Salmonella Contamination of Turkey from Processing to Final Product—A Process to Product Perspective

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Abstract

Food safety and the development of an understanding of issues surrounding pathogens associated with food is of considerable importance in modern-day food production. The design and use of risk models to estimate the likelihood of human illness has become an important part of our understanding of food safety issues. In order to quantify food safety risks, the first requirement is to estimate the occurrence of the pathogen associated with a particular production system. As such, an assessment of whether interventions significantly reduce these risks can be made. In light of recent events in food production including pathogens in ready to eat foods and outbreaks of *Escherichia coli* O157:H7 associated with produce, the development and implementation of risk models will continue to gain importance in the coming years. This paper focuses on some of the current research ongoing at North Dakota State University to develop a greater understanding of *Salmonella* as it pertains to turkey production and processing in the Midwest. This overview of studies of *Salmonella* in turkey production stems from a presentation of research made at the Integrated Risk Studies: Gate to Plate—Current Issues and Future Strategies Conference held in Fargo, ND, May 4–5, 2006.

Introduction

OUR CURRENT UNDERSTANDING of risk and foodborne pathogens is complicated by a lack of information and data gaps which do not lend themselves well to building a risk model. This review will focus on some projects carried out in our lab at North Dakota State University (NDSU) addressing issues associated with data gaps and information that could be applied to such risk models.

It is estimated that North Dakota produces almost 2 million turkeys for human consumption from its 17 regional growers (North Dakota Turkey Federation) annually. With the level of information as to the risk of salmonellosis as-

sociated with turkey production in the region and the links, if any, to human illness relatively unknown, we have been working on addressing protocols and methods for evaluating *Salmonella* contamination of production turkeys from the Midwest. This paper provides an overview of methods used in our studies to generate data. Some of this data could have application in risk models as well as refine our current knowledge of *Salmonella* in turkey. This paper is divided into a number of sections that focus on *Salmonella* at various stages of processing and on retail product destined for the consumer including sampling protocols, prevalence, effects of defeathering, antimicrobial susceptibility, and pathogenesis.

Development of Sampling Protocols for the Detection of *Salmonella* in Turkey

The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) requires that plants processing meat including poultry routinely test *Escherichia coli* levels present and verify that in-plant hazard analysis critical control point (HACCP) controls meet the pathogen reduction HACCP rule (Anonymous, 1996). In addition, FSIS routinely collects samples from carcasses as a means of verifying that in-plant HACCP controls are effective at reducing *Salmonella* below required performance standard levels. Although these protocols have been designed for broilers, turkey processors are subject to similar sample collection for *E. coli* verification. In contrast, whole turkey is currently not subject to the performance standards for *Salmonella* required for broiler production (Anonymous, 1996).

Sampling protocols for carcass testing typically consist of two approaches: nondestructive, which include techniques such as whole carcass rinsing or swabbing (Anonymous, 1996; Cox *et al.*, 1981; Logue *et al.*, 2003; Sarlin *et al.*, 1998), or destructive, which includes tissue removal (Avens and Miller, 1970; Cox *et al.*, 1978). Sampling protocols for the isolation and recovery of *E. coli* and pathogens from processed birds are relatively common. Most techniques developed have been applied to broilers and do not adequately address sampling issues for larger birds such as turkeys.

Turkey carcass sampling is typically carried out according to the original FSIS regulation designed for broilers and involves a whole carcass rinse procedure. The *carcass rinse* method consists of removing a carcass from the line and placing the carcass in a large bag to which a volume of rinse fluid is added. The carcass is rinsed by using a rocking motion in an "inverted arc" format to ensure that the liquid covers the carcass and rinses all surfaces of the bird. Once complete, the carcass is removed and the rinse fluid used for further analysis. The method has proven successful for whole carcass sampling and has greater sensitivity than sampling of limited areas (Cox *et al.*, 1976; Sarlin *et al.*, 1998; McEvoy *et al.*, 2005).

However, this method has proven to be a concern for turkeys due to the significantly larger size of the birds and the potential for cross contamination during sampling (McEvoy *et al.*, 2005). The method may not be useful for very large birds in particular from the point of the sampler because it may be difficult to assure full rinsing of the carcass. In addition, this method requires physical removal of a carcass from the line which may increase the potential for cross contamination during collection of the rinse fluid.

