DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500, MSC 6910
Bethesda, Maryland 20892-6910
Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

FOR EXPRESS MAIL:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301)-480-3387

May 6, 2021

Re: Animal Welfare Assurance
#A3331-01 (OLAW Case N)

Ms. Sherrie Settle
Acting Executive Director of Sponsored Programs
and Regulatory Compliance Services
North Carolina State University
2601 Wolf Village Way - (b) (4)
Raleigh, NC 27607

Dear Ms. Settle,

The Office of Laboratory Animal Welfare (OLAW) has received your April 30, 2021 letter responding to our request for information regarding an anonymous allegation of possible non-compliances with the PHS Policy on Humane Care and Use of Laboratory Animals at North Carolina State University. It is understood that you have completed an internal investigation and determined that the relevant IACUC protocols and safety approvals were in place for the work conducted and that there was no evidence to suggest that improper practices occurred which would substantiate these allegations. It is further understood that the exact same allegations were submitted anonymously to the NC State Institutional Biosafety Committee on January 22, 2019 and were investigated by the Committee and Environmental Health and Safety at that time, with the same result.

Regarding the specific allegations, it is understood that: no recombinant organisms were approved or used on this project or at the site, as alleged; there are no protocols at the location currently approved for the use of the agents listed in the manuscript, and; your biosafety committee and environmental health and safety team work closely with investigators at all locations (farms and research facilities) where animals are used for teaching, testing, or research, and biosafety approval is a requirement before any IACUC proposal is approved that includes the use of biological agents.

OLAW appreciates the prompt consideration of these matters by North Carolina State University. We especially want to recognize your part in providing transparency between your Office and OLAW. We appreciate your cooperation as Institutional Official regarding this matter in particular and find no cause for further action by this office.

Sincerely,

Brent C. Morse -S Digitally signed by Brent C. Morse -S
Date: 2021.05.06 15:26:44 -04'00'

Brent C. Morse, DVM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC contact

NC STATE UNIVERSITY

Office of Research & Innovation
Sponsored Programs & Regulatory Compliance Services

research.ncsu.edu/sparcs

Campus Box 7514
Raleigh, NC 27695-7514
P: 919.515.2444

April 30, 2021

Brent C. Morse, DVM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare

Re: Animal Welfare Assurance #A3331-01 (OLAW Case N)

Dear Dr. Morse,

The NC State IACUC committee met on April 15, 2021, and reviewed the allegations based on the paper published in the Journal of Applied Poultry Research - volume 25:591-609; 2016.

The IACUC Committee, Environmental Health and Safety personnel, and the Institutional Biosafety Committee Chair discussed the allegations and determined that the relevant IACUC protocols and safety approvals were in place for the work conducted and that there was no evidence to suggest that improper practices occurred which would substantiate these allegations.

It should be noted that the exact same allegations were submitted anonymously to the NC State Institutional Biosafety Committee on Jan 22, 2019, and were investigated by the Committee and Environmental Health and Safety at that time, with the same result.

Regarding the specific allegations:

"It is clear that the authors were inoculating antibiotic resistant organisms into a Poultry House without proper containment because they successfully isolated the organisms from pests found inside and outside the Poultry House.

Releasing recombinant organisms is against the national institute of health guidelines".

The IACUC Committee, Environmental Health and Safety Personnel, and the IBC Chair determined that the project had been appropriately submitted by the PI, reviewed by the IBC, inspected by EHS, and approved at Animal Biosafety Level 2 (ABSL-2) containment prior to commencing in 2011. No recombinant organisms were approved or used on this project or at the site, as alleged.

"I understand similar work is ongoing at the NC State Poultry Farm, and it is ongoing with the full support of the institution. How can organisms legally be released into an uncontained facility, and released to the environment?"

In reviewing the approved IACUC protocols for this facility, there are no protocols at the location currently approved for the use of the agents listed in the manuscript. The facility was last inspected on March 8, 2021, by the IACUC with no deficiencies noted.

"Presently, on campus, researchers are held to an exceedingly high standard at NC State; yet it appears that those at the farm can endanger the public health by releasing antibiotic resistant organisms not only into the poultry house; but also to the surrounding environment.

Why is the farm held to a different standard than laboratory research?"

NC STATE UNIVERSITY

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Our biosafety committee and environmental health and safety team work closely with investigators at all locations (farms and research facilities) where animals are used for teaching, testing, or research, and biosafety approval is a requirement before any IACUC proposal is approved that includes the use of biological agents.

