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ORIGINAL ARTICLE

Environmental *Salmonella* in Agricultural Fair Poultry Exhibits in Colorado

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Impacts

- *Salmonella* can be detected in the environment at agricultural fair poultry exhibits, and the contamination may be widespread throughout the fair exhibit.
- Human contact with exhibited poultry and the exhibition environment could potentially result in transmission of *Salmonella* to humans.
- Hygiene practices, including hand washing, and practices limiting contamination of food and drink should be emphasized at agricultural fairs with poultry exhibits to prevent zoonotic *Salmonella* transmission.

Keywords:
Salmonella; poultry; agricultural fair; zoonoses; environmental sampling

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Summary

Salmonella enterica is a common zoonotic pathogen in humans. Transmission typically occurs through consumption of contaminated food products or contact with infected animals, including poultry or their environment. The objective of this study was to estimate the frequency of *Salmonella* contamination in the environment in poultry exhibits at agricultural fairs. Samples were collected from cages, feed, floors and tables in the exhibit and cultured for *Salmonella*. At least one environmental sample was positive for *Salmonella* in 10 of 11 fairs (91%), and *Salmonella* was isolated from 28 of 55 environmental samples (50.9%). Eleven different serotypes were detected. Results of this study demonstrate that environmental surfaces at agricultural fairs can be contaminated with *Salmonella* and could potentially serve as a route of transmission to bird owners and the general public. Poultry owners and the general public should be educated about the risks of *Salmonella* infection from the poultry exhibit environment. Agricultural fairs should consider instituting policies and practices to improve hygiene and mitigate the risk of zoonotic salmonellosis.

Introduction

Poultry infected with *Salmonella* bacteria play an important role in disease transmission to humans. In poultry, *Salmonella* first infects the intestinal tract and some serotypes, such as Enteritidis, may invade other organs including the ovary, spleen and liver (Shivaprasad et al., 1990; Gast et al., 2004; Gast, 2008). *Salmonella* shed in the faeces of birds contaminates their environment (Davies and Breslin, 2004), and it is common for infected adult birds to be intermittent shedders of this organism without showing any clinical signs of illness (Barrow, 2000). Humans can become infected through consumption of contaminated food or water, contact with a contaminated environment or contact with infected animals (Greene, 2006; CDC, 2010b).

There are an estimated 1.4 million human cases and 400 deaths caused by *Salmonella* infection each year in the US (CDC, 2010b), and in 2011, 522 human cases of *Salmonella* infection were reported in Colorado (CDPHE, 2012). Most individuals affected by salmonellosis develop diarrhoea, fever and abdominal cramps 12–72 h after infection. Illness usually lasts 4–7 days. Most cases of human salmonellosis are not reported because people experience only moderate symptoms (Scallan et al., 2011), and many of those affected recover without treatment. However, this organism can cause severe illness when it spreads from the intestine to the

bloodstream in infants, elderly and immunocompromised individuals (CDC, 2010b).

Humans are at risk of contracting *Salmonella* from animals in public settings such as agricultural fairs. Enteric disease outbreaks at fairs, farms, petting zoos and similar venues from pathogens such as *Salmonella* are well documented. It is estimated that approximately 127 000 *Salmonella* infections per year are due to contact with animals (Hale et al., 2012). A recent study suggests that up to 7.6% of laboratory-confirmed cases of *Salmonella* in the United States are due to farm animal contact on farms and other public settings (Cummings et al., 2012). Steinmuller et al. (2006) documented 12 outbreaks of *Salmonella* due to animal contact in public settings from 1991 to 2005 in the US. In two multistate *Salmonella* Montevideo outbreaks in 2007, two cases reported exposure to chicks or ducklings at a fair and a petting zoo (CDC, 2009). Poultry, including geese and turkeys, were determined to chronically shed the organism in an outbreak of *Salmonella* Typhimurium among animals at a children's zoo (Sato et al., 1999).

