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Subtype Analysis of *Salmonella* Isolated from Subclinically Infected Dairy Cattle and Dairy Farm Environments Reveals the Presence of Both Human- and Bovine-Associated Subtypes

L.D. Rodriguez-Rivera[1], **E.M. Wright**[1], **J.D. Siler**[2], **M. Elton**[2], **K.J. Cummings**[2,3], **L.D. Warnick**[2], and **M. Wiedmann**[1,*]

[1]Department of Food Science, Cornell University, Ithaca, NY 14853

[2]Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY 14853

[3]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843-4458

Abstract

While it is well established that clinically ill livestock represent a reservoir of *Salmonella*, the importance of subclinical shedders as sources of human salmonellosis is less well defined. The aims of this study were to assess the subtype diversity of *Salmonella* in healthy dairy cattle and associated farm environments and to compare the subtypes isolated from these sources with the *Salmonella* subtypes associated with clinical human cases in the same geographic area. A total of 1,349 *Salmonella* isolates from subclinical dairy cattle and farm environments (46 farms) were initially characterized by traditional or molecular serotyping and tested for antimicrobial susceptibility. A set of 381 representative isolates was selected for further characterization by pulsed-field gel electrophoresis (PFGE); these isolates represented unique combinations of sampling date, serovar, antimicrobial resistance pattern, farm of origin, and source, to avoid overrepresentation of subtypes that were re-isolated from a given source. These 381 isolates represented 26 *Salmonella* serovars; the most common serovars were Cerro [(38.8%, 148/381) isolated from 21 farms], Kentucky [16.3%; 10 farms], Typhimurium [9.4%; 7 farms], Newport [7.6%; 8 farms], and Anatum [6.3%; 6 farms]. Among the 381 isolates, 90 (23.6%) were resistant to between 1 and 11 antimicrobial agents, representing 50 different antimicrobial resistance patterns. Overall, 61 *Xba*I-PFGE types were detected among these 381 isolates, indicating considerable *Salmonella* diversity on dairy farms without evidence of clinical salmonellosis. Fourteen PFGE types, representing 12 serovars, exactly matched PFGE types from human isolates, suggesting that subclinically infected dairy cattle could be sources of human disease-associated *Salmonella*.

[*]Corresponding author: Martin Wiedmann, Dr. med. vet., Ph.D. Department of Food Science, 347 Stocking Hall, Cornell University, Ithaca, NY 14853. Phone: 607-254-2838. Fax: 607-254-4868. mw16@cornell.edu.

Keywords

Salmonella; cattle; subclinical; dairy; subtyping; PFGE

Introduction

Salmonella is a zoonotic foodborne pathogen and the etiologic agent of salmonellosis. Salmonellosis is a major health concern as it is one of the leading causes of foodborne illness in the United States. It has been estimated that approximately 1.0 million nontyphoidal *Salmonella* illnesses, 20,000 hospitalizations and 370 deaths occur through foodborne transmission in the U.S. each year (Scallan et al., 2011). *Salmonella* is comprised of two species, *Salmonella bongori* and *Salmonella enterica*, and more than 2,600 recognized serovars (Guibourdenche et al., 2010). However, most human salmonellosis cases are caused by relatively few serovars within *S. enterica* (Jones et al., 2008). *Salmonella* serovars have different hosts and reservoirs ranging from cold-blooded (e.g., reptiles) to warm-blooded animals (e.g., mammals) (Hoelzer et al., 2011b). Furthermore, *Salmonella* can survive in farm and other environments for prolonged periods of time (Holley et al., 2006; Cummings et al., 2009a). Some previous studies have established that certain serovars may be overrepresented among specific hosts and/or associated with specific hosts; for example, while serovar Dublin has been isolated from both bovine and human hosts, it is most commonly isolated from cattle and rarely found in other non-primate hosts and is thus typically considered “bovine associated”.

Salmonella is transmitted to animals and humans through the fecal-oral route. Animals can become infected after ingestion of feed and water contaminated with *Salmonella.* Similarly, humans can become infected by foodborne transmission or after direct or indirect contact with infected animals (Hoelzer et al., 2011b). In livestock, clinical signs typically appear 6 to 24 hours after exposure and include profuse diarrhea, fever, dehydration, inappetence, foul-smelling feces, and mucus or blood in feces (Cummings et al., 2010a). Disease manifestations in people include diarrhea, fever, abdominal cramps and septicemia in severe cases, appearing 12 to 72 hours after ingestion. *Salmonella* can also be carried subclinically by both humans and animals (Murase et al., 2000; Perron et al., 2008; USDA-NAHMS, 2011). The purpose of this study was to investigate the phenotypic and genotypic diversity and distribution of *Salmonella* serovarsisolated from subclinically infected cattle and associated farm environments within New York dairy farms.

2. Materials and Methods

2.1. Study design

Bovine fecal and environmental samples were collected from dairy farms in New York between October 2007 and August 2009 as described by Cummings et al. (2010b); 44 of the farms detailed in that study (Cummings et al., 2010b) as well as 2 additional New York dairy herds included here yielded *Salmonella*-positive samples, for a total of 46 farms with *Salmonella*-positive samples. Briefly, *Salmonella* surveillance included both environmental screening and disease monitoring within each herd for a period of at least 12 months. A

positive *Salmonella* culture result arising from either surveillance method would trigger subsequent visits for cattle sampling, and 50–70 fecal samples were collected from apparently healthy cattle at each visit depending on herd size. Overall, 8,948 samples (1,420 environmental and 7,528 fecal) were collected from the 46 dairy herds.

