

POULTRY

FROM EGG TO CARCASS: TRACKING THE ENTRY OF POULTRY FOODBORNE PATHOGENS INTO THE FOOD CHAIN (J. J. Maurer, D. Cole, C. Hofacre, and M. P. Doyle)

The federal government has invested significant resources in diagnosis and prevention of foodborne disease as well as food inspection and compliance with slaughter and processing plant sanitation procedures intended to reduce pathogen levels in food. However, these surveillance networks do not address preharvest food safety and therefore a major approach to *Salmonella* control is neglected. The long-term goal of the work proposed in this application is the reduction of *Salmonella* contamination levels in poultry so as to improve the safety of poultry intended for human consumption. The central hypothesis of this proposal is that vertical transmission through the poultry production pyramid is a significant contributor to carcass contamination by *Salmonella*. By collaborating with the poultry industry, we propose to develop an epidemiological database containing the prevalent strains of *Salmonella* present in parental breeder poultry flocks in order to quantitate the risk factors that contribute to the transmission of these strains to meat birds. To accomplish these goals and to further the goals of the United States Department of Agriculture, our multi-disciplinary epidemiology, diagnostic and research group offer the following aims to: 1) develop an epidemiologic model of *Salmonella* transmission through integrated poultry companies from pedigree chickens down to the meat processing plant and finished, poultry product; 2) establish a molecular database of *Salmonella* strains present in the parental poultry flocks and their progeny in order to quantitate vertical transmission of specific serotypes and pathotypes through the poultry production pyramid; and 3) develop a stochastic quantitative risk assessment (QRA) model of foodborne pathogens in processor-packaged poultry products.

CONTAMINATION OF COMMERCIAL BROILER BREEDER ROOSTERS BY CAMPYLOBACTER, SALMONELLA, AND CLOSTRIDIUM PERFRINGENS (N. A. Cox, C. L. Hofacre, R. J. Buhr, J. L. Wilson, J. S. Bailey, L. J. Richardson, D. E. Cosby, M. T. Musgrove, K. L. Hiatt, and S. M. Russell)

The present study was conducted to determine if several foodborne pathogens (*Campylobacter*, *Salmonella*, and *Clostridium perfringens*) could be isolated from the ductus deferens, testes, and ceca of 45- to 65-wk old commercial broiler breeder roosters. Aseptic necropsy was performed on 15 roosters (five roosters from 3 separate commercial breeder farms) to remove the ductus deferens, testes, and ceca without surface contamination from blood and other tissues. None of the foodborne pathogens were isolated from the testes of the roosters. In the ductus deferens, *C. perfringens* was isolated from 1 of the 15 roosters, whereas no *Campylobacter* or *Salmonella* was isolated from this tissue. *Campylobacter* was cultured from the ceca of all 15 roosters, *C. perfringens* was isolated from 14 of 15 roosters, and *Salmonella* from 2 of 15 roosters. These data suggest that the contamination of semen by these foodborne pathogens is via fecal or cecal contamination as the semen passes through the cloaca and not from bacterial colonization of the testes and ductus deferens.

INTESTINAL COMMUNITY STRUCTURE OF CHICKENS IN RESPONSE TO ORALLY ADMINISTERED TETRACYCLINE (A. S. Fairchild, J. L. Smith, U. Idris, J. Lu, S. Sanchez, L. B. Purvis, C. Hofacre, and M. D. Lee)

Tetracyclines are common therapeutic antibiotics used in poultry production. This study sought to evaluate the effects of oral administration of tetracyclines on the resistance of poultry commensal bacteria and the intestinal bacterial community structure. The diversity indices calculated from terminal restriction

fragment length polymorphism analysis of 16S rRNA amplicons did not indicate significant changes in the cecal bacterial community in response to oxytetracycline. *Enterococcus* spp. and *E. coli* expressed tetracycline MICs of $>8 \mu\text{g/ml}$ and harbored a variety of *tet* resistance determinants regardless of the tetracycline exposure history of the birds. The enterococcal isolates possessed *tetM* (61%), *tetL* (25.4%), and *tetK* (1.3%), as well as *tetO* (52.5%), the determinant known to confer a tetracycline resistance phenotype in *Campylobacter jejuni*. *E. coli* isolates harbored *tetA* (32.2%) or *tetB* (30.5%). Tetracycline MICs remained at $< 2 \mu\text{g/ml}$ for *Campylobacter* isolates before and after tetracycline treatment of the chickens, even though isolates expressing MICs of $>16 \mu\text{g/ml}$ were commonly cultured from flocks that did not receive oxytetracycline. The results imply that complex ecological and genetic factors contribute to the prevalence of antibiotic resistance arising from resistance gene transfer in the production environment.

RESIDENT POPULATIONS OF ENTEROCOCCI ON POULTRY FARMS IN RESPONSE TO ANTIMICROBIAL USAGE

(A. L. Debnam, C. R. Jackson, G. E. Avellaneda, J. B. Barrett, and C. L. Hofacre)

Enterococci isolated from four poultry houses during six grow-outs was determined. In two houses, flavomycin, virginiamycin, and bacitracin were used during different poultry grow-outs, whereas the other two houses did not use any antimicrobials. Of the nine species of *Enterococcus* isolated (*Enterococcus faecalis*, *E. faecium*, *E. avium*, *E. casseliflavus*, *E. cecorum*, *E. durans*, *E. gallinarium*, *E. hirae*, and *E. malodoratus*), *E. faecalis* was isolated most frequently from chick boxliners and carcass rinses whereas *E. faecium* was the most frequent isolate in litter and feed. *E. faecalis* and *E. faecium* was isolated most often from the farm and houses, regardless of antimicrobial treatment, indicating that antimicrobial usage had no effect on the resident population of enterococci.