Many factors may increase the chance of disease transmission from poultry to humans, other poultry or waterfowl, and animals at agricultural fairs. Birds are more likely to shed *Salmonella* because of stress induced by transport, crowding, handling and confinement (Gast, 2008). The risk of transmission from birds to humans is increased by certain human behaviours, including lack of awareness of disease, inadequate hand washing, lack of supervision of children and contaminated hand to mouth activities (Olson and Gray, 2006; McMillian et al., 2007; Weese et al., 2007; Anderson and Weese, 2011; CDC, 2011). One study observed children picking up faecal material and frequent touching of hands to face at petting zoos that displayed chicks and other animals in Kansas and Missouri (Erdozain et al., 2013). This study also found that overall hand hygiene compliance was poor (37%) among visitors exiting petting zoos in the study. Children can be the most severely affected by infection and are often the focus of events such as petting zoos (Friedman et al., 1998; Budgell et al., 2004; Smith et al., 2004; CDC, 2010b).

Poultry are commonly competitively shown at agricultural (county/state) fairs by children participating in 4-H, a United States national agricultural youth organization, and by poultry fanciers and breeders. The goal of this study was to measure the frequency of isolation of *Salmonella* from the environment of poultry exhibits at agricultural fairs in Colorado.

Materials and Methods

Study design and sample collection

A convenience sample of 11 agricultural fairs that were known to have poultry exhibits were selected for the study. These fairs were held in 11 different counties in Colorado and ranged in attendance between 25 000 and 85 000 people (Kelley, 2011). The poultry exhibits ranged in size from 4 to 842 birds and included chickens, turkeys, ducks and geese. For the purposes of this study, the term 'waterfowl' will include ducks and geese and the term 'poultry' will include chickens, turkeys, ducks and geese. Colorado State University Extension Agents and Fair Superintendents were contacted to gain consent for environmental sampling of the poultry exhibits. Samples were obtained over a 3-month period from June to August of 2011.

The time that poultry exhibits were displayed during fairs varied. In order to obtain samples after maximal potential environmental contamination had occurred, research personnel obtained environmental samples as close to the last day of the poultry exhibition as possible. Most poultry exhibits lasted between 3 and 7 days; therefore, samples were collected on day 4 ± 1 day. Poultry inventory and size and layout of each poultry exhibit were recorded at the time of sampling. Source of litter and feed (provided by fair or owner) as well as types of adjacent animal and human food service exhibits were also recorded.

A visible contamination score was assigned based on a subjective assessment of cleanliness of the poultry exhibit environment. All research personnel (three people) involved in the study were trained on evaluation of contamination of the exhibits, in order to harmonize the assessment between personnel. Two scores were assigned to each fair, one for floors and one for poultry cages. The range of scores was 1 (very clean, no visible contamination) to 5 (very dirty, significant visible faecal and poultry debris contamination).

The total number of occupied cages was enumerated and 10% of cages were sampled in a non-random manner by sequentially counting the cages while walking through the exhibit. Using this method, at least one bird from almost every participant at the show (most participants entered more than one bird in the exhibition) was sampled. Composite samples of used chicken and turkey litter and used waterfowl litter were collected at each fair by removing approximately 1 g of litter from each sampled cage using sterile tongs. Composite samples of feed were obtained by collecting 1 g of feed using gloved hands from 10% of available feed sources, including owner-provided and fair-provided feed.

Floor and table/counter samples were collected using drag swabs. Drag swabs were assembled using two 4×4 inch sterile gauze pads fastened, in parallel, to nylon monofilament fishing line. Each swab was saturated in double-strength skim milk. Assemblies were placed into aluminium foil, sealed in autoclave bags and sterilized. Environmental samples were collected using the drag swabs to sample walkways, floors and tables in poultry exhibition areas. The

size of the exhibit area was estimated, and a minimum of 10% of each environmental surface was sampled.

Drag swabs and feed and litter samples were placed into Whirl-Pak® (Nasco, Fort Atkinson, WI, USA) bags and stored on ice. In total, five samples were collected from each fair, for a total of 55 samples. Samples were transported to the Colorado State University Veterinary Diagnostic Laboratory for analysis within 48 h of collection.