All *Salmonella* isolates obtained from the 46 farms were used here for phenotypic characterization (i.e., serotyping and antimicrobial susceptibility testing, as detailed below), totaling 1,349 isolates obtained from environmental (n = 402) and fecal samples (n = 947) (see Supplemental Table 1 for a list of all isolates). Isolates were obtained over multiple sample collections at the same farm, with a median number of 4 sample collection dates with *Salmonella* positive samples per farm (range: 1–18); the wide range reflects that some farms might have only had a single sample collection that yielded *Salmonella*, while others might have had a large number of sample collections that yielded *Salmonella* (for details see Cummings et al., 2010b).

2.2. Salmonella isolation from environmental and bovine fecal samples

Salmonella isolation procedures have previously been reported (Cummings et al., 2009a, 2010a, and 2010b). Briefly, environmental samples were taken from four different locations on each farm (i.e., calf housing, cow housing, sick pen, and manure storage area), using sterile 4×4 gauze swabs saturated in double-strength skim milk. Gauze swabs were placed into a sterile flip98 top container, and samples were stored at 4°C and brought to the laboratory for *Salmonella* isolation. Fecal samples from cattle without clinical signs of salmonellosis (i.e., animals that do not show diarrhea, fever, etc.) were collected via rectal retrieval using a new sleeve for each sample. Ten grams of fecal matter were placed into a Para-Pak bottle, sealed, and sent to the laboratory for culture and further analyses. Cultural testing of these environmental and fecal samples yielded 1,349 *Salmonella* isolates that were included in the study reported here.

2.3. Serotyping

Traditional serotyping was performed by agglutination (as described by Edwards and Ewing, 1986) at the National Veterinary Services Laboratories (NVSL), a division of the United State Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS, Ames, Iowa).

2.4. Molecular serotyping

Molecular serotyping was performed on selected isolates if (i) the isolates were not serotyped by traditional serotyping, (ii) the isolates were classified as untypeable by traditional serotyping, or (iii) serovars predicted by PFGE did not match the serovars reported based on traditional serotyping (Supplemental Table 2). Molecular serotyping was performed, as reported by Ranieri et al. (2013), using a combination of (i) PCR-based characterization of O antigens and (ii) sequencing-based characterization of the genes encoding the H1 and H2 antigen (i.e., *fli*Cand *flj*B, respectively). Briefly, PCR-based characterization of O antigens used a multiplex PCR targeting genes specific for serogroups B, C1, C2-C3, D1, and E1 (Herrera-León et al., 2007) as well as separate PCRs detecting genes specific for serogroups G and K (Fitzgerald et al., 2007, 2006). *fli*C and *flj*B

sequences obtained were aligned and compared to 236 sequences representing 131 *Salmonella* serovars (Ranieri et al., 2013) in order to predict H1 and H2 antigens. O antigen PCR and H1 and H2 antigen sequencing results were combined to assign serovar designation consistent with the White-Kauffman-Leminor scheme designations.

2.5. Antimicrobial susceptibility testing

Susceptibility testing was performed at Cornell University's Animal Health Diagnostic Center using the Sensititre® system (Trek Diagnostic Systems Ltd., Cleveland, OH). All 1,349 isolates were examined for susceptibility to 15 antimicrobial agents included in the National Antimicrobial Resistance Monitoring System (NARMS) Gram-negative panel: amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Clinical and Laboratory Standards Institute guidelines were used for the interpretation of MIC values when available (CLSI, 2008). Otherwise, MIC values were interpreted using NARMS breakpoints (FDA, 2012).

2.6. Pulsed field gel electrophoresis (PFGE)

PFGE typing was performed on 381 representative *Salmonella* isolates. We defined representative isolates as isolates with a unique combination of sampling date, serovar, antimicrobial resistance pattern, farm of origin, and source (i.e., environmental or bovine fecal). If two or more isolates shared a unique combination of the aforementioned criteria, then only one was randomly selected for PFGE typing (using www.random.org) to avoid overrepresentation of subtypes that were re-isolated on the same farm. If a serovar and its variants (e.g., *S.* Typhimurium and *S.* Typhimurium var. O 5 –) were isolated from the same farm on the same date, one representative of each was selected for PFGE typing. PFGE typing was performed using the standard CDC PulseNet protocol for *Salmonella* (Ribot et al., 2006). Bacterial cultures were embedded in 1% agarose plugs (Lonza SeaKem Gold Agarose, Rockland, ME) and digested with 50 U/plug of *Xba*I (Roche Applied Science, Indianapolis, IN) at 37°C. A subset of *Salmonella* Cerro isolates (n = 10) were also digested with 40 U/plug of *Not*I at 37°C, to improve the resolution (Zheng et al., 2007) of the subtyping results by *Xba*I. The restriction fragments were separated by agarose PFGE using the Chef Mapper® XA or the CHEF-DR II® electrophoresis systems (Bio-Rad, Hercules, California). The initial switch time was 2.16 s and the final switch time was 63.8 s for *Xba*I, and 2 s and 20 s for *Not*I. The gel images were captured using Gel Doc equipment (Bio-Rad, Hercules, California). The tiff images were analyzed using the BioNumerics software program version 5.1 (Applied Maths, Sint-Martens- Latem, Belgium). *Xba*I-PFGE type numbers were assigned after a comparison against 5,828 PFGE types in the BioNumerics database of the Food Safety Laboratory. Clustering analyses were performed using the unweighted pair group method with arithmetic mean algorithm (UPGMA) based on the DICE similarity coefficient with 1.5% position tolerance.