A second approved method recommended by the USDA FSIS as an alternative to carcass rinsing for *E. coli* testing is the *two-site swab* (2S) method (Anonymous, 1997). The principle of the technique is based on sampling a 50-cm² area of the thigh and back on a carcass and homogenizing the swab in diluent prior to analysis. The method itself is easy to perform requiring no specific training and can be carried out online without the need to physically remove the carcass from the process line. One of the major disadvantages of the method, however, is the limitation in only sampling specific areas of a carcass rather than the whole bird which may limit the sensitivity of the method. Bodnaruk *et al.* (1998) evaluated the use of the 2S protocol for the recovery of *E. coli* from turkey carcasses but failed to compare data obtained with the whole carcass rinse technique. No such comparison as a means for testing *Salmonella* protocols on turkeys has been established.

The final method often used in carcass sampling is destructive and includes *excision* of neck flap skin or skin from other carcass sites. This method is considered more sensitive for the recovery of bacteria from carcass surfaces than swabbing or rinsing (Cox *et al.*, 1976; Sarlin *et al.*, 1998).

One of our primary research efforts has evaluated appropriate methods for assessing the quality and pathogen presence on turkey carcasses. Evaluation of these methods was based on a need to replace or enhance the current USDA FSIS approach and included evaluation of whole carcass rinsing and a 2S method sampling the thigh and back of the bird.

In studies carried out in our lab (McEvoy *et al.*, 2005), carcass sampling methods were eval-

uated as a means for obtaining data that could have application in assessing the quality and *Salmonella* status of turkey carcasses by the most efficient means possible. One of our primary challenges was to develop a better physical method for whole carcass rinsing. A *modified carcass rinse* (MCR) procedure for rinsing whole carcasses was developed by our team at NDSU, altered how the rinse procedure was carried out (McEvoy *et al.*, 2005). The modified method uses a free-standing swing to achieve the same inverted arc motion as manual swinging, thus reducing operator fatigue. The method was otherwise carried out in a similar manner to the USDA carcass rinse method and was demonstrated to have similar detection efficiency (McEvoy *et al.*, 2005).

In other studies, four methods were evaluated for efficiency in detecting *Salmonella*: a modified whole carcass rinse procedure (MCR); whole carcass swab (WS); a single-site swab (1S) (swabbing an undelimited area of approximately 50 cm² under the wing area of the carcass); and a 2S, which sampled a 50-cm² area of the thigh and a 50 cm² area of the back. In addition, excision sampling (tissue removal) was included as a comparison standard.

When the methods were assessed for recovery of *Salmonella*, the recovery rates of the pathogen varied significantly between the two sampling periods investigated (Table 1)—an effect that may be related to overall *Salmonella*

levels in the flock sampled during the period 1 visit. *Salmonella* was recovered from 2%, 2%, and 6% of the WS, MCR, and excision samples tested but was not recovered from any carcasses using 2S and 1S swabs for period 1. In contrast, during period 2 the recovery of *Salmonella* was significantly greater ($p < 0.05$) for WS and MCR samples than those of excision, 2S or 1S swabs. It was interesting to note that the standard excision method was not significantly different than MCR or 2S samples.

The data suggest that isolation and recovery methods for pathogens are different from those of indicator organisms and should be taken into account when approaching protocols for sampling of large birds. Sampling protocols may also have a significant impact in application for a risk model as the distribution of the pathogen may not be homogeneous on the carcass being sampled. Therefore protocols that fail to sample the carcass adequately may considerably underestimate pathogen levels or prevalence of the target organism. This study concluded that the use of a whole carcass swabbing method was as efficient as whole carcass rinsing. Whole carcass swabbing offers an efficient online sampling method, reducing the need for carcass removal from the process line and avoiding possible cross contamination events.

This study highlighted the fact that sensitive, specific, and valid methods should be used for estimating contamination rates and exposure levels which could be applied to risk models. The results from this study provided a suitable alternative to the cumbersome carcass rinsing procedure—the WS which we subsequently used for determining *Salmonella* occurrence at different processing stages.