Thank you for the opportunity to review and respond to this allegation. Please let us know if you require further information.

Sincerely,

(b) (6) Digitally signed by Sherrie Settle
Date: 2021.04.30 15:00:03 -04'00'

Sherrie Settle
Acting Executive Director of Sponsored Programs and Regulatory Compliance Services
North Carolina State University

DEPARTMENT OF HEALTH & HUMAN SERVICES

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Telephone: (301) 496-7163
Facsimile: (301)-480-3387

April 8, 2021

Re: Animal Welfare Assurance
#A3331-01 (OLAW Case N)

Ms. Sherrie Settle
Acting Executive Director of Sponsored Programs
and Regulatory Compliance Services
North Carolina State University
2601 Wolf Village Way - (b) (4)
Raleigh, NC 27607

Dear Ms. Settle,

The Office of Laboratory Animal Welfare (OLAW) has become aware of an anonymous allegation of possible non-compliances with the PHS Policy on Humane Care and Use of Laboratory Animals at North Carolina State University. Below is an unedited transcript of the allegation:

"Attached is a manuscript from work performed in the Prestage Department of Poultry Science at North Carolina State University

It is clear that the authors were inoculating antibiotic resistant organisms into a Poultry House without proper containment because the successfully isolated the organisms from pests found inside and outside the Poultry House.

Releasing recombinant organisms is against national institute of health guidelines.

It is not clear whether the strains were recombinant. It is clear that the strains were antibiotic resistant; which is a major human health concern, and releasing antibiotic resistant bacteria into the environment cannot be a good thing for the public health. However, the genes the organisms exhibit resistance make it highly likely they are recombinant.

I understand similar work is ongoing at the NC State Poultry Farm, and it is ongoing with the full support of the institution. How can organisms legally be released into an uncontained facility, and released to the environment?

Presently, on campus, researchers are held to an exceedingly high standard at NC State; yet it appears that those at the farm can endanger the public health by releasing antibiotic resistant organisms not only into the poultry house; but also to the surrounding environment. Why?

Why is the farm held to a different standard then laboratory research?"

The referenced manuscript is enclosed.

Please instruct the IACUC, avoiding any conflict of interest, to investigate this concern and if substantiated but not reported please state why and provide further information regarding the incident and all corrective/preventive actions.

We appreciate your cooperation and ask that you please provide the requested information **by May 28, 2021**. Please contact me if I can be of assistance.

Sincerely,

Brent C. Morse -S Digitally signed by Brent C. Morse -S
Date: 2021.04.08 14:20:19 -04'00'

Brent C. Morse, DVM
Director
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC contact
Encl

A3331 - N

JAN 2 4 2019

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It is clear that the authors were inoculating antibiotic resistant organisms into a Poultry House without proper containment because the successfully isolated the organisms from pests found inside and outside the Poultry House.

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Why is the farm held to a different standard then laboratory research?

Walker, Keri (NIH/OD) [E]

From: Morse, Brent (NIH/OD) [E]
Sent: Thursday, March 18, 2021 10:45 AM
To: Walker, Keri (NIH/OD) [E]
Subject: FW: DPI Case #2019-006— Prestage Department of Poultry Science at North Carolina State University
Attachments: Allegation #2019-006.pdf

Follow Up Flag: Follow up
Flag Status: Flagged

Keri,

Please open a case under A3331 using this email and attached file. Please assign it to me. Thank you. Brent

Brent C. Morse, DVM, DACLAM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Sent: Wednesday, March 17, 2021 1:31 PM
To: Peyton, Roderick (NIH/OD) [E] <roderick.peyton@nih.gov>
Cc: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>
Subject: FW: DPI Case #2019-006— Prestage Department of Poultry Science at North Carolina State University

Dear Mr. Peyton,

This is to acknowledge receipt of the allegation. The OLAW Division of Compliance Oversight will review and take appropriate action.

Sincerely,

Patricia Brown, VMD, MS
Director, Office of Laboratory Animal Welfare,
Office of Extramural Research, Office of the Director, NIH
301-451-4209, brownp@mail.nih.gov

From: Peyton, Roderick (NIH/OD) [E] <roderick.peyton@nih.gov>
Sent: Wednesday, March 17, 2021 1:08 PM
To: Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Subject: DPI Case #2019-006— Prestage Department of Poultry Science at North Carolina State University

Good afternoon,

The Division of Program Integrity in the Office of Management Assessment (OMA) at the National Institutes of Health (NIH) has received the attached allegation. In accordance with NIH Manual Chapter 1754, this allegation is outside of our purview. Therefore, we are referring this case to your office. OMA is not responsible for reporting your actions to another office; therefore, we do not need to receive a report. We are closing this case and plan to take no further action.

