

Date: 4/5/22

Name of Project: Verification of *Coryza* negative status and understanding risk of backyard birds in an ongoing *Coryza* outbreak

Name of Researcher: Pierdon

Infectious *Coryza* Flock Sampling in Southeast Pennsylvania

Introduction

Infectious *Coryza* (IC) is a respiratory disease of chickens caused by the bacterial organism *Avibacterium paragallinarum*. The disease affects both pullets and layers, and less commonly, broilers around the world. Clinical signs can include facial swelling, serous nasal discharge, decreased feed and water consumption, and slightly increased mortality. A 10 to 40% drop in egg production can often be seen in layers as well. Bacterial culture or PCR assay are needed for a definitive diagnosis. Both diagnostic techniques have similar accuracy, but PCR is much faster (Blackall, 1999). Birds that have recovered from the disease can act as reservoirs and continue the spread within an area. On farms where different age groups are present, IC is able to maintain its presence in that location by continuously spreading to the younger flocks (Blackall and Soriano, 2020).

Infectious *Coryza* has been prevalent in California and the Southeastern United States for decades but has generally not been seen in the Northeastern United States until relatively recently. Starting in early of 2019, an outbreak of Infectious *coryza* has been occurring in poultry flocks across Pennsylvania (Byukusenge et al., 2020).

Risk factors associated with the spread of IC are poor biosecurity, stress from other disease, and improperly or unvaccinated flocks. Vaccination is a common way of avoiding the harmful clinical signs of Infectious *coryza*. There are currently both commercial and autogenous vaccines in use with administration timelines and number of doses varying by company.

Due to the loss in egg production of layers and slowed growth of broilers associated with Infectious *Coryza*, it is of high economic significance. A highly sensitive testing protocol is integral to ensure a flock is negative or confirm its positive status. The results of this study could have a major impact on the way pullets and layer chickens are tested for *coryza* prior to shipment.

Materials and Methods

Animals and Housing

14 Commercial layer chicken flocks ranging from 30 weeks to 78 weeks old and containing from 11,570 to 421,097 birds were used for this study. Of the 14 flocks tested, nine were housed in cage-free facilities, five were in conventional cages, and one was free-range. 12 flocks were vaccinated for Infectious *coryza*, and two flocks were not. Two of the flocks were known positive before testing, all others were thought to be negative (Table 1).

Table 1.

Flock #	Production Type	House Type	Breed	# of Birds	Age	Vaccination status	Presumed Status
1	Layer	Caged	Lohmann LSL Lite	413,557	47 Weeks	Vaccinated	Neg
2	Layer	Caged	Hyline Brown	184,793	66 Weeks	Vaccinated	Neg
3	Layer	Caged	Lohmann LSL Lite	207,894	68 Weeks	Vaccinated	Neg

4	Layer	Caged	Dekalb White	421,097	49 Weeks	Vaccinated	Neg
5	Layer	Free Range	Lohmann Brown	17,981	58 Weeks	Not Vaccinated	Pos
6	Layer	Caged	Hyline W-36	53,000	62 Weeks	Not Vaccinated	Pos
7	Embryo	Cage Free	Dekalb White	42,253	30 Weeks	Vaccinated	Neg
8	Embryo	Cage Free	Dekalb White	42,342	30 Weeks	Vaccinated	Neg
9	Layer	Cage Free	Bovan Brown	20,059	30 Weeks	Vaccinated	Neg
10	Layer	Cage Free	Bovan Brown	20,086	30 Weeks	Vaccinated	Neg
11	Layer	Cage Free	Lohmann Brown	19,198	48 Weeks	Vaccinated	Neg
12	Layer	Cage Free	Lohmann Brown	19,302	48 Weeks	Vaccinated	Neg
13	Layer	Cage Free	Hyline Brown	15,257	53 Weeks	Vaccinated	Neg
14	Layer	Cage Free	Hyline Brown	19,614	53 Weeks	Vaccinated	Neg

Sample Collection and Testing

Samples were taken from randomly selected chickens while moving throughout the facility. Care was taken to ensure birds from all areas of the flock were sampled. SteriPack 3" Sterile Polyester Spun Swabs (SteriPack USA Ltd LLC; Lakeland, FL, USA) were used to obtain oropharyngeal samples from 75 chickens in each flock. The samples used in this study were collected by a team of two people, one handler and one sampler. The handler caught and restrained birds while the sampler used one hand to open the mouth of the subject and place the swab into the choanal cleft, spinning it slowly to achieve adequate sampling.