***Salmonella* Prevalence on Turkeys During Processing**

Nde *et al.* (2006) investigated the changes in *Salmonella* contamination on a turkey processing line by determining the pathogen prevalence at eight specific stages during processing from slaughter to final boned-out product (see Fig. 1 for a typical layout of a process line). It was interesting to note that simple interventions such as cold water washing had a significant effect in

TABLE 1. *SALMONELLA* RECOVERY FROM TURKEY CARCASSES USING WHOLE CARCASS SWAB (WS), MODIFIED CARCASS RINSE (MCR), NECK EXCISION (EXCISION), TWO-SITE SWAB (2S), AND SINGLE-SITE SWAB (1S)

Time	Method	No. of positive samples (%)
Period 1	WS	1 (2)
	MCR	1 (2)
	Excision	3 (6)
	2S	0
	1S	0
Period 2	WS	34 (68) ^a
	MCR	29 (58) ^{ab}
	Excision	23 (46) ^{bc}
	2S	18 (36) ^{cd}
	1S	10 (20) ^d

A total of 50 samples were evaluated for each method tested. Values followed by different letters are significantly different ($p < 0.05$).

FIG. 1. Flow diagram of a typical turkey slaughter line.

reducing the overall carcass prevalence of *Salmonella* (Fig. 2). In contrast, further processing stages resulted in recontamination of the carcass—an effect that was also reported by Morris and Wells (1970). It was noted that there were considerable differences in pathogen contamination rates following certain stages of processing, in particular, after defeathering, after washing, and after handling following the

final wash (prechill). *Salmonella* carcass prevalence rates reached a maximum of 16% for any single processing stage sampled. The incidence of the organism on carcasses prescald was relatively low at 7%, increasing to 16% post defeathering. Significant reductions ($p < 0.05$) were noted for carcasses postwash where levels dropped to 1%. A significant increase ($p < 0.05$) in prevalence occurred between postwash and

FIG. 2. Cumulative percent prevalence of *Salmonella* spp. at eight processing stages over five sampling visits.

prechill (1–11% increase) suggesting the influence of worker handling on carcass contamination during final removal of the necks and remaining tail feathers with little or no disinfection. Similar results have been obtained for pork, beef, and poultry workers (Rahkio and Korkeala, 1996; de Wit and Kamplmacher, 1982). The increase in contamination rates observed prechill did not significantly change after chilling and after the carcasses were deboned.

When the carcass contamination rates on entry to the plant were compared overall with contamination rates following final processing at the end of the line (after the backframe had been removed), no significant difference ($p > 0.05$) was observed. This study suggests that despite a series of interventions and processing stages, *Salmonella* prevalence on incoming birds was relatively similar to that found on finished product. The implications of this result are not desirable especially if heavily contaminated flocks are slaughtered.

Traditional risk assessments do not incorporate information pertaining to the characteristics of the pathogens isolated. However, further molecular characterization of foodborne pathogens provides greater insight into the movement of the pathogen, its ability to cause disease, and effective treatment options. These characteristics are often considered important especially in a dose–response facet of a risk assessment as some serotypes may be host adapted and will not apply to causing disease in humans.

The use of a serotype and molecular approach was used as a means to further evaluate the

effects of processing on carcass contamination. Molecular methods and serotyping provide valuable information in tracking the movement of pathogens through a processing line. Such studies have been used by a number of investigators and provide useful information in studying the movement of *Salmonella* serotypes or tracing outbreaks associated with foods (Nayak *et al.*, 2004; Olsen *et al.*, 1993; Liebana *et al.*, 2001; Cormican *et al.*, 2002). Nivas and colleagues (1973) used serotype analysis to demonstrate that *Salmonella* serotypes introduced into a processing plant spread throughout the plant, thus providing valuable information on sources for cross-contamination. In studies carried out by Nde *et al.* (2006) serotyping provided useful information regarding the spread and types of *Salmonella* prevalent on turkey carcasses at specific sampling points on the processing line. It was noted that strains that were detected on the carcasses at earlier points of the process line were carried through the line while new serotypes were also found at later points on the line during processing, suggesting that there were mixed serotypes present on some carcasses or that some strains that were located internally became external during further processing (Table 2). Alternatively, sources such as the hands of workers (Rahkio and Korkeala, 1996; de Wit and Kamplmacher, 1982) could also be a contributing factor.

