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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Application of Commercial and Backyard Poultry Geographic Information System Databases for the Identification of Risk Factors for Clinical Infectious Laryngotracheitis in a Cluster of Cases on the Delmarva Peninsula

Y.J. Johnson<sup>1\*</sup>, M.M. Colby<sup>1,2</sup>, N.L. Tablante<sup>1</sup>, F.N. Hegngi<sup>3</sup>, M. Salem<sup>4</sup>, N. Gedamu<sup>4</sup> and C. Pope<sup>4</sup>

<sup>1</sup>Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland,  
College Park, MD 21801 USA

<sup>2</sup>American Academy for the Advancement of Science Global Security Fellow Office of Science and  
Technology Policy, Executive Office of the President, Washington, DC 20502 USA

<sup>3</sup>Assistant State Veterinarian, Maryland Department of Agriculture, Annapolis, MD 21401 USA

<sup>4</sup>Department of Animal and Food Science, University of Delaware, Newark Delaware 19717 USA  
E-mail: yj16@umail.umd.edu

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**Abstract:** Between May 4, 1998 and June 26, 1998 one broiler flock and six roaster flocks, all contracting with the same poultry integrator were reported positive for clinical Infectious Laryngotracheitis. A three-part study applying both standard outbreak investigation techniques and GIS tools was implemented to determine prevalence, distribution and risk factors for ILT. A field investigation, a case-control study, and seroprevalence survey were conducted. The case-control study used a sample of 7 case flocks and 14 control flocks from the affected integrator. Risk factors analyzed include proximity to: broiler-breeder flocks, backyard flocks and game bird facilities. A systematic-random sample of 168 flocks were selected for the seroprevalence study. Case flocks were 36.00 (95% C.I. 2.69, 481.21) times more likely to be located within 1 mile of a backyard than control flocks. The prevalence of sero-positive flocks was 57.1%. Flocks from the affected integrator were significantly older than those from other integrators. Increased flock age was significantly associated with negative titers for Newcastle Disease, Infectious Bronchitis, and Infectious Bursal Disease. The development of commercial and backyard poultry flock databases facilitated the quantification of the risk imposed by backyard flocks to local commercial flocks. Immunological status of the bird may be an important risk factor for clinical ILT and production systems with older birds that are not managed as "all-in-all-out" may be at much greater risk of clinical ILT and perhaps other infectious diseases.

**Key words:** Infectious laryngotracheitis, epidemiology, risk factors, geographic information systems

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### Introduction

Since its first reported discovery in 1925, several studies have been conducted on the pathogenesis and epidemiology of Infectious Laryngotracheitis (ILT) (May and Tittler, 1925; Linares *et al.*, 1994; Keeler *et al.*, 1993; Andreasen *et al.*, 1990; Davison and Miller, 1988; Guy *et al.*, 1989; Cover, 1996). Despite these efforts however, outbreaks of the disease continue to plague the poultry industry resulting in severe economic losses. Losses attributable to clinical ILT include: increased poultry mortality rates, reduced feed efficiency and rate of gain, reduced egg production, and loss of export markets. Additional losses incurred by the poultry industry as a result of ILT outbreaks include the costs associated with control efforts: vaccination, restricted vehicle and personnel movement, disinfection costs, and vaccine induced morbidity (Zellen *et al.*, 1984; Linares *et al.*, 1994; Davison and Miller 1988). The outbreaks of ILT in the Delmarva Peninsula in 1998-2000 alone were estimated to have cost the regional poultry industry in excess of 4 million dollars (Ritter, 2000).

Infectious Laryngotracheitis is caused by Gallid Herpesvirus-1, an Alpha-Herpes Virus (Biggs, 1982; Hanson and Bagust, 1991). As with other Herpes viruses it has the potential to produce latent infections with sporadic viral shedding. The virus has a tropism for the trigeminal ganglia. ILT is transmitted via the upper respiratory system. The virus is shed in ocular and oral secretions. The characteristic clinical signs include dyspnea, rales, conjunctivitis, and facial edema. Gross pathological lesions include hemorrhagic tracheitis, conjunctivitis, and muco-purulent caseous bronchial exudates.

Recent outbreaks of ILT on the Delmarva Peninsula have been atypical with respect to the time of year and distribution of cases over time and space. It is not known whether these changes in the epidemiology of the disease are due to a genetic shift in the virus, genetics of the birds, routine farm management practices or outbreak response strategies. One of these atypical clusters of cases occurred in the spring of 1998. Between May 4, 1998 and June 26, 1998 one broiler flock and six roaster flocks, all contracting with the same

poultry integrator were reported positive for clinical Infectious Laryngotracheitis.