Once samples were obtained, 5 swabs each were placed into 15 tubes to make 15 pooled samples from the 75 birds per flock. Samples were stored at -80° before being sent to The Pennsylvania State University Animal Diagnostic Lab for Real time PCR. Results were provided on a per tube basis as positive, negative, or suspicious along with a cycle threshold (Ct) value.

This protocol took between 30 to 40 minutes per flock, depending on the housing type. Birds in caged systems were easier to catch, making them take less time to sample than cage free. PCR testing cost \$35 per tube, making the total cost for testing 15 tubes (75 birds in a flock) \$525.

Results

Table 2.

Flock #	House Type	Vaccination Status	Previous Infection	Presumed Status	Results
1	Caged	Yes	Yes	NEG	POS
2	Caged	Yes	Yes	NEG	SUS
3	Caged	Yes	Yes	NEG	POS
4	Caged	Yes	Yes	NEG	SUS
5	Free Range	No	Yes	POS	POS
6	Caged	No	Yes	POS	POS
7	Cage Free	Yes	No	NEG	NEG
8	Cage Free	Yes	No	NEG	NEG
9	Cage Free	Yes	No	NEG	NEG

10	Cage Free	Yes	No	NEG	NEG
11	Cage Free	Yes	No	NEG	NEG
12	Cage Free	Yes	No	NEG	NEG
13	Cage Free	Yes	No	NEG	NEG
14	Cage Free	Yes	No	NEG	NEG

Two of the positive flocks were known positive due to clinical signs and testing prior to this study taking place. Four of the presumed negative flocks, all of which were caged layers, had either a suspicious or positive result, indicating the bacteria was likely present at a low level.

All cage-free were presumed negative prior to the study and were found to be negative using the testing protocol above.

Discussion

Caged layer flocks were the only ones to show a different disease status than the presumed status prior to testing. Because modern caged layer houses are often set up in multi house facilities, frequently holding more than one flock under the same roof, infectious coryza may be able to sustain a presence on the farm continuously transferring to different flocks. This is despite flock turnover and vaccination programs. Considering history of infection on farms, even with multiple flock turnover, great care needs to be taken if sites plan to fully render a facility infectious coryza-free after testing positive.

From the results of this study, we can conclude that a flock should not be assumed negative based solely on clinical signs. One of the many goals of this study was to verify the disease status of layer flocks around Pennsylvania and compare it to the status assumed by the producer, which may be based more on production and clinical signs than a systematic testing protocol. Production can still be very high with no external signs of disease in Coryza positive flocks. Vaccination also likely suppresses clinical signs associated with IC, while still allowing the birds to spread the disease.

The large sample size of 75 birds per flock helped to get a clearer picture of the disease status of the birds on an individual level and as a flock. While swabbing is taking place, the sampler is able to visually inspect each bird for signs that may be indicative of malady. Testing a large portion of the flock, in relation to traditional testing techniques, also allows for finding pockets of positivity within the flock.

The surveillance technique used in this study proved to be very helpful in confirming flocks as either positive or negative. This can be tremendously useful in transportation of birds within or outside of the state. It can also be a factor in the decision-making process of whether to ship the birds and clean out, or attempt to maintain the flock. In many cases, prevention is more important than treating a disease.

Further studies are required to determine if this testing protocol would be useful in other types of systems, like organic layers or broiler breeders.

References

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Objective:

The objective of this study was to establish locations of backyard birds within a 2-mile radius of 22 randomly selected *coryza* positive and control poultry farms.

Materials and Methods

Farms

11 of the farms were positive for *coryza*, and 11 of the farms were control farms. Using ArcMap, each farm was plotted with its 2-mile radius.

Verifying backyard bird locations

There were 9 total groups of farms that had overlapping radii and could be driven in the same day. Backyard birds were spotted using the naked eye and binoculars while driving past houses. GPS coordinates were taken where there were any signs of backyard birds, including the spotting of one or more chickens, ducks, pigeons, guinea hens, turkeys, and geese, as well as indicators like hen houses or “eggs for sale” signs. Driving down every road within each 2-mile circle, on average, required 53.5 miles of driving. To confirm we drove down every road within the 2-mile circle, both physical map tracing, as well as the app GPX Tracker, tracked where we had driven during the day.