Serotyping alone may not, however, indicate the genetic relatedness of strains and requires analysis at the molecular level using techniques such as pulsed-field gel electrophoresis (PFGE),

TABLE 2. *SALMONELLA* SEROTYPES RECOVERED FROM PROCESSED TURKEY AT EACH PROCESSING STAGE

Visit	Prescald	Post-defeathering	Pre-evisceration	Prewash	Postwash	Prechill	Postchill	Post-back removal	Total
1	—	a (8)	—	a (2)	—	a (4)	a (9) f (1)	a (12)	36
2	a (1)	a (5) e (1)	a (1)	d (1)	a (1)	a (4)	—	—	14
3	a (2) f (2)	—	f (1)	a (6) b (1)	—	—	a (1) b (3)	a (1)	17
4	—	—	—	—	—	—	—	—	—
5	f (2)	f (2)	f (3)	f (1)	—	f (2)	c (1) f (1)	b (1)	13
Total	7	16	5	11	1	10	16	14	80

Lowercase letters represent *Salmonella* serotypes: a = Hadar; b = Heidelberg; c = Montevideo; d = Newport; e = Schwarzengrund; f = Senftenberg. Numbers in parentheses indicate number of isolates recovered at each sampling stage.

amplified fragment length polymorphism, multilocus sequence typing, etc., to determine the relationship between serotype and strain type (Fakhr *et al.*, 2005). Nde *et al.* (2006) demonstrated the movement of *Salmonella* serotypes during processing (Table 2) and used PFGE to show the relationship between *Salmonella* serotypes and pulse types (from PFGE) and processing stages (Fig. 3). It was evident that incoming birds were a significant source of *Salmonella* for the processing line and that *Sal-*

monella had the potential to be transferred between birds and to the line itself. It was also noted that there was wide diversity in the number of stains isolated as indicated by the number of clusters into which the isolates fell. Also, identical serotypes were found to cluster together regardless of the date of the visit or processing stage from which the isolates were collected. Although all samples were collected from birds originating on different farms, the relatedness of strains as linked to the hatchery

FIG. 3. Dendrogram showing pulsed-field gel electrophoresis (PFGE) profiles of *Salmonella* isolates from visit 2. Pre = pre-defeathering; Post = post-defeathering; Fingers = Fingers of picker machines.

source could not be excluded at the time of the study. Hatcheries have been suggested as a source of vertical transmission for *Salmonella* spp. (Kumar *et al.*, 1971; Heyndrickx *et al.*, 2002) and this source for different farms in the same region could not be discounted. This study also highlighted the spread of the same serotype and pulse types from prescalding to pre-evisceration, prechill, and postchill thus providing significant evidence that incoming birds represent a *Salmonella* source for the processing line.

These studies also served to highlight processing stages where contamination was greatest and could have implications in the development of strategies for the control of *Salmonella* on a process line. The use of serotype in conjunction with PFGE provided useful data for tracking contamination within the plant.

Effects of Defeathering on *Salmonella* Carcass Contamination

In studying the changes in *Salmonella* prevalence during processing, of significant importance was the effects of the defeathering process on carcass cross-contamination. *Salmonella* on the feathers and skin of live birds can be a source of contamination for the underlying skin and ultimately the carcass. This possibility has not previously been investigated. In an effort to develop a greater understanding, studies were carried out to examine the effect of defeathering on carcass skin contamination and the overall effects of the defeathering process (scalding and picking).

Defeathering has always been considered a significant source of carcass contamination which can occur through series of processes including, direct contact between contaminated and uncontaminated carcasses (Mulder *et al.* 1978), compression of the carcass, which results in expulsion of internal feces to the carcass surface (Berrang *et al.*, 2001), aerosol contamination generated during spraying, and from the picker fingers (Allen *et al.*, 2003a, 2003b; Clouser *et al.* 1995a, 1995b; Berrang *et al.*, 2001) which contribute to cross-contamination between carcasses. Nde *et al.* (2007) evaluated the feathers as a contamination source for carcasses during defeathering. The study evaluated defeathering and

carcass contamination for individually tagged birds at pre- and post-defeathering stages.

No strict correlation was found between incoming contamination rates and those observed on carcasses post-defeathering. Generally, if the prevalence was high on incoming birds it tended to be high on birds post-defeathering. Similarly, situations where incoming bird contamination rates were relatively low tended to result in low contamination rates on defeathered birds. When data from all four visits of the study was combined, a significant increase was observed in the rate of *Salmonella* carcass contamination post-defeathering ($p < 0.05$) (Table 3). This study agrees with previous studies (Lillard *et al.* 1989, 1990; Clouser *et al.* 1995a, 1995b).