In accordance with the Privacy Act, all parties must maintain the confidentiality of this matter. Within the agency, information pertaining to this case may be shared only on a need-to-know basis. If you have any questions, please call me at 301-827-7962.

Thank you,

Roderick Peyton
Auditor/Analyst
Division of Program Integrity
(NIH/OD) [E]
Office 301-827-7962
Roderick.Peyton@NIH.gov
National Institutes of Health

Routes of transmission of *Salmonella* and *Campylobacter* in breeder turkeys

M. D. Crespo,* S. Kathariou,† J. L. Grimes,* N. A. Cox,‡ R. J. Buhr,‡ J. G. Frye,‡ W. G. Miller,§ C. R. Jackson,‡ and D. P. Smith*,[1]

**Prestage Department of Poultry Science North Carolina State University 2711 Founders Drive, Raleigh 27695; †Department of Food, Bioprocessing, and Nutrition Sciences North Carolina State University 400 Dan Allen Drive, Raleigh 27695; ‡USDA-ARS, Russell Research Center 950 College Station Road, Athens, GA 30605; and §USDA-ARS, Western Regional Research Center 800 Buchanan Street, Albany, CA 94710*

Primary Audience: Researchers, Flock Supervisors, Quality Assurance and Laboratory Personnel, Veterinarians

SUMMARY

Salmonella and *Campylobacter* are frequent colonizers of the intestinal tracts of poultry and have often been associated with human foodborne illness. The entry, transmission, and prevalence of both pathogens have been extensively studied in chickens but little information is available for turkeys. This project monitored turkey breeder hens and toms from d of hatch to 65 wk of age with the objective of determining routes of transmission for *Salmonella* and *Campylobacter* throughout the turkey production cycle. Breeder poults were separated by sex and then into 2 groups (control and inoculated) for each sex. The inoculated group was orally gavaged with marker strains of both *Salmonella* and *Campylobacter*. The inoculated groups (toms and hens) were placed on the opposite side of a growout house from the uninoculated groups. Fecal samples, intestinal samples and organs, feed, drinkers, and potential vectors such as insects and mice, were analyzed at different times until 65 wk. Monitoring showed that *Campylobacter* spread rapidly and cross-contaminated turkeys throughout the growout house. For both *Salmonella* and *Campylobacter*, naturally occurring strains that were first isolated in control groups at wk 3 and 4, respectively, outcompeted marker strains several wk post inoculation and persisted in the flock. The most common naturally occurring strains were *C. jejuni* (tetracycline resistant), *C. coli* (kanamycin resistant), and *S.* Agona. *Campylobacter* and *Salmonella* also were isolated from flies and from a mouse, confirming the importance of proper pest control and biosecurity to reduce the spread of the bacteria.

Key words: *Salmonella*, *Campylobacter*, breeder turkeys, routes of transmission

2016 J. Appl. Poult. Res. 25:591–609
http://dx.doi.org/10.3382/japr/pfw035

DESCRIPTION OF PROBLEM

Salmonella and *Campylobacter* are foodborne zoonotic pathogens of high public health relevance worldwide, both ranking among the top 5 pathogens contributing to foodborne disease in the United States [1–3]. Frequent colonization of intestinal tracts of poultry with these bacteria makes poultry meat an important vehicle for infection [1, 2, 4]. Furthermore,

[1]Corresponding author: dsmith@diamondv.com

pre-harvest colonization of the flock by these pathogens has been found to be associated with contamination of poultry carcasses during processing [5–13]. Since 2011, USDA Food Safety and Inspection Service (**FSIS**) regulations require turkey processing companies not to exceed certain levels of *Salmonella* and *Campylobacter* in raw products, as monitored by testing programs [14]. A number of methods, including chemical antimicrobial interventions, have been used to control these pathogens at the processing plant. However, reducing or eliminating both pathogens from birds prior to processing is potentially more beneficial than excessive plant interventions. Further understanding about entry, transmission, and overall prevalence of both pathogens in the production chain may help to determine risk factors and lead to methods for prevention and/or reduction of pathogenic bacteria colonizing poultry. Many different routes of transmission for these pathogens into the flock such as vertical transmission, pests, wild animals, feed and water, farm workers, and environment have been previously considered and investigated, especially in chickens [15–20]. However, there is little information regarding turkey flocks. The objective of this project was to determine routes of transmission for *Salmonella* and *Campylobacter* throughout turkey production and processing. The focus of the current paper was horizontal transmission while vertical transmission will be evaluated in a different manuscript.

