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Mini-review

Developing probiotics, prebiotics, and organic acids to control *Salmonella* spp. in commercial turkeys at the University of Arkansas, USA

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Abstract

In the United States, non-typhoidal *Salmonella* causes over one million foodborne infections every year and turkey meat contaminated with *Salmonella* has been associated from the farm to the processing plant. These outbreaks emphasize efforts on decreasing and preventing human illness associated with live poultry contact through comprehensive interventions from "farm-to-fork" levels. This review article revises the role of the turkey upper respiratory tract, which is now known to play a crucial role in colonization and as a source of contamination, for this remarkable bacterium that has co-evolved to infect plants and animals. Because agriculture represents over 60% of the economy of the state of Arkansas, the mission of our laboratory over the last 21 years has been directed to evaluate and develop applied research to help reduce the incidence of *Salmonella* spp. from commercial turkey operations. A summary of the published research is presented.

Keywords: Salmonellosis, turkeys, ground meat, nutraceuticals, vaccines

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Introduction

Foodborne or waterborne microbial pathogens are associated with diarrheal disorders killing an estimated two million people annually (Schlundt et al., 2004). In the United States, non-typhoidal *Salmonella* causes over one million foodborne infections every year (Scallan et al., 2011). Poultry meat, particularly ground turkey contaminated with *Salmonella*, has been associated from the farm to the processing plant (Arsenault et al., 2007; Loharikar et al., 2012). These outbreaks emphasize the necessity and efforts on decreasing and preventing human illness associated with live poultry contact through comprehensive interventions from "farm-to-fork" levels.

In current turkey processing, the organ or anatomical source of *Salmonella* contamination in products, especially ground turkey, is largely unreported. Hence, in a recent study, we evaluated the fate and organ distribution of *Salmonella enterica* serovar Reading in turkeys for up to three weeks confirming that *Salmonella* Reading is an emerging problem for the turkey industry (Ashcraft et al., 2021). The first report of *Salmonella* Reading in commercial turkeys in the USA was published in 1956 (Mitrovic, 1956). Even though *Salmonella* Reading is a serotype that is uncommonly associated with human illness, during 2018–2019, four recalls of turkey meat were declared in 42 states in the USA reported three hundred and fifty-six *Salmonella* Reading linked ground turkey meat (Hassan et al., 2019). In combination with previous works completed by our laboratory, the results of this work indicate that the turkey upper respiratory tract (Figure 1) may play a much significant role in *Salmonella* contamination during processing than previously known (Kallapura et al., 2014a). Laboratory and commercial trials have confirmed the respiratory route as a critical route for *Salmonella* spp. infection in chickens and turkeys (Kallapura et al., 2014b,c).