Of interest in this study was the use of serotyping and molecular analysis to determine the correlation between the defeathering process and carcass contamination. The study found all serotypes recovered from carcasses after defeathering were present before defeathering (Table 4). It was also noted that serotypes with similar PFGE profiles were detected at pre- and post-defeathering and from the picker fingers of the defeathering machines (Fig. 3). During one visit, the same serotype or pulse type was recovered from the birds before and after defeathering, the picker fingers, and the scald water. This study was one of the first to provide a clear link between *Salmonella* carcass contamination during defeathering and demonstrate the transfer of *Salmonella* from the feathers of live birds to carcass tissue. It was possible to genetically link contamination on incoming carcasses with the scald water and the picker

TABLE 3. PREVALENCE OF *SALMONELLA* POSITIVE BIRDS AT PRE- AND POST-DEFEATHERING

Visit number	No. positive birds/no. sampled	
	Pre-defeathering	Post-defeathering
1	14/24 (58%) ^a	24/24 (100%) ^b
2	14/50 (28%) ^a	16/50 (32%) ^a
3	30/50 (60%) ^a	25/50 (54%) ^a
4	24/50 (48%) ^a	45/50 (90%) ^b
Total	82/174 (47%) ^a	110/174 (63%) ^b

Percentages within a row lacking a common letter differ ($p < 0.05$).

TABLE 4. *SALMONELLA* SEROTYPES DETECTED BEFORE AND AFTER DEFEATHERING, IN SCALD WATER, AND ON THE PICKER FINGERS DURING EACH VISIT

Visit	Location	Serotypes	PFGE profile
1	Pre	Bredeney, Hadar	A, B
	Post	Bredeney, Hadar	A, B
	Scald water	—	
2	Picker fingers	Hadar	B
	Pre	Hadar, Heidelberg, Schwarzengrund, Typhimurium	B, E, D, C
	Post	Schwarzengrund, Typhimurium	D, C
3	Scald water	—	
	Picker fingers	Schwarzengrund	B
	Pre	Hadar, Muenster	F, G
4	Post	Hadar	F
	Scald water	—	
	Picker fingers	Hadar	F

Pre = pre-defeathering; Post = post-defeathering; Fingers = fingers of picker machines; PFGE = pulsed-field gel electrophoresis.

fingers demonstrating sources of contamination between incoming contaminated carcasses and other non-contaminated carcasses. These data could be of significant importance in situations where noncontaminated carcasses follow contaminated birds.

Antimicrobial Susceptibility and Pathogenesis of *Salmonella* Recovered from Processed Turkey

When investigating the relationship between human illness and *Salmonella* recovered from processed turkey it is important to assess relatedness on a number of levels. In follow-up studies, isolates recovered from online processed turkeys were assessed for antimicrobial susceptibility and pathogenesis traits as a means to evaluate their potential for causing human illness and to understand the influence of processing, if any, on selection of the strain types.

A total of 80 isolates recovered from processed turkeys from various stages of the process line were assessed for antimicrobial susceptibility to a range of antimicrobials using the National Antimicrobial Resistance Monitoring System panel. This panel assesses susceptibility to 17 antimicrobial agents using a broth microdilution technique. Antimicrobials assessed included ampicillin, amoxicillin-clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, tetracycline, amikacin, gentamicin, kanamycin, streptomycin, trimethoprim-sulfamethoxazole, sulfisoxazole, ciprofloxacin, and nalidixic acid (Table 5). Isolates were assessed for minimum inhibitory concentrations of agents inhibiting growth of the organism and defined as sensitive or resistant using the Clin-

TABLE 5. ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* FROM PROCESS LINE TURKEY

Class/antimicrobial	Concentration ($\mu\text{g/mL}$)	Breakpoint ($\mu\text{g/mL}$)	No. of resistant isolates (%)
Penicillins			
Ampicillin	1–32	32	1 (1.25)
Amoxicillin-clavulanic acid	1/0.5–32/16	32/16	1 (1.25)
Cephalosporins			
Cefoxitin	0.5–32	32	0 (0)
Ceftiofur	0.12–8	8	0 (0)
Ceftriaxone	0.25–64	64	0 (0)
Chloramphenicol	2–32	32	0 (0)
Tetracycline	4–32	16	61 (76.3)
Aminoglycosides			
Amikacin	0.5–64	64	0 (0)
Gentamicin	0.25–16	16	4 (5)
Kanamycin	8–64	64	2 (2.5)
Streptomycin	32–64	64	32 (40)
Folate pathway inhibitors			
Trimethoprim-sulfamethoxazole	0.12/2.38–4/76	4/76	0 (0)
Sulfonamides			
Sulfisoxazole	16–256	256	5 (6.25)
Quinolones-fluoroquinolones			
Ciprofloxacin	0.015–4	4	0 (0)
Nalidixic acid	0.5–32	32	0 (0)

ical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards [NCCLS]) guidelines (NCCLS 2002).