MATERIALS AND METHODS

Inoculation and Monitoring

This study was approved by the NCSU Institutional Animal Care and Use Committee (IACUC). A flock of 140 Nicholas turkey breeder poults [21] were placed in a growout house of the Turkey Research Unit at North Carolina State University, Raleigh [22]. Prior to beginning the project, the house was spray-disinfected [23], and environmental samples, including drinkers and feed, were analyzed for the presence of *Salmonella* and *Campylobacter*. At arrival, artificial straw from the transportation boxes that contained feces and fecal samples during the first wk of life were analyzed for both bacteria.

Poults were placed into pens separated by sex and then separated further into 2 groups: Inoculated (82 hens and 22 toms) and control (28 hens and 8 toms) (Figure 1). Inoculated and control groups were separated by plastic curtains; a tray containing quaternary ammonium disinfectant (PI quat 20®) for boot immersion was located at the end of the inoculated side for passing through when leaving this area. Control groups were serviced before inoculated groups to prevent cross-contamination. Moreover, boot covers and other personal protective equipment (gloves, coveralls) were required to enter the pens of inoculated turkeys and were removed when leaving the inoculated side of the house. Inoculated

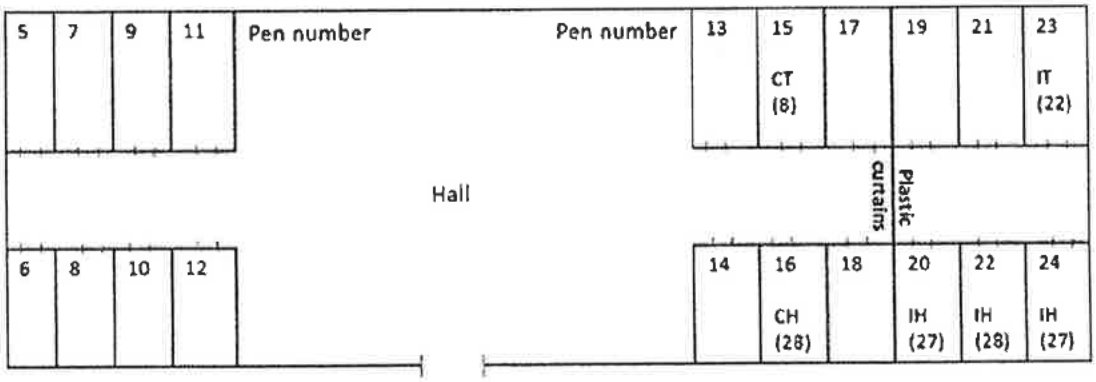

Figure 1. Distribution of pens of control and inoculated turkeys in the growout house, wk 0 to 12. The number of turkeys per pen is indicated in parentheses. CT, control toms; CH, control hens; IT, inoculated toms; IH, inoculated hens.

toms (**IT**) and inoculated hens (**IH**) were orally gavaged at 10 d of age with 0.1 mL of an inoculum containing the *Salmonella* marker strain (approx. 10^7 cfu/mL) and the *Campylobacter* strains (approx. 10^7 cfu/mL) (Table 1). *S. enterica* ser. Enteritidis resistant to nalidixic acid (*S.* Enteritidis NAL^R) and *C. coli* resistant to gentamicin and kanamycin (GK) [24] were administered to IH, while nalidixic acid-resistant *S. enterica* ser. Typhimurium (*S.* Typhimurium NAL^R) and a strain of *C. jejuni* resistant to tetracycline, streptomycin, kanamycin, and quinolones (nalidixic acid and ciprofloxacin) (TSKQ) were administered to IT. *S.* Enteritidis was chosen for inoculating hens due to the higher propensity of this serotype in colonizing the reproductive tract and transmission through shell eggs in layers [25, 26]. Control toms (**CT**) and hens (**CH**) were orally gavaged with the same volume of phosphate-buffered saline (**PBS**) [27] to simulate the same stressors, handling and gavage. Turkeys were fed diets formulated without antibiotics or growth promoters. At wk 12, birds in the same pen were distributed into 2 pens to reduce the number of turkeys per pen. The distribution of pens in the house and number of turkeys per pen is shown in Figure 2. Due to the reduction in number of positive fecal samples for the inoculated marker strains, IH and IT were inoculated again via gavage with 2 mL of an inoculum containing the same strains of bacteria used for the first inoculation but in higher concentration (approx. 10^8 cfu/mL) (Table 1).