Salmonella isolation and identification

All samples were cultured for *Salmonella* according to USDA National Poultry Improvement Plan procedures (USDA APHIS, 2011). Tetrathionate enrichment broth (BD Diagnostic Systems, Sparks, MD, USA) was added to the samples at a 1 : 10 sample to enrichment media ratio and incubated at 37°C for 24 h. After incubation, 100 μl of enriched culture was inoculated onto Modified Semi-Solid Rappaport Vassiliadis with Novobiocin (MSRV; Oxoid, Basingstoke, UK) plates and incubated at 42°C for 24 h. The plates were observed for growth travelling from the point of inoculation. If there was no growth observed at 24 h, the plates were re-incubated. If after the second incubation period no growth was observed, a sterile loop sample of the plate at the site of inoculation was inoculated onto Brilliant Green with Novobiocin (BGN; BD Diagnostic Systems) and Xylose-Lysine-Tergitol 4 (XLT4; Hardy Diagnostic Systems, Santa Maria, CA, USA), and the plates were streaked for isolation of any possible non-motile *Salmonella* spp. If growth was present, a sterile loop sample of the outer edge of growth was inoculated onto BGN and XLT4, and the plates were streaked for isolation. A maximum of five suspect *Salmonella* colonies were inoculated into triple sugar iron agar (TSI; BD Diagnostic Systems) and lysine iron agar (LIA; BD Diagnostic Systems) slants and incubated at 37°C for 24 h for identification. All *Salmonella* suspect colonies were serogrouped using grouping reagents (BD Diagnostic Systems). All *Salmonella* isolates were sent to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa for serotyping.

Results

The average visible contamination score for the poultry exhibits was 2.7 for both floors/other surfaces and for cages. One fair received a score of 1 for the poultry cages and no fairs received a score of 5. All other fairs received scores between 2 and 4 (Fig. 1).

Poultry exhibits were most commonly housed in the same building as the rabbit exhibit (6/11), followed by sheep (3/11) and goat (2/11) exhibits. One fair housed all livestock in one building. Other than this fair, swine and cattle exhibits were housed in a building separate from, but typically adjacent to, the poultry exhibit.

Salmonella spp. were recovered from at least one sample from 10 of the 11 fairs (91%). Of all of the samples collected, *Salmonella* spp. were recovered from 28/55 (50.9%). The most common positive sample type was waterfowl litter, with 8/11 fairs positive for *Salmonella*, followed by tables (7/11), chicken and turkey litter (6/11), floors (6/11) and feed (1/11) (Table 1). The association between fair cleanliness and *Salmonella* detection could not be evaluated because all but one fair were positive for *Salmonella*.

Five serogroups were identified: B, C, D, E and G (Grimont and Weill, 2007). *Salmonella* isolates of serogroup C (31.6%) were the most commonly identified. Eleven *Salmonella* serotypes were identified. Serotype Kentucky was the most commonly isolated serotype, with 13 of 30 isolates. This was followed by Meleagridis (4/30), Bredeney (3/30), Infantis (2/30), 8:20:-:z6 (2/30), Enteritidis (1/30), Montevideo (1/30), Thompson (1/30), Derby (1/30), Braenderup (1/30) and Cubana (1/30). Of the serotypes isolated, all but one (Derby) are commonly associated with poultry in the United States, based on data of serotypes detected in more than 6500 chicken and turkey *Salmonella* isolates sent to the NVSL in 2010 (NVSL, unpublished *Salmonella* data, 2010). Nine of the 11 serotypes have been associated with human illness, either through consumption of contaminated food products or through contact with infected animals or their environment (Doyle et al., 2008; ECDC, 2011; Table 2).

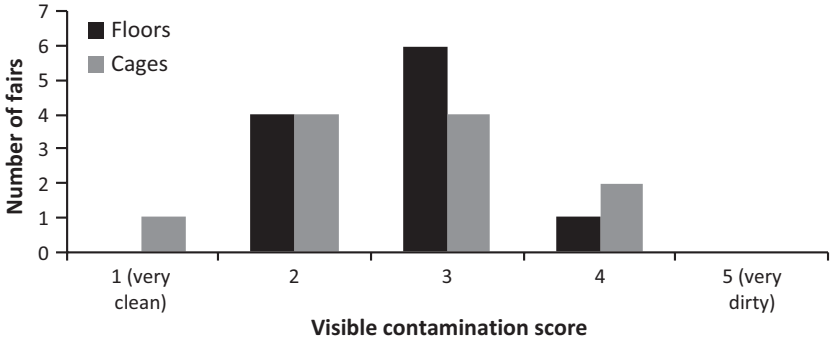

Fig. 1. Visible contamination score of floors and cages at 11 sampled poultry exhibits.