Results from these studies found that 76.3% of all isolates tested were resistant to tetracycline, and 40% to streptomycin, while lower levels of resistance were observed to ampicillin, amoxicillin-clauvalinic acid, gentamicin, kanamycin, and sulfisoxazole (see Table 5). Twenty-nine isolates displayed resistance to one single agent, 28 to two, and one isolate to three, three isolates to four agents, and one isolate to five antimicrobials. Thirty-three isolates of the 80 tested displayed multiresistance, which was defined as displaying resistance to more than one antimicrobial agent (results not shown).

When these isolates were assessed for genes associated with antimicrobial resistance using polymerase chain reaction (PCR), 58.8% were positive for the genes associated with integrase, and 8.8% were positive for the class I integron with sizes ranging from 200 to 800 bp. Other resistance genes were also evaluated including genes encoding for *aadA* (resistance to streptomycin, spectinomycin), found in 7.5% of all isolates tested; the streptomycin resistant genes *strA* and *strB* found in 73.8% of all isolates tested and *tetA* (tetracycline resistance gene) found in 72.5% of the tetracycline resistant strains (Table

TABLE 6. ANTIMICROBIAL RESISTANCE GENES OF *SALMONELLA* ISOLATES

Gene	No. positive/ no. tested (% prevalence)	Reference
<i>IntI</i>	47/80 (58.8)	Levesque and Roy, 1993
Conserved sequence	7/80 (8.8)	Levesque and Roy, 1993
<i>aadA</i>	6/80 (7.5)	Li <i>et al.</i> , 2007
<i>strA</i>	59/80 (73.8)	Li <i>et al.</i> , 2007
<i>strB</i>	59/80 (73.8)	Li <i>et al.</i> , 2007
<i>tetA</i>	58/80 (72.5)	Li <i>et al.</i> , 2007

6). Genotype confirmation of the resistance genes was not unexpected as 76% of the strains phenotypically expressed resistance to tetracycline and 41.3% to streptomycin (Table 6) (results not shown).

Isolates were also examined for virulence factors and their potential links to human illness. Using a multiplex PCR procedure (Skyberg *et al.*, 2006), isolates were screened for 17 virulence factors associated with host invasion, survival within macrophages, iron acquisition, etc. (Table 7). All (100%) of the isolates tested possessed genes *spiA*, *pagC*, *msgA*, *invA*, *sipB*, *prgH*, *spaN*, *orgA*, *tolC*, and *sopB*. Lower prevalences were observed for the genes *iroN*, *sifA*, *lpfC*, and *sitC*, and significantly lower prevalences for *spvB*, *cdtB*, and *pefA* (Table 7).

TABLE 7. *SALMONELLA* GENES USED IN VIRULENCE GENOTYPING AND PREVALENCE OF VIRULENCE GENES IN *SALMONELLA* RECOVERED FROM PROCESSED TURKEY

Gene	Virulence-related function	% Prevalence (n = 80)	Reference
<i>invA</i> ^a	Host recognition/invasion	100	Galan and Curtiss, 1989
<i>orgA</i> ^a	Host recognition invasion	100	Jones and Falkow, 1994
<i>prgH</i> ^a	Host recognition/invasion	100	Behlau and Miller, 1993
<i>spaN</i>	Entry into nonphagocytic cells, killing of macrophages	100	Chen <i>et al.</i> , 1996
<i>tolC</i> ^a	Host recognition/invasion	100	Skyberg <i>et al.</i> , 2003
<i>sipB</i>	Entry into nonphagocytic cells, killing of macrophages	100	Chen <i>et al.</i> , 1996
<i>sitC</i> ^a	Iron acquisition	95	Janakiraman and Schlauch, 2000
<i>pagC</i> ^a	Survival within macrophage	100	Miller <i>et al.</i> , 1989
<i>msgA</i> ^a	Survival within macrophage	100	Gunn <i>et al.</i> , 1995
<i>spiA</i> ^a	Survival within macrophage	100	Ochman <i>et al.</i> , 1996
<i>iroN</i>	Iron acquisition	83.8	Baumler <i>et al.</i> , 1996
<i>sopB</i> ^a	Host recognition/invasion	100	Parsot, 1994
<i>lpfC</i> ^a	Host recognition/invasion	98.8	Baumler <i>et al.</i> , 1998
<i>cdtB</i>	Host recognition/invasion	1.25	Haghjoo and Galan, 2004
<i>sifA</i> ^a	Filamentous structure formation	91.3	Skyberg <i>et al.</i> , 2003
<i>pefA</i> ^a	Host recognition/invasion	22.5	Stone and Miller, 1995
<i>spvB</i> ^a	Growth within host	33.8	Gulig <i>et al.</i> , 1993