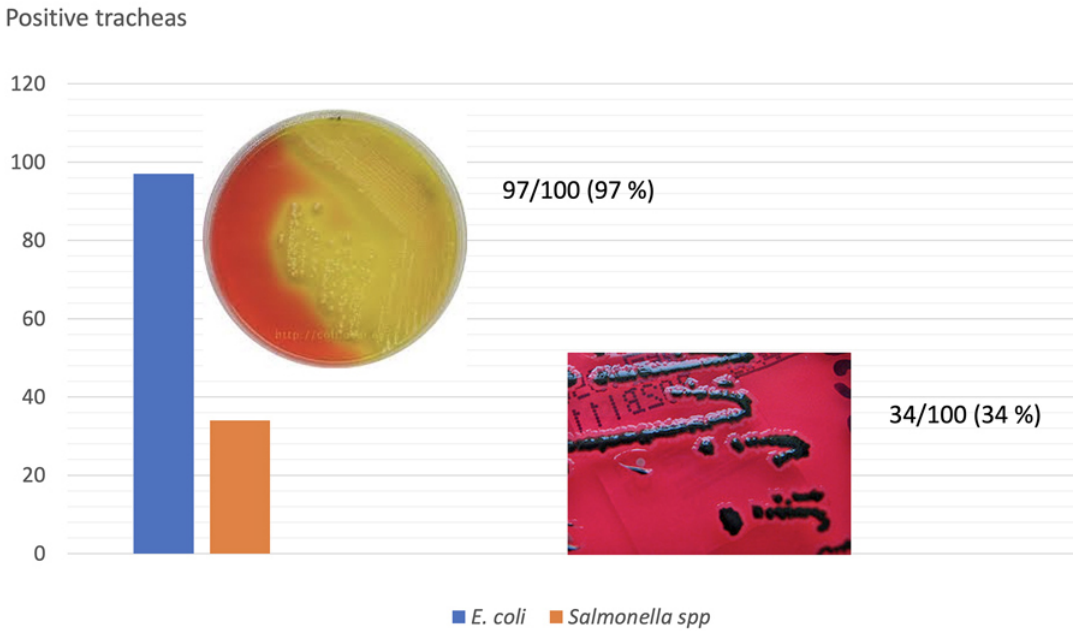

Figure 1: Evaluation of *Salmonella* spp. prevalence in trachea samples from commercially processed turkeys (Kallapura et al., 2014a).
Trachea and ceca were aseptically removed from 16-week-old commercial turkeys at the processing plant (n=100). After aseptic removal from the birds, tracheas were clamped on each end, and 20 mL of peptone water added for 8 hours of incubation at 37°C. The peptone water from each trachea was collected and enriched with an equal volume of 2X tetrathionate broth for overnight incubation. Samples were then streaked onto XLD-4 agar.

Salmonella incidence, virulence, under-cooking, food consumption behavior, and host resistance play important roles for this critical foodborne pathogen, as has been described by (Oscar, 2021). Through eating, direct contact, and via the environment, the human and the animal bacterial flora are in contact with each other. The foodborne route is probably the most important gateway for this contact. Most infections with enteric zoonotic bacterial pathogens, such as *Salmonella enterica* occurs through this route. Hence, food becomes an important essential vehicle for this zoonotic pathogen. In recent years, one of the major issues in food safety has been the lack of cross-sectoral cooperation among the food production chain, food control, human health sector, restaurants, and school cafeterias. The term "farm-to-fork" is a social movement that promotes serving local food at restaurants and school cafeterias with attitudes about food safety, including the food production chain. Therefore, science and education programs are necessary to reduce this zoonotic pathogen at relevant points of the "farm-to-fork" food production chain.

Holistic control methods for salmonellosis in turkeys

While antibiotics have saved millions of human and animal lives and have been used for decades as growth promoters, several studies have revealed that turkeys are a critical reservoir for multidrug-resistance (MDR) *Salmonella* strains in several countries around the world, reinforcing the need to develop alternative strategies to reduce the development of antimicrobial resistance (Poppe et al., 1995; Beier et al., 2011; Choudhary et al., 2018; Yeh et al., 2018). Turkeys and chickens are highly vulnerable to *Salmonella* infection; therefore, decreasing the incidence of *Salmonella* before processing is vital. *Salmonella enterica* serovars remain significant foodborne pathogens due to the remarkable ability of this bacteria to infect all forms of life (Hernández-Reyes and Schikora, 2013), as indicated in Figure 2. Moreover, scientific, consumers, and global commercial strategic positions force producers to find alternatives to antibiotic growth promoters.

Since 2017, *Salmonella enterica* serovar Heidelberg has been the most frequently reported Salmonella from turkey ground meat in the USA, and a substantial number of the isolates are resistant to several antimicrobial agents (Nair et al., 2017; Nisar et al., 2017). In 2011, the Centers for Disease Control and Prevention identified a multistate cluster of *Salmonella* Heidelberg infections and two multidrug-resistant isolates from raw ground turkey retail samples (Routh et al., 2015). The outbreak resulted in the largest recall of fresh ground meat in the USA, involving 36 million pounds of turkey meat (Bearson et al., 2017).