At wk 21 both IH and CH hens, were moved to a dark-out house where a step-down lighting program was applied. This house had solid side walls and light traps covering fans and air inlet systems for total light control. Hours of light were controlled by a time switch [28]. Hens received 8 h of light per d for the first five wk (wk 21 to 25), and then the h of light were reduced half an h per wkuntil wk 32, when they received 4.5 h of light per day. IH and CH were in 2 different rooms, but hens of each group coming from different pens in the breeder house (Figure 2) were placed together in a common pen. Thus, for this period of time fecal samples were reduced to 2 pooled samples for CH, and 3 to 4 pooled samples for IH.

Table 1. *Campylobacter* and *Salmonella* strains used for inoculation of turkeys.

Sex of inoculated turkeys	*Campylobacter* strain and antibiotic resistance profile	mL of inoculum gavaged per bird (cfu/mL)		*Salmonella* serotype (Nalidixic acid resistant)	mL of inoculum gavaged per bird (cfu/mL)	
		First inoculation (10 d)	Second inoculation (12 wk)		First inoculation (10 d)	Second inoculation (12 wk)
Toms	*C. jejuni* 10882 TSKQ[1]	0.1 (9.2×10^6)	2 (1.3×10^8)	*S.* Typhimurium NAL^R	0.1 (9.4×10^6)	2 (5.6×10^8)
Hens	*C. coli* 12456 GK[1]	0.1 (1.4×10^7)	2 (4.8×10^8)	*S.* Enteritidis NAL^R	0.1 (1.2×10^7)	2 (3.6×10^8)

[1]T, tetracycline; S, streptomycin; K, kanamycin; Q, (fluoro) quinolones (nalidixic acid and ciprofloxacin); G, gentamicin; NAL, nalidixic acid. Acronyms indicate that the strain was resistant to these specific antibiotics but not to others used in the testing panel. Thus, GK indicates that the strain was resistant to gentamicin and kanamycin but susceptible to tetracycline, streptomycin, erythromycin, nalidixic acid and ciprofloxacin, while TSKQ indicates resistance to tetracycline, streptomycin, kanamycin, nalidixic acid and ciprofloxacin but susceptibility to erythromycin and gentamicin.

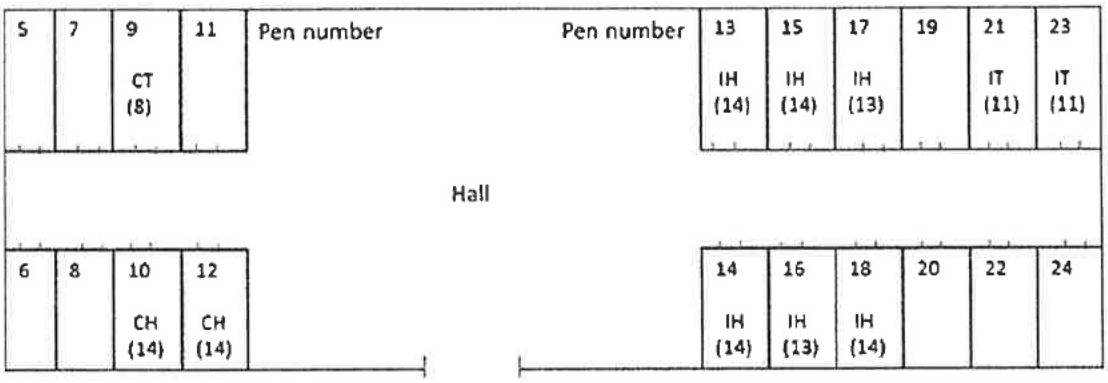

Figure 2. Distribution of pens of control and inoculated turkeys in the growout house after second inoculation (wk 12). In parenthesis is indicated the number of turkeys per pen. CT, control toms; CH, control hens; IT, inoculated toms; IH, inoculated hens.