CONTAMINATION OF INTERNAL ORGANS BY *CAMPYLOBACTER JEJUNI* FOLLOWING ORAL OR INTRACLOACAL INOCULATIONS OF BROILER CHICKS

(N. A. Cox, C. L. Hofacre, J. S. Bailey, R. J. Buhr, J. L. Wilson, K. L. Hiatt,
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Campylobacter jejuni was administered to day-old chicks ($n = 30$) through oral and intracloacal inoculations. After inoculation, broilers were aseptically opened and internal organs (thymus, spleen, liver/gallbladder, bursa of Fabricius, and ceca) individually analyzed for *C. jejuni*. Overall, *C. jejuni* was isolated after oral inoculation from 13%, 17%, and 28% of the 1-h, 1-day, and 1-wk samples, respectively. Following the intracloacal route of inoculation, *C. jejuni* was recovered from 32%, 8%, and 16% of the 1-h, 1-day, and 1-wk samples, respectively. *C. jejuni* was isolated from 10%, 8%, 19%, 25% and 40% of the thymus, spleen, liver/gallbladder, bursa of Fabricius, and ceca samples, respectively. The rapid movement of *Campylobacter* to internal organs following both oral and intracloacal inoculation may be significant, particularly if it persists in these organs as reservoirs throughout the 65-wk life cycle of breeding birds.

INHIBITORY EFFECT OF OXALIC ACID ON BACTERIAL SPOILAGE OF RAW, CHILLED CHICKEN

(D. M. Anang, G. Rusul, S. Radu, J. Bakar, and L. R. Beuchat)

Processing of poultry consists of several steps, some of which can result in increased microbial counts, leading to a reduction in shelf life and increased safety risk. Opportunities for microbial cross-contamination exist during transport of birds from the farm to the processing plant and during processing and packaging. An increase in the number of microorganisms on the surface of carcasses can occur during removal of feathers, evisceration, washing, and chilling. Contamination from aerosols generated

in the processing environment and from process water, ice, equipment, and the hands of workers can also occur. The use of organic acids and their salts for surface decontamination and extension of shelf life of poultry and beef has been investigated. Lactic acid and acetic acid also have been successfully used to reduce microbial populations on poultry. Other sanitizers evaluated have included lauric, palmitic, myristic, palmitoleic, stearic, oleic, linoleic, and linolenic acids, trisodium phosphate, potassium sorbate, and electrolyzed water but the use of oxalic acid to reduce populations of microorganisms on raw poultry has not been described. Oxalic acid occurs naturally in many fruits and vegetables and the human body synthesizes oxalic acid from ascorbic acid. Vegetables and herbs containing highest amounts of oxalic acid include parsley (1.70%, dry weight basis), chives (1.48%), spinach (0.48%), beet leaves (0.61%), carrots (0.50%), and radish (0.48%). Commercial soy foods contain up to 2.06 mg of oxalate/g. Eaten in large amounts, oxalic acid may combine with calcium and other minerals to form less soluble oxalates. The predicted LD₅₀ in rats is 375 mg/kg. Extrapolating from this dose, for a person weighing 150 lb (68.1 kg), consumption of 25.5 g of oxalic acid would be required for an LD₅₀, although smaller amounts may cause illness.

We did a study to evaluate oxalic acid for its effectiveness in killing microflora on the surface of raw chicken breasts, to identify the predominant bacteria that survive on chicken treated with oxalic acid, to evaluate the inhibitory effects of oxalic acid on growth of predominant spoilage microorganisms on treated breasts, and to determine the effects of treatment with oxalic acid on the color of breasts during subsequent storage at 4°C. Raw chicken breasts were dipped in solutions of oxalic acid (0, 0.5, 1.0, 1.5 and 2.0% [w/v]) for 10, 20, and 30 min, individually packed in oxygen-permeable polyethylene bags, and stored at 4°C. Total plate counts (TPC) and populations of *Pseudomonas* spp. and Enterobacteriaceae on breasts were determined before treatment and after storage for 1, 3, 7, 10, and 14 days. pH and Hunter L, a, and b values of the breast surface were measured. The TPC were ca. 1.5 and 4.0 log₁₀ CFU/g higher on untreated chicken breasts after storage for 7 and 14 days, respectively, compared to TPC on breasts treated with 0.5% oxalic acid, regardless of dip time. Differences in counts on chicken breasts treated with water and 1.0 - 2.0% of oxalic acid were greater. Populations of *Pseudomonas* spp. on chicken breasts treated with 0.5 - 2.0% oxalic acid and stored at 4°C for 1 day were less than 2 log₁₀ CFU/g (detection limit), compared to 5.14 log₁₀ CFU/g of untreated breasts. *Pseudomonas* grew on chicken breasts treated with 0.5% oxalic acid to reach counts not exceeding 3.88 log₁₀ CFU/g after storage for 14 days. Counts on untreated chicken exceeded 8.83 log₁₀ CFU/g at 14 days. Treatment with oxalic acid caused similar reductions in Enterobacteriaceae counts. *Kocuria rhizophila* was the predominant bacterium isolated from treated chicken. Other prominent bacteria included *Escherichia coli* and *Empedobacter brevis*. Treatment with oxalic acid caused a slight darkening in color (decreased Hunter L value), retention of redness (increased Hunter a value), and increase in yellowness (increased Hunter b value). Results show that oxalic acid has potential for use as a sanitizer to reduce populations of spoilage microorganisms naturally occurring on raw chicken, thereby extending the shelf life.