Veterinary Microbiology 179 (2015) 109–118

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Molecular characterization of *Salmonella enterica* isolates associated with starling–livestock interactions

James C. Carlson [a,*], Doreene R. Hyatt [b], Kevin Bentler [a], Anna M. Mangan [a], Michael Russell [b], Antoinette J. Piaggio [a], George M. Linz [c]

[a] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA
[b] Colorado State University, Veterinary Diagnostic Laboratories, College of Veterinary Medicine and Biomedical Science, 1644 Campus Delivery, Fort Collins, CO 80523-1644, USA
[c] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 2110 Miriam Circle, Suite B, Bismarck, ND 58501-2502, USA

ARTICLE INFO

Keywords:
Antimicrobial resistance
European starlings
Bacteria
Enterobacteriaceae
Salmonella enterica

ABSTRACT

Bird–livestock interactions have been implicated as potential sources for bacteria within concentrated animal feeding operations (CAFO). In this study we characterized *Xba*I-digested genomic DNA from *Salmonella enterica* using pulsed-field gel electrophoresis (PFGE). The PFGE analysis was conducted using 182 *S. enterica* isolates collected from a single CAFO between 2009 and 2012. Samples collected in 2012 were subjected to antimicrobial susceptibility testing. The analysis was limited to *S. enterica* serotypes, with at least 10 isolates, known to occur in both European starlings (*Sturnus vulgaris*) and cattle (*Bos taurus*) within this CAFO. A total of five different serotypes were screened; *S.* Anatum, *S.* Kentucky, *S.* Meleagridis, *S.* Montevideo, *S.* Muenchen. These samples were recovered from five different sample types; starling gastrointestinal tracts (GI), starling external wash, cattle feces, cattle feed and cattle water troughs. Indistinguishable *S. enterica* PFGE profiles were recovered from isolates originating in all sample types. Antimicrobial resistance (AMR) was also associated with indistinguishable *S. enterica* isolates recovered from all samples types. These data suggests that AMR *S. enterica* is transmitted between cattle and starlings and that shared feed sources are likely contributing to infections within both species. Moreover we isolated indistinguishable PFGE profiles across all years of data collection, suggesting long-term environmental persistence may be mediated by starling visits to CAFO.

Published by Elsevier B.V.

1. Introduction

Salmonella enterica is recognized as one of the most common causes of foodborne illness worldwide (Zhao et al., 2006). Within the United States of America, *S. enterica* infections are responsible for 1.3 million human cases of foodborne salmonellosis, resulting in 15,600 hospitalizations and 550 deaths each year (Mead et al., 1999; Zhao et al., 2006). Globally, *S. enterica* infections are responsible for 93.8 million human cases of salmonellosis, resulting in 155,000 deaths annually and 85.6% of all cases were foodborne (Majowicz et al., 2010). Human infections with *S. enterica* are primarily associated with the consumption of animal derived food products (Mead et al., 1999; Pang et al., 1995; Zhao et al., 2006).

S. enterica contributes to morbidity and mortality in livestock (Fedorka-Cray et al., 1998; Dargatz et al., 2000). In concentrated animal feeding operations (CAFO), cattle

* Corresponding author. Tel.: +1 970 266 6127; fax: +1 970 266 6138.
E-mail address: James.C.Carlson@aphis.usda.gov (J.C. Carlson).

http://dx.doi.org/10.1016/j.vetmic.2015.03.015
0378-1135/Published by Elsevier B.V.

(*Bos taurus*) typically acquire *S. enterica* from other infected livestock which spread the pathogen throughout the herd via contaminated cattle feces (Wray and Davies, 2000), cattle feed (Maciorowski et al., 2006), and water (Kirk et al., 2002a). There is evidence that the ecological interactions between synanthropic birds and cattle also contribute to increased cattle fecal shedding and environmental contamination of CAFO with *S. enterica*, *Escherichia coli* O157:H7 and *Mycobacterium avium* spp. *paratuberculosis* (Daniels et al., 2003; Carlson et al., 2011a; Kauffman and LeJeune, 2011; Shwiff et al., 2012). European starlings (*Sturnus vulgaris*) stand out as a potential source for *E. coli* O157:H7 and *S. enterica* in CAFO (Carlson et al., 2011b; Cernicchiaro et al., 2012). In one instance, captured starlings shared genetically indistinguishable *E. coli* O157 subtypes with cattle in two isolated dairies visited by the foraging flock (Williams et al., 2011). Based upon the published literature and our behavioral observations of starling–cattle interactions we hypothesize that cross-species transmission of *S. enterica* occurs between starlings and cattle in CAFO. We predict that indistinguishable PFGE profiles would be found in starling and cattle samples involved in the cross-species transmission of *S. enterica*; starling feces (GI samples), cattle feces, external starling, cattle feed and cattle water trough samples.