3. Results

3.1. Initial phenotypic subtyping data suggest frequent re-isolation of isolates characterized by a combination of identical serovar and antimicrobial resistance patterns on a given farm

The initial isolate set included 1,349 *Salmonella* isolated from 46 New York dairy farms; 70.2% and 29.8% of isolates were obtained from subclinical dairy cattle and farm environments, respectively (Supplemental Table 1). The median herd size for all 46 positive farms was 978 female dairy cattle (range: 245–7,412). Traditional serotyping was performed for 1,344 isolates; ten of these isolates were classified as untypeable by the NVSL (Supplemental Table 1). These *Salmonella* isolates were used to initially select 403 representative isolates for PFGE typing; these isolates represented a unique combination of sampling date, serovar, antimicrobial resistance pattern, farm of origin, and source. For example, on farm 1, a total of 8 pansusceptible serovar Meleagridis isolates were obtained from bovine fecal samples on 12/11/2007; one isolate was randomly selected to be included in the set of representative isolates (Supplemental Table 1).

Initial PFGE analysis of the 403 representative isolates identified 47 isolates where the PFGE type for a given isolate matched one or more isolates that were classified into a different serovar (Supplemental Table 2). For 16 of these 47 isolates, additional isolates with a matching PFGE type were isolated from the same farm as a given isolate. These 16 isolates thus were reclassified as the serovar they were predicted to have, based on PFGE, since the other isolates with the matching PFGE pattern also showed this PFGE predicted serovar. For example, isolate FSL R8-346 from farm 1 was predicted by PFGE to represent serovar Meleagridis but was initially classified as serovar Kentucky by traditional serotyping; as 14 other isolates with the same PFGE isolated from the same farm had been classified as serovar Meleagridis (by classical serotyping), isolate FSL R8-346 was reclassified as Meleagridis (Supplemental Table 1). For the other 31 of these 47 isolates, molecular serotyping was performed (see Supplemental Table 2 for details). In addition, 10 isolates classified as untypeable and 5 isolates that had not been serotyped were characterized by molecular serotyping (see Supplemental Table 2 for details). After completion of molecular serotyping and PFGE analysis, a total of 381 isolates were identified as representative; these isolates were used for the subsequent analyses described below; some of the 403 isolates that were initially identified as representative were removed from the set of representative isolates as the additional characterization described here revealed that the initial serovar represented a misclassification and that after reclassification this given isolate matched another isolate also included among the isolate set that represented unique subtypes. Interestingly, as part of this analysis we also identified a number of isolates where agiven PFGE type represented two different serovars, including (i) PFGE type NYCU.JAAX01.1028, representing serovars Orion 15+ 34+ and Meleagridis; (ii) PFGE type NYCU.JAAX01.0096, representing Kentucky and 8,20:–:z6; and (iii) PFGE type NYCU.JAAX01.0007, representing Muenster and 3,10:–:1,5 (see Figure 1 for examples).

3.2. More than 50% of representative environmental and subclinical isolates represented serovars Cerro and Kentucky

Among the 381 representative *Salmonella* isolates, 61.6% (235/381) and 38.3% (146/381) were obtained from the farm environment and subclinical dairy cattle, respectively. These isolates represented 26 serovars (Table 1). The 5 most common serovars were Cerro (38.8%; 148 isolates obtained from 21 farms); Kentucky (16.3%; 62 isolates from 10 farms); Typhimurium, including variant O 5– (9.4%; 36 isolates from 7 farms); Newport (7.6%; 29 isolates from 8 farms); and Anatum, including variant 15+ (6.3%, 24 isolates from 6 farms). The remaining serovars accounted for 21.5% (82/381). The number of *Salmonella* serovars isolated per farm ranged from 1 to 6 (Table 2); for example, farm 19 yielded isolates representing six different serovars, including Typhimurium (including Typhimurium var. O 5–), Kentucky, Newport, Oranienburg, Anatum, and 6,7:–:1,5.

3.3. About 25% of environmental and subclinical isolates were resistant to one or more antimicrobial agents, with Salmonella Typhimurium representing the most common drug resistant serovar

While 76.4% of the 381 isolates designated as representative were pansusceptible, 23.6% (90 isolates) showed resistance to 1 to 11 antimicrobial agents (see Supplemental Table 1). These isolates represented 50 different antimicrobial resistance patterns (see Supplemental Table 3). All isolates were susceptible to amikacin and ciprofloxacin (Table 3). Of the 90 resistant isolates, 42were isolated from subclinical cattle and 48 were isolated from the farm environment. Among these 90 isolates, the most commonly observed resistances were to ampicillin (found in 72% of these 90 isolates), tetracycline (63% of isolates), and amoxicillin/clavulanic acid (58% of isolates) (Table 3). Both pansusceptible as well as resistant *Salmonella* isolates were obtained from 48% (22/46) of the farms; 46% and 6% of farms yielded only pansusceptible or resistant isolates, respectively. Interestingly, in some cases isolates resistant to antimicrobial agents shared the same serovar-*Xba*I-PFGE-type combination as pansusceptible isolates suggesting resistance gene acquisition and/or deletion events that did not affect the PFGE banding pattern.

The 90 isolates that showed resistance to at least one antimicrobial included 14 different serovars, including Typhimurium (25 isolates, representing 20 different resistance patterns), Cerro (13 isolates, representing 12 different resistance patterns), Newport (22 isolates, representing 7 different patterns), Kentucky (8 isolates representing 6 different resistance patterns) (Supplemental Table 3).

3.4. PFGE typing of 381 representative environmental and subclinical isolates revealed presence of PFGE types that exactly match human clinical isolates

Among the 381 isolates, a total of 61 different *Xba*I-PFGE types were identified (Figure 2). On 27 farms, we identified isolates from both environmental samples and fecal samples that shared the same PFGE type (see Figure 3 for two examples). *Salmonella* Cerro *Xba*I-PFGE type NYCU.JAAX01.0213 was the most widely distributed PFGE pattern; this pattern represented 134 of the 148 *S.* Cerro isolates (90.5 %), and isolates with this pattern were obtained from 19 farms in 11 different counties in New York. Furthermore, *Not*I-PFGE analyses of 10 randomly chosen serovar Cerro isolates (representing 4 *Xba*I-PFGE types,

including 7 isolates with *Xba*I- PFGE type NYCU.JAAX01.0213) yielded two *Not*I-PFGE types with 9 isolates sharing the same *Not*I-PFGE type (Supplemental Figure 1).