Inoculum Preparation

S. enterica ser. Enteritidis and Typhimurium, both NAL^R, were grown overnight in brain heart infusion (**BHI**) broth [29] at 37°C in a water bath with agitation. *Campylobacter* strains were initially grown in Mueller-Hinton agar (**MHA**) plates [30] at 42°C/48 h in microaerobic conditions using zip-top bags filled with a gas mixture (5% O_2, 10% CO_2, 85% N) [31], and then transferred into Mueller-Hinton broth (**MHB**) [29] and incubated at 42°C /24 h in microaerobic conditions. To enumerate the number of bacteria in the inoculum, inoculum was serially diluted and plated (100 μL) onto brilliant green sulfa agar plates (**BGS**) [32] for *Salmonella* and on MHA for *Campylobacter*. For the first inoculation (d 10), after overnight growth, both marker inocula were diluted 1:10 in BHI (10^8-10^9 cfu/mL). Then, one mL of each *S.* Enteritidis and *C. coli* inocula was added into 8 mL of PBS (dilution 1:10). The same dilution (1:10) was made for *S.* Typhimurium and *C. jejuni*. For inoculation at 12 wk, after overnight growth, both inocula were diluted 1:2 in BHI. Then, the same volumes of *Salmonella* and *Campylobacter* inocula were mixed.

Fecal Analysis

Pooled fecal droppings from each pen were analyzed weekly until wk 15 (from April 2012 to July 2012), biweekly in July (wk 17 and 19) and August (wk 21 and 23), and in September (wk 27), October (wk 32), December (wk 39) 2012, and May 2013 (wk 61). In wk 19 individual fecal droppings per pen were analyzed for *Campylobacter*, which increased the number of samples analyzed for *Campylobacter* (n = 200) in comparison with *Salmonella* (n = 184). For the last sample analyzed at wk 61, only the hens were available; toms were sacrificed and sampled at wk 50 (March 2013). Fecal droppings from the same pen were collected in a 50 mL centrifuge plastic tube, mixed with a sterile cotton swab, and directly streaked onto Campy Cefex agar (**CCA**) [32] containing gentamicin (200 μg/mL) or nalidixic acid (20 μg/mL), respectively, for the selective identification of marker strains of *Campylobacter* inoculated into the poults. CCA was no longer used after wk 21 due to excessive growth of background microflora, and modified cefoperazone charcoal deoxycholate agar (**mCCDA**) [30] was used instead. Enumeration of *Campylobacter* in fecal droppings was performed for occasional samples (Table 2). For enumeration, one g of feces was suspended in 9 mL of buffered peptone water (**BPW**) and serial dilutions were plated (0.1 mL) onto mCCDA; plates were incubated at 42°C for 48 h under microaerobic conditions. The detection limit was 100 cfu/g. One *Campylobacter* colony per plate was sub-cultured on MHA for purification and further characterization, including antibiotic susceptibility tests and species determination.

For *Salmonella* identification, fecal samples were diluted in BPW in a ratio of 1:10 for a first pre-enrichment step [30]; the sample was stomached for 60 s and incubated at 37°C for

Table 4. Vectors and other environmental samples analyzed during the project.

Sampling month and year	Vectors-environmental sample	*Campylobacter* and/or *Salmonella* detected
Mar 12	Feed (25g), drinkers (2 swabs/drinker/pen), wood shavings	Negative
May 12	Flies (10), feed (25g)	*Salmonella* (flies)
Sep 12	Flies (5), cricket (1), roaches (3)	Negative
Oct 12	Roaches (4), cricket (3), camel crickets (3)	Negative
Oct 12	Flies (1), roaches (7), mouse (1), camel crickets (3)	Negative
Oct 12	Flies (5), roaches (11), spiders (2), feed (25g), drinkers (2 swabs/drinker/pen)	*Campylobacter* (flies)
Feb 13	Feed (25g), drinkers (2 swabs/drinker/pen), wood shavings	Negative
Mar 13	Mouse (1)	*Salmonella*
Jun 13	Flies (5), roaches (5)	Negative
Jun 13	Flies (6), roaches (4)	Negative

Table 5. Minimum inhibitory concentration of antibiotics tested for *Campylobacter* isolates. Isolates that yielded confluent growth at the indicated concentrations were considered resistant.

Antibiotic	Concentration (μg/mL)
Kanamycin[1]	25
Tetracycline[2]	16
Erythromycin[2]	10
Streptomycin[2]	15
Nalidixic Acid[1]	20
Ciprofloxacin[1]	4
Gentamicin[1]	200

[1][28].
[2][78].

their growth in the presence of specific amounts of the indicated antibiotic (Table 5), as described [52]. For gentamicin, the level of resistance of the marker strain *C. coli* was used (200 μg/mL). Plates were spotted in duplicate and examined after 48 h of microaerobic growth on MHA at 42°C; isolates yielding confluent growth in both spots were considered resistant. All isolates were simultaneously also spotted on MHA to ensure viability. *C. jejuni* ATCC 33560 (purchased from the American Type Culture Collection; sensitive to all tested antibiotics) was included each time as quality control strain.