Results

Covering all 22 farms and their 2-mile radii required a full 10 days of driving.

We calculated backyard flocks within 2 miles, 1 mile, and a half mile radius of each study farm. Figure 1 represents the locations of backyard birds within 2 miles of each study farm. Figure 2 is a heat map of backyard bird locations within 2 miles of each study farm, with lighter colors representing a higher density of backyard bird flocks.

The closest backyard flock to a study farm was a mere 85.6 meters (0.05 miles) away, and on average there were 25.2 backyard flocks within a 2-mile radius of the farms included in the study. Table 1 depicts the full data gathered for the study. There were no statistically significant values that implied positive *coryza* farms had increased numbers of backyard bird flocks within a 2-mile, 1 mile, or half mile radius compared to control farms.

Discussion

These methods may be useful in alerting community members with backyard birds in the case of future disease outbreaks or finding backyard flocks in control zones. In future, these numbers may be used to predict numbers of backyard birds in different locations to examine the influence of backyard poultry in different scenarios. We found no evidence that backyard birds were a risk factor for infectious *coryza*.

Table 1: Counts of backyard birds and poultry farms within varying radii of study farms

Variable	Coryza	mean	sd	min	max	n
2 miles: # backyard flocks	control	24.5	6.1	14	34	11
	positive	25.9	11.9	11	41	11
	total	25.2	9.2	11	41	22
1 mile: # backyard flocks	control	7.6	4.4	2	17	11
	positive	5.6	3.9	1	13	11
	total	6.6	4.2	1	17	22
half mile: # backyard flocks	control	1.0	1.5	0	5	11
	positive	2.1	1.6	0	5	11
	total	1.5	1.6	0	5	22
closest backyard bird flock (miles)	control	0.49	0.22	0.16	0.82	11
	positive	0.36	0.20	0.05	0.71	11
	total	0.43	0.21	0.05	0.82	22