The published data implicating starlings as a source for *S. enterica* contamination within CAFO has relied primarily upon direct plating and serotyping to demonstrate that *S. enterica* contamination is associated with starling–livestock interactions (Kirk et al., 2002b; Gaukler et al., 2009; Carlson et al., 2011a,b). These data have been useful at identifying associations between foraging flocks of starlings and *S. enterica* contamination of the CAFO environment, but these data cannot show that transmission is occurring between species or that shared feed sources are contributing to the infection process. Genetic identification is necessary to determine if *S. enterica* isolates obtained from starlings, livestock, and their shared feed and water sources are epidemiologically linked.

In this study we characterized patterns of *Xba*I-digested genomic DNA from *S. enterica* isolates collected from starlings and CAFO using pulsed-field gel electrophoresis (PFGE). PFGE profiling was completed for 182 *S. enterica* isolates collected from a single CAFO between 2009 and 2012. All serotypes of *S. enterica* isolated from starling gastrointestinal tracts (GI), external starling wash, cattle feces, cattle feed and cattle water trough samples were included in the PFGE analysis. The objectives of this study were to: (1) determine if starling GI and cattle fecal samples share indistinguishable *S. enterica* profiles based upon *Xba*I-digested genomic DNA patterns; (2) determine if *S. enterica* isolates from starling GI and external wash samples are phylogenetically related to isolates originating from cattle feed and water sources; (3) determine if antimicrobial resistance (AMR) in *S. enterica* is associated with starling–cattle interactions.

2. Methods

All *S. enterica* samples used in this analysis originated from a single CAFO in Moore County, TX, USA. The CAFO produced feeder cattle and had a herd size of approximately 50,000 head. No other livestock were present and the CAFO had extremely high visitation rates of starlings ($\geq$10,000 starlings/day).

Detailed methodologies for collection of external starling washes, starling GI, cattle fecal, feed and water trough samples, and Colorado State University, Veterinary Diagnostic Laboratory (CSU-VDL) procedures for *Salmonella* culture, serotyping and the antimicrobial susceptibility testing have been described by Carlson et al. (in review). Briefly, 182 *S. enterica* isolates were used for PFGE analysis. Among these isolates 7 were collected in 2009, 35 were collected in 2010 and 140 were collected in 2012. Number of isolates included in PFGE analysis differed by serotype and source (Table 1). PFGE analysis was conducted following the PulseNet protocol developed by the Centers for Disease Control (CDC, 2013; Ribot et al., 2006). Standardized methods for molecular subtyping by PFGE are described below.

2.1. PFGE plug preparation

Frozen bacterial stock was cultured on Trypticase soy agar plates with 5% sheep blood (TSA-SB; BD Diagnostics, Sparks, MD 21152). A single colony from each TSA-SB plate was removed and transferred to falcon 2054 tubes containing 2 mL of cell suspension buffer (CSB). Cell suspension concentration was adjusted to the desired optical density of 1.3–1.4 nm through incremental additions of CSB. Absorbance (optical density) measurements were made using a spectrophotometer.

2.2. Casting plugs and digestion of genomic DNA

A 200 μL aliquot of cell suspension was transferred to a 1.5 mL microcentrifuge tube containing 10 μL of proteinase K. Agarose mixture (200 μL 1% SeaKem Gold; SKG) was added to each microcentrifuge tube, mixed gently and then dispensed into disposable PFGE plug molds.

Table 1
Salmonella enterica serotypes isolated by source. All samples were collected in a concentrated animal feeding operation in TX, USA between 2009 and 2012.

Sample source	*Salmonella* Anatum	*Salmonella* Kentucky	*Salmonella* Montevideo	*Salmonella* Muenchen	*Salmonella* Meleagridis	Total
Cattle fecal	18	28	11	1	2	60
Water	21	8	14	3	3	49
Feed	6	12	4	3	4	29
Starling gastrointestinal	15	3	6	2	1	27
Starling external wash	5	3	2	5	2	17
Total	65	54	37	14	12	182