The 61 *Xba*I-PFGE types identified here were also compared to PFGE types represented among 1,849 human isolates (provided by the New York state Department of Health) in our database; these human isolates were obtained between January 2001 and December 2010, while the 381 isolates characterized here were obtained between October 2007 and August 2009. Human isolates represented a longer time frame to capture a larger number of PFGE types; the goal of this comparison was not to determine whether the farms sampled here were the specific source of human infections but rather to determine whether the PFGE types found among healthy cattle and associated farm environments and human isolates represent overlapping populations. Overall, 14 of the PFGE types represented among the 381 isolates from dairy farms exactly matched PFGE types found among human isolates. These PFGE patterns represent 12 different serovars (Table 4). A total of 143 human isolates (7.7% of the 1,849 human isolates) were represented by PFGE types that were identical to PFGE types found among *Salmonella* isolates from dairy farms (Table 4).

4. Discussion

4.1. Salmonella serovars Cerro and Kentucky were the most common serovars isolated from subclinical dairy cattle and dairy farm environments in New York

Salmonella serovars Cerro and Kentucky were the two most common serovars isolated from subclinical dairy cattle and dairy farm environments. While farms that yielded *Salmonella* isolates on multiple sample collection dates could drive serovar and subtype frequencies (as isolates with a given serovar may have been included for multiple sampling dates from a given farm), re-isolation of a serovar also makes exposure and dispersal of this serovar more likely. Our data are consistent with the NAHMS Dairy 2007 study, which found serovars Cerro and Kentucky to be the most common serovars isolated from healthy cattle on U.S. dairy operations; in this study, 121 dairy farms were sampled in 17 states that host 79.5% of U.S. dairy herds and82.5% of U.S. dairy cows (USDA-NAHMS, 2011). *Salmonella* Cerro was also the most common serovar found in bulk tank milk and milk filters tested as part of the NAHMS study (van Kessel et al., 2011). van Kessel et al. (2007) previously suggested that *S.* Cerro might behave as a commensal organism in dairy cattle, based on a protracted outbreak of subclinical *S.* Cerro infection. However, *S.* Cerro has been described as an emerging pathogen among dairy cattle in the northeastern U.S. over the last few years, as evidenced by a sharp rise in *S.* Cerro isolations from bovine clinical cases (Cummings et al., 2010b; Hoelzer et al., 2011a; Tewari et al., 2012). These studies have also reported that, based on PFGE analysis, U.S. *Salmonella* Cerro isolates represent a highly clonal population, consistent with our PFGE data reported here. While our data support frequent isolation of *S.* Cerro from cattle that are subclinically infected, experimental infection studies will be needed to further characterize this serovar's behavior as a commensal vs. pathogenic organism.

In addition to serovars Cerro and Kentucky, a number of other serovars commonly isolated from the New York dairy farms in our study, were also reported by the 2007 NAHMS study (USDA-NAHMS, 2011) as commonly isolated from dairy operations and healthy cattle

around the U.S. (e.g., Muenster, Meleagridis, and Mbandaka). Interestingly, none of these serovars (i.e., Cerro, Kentucky, Muenster, Meleagridis, and Mbandaka) are among the 20 most common human disease-associated serovars reported by CDC (CDC, 2011). These findings suggest that a number of *Salmonella* serovars that are common among subclinically infected cattle are rare among human clinical cases. Future phenotypic and genotypic studies on these serovars may be warranted to identify possible mechanisms that may explain an association of these serovars with bovine hosts.

4.2. Serovars resulting from traditional serotyping could be confirmed using a combination of PFGE typing and molecular serotyping

PFGE typing has previously been demonstrated to be useful in serovar prediction (Zou et al., 2010), particularly if large databases are available to facilitate serovar prediction (Ranieri et al., 2013). We thus used the PFGE data for our isolates to determine whether serovar identification by classical serotyping matched the serovar predicted based on a comparison of PFGE types to a database of previously characterized isolates with both PFGE and classical serovar data. Here, this approach successfully identified a number of isolates that did not have the correct serovar assigned to them. We also identified a number of instances where different serovars with similar antigenic formula were found to have indistinguishable PFGE types, such as Kentucky (8,20:i:z6) and 8,20:–:z6, among others. This is consistent with previous reports (e.g., Soyer et al., 2010; Torpdahl et al., 2005) that also identified isolates, with similar antigenic formula, that shared indistinguishable PFGE types. These findings support that prediction of serovar using banding pattern-based subtyping methods is likely to yield at least some inconsistent results. In contrast, use of a molecular serotyping approach based on PCR amplification of O-antigen specific genes and sequencing of *fliC* and *fljB* (Ranieri et al., 2013) provided good differentiation of isolates and clarification of ambiguous traditional serotyping results (e.g., where traditional serotyping data and PFGE-based prediction of serovars did not match), as well as for improved classification of untypeable isolates. These findings suggest that the previously described (Ranieri et al., 2013) molecular serotyping approach used here provides a suitable tool for the assessment of *Salmonella* serovars, particularly as PCR reactions that identify additional O antigens are developed.