^aRequired for full virulence in a murine model.

The low prevalence of genes *pefA*, *cdtB*, and *spvB* was not considered unusual as these genes appear to occur in a limited number of serovars. In similar studies, Skyberg *et al.* (2006) found a low prevalence of *cdtB*, *pefA*, and *spvB* (<17%) observed for *Salmonella* from sick and healthy birds. However, our studies (Nde *et al.*, unpublished observations) only examined these genes in isolates from healthy birds presented for processing and found the levels to range from 1.25% (*cdtB*) to 22.5% (*pefA*) and 33.8% (*spvB*). *pefA* and *spvB* have been reported as being usually carried on plasmids (Gulig *et al.*, 1993; van del Velden *et al.*, 1998) which may also account for their limited detection and these plasmids (Gulig *et al.*, 1993; van den Velden *et al.*, 1998) can be serovar specific (Rotger and Casadesus, 1999). In addition, not all plasmid-bearing serovars contain these virulence plasmids (Rotger and Casadesus, 1999). *spvB* and *pefA* may also be encoded by the same plasmid but are not always detected together, which may account for differences in their prevalence. In contrast, *cdtB* is located elsewhere on the genome (Baumler *et al.*, 1998; Haghjoo and Galan, 2004; Stone and Miller, 1995). In our studies, *cdtB* was found in very low prevalence which may be linked to the fact the gene is limited to certain *Salmonella* serovars; previous studies have linked this gene to *S. typhi* strains only (Haghjoo and Galan, 2004), our studies as well as recent reports (Skyberg *et al.*, 2006) have shown the presence of this gene in serovars not exclusive to humans.

These studies suggest that *Salmonella* isolates from turkeys possess significant pathogenic potential for causing human illness. This evidence is based on the association between possession of these genes and virulence in a murine model (Skyberg *et al.*, 2006).

Salmonella on Retail Products

Of importance in evaluating *Salmonella* at the retail level is the use of methods which allow optimum recovery of the pathogen in as short a time as possible. Studies in our lab have examined the use of isolation protocols for the recovery of *Salmonella* from naturally contaminated premarket turkey meat and retail product in an effort to investigate rapid methods for the detection of the pathogen in naturally contaminated products and to evaluate effective recovery. Our studies compared the ability of conventional culture, the iQ-Check™ real-time PCR kit (BioRad, Hercules, CA) and conventional PCR (*invA*) for the detection of *Salmonella* in premarket turkey meat (product at the plant prior to dispatch) and retail turkey meat products (Nde *et al.*, unpublished observations, 2007). When all three methods were compared for premarket and retail product, significant differences were observed in the recovery of the pathogen by conventional method and the two molecular methods. In the case of the premarket product, significantly fewer positive samples ($p < 0.05$) were detected using conventional methods compared to using molecular procedures. In contrast, a significantly ($p < 0.05$) greater number of samples tested positive by the conventional method when compared to one molecular method (*invA*) for retail product (Table 8). Molecular methods such as PCR rely on the detection of target DNA and will detect dead, sublethally injured, or viable but non-culturable cells that standard protocols may not (Knight *et al.*, 1990; Whyte *et al.*, 2002). It is also possible that *Salmonella* may exhibit atypical biochemical profiles and or morphology that may not be detectable by conventional bacteriological techniques (Bennett *et al.*, 1998;

TABLE 8. PREVALENCE OF *SALMONELLA* USING CONVENTIONAL CULTURE, THE iQ-CHECK™ KIT, AND POLYMERASE CHAIN REACTION (PCR) (*INV A*)

Sample type	No. of samples	Cultural method	% Positive	
			iQ-Check™	PCR (<i>invA</i>)
Premarket	120	51/120 (42.5%) ^a	70/120 (58.3%) ^b	74/120 (61.7%) ^b
Retail	119	93/138 (67.4%) ^a	88/138 (63.8%) ^a	81/38 (58.7%) ^b

Values in rows which are significantly different ($p < 0.05$) are followed by a different letter.