Statistical Analysis

Frequencies of detection observed for both pathogens were reported. Frequencies of detection in ceca and jejuna were compared using 2-sided Fisher's exact test. Fisher's test was performed using JMP 11 software [53]. Significance was defined at $P \leq 0.05$. Clonal relationships of *Campylobacter* isolates based on PFGE banding patterns were calculated using BioNumerics [54].

RESULTS AND DISCUSSION

Monitoring of Salmonella in Fecal Samples

Prior to the placement of the poults in the pens, environmental samples, drinkers, and feed samples analyzed were negative for *Salmonella*, as were samples of artificial straw containing feces from the boxes where the birds were shipped, and fecal samples during the first 2 weeks. However, nalidixic acid-susceptible strains of *Salmonella (S.* Agona*)* were isolated from CH at wk 3 (wk 1 after the first inoculation), and at wk 9 from CT (wk 7 after inoculation). The same serotype (*S.* Agona) was first detected in IH at wk 7 (wk 6 after inoculation). In the IT, nalidixic acid-susceptible *Salmonella* was detected at wk 10 (wk 8 after inoculation); however, this isolate was not subtyped. Table 6 shows the number of positive fecal samples for *Salmonella* per group. A total of 184 fecal samples was analyzed for *Salmonella* from wk 3 to 61. Of those, 102 (55.4%) were positive. Marker strains (nalidixic acid-resistant) were isolated from 45 (44.1%) of the positive samples while the remaining 57 (55.9%) were susceptible to nalidixic acid (NAL^S) and presumed to be naturally occurring strains (Table 6). A subset of 29 representative NAL^S isolates, including isolates from each group and from different dates was selected for subtyping at the Russell Research Center (USDA-ARS, Athens, GA). Serotyping of these 29 isolates revealed

Table 6. Marker and naturally occurring *Salmonella* recovered from fecal samples analyzed from the different groups of turkeys (hens and toms, control and inoculated) from wk 3 (April 2012) to wk 61 (May 2013).

Group[1]	Fecal samples	POS (%)	Marker (% of isolates from positive samples)	Naturally occurring (susceptible to NAL[2]) (% of isolates from positive samples)
CT	20	14 (70)	0	14 (100)
CH	36	21 (58)	0 (0)	21 (100)
IH	96	50 (52)	31 (62)	19 (38)
IT	32	17 (53)	14 (82)	3 (18)
Total	184	102 (55)	45 (44)	57 (56)

[1]CT, control toms; CH, control hens; IH, inoculated hens; IT, inoculated toms.

[2]NAL, nalidixic acid 200 μg/mL.

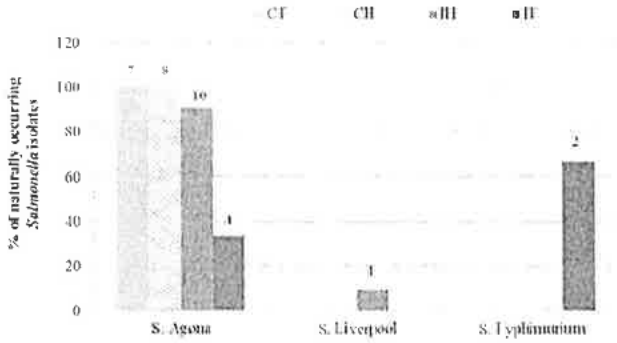

Figure 3. Naturally occurring *Salmonella* serotypes isolated from fecal samples of each group of breeder turkeys. Control toms (CT), control hens (CH), inoculated hens (IH), and inoculated toms (IT). Relative frequencies were calculated from the total number of naturally occurring isolates per group (CT = 7, CH = 8, IH = 11, IT = 3).