4.3. Some Salmonella serovars and subtypes frequently recovered from human clinical cases were also regularly found among healthy cattle and dairy farm environments

In addition to *Salmonella* serovars that were frequently isolated from healthy cattle and dairy farm environments but rare among human clinical cases, we also identified a number of serovars commonly associated with human clinical cases. For example, the 3rd and 4th most commonly isolated serovars in this study were Typhimurium and Newport, which are among the top 3 *Salmonella* serovars isolated from laboratory-confirmed human cases in the U.S. (CDC, 2011). Furthermore, 10 of the 26 serovars isolated here were reported among the 20 most commonly isolated serovars from human clinical cases in 2009 (CDC, 2011). When the PFGE types for 381 isolates from subclinical cattle and farm environments were compared to 1,849 PFGE types from human isolates collected in New York, a total of 14 PFGE types representing 12 serovars were indistinguishable from PFGE types from human isolates. Even though *Salmonella* Cerro is rarely associated with human illness, three human

isolates matched Cerro isolates from healthy cattle and dairy farm environments by PFGE, indicating that Cerro may, in rare cases, cause human illnesses, consistent with a few reports of *Salmonella* Cerro-associated human salmonellosis (CDC, 1985; Mammina et al., 2000; Tewari et al., 2012). While our data do not specifically show that the farms sampled here were sources of human cases, our data support that subclinically infected cattle as well as farm environments may represent reservoirs or sources of *Salmonella* serovars and PFGE types commonly associated with human disease. Similarly, other reports have shown that a considerable proportion of *Salmonella* isolates from dairy cattle with clinical signs can match human clinical isolates by PFGE (Gupta et al., 2003).

4.4. Subclinical cattle and dairy farm environments are sources of drug-resistant Salmonella

Our data also showed that a number of *Salmonella* isolates from healthy cattle and farm environments are resistant to antimicrobial drugs, including some isolates resistant to 8 or more drugs. Identification of a number of MDR *S.* Newport is consistent with previous studies that found a considerable number of MDR *S.* Newport among cattle with clinical salmonellosis (Cummings et al., 2009b; Rankin et al., 2002). Previous studies have also found antimicrobial drug-resistant *Salmonella*, including MDR isolates, in healthy cattle and dairy farms in Thailand and the U.S. (Chuanchuen et al., 2010; Cobbold et al., 2006). In another study, Perron et al. (2008) showed that *Salmonella* from subclinically infected livestock representing species otherthan cattle (i.e., swine) can also be drug-resistant. Overall, these data suggest that not only clinically affected animals, but also healthy animals and farm environments not associated with animal salmonellosis cases or outbreaks, can be possible sources of MDR *Salmonella*.

5. Conclusion

Our data indicate that healthy livestock and farm environments may represent a potentially important reservoir of *Salmonella* serovars and subtypes associated with human infections, particularly considering that nearly 25% of *Salmonella* isolates were resistant to multiple antimicrobial agents. Subclinical shedding of *Salmonella* by dairy cattle thus represents a potential public health issue, particularly because fecal shedding results in widespread environmental contamination and an increased risk of within-herd transmission, both of which can promote zoonotic transmission through foodborne exposure or direct contact (e.g., for farm visitors). In addition, shedding animals cannot be recognized through clinical signs, which reduces the likelihood of adequate biosecurity efforts and quarantine efforts for these non- clinical shedders. While some studies have suggested that direct contact with cattle or other livestock is a risk factor for acquiring human salmonellosis (Cummings et al., 2012), further studies will be needed to quantitatively define the risk of transmission from healthy dairy cattle or cattle with clinical signs of salmonellosis and dairy-associated environments to humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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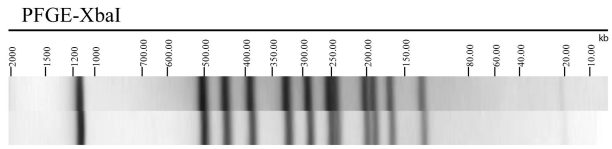
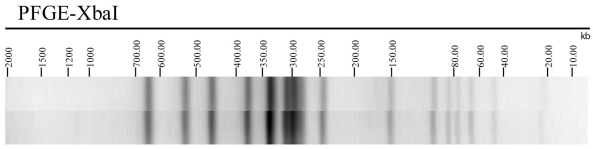
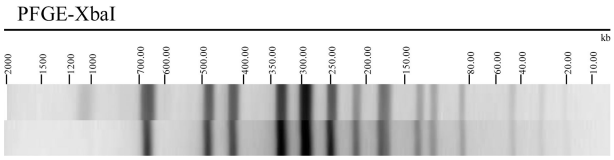


FSL No.	Serovar	Source	*Xba*I PFGE Type No.	Farm ID
FSL R8-3193	3,10:-:1,5	Bovine Non-clinical	NYCU.JAAX01.0007	47
FSL R8-4029	Muenster	Environmental	NYCU.JAAX01.0007	62