Clearly, *Salmonella* has established a commensal-like condition in livestock and poultry, contributing to the asymptomatic carrier status of the human foodborne pathogen in our animal food supply. This is a critical situation if we consider that acquisition of resistance plasmids to ceftiofur from *Salmonella* isolates from animals, including poultry, have been re-

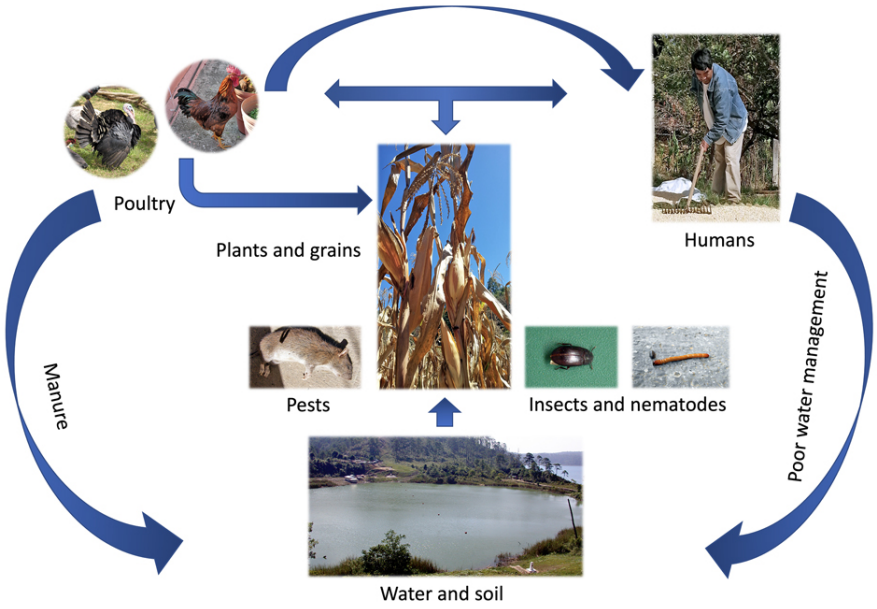

Figure 2: *Salmonella* cross-kingdom infection.

ported. Hence, in recent years, this reality has become a public, scientific, and health concern considering that third-generation cephalosporins are the antimicrobials of choice for treatment of salmonellae infections in humans (Frye and Fedorka-Cray, 2007).

Figure 3 shows the infection routes of *Salmonella* in turkeys. Under this scenario, several control methods must be implemented to reduce the number of reproductive cases. Several studies have shown promising results using *Salmonella* spp. vaccines in turkeys (Zhang-Barber et al., 1999; Penha Filho et al., 2012; Hesse et al., 2017, 2018). The *Salmonella* Typhimurium BBS 866 vaccine strain has demonstrated cross-protection against diverse *Salmonella* serovars, not only in poultry but also in mammals (Hesse et al., 2016).

University of Arkansas research

Agriculture represents over 60% of the economy of the state of Arkansas, and poultry plays a critical role in this sector. As part of the Center of Excellence in Poultry Science, the John Kirkpatrick Skeeles Poultry Health Laboratory has the mission to listen the demands and health problems of the poultry companies and to develop applied research programs to solve those issues. As a result, over the last 21 years, our laboratory has developed and evaluated several probiotics, prebiotics, organic acids, and vaccines on commercial turkey farms with a high incidence of *Salmonella* spp. Those studies were part of the master thesis and Ph.D. dissertations that were published at the University of Arkansas. More recently, we have also been evaluating phytogenic compounds. A summary of these experiences is described below.

In three commercial trials, the effects of selected probiotic bacteria or antibiotics on the performance of poults within idiopathic diarrhea and stunting was evaluated. Turkey poults that received antibiotics followed by a probiotic culture showed a significant improvement in body weight gain than non-treated probiotic control poults (Higgins et al., 2005).

Following this trial, two probiotic cultures combined with a formulation of a commercial organic acid (OA) product were tested in turkey houses that had a historically high incidence of *Salmonella* spp. Two weeks after treatment, environmental *Salmonella* recovery was reduced up to 100%, suggesting that the use of selected probiotic cultures in combination with OA is a reliable and effective method to reduce environmental *Salmonella* in turkey houses before processing (Jarquin et al., 2007; Vicente et al., 2007). These results were further confirmed in another field trial (Vicente et al., 2008).

