (%)
	Region	Host	Attributes					
Recent isolates	Northeast	Cattle	Clinical	2007–2009	0.26 (0.12 – 0.40)‡	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) ‡	32	CU-213	31 (97%)
							CU-843	1 (3%)
	Northeast	Farm environment		2007–2009	0.25 (0.08 – 0.42) ‡	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 & Non-clinical	1986–2007	0.43 (0.30 – 0.55) ‡	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%)

* Simpson's Index of Diversity value for all recent isolates combined: 0.21 (0.12 – 0.31);

† none of the isolates was resistant to any of the antimicrobial drugs tested, and none of the tested isolates was positive for *spvA*;

‡ 95% Confidence Interval;