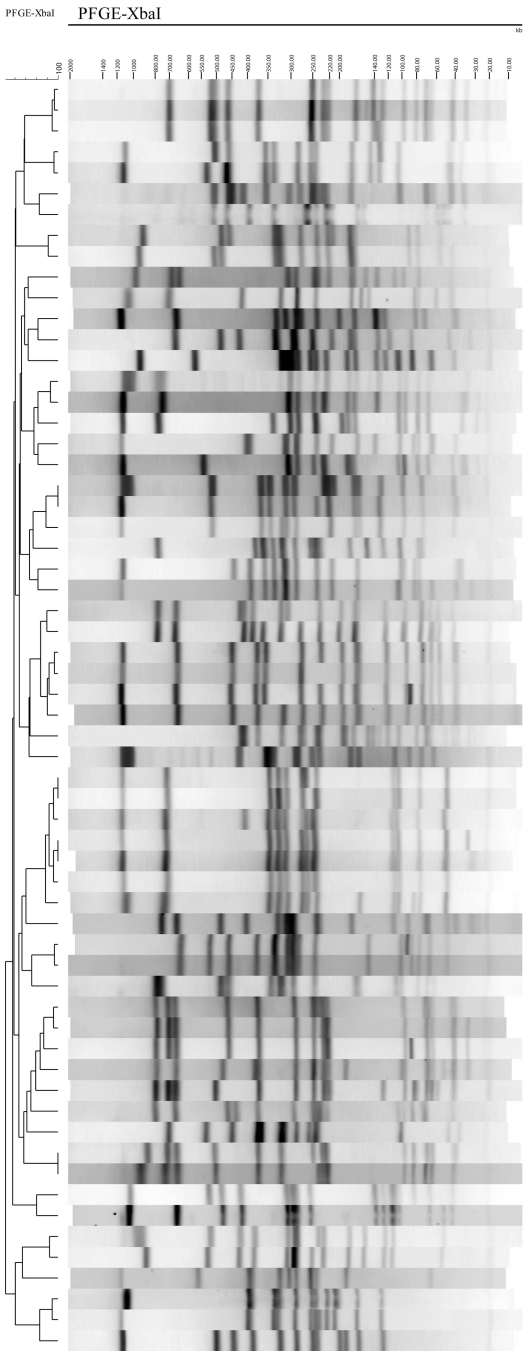


FSL No.	Serovar	Source	*Xba*I PFGE Type No.	Farm ID
FSL R8-061	8,20:-:z6	Environmental	NYCU.JAAX01.0096	16
FSL R8-065	Kentucky	Bovine Non-clinical	NYCU.JAAX01.0096	26



FSL No.	Serovar	Source	*Xba*I PFGE Type No.	Farm ID
FSL R8-2244	Meleagridis	Environmental	NYCU.JAAX01.1028	11
FSL R8-3428	Orion var.15+ 34+	Environmental	NYCU.JAAX01.1028	64

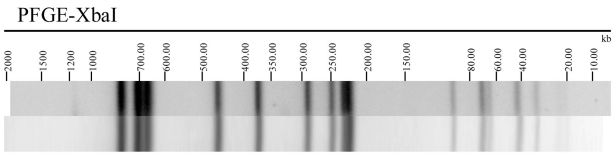
Figure 1.
Three instances where *Salmonella* isolates with different serovars shared the same *Xba*I-PFGE type.



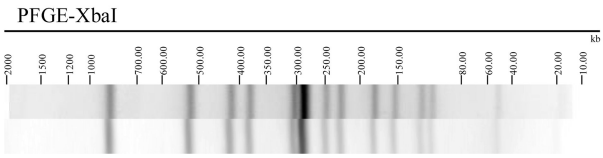
FSL No.	Serovar	Source	*Xba*I-PFGE Type No.	Farm ID
FSL R8-1623	Typhimurium var. O 5 -	Environmental	NYCU.JAAX01.0104	25
FSL R8-1642	Typhimurium var. O 5 -	Environmental	NYCU.JAAX01.1031	25
FSL R8-1622	Typhimurium	Environmental	NYCU.JAAX01.1034	25
FSL R8-2014	Mbandaka	Environmental	NYCU.JAAX01.0757	10
FSL R8-2007	Mbandaka	Environmental	NYCU.JAAX01.0758	52
FSL R8-2241	Heidelberg	Environmental	NYCU.JAAX01.1016	46
FSL R8-900	Kentucky	Environmental	NYCU.JAAX01.0184	16
FSL R8-2648	Minnesota	Bovine Non-clinical	NYCU.JAAX01.0523	62
FSL R8-2694	Minnesota	Bovine Non-clinical	NYCU.JAAX01.1029	49
FSL R8-1403	Paratyphi B var. L- tartrate+	Environmental	NYCU.JAAX01.0187	20
FSL R8-821	Senftenberg	Bovine Non-clinical	NYCU.JAAX01.0372	46
FSL R8-2812	Montevideo	Environmental	NYCU.JAAX01.0156	41
FSL R8-3428	Orion var. 15+ 34+	Environmental	NYCU.JAAX01.1028	64
FSL R8-3417	Montevideo	Environmental	NYCU.JAAX01.1006	60
FSL R8-2249	Newport	Environmental	NYCU.JAAX01.1032	35
FSL R8-3136	Newport	Environmental	NYCU.JAAX01.0126	35
FSL R6-961	Newport	Bovine Non-clinical	NYCU.JAAX01.0328	18
FSL R6-932	Infantis	Environmental	NYCU.JAAX01.0114	36
FSL R8-2900	Newport	Bovine Non-clinical	NYCU.JAAX01.0121	62
FSL R6-965	Newport	Bovine Non-clinical	NYCU.JAAX01.1015	19
FSL R8-2649	Newport	Bovine Non-clinical	NYCU.JAAX01.1014	60
FSL R8-1683	Newport	Bovine Non-clinical	NYCU.JAAX01.1017	60
FSL R8-060	Agona	Environmental	NYCU.JAAX01.0807	46
FSL R8-1392	Infantis	Environmental	NYCU.JAAX01.0261	10
FSL R6-791	Infantis	Environmental	NYCU.JAAX01.0107	18
FSL R8-2634	Anatum	Bovine Non-clinical	NYCU.JAAX01.1026	4
FSL R8-350	Anatum	Environmental	NYCU.JAAX01.0430	56
FSL R6-928	Anatum	Environmental	NYCU.JAAX01.0424	39
FSL R6-929	Anatum var. 15+	Environmental	NYCU.JAAX01.0423	39
FSL R8-264	Anatum	Bovine Non-clinical	NYCU.JAAX01.1025	39
FSL R8-855	Anatum	Bovine Non-clinical	NYCU.JAAX01.0431	21
FSL R8-1646	6,7:-:1,5	Environmental	NYCU.JAAX01.1027	19
FSL R8-2240	Tennessee	Environmental	NYCU.JAAX01.0442	46
FSL R8-2856	Cerro	Environmental	NYCU.JAAX01.0840	54
FSL R8-4032	Cerro	Environmental	NYCU.JAAX01.0974	65
FSL R8-2237	Cerro	Environmental	NYCU.JAAX01.0839	49
FSL R8-1721	Cerro	Bovine Non-clinical	NYCU.JAAX01.0841	30
FSL R8-3155	Cerro	Environmental	NYCU.JAAX01.0973	49
FSL R8-1413	Cerro	Environmental	NYCU.JAAX01.0213	61
FSL R8-2639	Cerro	Bovine Non-clinical	NYCU.JAAX01.0843	52
FSL R8-835	Dublin (Untypeable)	Bovine Non-clinical	NYCU.JAAX01.0585	15
FSL R8-815	Kentucky	Environmental	NYCU.JAAX01.0834	17
FSL R8-912	Kentucky	Environmental	NYCU.JAAX01.0096	18
FSL R8-2245	Thompson	Environmental	NYCU.JAAX01.0157	57
FSL R6-786	Typhimurium var. O 5 -	Environmental	NYCU.JAAX01.0914	19
FSL R8-231	Typhimurium	Bovine Non-clinical	NYCU.JAAX01.0071	22
FSL R6-954	Typhimurium	Bovine Non-clinical	NYCU.JAAX01.0655	1
FSL R6-794	Typhimurium	Environmental	NYCU.JAAX01.0067	25
FSL R8-920	Typhimurium	Environmental	NYCU.JAAX01.0723	51
FSL R8-2381	Typhimurium var. O 5 -	Bovine Non-clinical	NYCU.JAAX01.1171	60
FSL R6-774	Agona	Environmental	NYCU.JAAX01.0812	28
FSL R8-063	4,5,12:i:-	Bovine Non-clinical	NYCU.JAAX01.0089	26
FSL R8-915	4,5,12:i:-	Environmental	NYCU.JAAX01.1024	52
FSL R8-1416	Oranienburg	Environmental	NYCU.JAAX01.0844	10
FSL R8-169	Oranienburg	Bovine Non-clinical	NYCU.JAAX01.0040	19
FSL R6-955	Muenster	Bovine Non-clinical	NYCU.JAAX01.1030	1
FSL R8-1633	Meleagridis	Environmental	NYCU.JAAX01.0175	1
FSL R6-797	Muenster	Environmental	NYCU.JAAX01.0170	23
FSL R8-822	Muenster	Bovine Non-clinical	NYCU.JAAX01.0836	3
FSL R6-780	Muenster	Environmental	NYCU.JAAX01.0173	26
FSL R8-4029	Muenster	Environmental	NYCU.JAAX01.0007	62