Backyard Birds and Farms

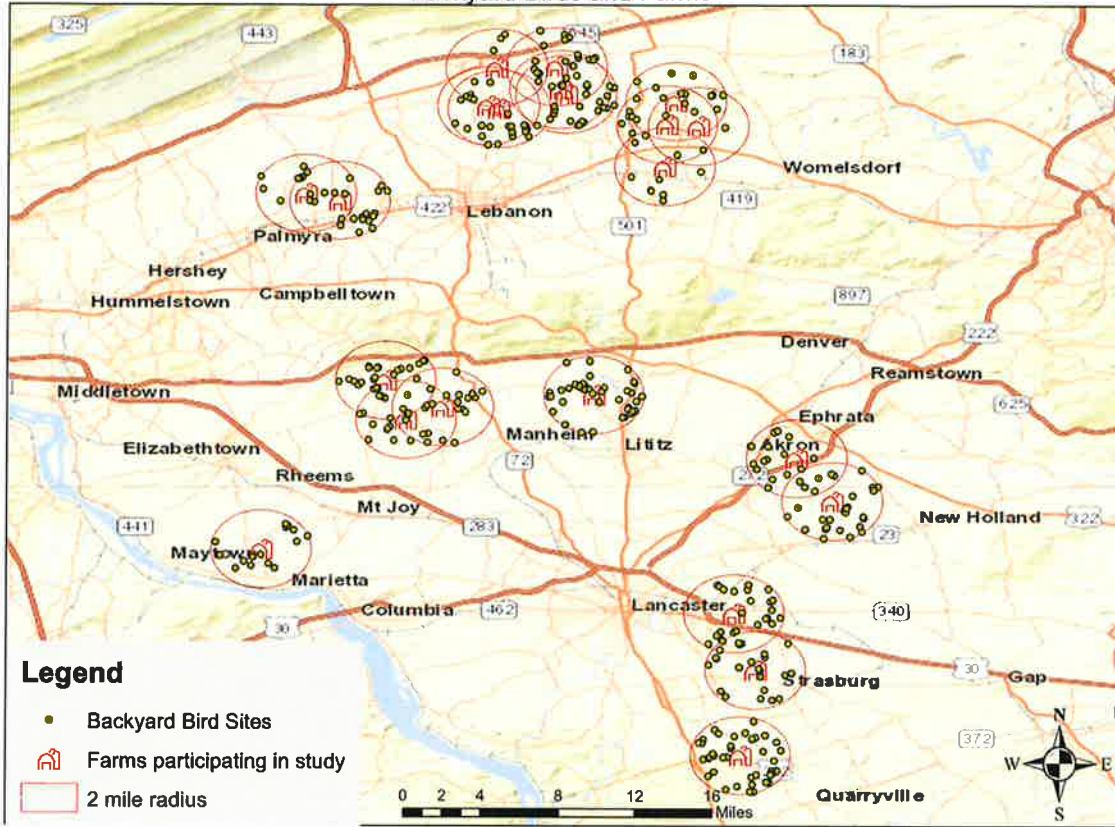
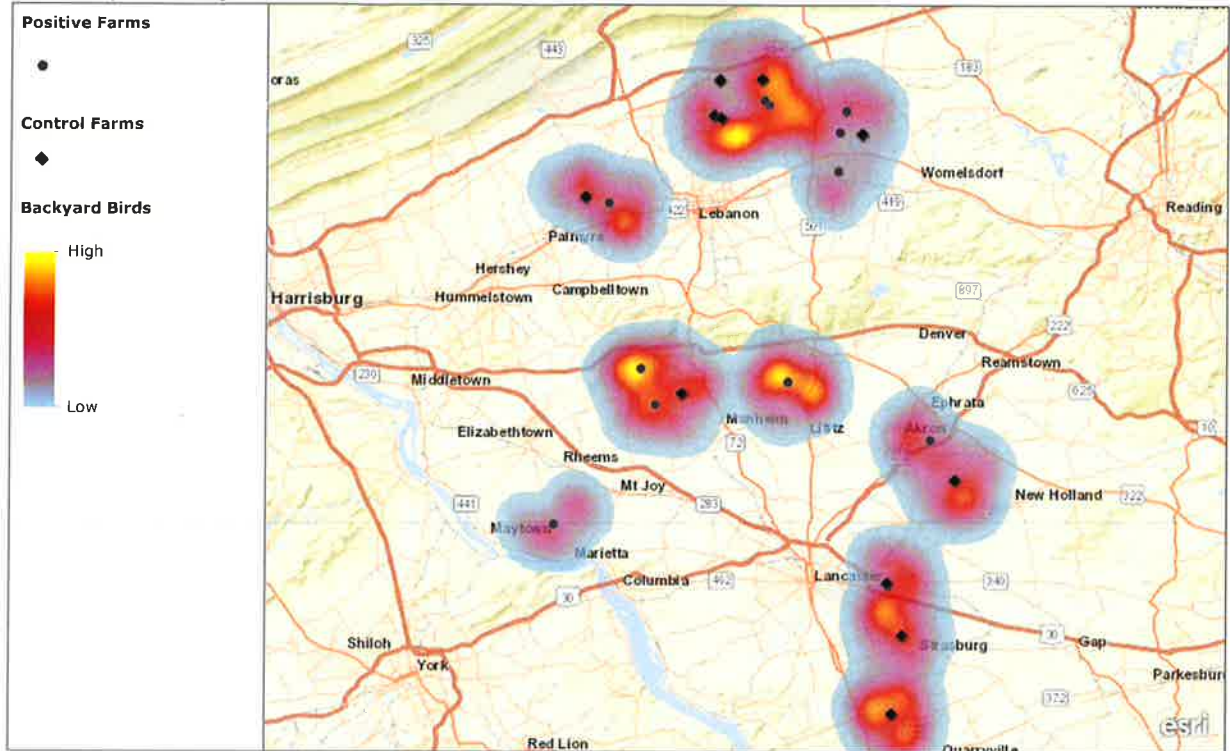


Figure 1: Locations of Backyard Bird Flocks within 2 miles of each study farm

Heat Map of Backyard Birds



Esri, HERE, Garmin, NGA, USGS, NPS

Figure 2: Heat map of Backyard Birds within 2 miles of study farms