TABLE 9. COMPARISON OF *SALMONELLA* DETECTION FROM RETAIL TURKEY MEAT PRODUCT SAMPLES USING CONVENTIONAL CULTURE METHODOLOGY, RAPIDCHECK[®] *SALMONELLA* IMMUNOCAPTURE ASSAY, AND IQ-CHECK[™] *SALMONELLA* REAL-TIME POLYMERASE CHAIN REACTION (PCR)

Sample type	No. of samples	Conventional culture method no. positive	Rapid Check no. positive	iQ Check [™] real-time PCR	
				Pre-enrichment no. positive	Selective enrichment no. positive
Turkey parts	25	1	0	1	6
Ground turkey	74	48	38	55	60
Total	99	49	38 ^a	56 ^b	66

^aAll of the 38 positive *Salmonella* samples detected by the rapid check *Salmonella* method were also positive by the culture method and the iQCheck *Salmonella* real-time PCR (pre-enrichment and selective enrichment).

^bAll of the 56 positive *Salmonella* samples detected but the iQ Check *Salmonella* real-time PCR immediately after the pre-enrichment were also positive after selective enrichment but only 46 of these samples were positive by the culture method.

Eyigor *et al.*, 2002). These differences have also been noted by other researchers (Fratamico, 2003).

In a similar study, Fakhr *et al.* (2006) found that the addition of a selective enrichment step improved the detection of *Salmonella* in naturally contaminated retail turkey meat. The study compared the use of real-time iQ-Check PCR with an immunocapture kit (RapidCheck; Strategic Diagnostics Inc., Newark, DE) and conventional culture method. *Salmonella* was recovered from 49 of 99 retail meat samples tested by conventional method, the RapidCheck kit failed to detect the pathogen in 11 samples that were positive by the conventional method while the iQCheck kit failed to detect the pathogen in three samples positive by conventional culture protocol (Table 9). When the iQ Check protocol was modified to include an additional selective enrichment step, the kit detected the pathogen in all 49 samples that were positive by standard cultural methods, but also detected the pathogen in samples that were not positive by the standard method. Our study found that the incorporation of a selective enrichment step helped to significantly reduce the discrepancy between conventional methods and the iQ-Check kit. This finding further emphasizes the need for sensitive methods for *Salmonella* detection, especially if the data is to be incorporated into a risk assessment.

Summary

These studies have demonstrated that the whole carcass swab (WS) is a suitable, less

cumbersome alternative to turkey carcass rinsing for *Salmonella* testing. This method will augment current sampling procedures that can be used to determine the *Salmonella* contamination levels on processed turkeys. The WS can therefore, be applied in the exposure assessment facet of a risk assessment for *Salmonella* on turkeys. These studies have also demonstrated that simple in-plant interventions such as washing play a major role in reducing contamination rates. However, for the period studied, the *Salmonella* levels on slaughtered birds were not significantly different from the levels on carcass parts at the end of the line. Effective interventions especially towards the end of the line are, therefore, necessary to control *Salmonella* levels in the final product.

By using molecular and serological techniques it was possible to demonstrate the movement of *Salmonella* through the process line to final product. Studies of defeathering also highlighted the effect of contaminated feathers on carcass contamination rates and the ability of machinery and scald water to facilitate cross-contamination. Our studies have also indicated the potential pathogenicity of *Salmonella* from turkey and the antimicrobial resistance patterns they exhibit. The virulence and drug resistance capacities of isolates are also significant characteristics to be considered in a potential dose-response assessment and risk management options for human illness from Midwest-produced turkeys. By comparing conventional culture to molecular detection of *Salmonella*, our studies have shown that real-time detection of *Salmonella* is a useful alternative for determining

Salmonella contamination rates in premarket turkey parts.

This paper offers a comparative perspective on *Salmonella* detection methods in poultry. Characterization of isolates facilitated our understanding of the movement, nature, and pathogenesis of *Salmonella* from turkey. Data presented in this paper provide valuable information for risk assessors and poultry researchers investigating the safety of the food supply.

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