Figure 2.

The 61 *Xba*I-PFGE types found among the 381 representative *Salmonella* isolates characterized here. These isolates were obtained from subclinical dairy cattle and associated dairy farm environments samples collected in 46 New York state dairy farms.



FSL No.	Serovar	Source	*Xba*I PFGE Type No.	Farm ID
FSL R8-171	Typhimurium	Environmental	NYCU.JAAX01.0071	22
FSL R8-271	Typhimurium	Bovine Non-clinical	NYCU.JAAX01.0071	22



FSL No.	Serovar	Source	*Xba*I PFGE Type No.	Farm ID
FSL R6-810	Meleagridis	Bovine Non-clinical	NYCU.JAAX01.0175	1
FSL R8-1406	Meleagridis	Environmental	NYCU.JAAX01.0175	1

Figure 3.

Examples where *Salmonella* isolated from different sources within the same farm shared undistinguishable *Xba*I-PFGE types. Shown here are environmental and fecal isolates from Farms 1 and 22 that shared the same PFGE type; overall (including these two farms), there were 27 farms where isolates from different sources within the same farm shared undistinguishable *Xba*I-PFGE types.

Table 1

Serovar diversity (by source and farm) among the 381 representative *Salmonella* isolates obtained from dairy cattle and farm environments in NY state.

Serovar[a]	Number of environmental isolates	Number of bovine non-clinical isolates	Total Number of isolates	Total Number of farms	Farm ID
Agona	3	—	3	3	10, 28, 46
Anatum	13	7	20	6	4,19,21,39,52,56
Anatum var. 15+	2	2	4	1	39
Cerro	92	56	148	21	15,17,18,26,27,28,30,45,47,49,50,52, 53,54,55,57,59,60,61,62,65
Heidelberg	1	—	1	1	46
Infantis	4	—	4	4	10,18,36,55
Kentucky	36	26	62	10	14,15,16,17,18,19,21,26,42,53
Mbandaka	6	—	6	4	10,40,48,52
Meleagridis	13	6	19	2	1,11
Minnesota	1	3	4	2	49,62
Montevideo	5	1	6	2	41,60
Muenster	5	3	8	5	1,3,23,26,62
Newport	13	16	29	8	14,17,18,19,29,35,60,62
Oranienburg	4	—	4	2	10,19
Orion var. 15+, 34+	2	—	2	1	64
Paratyphi B var. L-tartrate+	1	—	1	1	20
Senftenberg	—	1	1	1	46
Typhimurium	13	14	27	7	1,17,19,22,25,51
Typhimurium var. O 5– (Copenhagen)	6	3	9	4	1,19,25,60
Tennessee	1	—	1	1	46
Thompson	1	—	1	1	57
3,10:–:1,5	2	2	4	1	47
3,10:–:l,w	1	—	1	1	1
3,10:e,h:–	1	—	1	1	11
4,5,12:i:–	1	1	2	2	26,52
6,7:–:1,5	1	—	1	1	19
8,20:–:z6	1	—	1	1	16

Serovar[a]	Number of environmental isolates	Number of bovine non-clinical isolates	Total Number of isolates	Total Number of farms	Farm ID
Untypeable[b]	5	5	10	8	1,15,16,19,39,42,57,65
Total	235	146	**381**	—	

[a] A total of 26 serovars were found in the 46 farms in this study. Serovar variants (e.g., *S*. Typhimurium and *S*. Typhimurium var. O 5 –) were not considered as individual serovars.