Table 1. Summary of *Salmonella* serotypes isolated from poultry exhibits

		Sample location				
Fair ID	Number birds in exhibit	Floors	Tables	Feed	Chicken and turkey litter	Waterfowl litter
1	4	None	None	None	None	None
2	48	Meleagridis	Meleagridis	Meleagridis	None	Meleagridis
3	266	Kentucky	8,20:-:z6	None	None	8,20:-:z6
4	290	Kentucky	Kentucky	None	Kentucky	Kentucky and Enteritidis
5	353	Kentucky	Kentucky	None	Bredeney	Kentucky
6	268	Kentucky	Kentucky	None	Bredeney	Bredeney and Kentucky
7	164	None	None	None	Braenderup	None
8	842	None	None	None	None	Thompson
9	147	None	Infantis	None	Infantis	Derby
10	34	None	None	None	None	Cubana
11	606	Kentucky	Montevideo	None	Kentucky	None

Discussion

This study demonstrates that the environment of agricultural fair poultry exhibits can be contaminated with *Salmonella*, and a variety of serotypes may be isolated. This is particularly concerning when the *Salmonella* serotypes detected have been frequently associated with outbreaks of human salmonellosis. Because a number of outbreaks of *Salmonella* in humans have been associated with petting zoos, poultry exhibits should be examined as a potential source of an outbreak.

None of the fairs in Colorado required pathogen testing or veterinary health certificates for poultry prior to fair entry. While some fairs did require a veterinary inspection prior to entry, this type of health examination was limited in scope and was often not conducted by a veterinarian. Because *Salmonella* is typically shed by clinically normal poultry, it would be impossible to detect *Salmonella* infected birds without testing prior to the fair.

For the fairs evaluated, the poultry exhibits were typically in place for most of or the entire duration of the fair. Poultry movement between fairs was uncommon, with the exception of movement of poultry from their respective county fair to the Colorado State Fair, which usually occurred one or more weeks after the county fair. Most county fair attendees live in the geographical area surrounding the fair. Thus, movement of people and poultry between fairs was limited so the *Salmonella* isolates detected at each fair were likely not due to fair-to-fair movement.

The average visible contamination score for the poultry exhibits sampled was 2.7. Most poultry exhibits were held in buildings with dirt floors that are difficult to clean and many had few designated facility cleaning staff on site. No fairs received a score of 5 (very dirty conditions). However, only one fair received a score of 1 (very clean conditions) for cage cleanliness. Fairs with higher visible contamination scores (3 or 4) had greater visible faecal and poultry debris contamination of floors and other surfaces or litter in the

Table 2. Summary of *Salmonella* serotypes isolated

Serotype	Serogroup	*n* (%)[a]	Frequency of serotype[b]	Associated with human illness?
Braenderup	C	1 (3.3)	Chicken (#14); no turkey isolates	Yes
Bredeney	B	3 (10)	Rare in chickens; turkey (#20)	Yes
Cubana	G	1 (3.3)	Chicken (#29); rare in turkeys	Yes
Derby	B	1 (3.3)	Rare in chickens; turkeys common in swine	Yes
Enteritidis	D	1 (3.3)	Chicken (#1); rare in turkeys	Yes
Infantis	C	2 (6.7)	Chicken (#8); turkey (#22)	Yes
Kentucky	C	13 (43.3)	Chicken (#2); turkey (#7)	Yes
Meleagridis	E	4 (13.3)	Chicken (#38); no turkey isolates; common in cattle	No
Montevideo	C	1 (3.3)	Chicken (#13); turkey (#5)	Yes
Thompson	C	1 (3.3)	Chicken (#17); rare in turkeys	Yes
8,20:-:z6 (unsubtypable)	C	2 (6.7)	Chicken (#26); no turkey isolates	No

[a]Percentage is calculated using total number of isolates ($n = 30$).