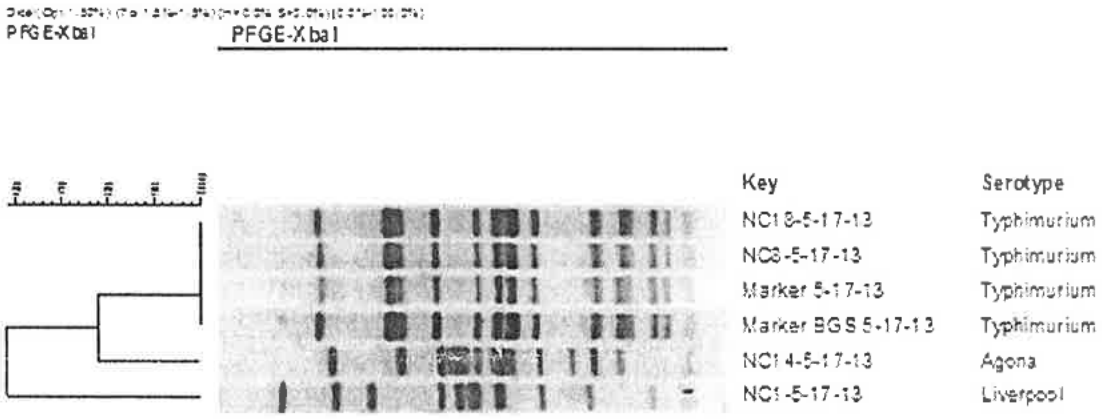

Figure 4. PFGE results showing the band pattern of *S.* Typhimurium marker strains (Marker), the two nalidixic acid sensitive *S.* Typhimurium isolates (NC18 and NC8), both isolated on wk 13 from fecal samples collected in 2 different pens of inoculated toms, and 2 other isolates (*S.* Agona-NC14 and *S.* Liverpool-NC1), isolated from fecal samples collected in 2 different pens of inoculated hens on wk 10. PFGE performed at the Russell Research Center (USDA-ARS), Athens, GA.

that the majority (26/29, 90%) were serotype Agona while 2 (7%) and one (3%) were serotypes Typhimurium and Liverpool, respectively (Figure 3). Further characterization by PFGE revealed that the 2 NAL^S *S.* Typhimurium isolates from IT had the same PFGE profile as the NAL^R marker strain inoculated into the toms (Figure 4), suggesting that they may have been derived from the inoculated marker strain upon loss of nalidixic acid resistance. Although nalidixic acid resistance of *Salmonella* is frequently associated with point mutations in the quinolone resistance

Morse, Brent (NIH/OD) [E]

From: Peyton, Roderick (NIH/OD) [E]
Sent: Thursday, March 25, 2021 9:43 AM
To: Morse, Brent (NIH/OD) [E]
Subject: RE: DPI Case #2019-006— Prestage Department of Poultry Science at North Carolina State University
Attachments: Manuscript.pdf

Follow Up Flag: Follow up
Flag Status: Completed

Yes.

Please see the attached manuscript.

Thanks

From: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>
Sent: Wednesday, March 24, 2021 8:07 AM
To: Peyton, Roderick (NIH/OD) [E] <roderick.peyton@nih.gov>
Subject: RE: DPI Case #2019-006— Prestage Department of Poultry Science at North Carolina State University

Hello Mr. Peyton,

Did you receive a copy of the manuscript referenced in the first sentence of the attached allegation? If so, would you please forward it to me? Thank you.

Best regards, Brent Morse

Brent C. Morse, DVM, DACLAM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Sent: Wednesday, March 17, 2021 1:31 PM
To: Peyton, Roderick (NIH/OD) [E] <roderick.peyton@nih.gov>
Cc: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>
Subject: FW: DPI Case #2019-006— Prestage Department of Poultry Science at North Carolina State University

Dear Mr. Peyton,

This is to acknowledge receipt of the allegation. The OLAW Division of Compliance Oversight will review and take appropriate action.

Sincerely,

Patricia Brown, VMD, MS
Director, Office of Laboratory Animal Welfare,

Office of Extramural Research, Office of the Director, NIH
301-451-4209, brownp@mail.nih.gov

From: Peyton, Roderick (NIH/OD) [E] <roderick.peyton@nih.gov>
Sent: Wednesday, March 17, 2021 1:08 PM
To: Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Subject: DPI Case #2019-006— Prestage Department of Poultry Science at North Carolina State University

Good afternoon,

The Division of Program Integrity in the Office of Management Assessment (OMA) at the National Institutes of Health (NIH) has received the attached allegation. In accordance with NIH Manual Chapter 1754, this allegation is outside of our purview. Therefore, we are referring this case to your office. OMA is not responsible for reporting your actions to another office; therefore, we do not need to receive a report. We are closing this case and plan to take no further action.

In accordance with the Privacy Act, all parties must maintain the confidentiality of this matter. Within the agency, information pertaining to this case may be shared only on a need-to-know basis. If you have any questions, please call me at 301-827-7962.

Thank you,

Roderick Peyton
Auditor/Analyst
Division of Program Integrity
(NIH/OD) [E]
Office 301-827-7962
Roderick.Peyton@NIH.gov
National Institutes of Health