The University of Arkansas then licensed the technology, and the lactic acid bacteria probiotic culture (FloraMax B-11) was tested alone or in combination with lactose as a prebiotic in commercial turkey houses during the brooding and grow-out phases. Results showed a remarkable increase in body weight gain during the starter phase that was maintained or enhanced during the grow-out phase (Torres-Rodriguez et al., 2007b). These results encouraged the turkey company to confirm and extend the trials to 118 commercial turkey houses, ranging from 1,542 to 30,390 hens per lot, with Nicholas or Hybrid genetic lines. The overall results indicated that the probiotic culture increased market bodyweight, showing a significant cost-benefit ratio (Torres-Rodriguez et al., 2007a).

In more recent studies with other lactic acid bacteria probiotic candidates, we have shown significant improvement in transepithelial electrical resistance *in vitro* and intestinal colonization of *Salmonella* Typhimurium in neonatal turkey poults (Yang et al., 2018) and *Salmonella* Enteritidis (Arreguin-Nava et al., 2019).

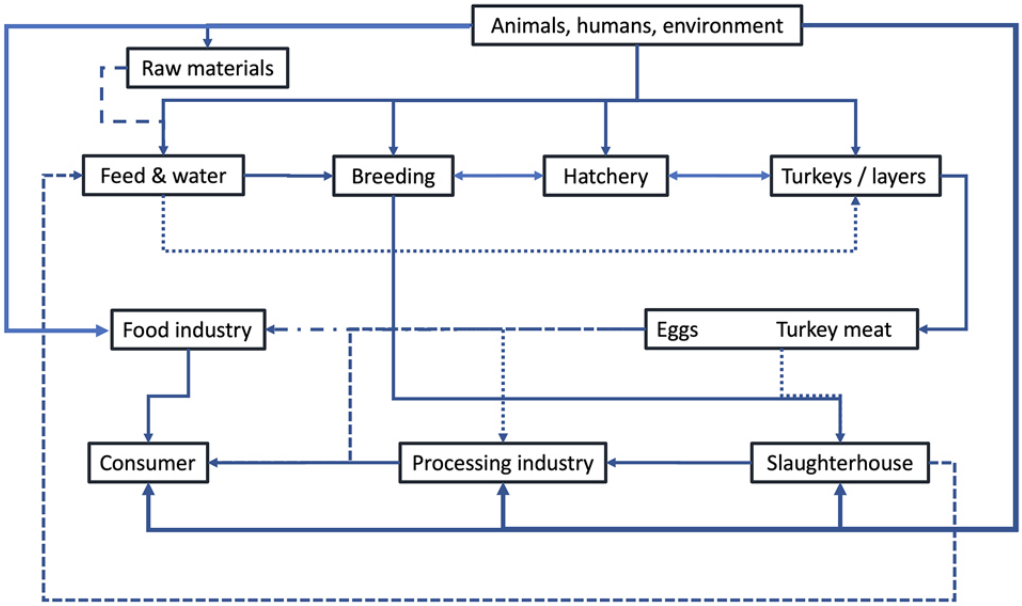

Figure 3: Infection routes of *Salmonella* in turkeys.

Phytogenic compounds

Phytogenic compounds and organic acids have also been extensively studied for their antioxidant, anti-inflammatory, and immune-modulating properties against *Salmonella* spp. (Friedman et al., 2002, 2003, 2004; Bajpai et al., 2012). Our laboratory has also confirmed the benefits of these natural compounds confirming that they are safe and serve as holistic alternatives to antibiotics growth promoters in poultry (Hernandez-Patlan et al., 2018a,b, 2019; Leyva-Diaz et al., 2021).

Vaccines

Our laboratory developed a recombinant oral vaccine using the conserved *fli*C that protects animals against multiple *Salmonella* serotypes. This vaccine was evaluated in three independent commercial turkey operations and has induced humoral response and reduction to *Salmonella* spp. infection (Wolfenden et al., 2010; Kremer et al., 2011).

Conclusions

No single "silver bullet" has been identified, which can be ubiquitously applied commercially to eliminate *Salmonella* from commercial poultry. Successful poultry companies that have eliminated AGP and have been able to reduce the incidence of *Salmonella* from their operations have used several nutraceuticals and vaccines simultaneously, in combination with strict biosecurity programs and genetic sources free of *Salmonella* spp. and *Mycoplasma* spp. with outstanding results and profitability.

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Conflict of Interest.The authors declare no conflict of interest.

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