[b]Based on frequency of serotype among total samples submitted in 2010 to the National Veterinary Services Laboratories in Ames, Iowa. Data are listed by numbered ranking and includes 5257 isolates and 151 serotypes from chickens; 1283 isolates and 63 serotypes from turkeys.

birdcages. Poultry exhibit areas that had concrete floors tended to be cleaner than those with dirt floors, possibly because concrete is easier to clean.

While conducting this study, we observed husbandry practices that might increase the risk of salmonellosis and other zoonotic diseases at poultry exhibits. Owners were designated as the personnel responsible for maintaining cage hygiene at most fairs. This task can be difficult as poultry and waterfowl are inherently messy when kept in confinement and owners may only visit the exhibition once per day. At multiple fairs, owners were observed to be bathing birds and washing poultry equipment in restroom sinks designated for human use. At all fairs, Superintendents or exhibition participants were observed to be eating while handling birds or placing food or utensils on table surfaces contaminated with poultry faeces. In addition, while owners and fair personnel were observed to sweep floors and wipe tables, disinfectants were not commonly used. Cleaning and disinfection of used (vacant) cages between birds was rarely performed.

Most poultry exhibits were located in the same building as or adjacent to other animal exhibits. The proximity of poultry exhibits to other animal exhibits allows for the potential transmission of *Salmonella* between animal species. In addition, in one fair building, the poultry exhibit was located immediately adjacent to the food service area, increasing the potential for exposure of humans to poultry faeces and contaminated environments.

Salmonella was detected in at least one sample from all but one of the fairs. The negative fair had only four birds from two different owners thus decreasing the potential for *Salmonella* detection due to the small exhibit size. The most common sample positive for *Salmonella* was waterfowl litter, followed by tables, chicken and turkey litter and floors. The finding of multiple *Salmonella* serotypes in waterfowl litter is interesting, as shedding of *Salmonella* from waterfowl is poorly characterized in the US. In all but two instances, the *Salmonella* serotypes detected in floor and table samples were the same as those detected in chicken and turkey litter, waterfowl litter or both. These results are concerning as they demonstrate the potential for shedding by poultry and subsequent environmental contamination. *Salmonella* detected on floor or table samples with no link to litter samples could be contaminants brought into the poultry exhibit from other livestock exhibits via human movement, from poultry that were not sampled during litter collection, or from residual contamination from previous activities at the fairgrounds. Six *Salmonella* isolates were detected in poultry or waterfowl litter that were not detected in the floor or table environmental samples of the respective fair. It is possible that personnel maintained better hygienic practices in these areas, although this was not specifically evaluated.

Feed is a commonly reported source of *Salmonella* introduction in commercial poultry operations (Davies et al., 1997; Crump et al., 2002; Davies and Wales, 2010), but only one of 11 feed samples was positive for *Salmonella* in this study. Interestingly, *Salmonella* Meleagridis was isolated from the single positive feed sample from a fair with four of five environmental samples positive for the same serotype. It cannot be determined from our results if the feed was the original source of the *Salmonella* or if the feed was contaminated with the organism along with the exhibit environment. All bird owners at all fairs were feeding a commercially produced feed marketed for small flock owners which may not have the same *Salmonella* contamination issues that have been documented in large-scale commercial poultry production feed.

In most fairs, one *Salmonella* serotype predominated. This may be caused by one bird or one flock shedding *Salmonella*, with subsequent spread of organisms throughout the fair environment or to other birds at the fair; especially with some of the more common serotypes, more than one bird or flock may have been infected and shedding the organism, resulting in widespread environmental contamination.