[b] All untypeable isolates were characterized by molecular serotyping, and then classified as Anatum (*n*=2), Cerro (*n*=2), Kentucky (*n*=4), Dublin (n=1), and Meleagridis (*n*=1) (See Supplemental Table 2).

Table 2

Sample collection dates when *Salmonella* positive samples were obtained from each of the 46 farms.

Farm ID	No. of serovars per farm (no. of untypeable isolates)	No. of sample collection dates	Sample collection dates
1	4 (1)	11	10/2/2007–10/22/2008
3	1	1	02/07/2008
4	1	2	07/15/2008–08/18/2008
10	4	4	11/27/2007–09/01/2008
11	2	5	10/31/2007–10/30/2008
14	2	5	10/11/2007–08/30/2008
15	2 (1)	6	10/11/2007–12/09/2008
16	2 (2)	6	12/04/2007–11/11/2008
17	4	9	10/11/2007–09/10/2008
18	4	7	10/18/2007–09/08/2008
19	6 (1)	7	10/18/2007–10/02/2008
20	1	1	06/10/2008
21	2	4	10/19/2007–06/16/2008
22	1	3	12/17/2007–03/14/2008
23	1	1	10/19/2007
25	1	9	10/19/2007–09/03/2008
26	4	9	10/24/2007–10/27/2008
27	1	2	10/24/2007–01/25/2008
28	2	1	10/24/2007
29	1	1	10/24/2007
30	1	18	10/24/2007–10/15/2008
35	1	4	10/23/2008–12/09/2008
36	1	1	11/13/2007
39	1 (1)	5	11/13/2007–12/03/2008
40	1	2	11/14/2007–1/10/2008
41	1	2	08/04/2008–10/03/2008
42	1 (1)	7	11/15/2007–09/17/2008
45	1	1	11/29/2007
46	4	3	11/29/2007–09/25/2008
47	2	3	09/11/2008–01/06/2009
48	1	1	03/2008
49	2	16	01/31/2008–02/23/2009
50	1	4	11/01/2008–02/09/2009
51	1	1	05/09/2008
52	4	6	04/26/2008–02/14/2009
53	2	4	11/06/2008–03/03/2009
54	1	1	11/26/2008
55	2	3	09/29/2008–03/12/2009

Farm ID	No. of serovars per farm (no. of untypeable isolates)	No. of sample collection dates	Sample collection dates
56	1	5	02/22/2008–03/25/2009
57	2 (2)	12	03/19/2008–03/24/2009
59	1	1	09/2008
60	4	7	06/17/2008–04/13/2009
61	1	6	06/17/2008–04/12/2009
62	4	10	06/22/2008–01/22/2009
64	1	2	09/27/2008–02/17/2009
65	1 (1)	10	09/12/2008–08/11/2009

Table 3

Antimicrobial resistance observed among the 381 representative *Salmonella* isolated from healthy dairy cattle and associated farm environments in New York.

	Resistant		Intermediate Resistance	
Antimicrobials	**Concentration (mg/mL)**	**No. of isolates**	**Concentration (mg/mL)**	**No. of isolates**
Amikacin	≥ 64	0	32	0
Amoxicillin/Clavulanic Acid	≥32/16	52	16/8	5
Ampicillin	≥ 32	65	16	2
Cefoxitin	≥ 32	43	16	2
Ceftiofur	≥ 8	48	4	5
Ceftriaxone	≥ 64	5	16–32	28
Chloramphenicol	≥ 32	32	16	13
Ciprofloxacin	≥ 4	0	2	0
Gentamicin	≥ 16	0	8	1
Kanamycin	≥ 64	29	32	0
Nalidixic Acid	≥ 32	1	-	0
Streptomycin	≥ 64	41	-	0
Sulfisoxazole	≥ 512	48	-	0
Tetracycline	≥ 16	57	8	2
Trimethoprim/Sulfamethoxazole	≥ 4/76	2	-	0

Table 4

PFGE types found among both, isolates from subclinical dairy cattle and farm environments, (collected between October 2007 and August 2009) and of isolates from human clinical cases (reported to the New York State Department of Health between January 2001 and December 2010).

PFGE type	Serovar	No. of human isolates	No. of isolates obtained from farm environments and subclinical animals
NYCU.JAAX01.0040	Oranienburg	13	1
NYCU.JAAX01.0071	Typhimurium	31	14
NYCU.JAAX01.0089	4,5,12:i:–	31	1
NYCU.JAAX01.0096	Kentucky	1	66
NYCU.JAAX01.0114	Infantis	1	1
NYCU.JAAX01.0121	Newport	4	15
NYCU.JAAX01.0126	Newport	8	8
NYCU.JAAX01.0156	Montevideo	11	3
NYCU.JAAX01.0157	Thompson	31	1
NYCU.JAAX01.0213	Cerro	3	136
NYCU.JAAX01.0442	Tennessee	5	1
NYCU.JAAX01.0757	Mbandaka	2	1
NYCU.JAAX01.0914	Typhimurium	1	2
NYCU.JAAX01.1016	Heidelberg	1	1