All but one of the serotypes isolated are routinely associated with poultry. Serotype Kentucky was the most frequently isolated serotype in this study; it was the second most common serotype detected in chickens and seventh most common in turkeys by the NVSL in 2010 (NVSL, unpublished *Salmonella* data, 2010). In contrast, serotype Enteritidis was detected in only one sample (waterfowl litter) from one fair; it was the most common serotype detected in chickens by NVSL in 2010. This disparity is likely due to sampling bias in the serogroups of samples sent to NVSL for typing as regulatory poultry programs emphasize the detection of *Salmonella* Enteritidis. This results in a disproportionate submission of serogroup D isolates sent to NVSL for serotyping. Importantly, *Salmonella* Enteritidis is highly associated with human illness (CDC, 2010a). *Salmonella* Derby was isolated from waterfowl litter but is rarely detected in chickens and turkeys. Because the NVSL receives very few *Salmonella* isolates from waterfowl, it is unknown if this serotype is common in ducks and geese. *Salmonella* Derby is common in swine (NVSL, unpublished *Salmonella* data, 2010), which may have been the source of infection for the waterfowl, as many of the fair flock owners are 4-H participants and raise multiple species of poultry and livestock on their farms.

Nine of the 11 serotypes detected in this study have been associated with human illness through consumption of contaminated food products or handling of live poultry (Doyle et al., 2008; ECDC, 2011). Poultry are frequently handled at fairs by their owners and are occasionally handled by the general public via touching the birds through

their cages, petting birds that are on display and visiting fair-sponsored petting zoos. These practices may result in human exposure to *Salmonella* through contact with infected birds or environmental exposure. Cummings et al. (2012) found associations between human *Salmonella* infection and contact with farm animals and their environment. While these researchers did not include poultry in their study, direct contact with poultry and their environment may have the potential for similar risk of *Salmonella* transmission. The potential for human exposure to *Salmonella* via environmental contamination is difficult to measure but should be considered as a possibility, particularly when people are not practicing good hygiene through behaviours such as touching faecal-contaminated tables or eating while walking through animal exhibits. Of particular concern is the apparently common practice of bathing birds and cleaning soiled equipment in the same restroom sink that is designated for hand hygiene. This practice should be discontinued at all fairs; dedicated and separate facilities for bathing birds and washing equipment should be provided. In addition, because children, the elderly and the immunocompromised are at increased risk for severe *Salmonella* infection (CDC, 2010b), hygienic practices at poultry exhibits should be improved, in light of the fact that children are the primary participants in 4-H poultry shows.

Agricultural fairs should consider instituting policies and practices to improve hygiene and mitigate the risk of zoonotic salmonellosis. These efforts could include educational programs targeted to fair staff, including Extension Agents and Superintendents, poultry exhibit participants and public visitors to the fair. These efforts should stress the use of personal protective measures to minimize human exposure when handling birds. Also, general hygiene practices should be a focus, including guidelines on hand washing, cleaning and disinfection of hand contact surfaces, restricting food and drink to designated areas well separated from animal exhibits, and measures to prevent contamination of food products at the fair.

In this study, the sample size was limited because the geographical area of this study was restricted to Colorado, and not all Colorado agricultural fairs held poultry exhibits. Therefore, the results of the current study may not be representative of all agricultural fairs. The small sample size limited the ability to find differences among fair variables such as number of birds and size of exhibit. In addition, not all *Salmonella* present in the environment may have been detected. While the drag swab and litter and feed collection methods of sampling and testing used in this study are widely used throughout the commercial poultry industry and are considered sensitive methods of sampling (FDA, 2009; USDA APHIS, 2011), *Salmonella* organisms may have been missed because the samples did not include the entire environment. Also, organisms may have been missed during culture or excluded when one specific *Salmonella* serotype grew in higher numbers than another, so *Salmonella* prevalence may be underestimated. The detection of a single serotype may also be due to laboratory practices, where the potential exists that not all isolated *Salmonella* organisms are selected from the culture plate for further typing. Additional studies should be conducted to determine prevalence of *Salmonella* shedding by individual birds exhibited to measure the association between individual bird shedding and environmental contamination.

We determined that poultry shed *Salmonella* during fairs and environmental contamination can occur. This represents potential risk for zoonotic disease transmission and dissemination of disease in backyard poultry populations that are involved in poultry exhibition.

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