In response to this cluster of cases, a three-part study was implemented to determine the prevalence and distribution of ILT sero-positive poultry flocks and to identify risk factors for clinical laryngotracheitis in the local commercial poultry flocks. While an initial field investigation was conducted at the time of the outbreak, the subsequent development of geographic information system (GIS) databases of the commercial and backyard poultry flocks in the region allowed for more extensive analysis of geographic and spatial risk factors. The aims of this study were to apply both standard outbreak investigation techniques and GIS tools to identify probable sources, routes of transmission, and risk factors for clinical laryngotracheitis from an outbreak investigation of a cluster of cases occurring in a single integrator on the Delmarva Peninsula.

### Materials and Methods

**Field investigation:** The index case occurred on May 4, 1998 in a flock of 5-week old broilers. The characteristic clinical signs were presented with a daily mortality rate of 9/1,000. Upon necropsy, lesions included tracheitis, conjunctivitis, and muco-purulent to caseous bronchial exudates. Six subsequent cases occurred between May 21 and June 26, 1998. Each of these cases occurred in roaster flocks of 6 to 8.5 weeks of age. Daily mortality rates ranged from 4 – 8 birds per 1,000 at diagnosis. The clinical signs were the same as in the index case. All farms were located in Delaware and all affected flocks contracted with the same poultry integrator.

Each case flock was visited by the investigators subsequent to presumptive diagnosis by a company veterinarian based upon clinical signs. Diagnosis was confirmed by virus isolation and histopathology at the University of Delaware Lasher Laboratory.

**Case-Control Study:** A case-control study design was employed to identify risk factors for clinical ILT. Since all of the clinical cases in this cluster were with growers contracting for the same integrator, 14 control flocks from the same integrator were selected from within the same geographic region as the 7 case flocks (Fig. 1). Risk factors analyzed include proximity to: broiler-breeder flocks, backyard flocks and game bird facilities. Distances were measured using ArcView software (ESRI Redlands, CA 92373) from GIS databases of commercial and backyard poultry operations within the region and from data obtained during field investigations of the outbreaks. The two-sample t-test was used to test for significant differences in the distances between flocks and risk factors in question.

**Sero-prevalence Study:** A systematic-random sample of commercial poultry houses was selected for

sampling. Poultry company personnel from each integrator on the Delmarva Peninsula selected random lots for sampling at the processing plant. The number of lots sampled for each integrator was representative of the company's market share in the region. The first bird in the lot to be sampled was selected randomly and each subsequent bird in the processing line was sampled until 30 birds from the same lot were sampled. Birds from a total of 221 poultry lots representing 168 separate flocks were sampled. Blood samples were collected in serum tubes and submitted to University of Delaware Lasher Laboratory, Georgetown Delaware. Additional data collected included farm name, grower name, contracting poultry integrator, flock age, and farm location. The KPL ELISA (Kirkegaard and Perry Laboratories Inc. Gaithersberg, MD 20879) was used to test for titers to ILT virus. Samples were then transferred to Maryland Department of Agriculture Salisbury Animal Health Laboratory for further serological testing. A random sample of 78 of these flocks were tested for antibodies to Newcastle Disease, Infectious Bursal Disease, and Infectious Bronchitis. The Wilcoxon-Mann-Whitney-U test was used to test for significant differences in geometric mean titers between case and control flocks and between the affected poultry integrator and the other companies (SAS NPAR1WAY Procedure Median Scores) (SAS Institute Inc. Cary, NC 27513).

### Results

**Field Investigation:** The index case occurred in a broiler flock. The next six cases occurred in roaster flocks. Briefly, the roaster production system employed by the affected integrator differs from standard broiler production in important ways. Growers are supplied with broiler chicks at the normal bird placement density. Approximately six weeks after placement one-third of the birds are removed from the house and sent for processing. These birds are known as "fillers". The remaining two-thirds of the birds in the house are known as "roasters". They are reared for an additional three weeks to produce a larger bird.

Clinical signs in the roaster flocks occurred 1 - 3 weeks after filler removal. The incubation period for ILT is 1 to 2 weeks. Vehicles and personnel associated with filler removal may serve as mechanical vectors. There was no association between flock service-people or feed trucks and case flocks. Case numbers 2,4, and 6 had the same personnel and vehicles for filler removal. Case numbers 5 and 7 also had the same personnel and vehicles for filler removal. Case numbers 2 and 3 had the same vehicles for filler removal but different personnel. The personnel and vehicles associated with these cases also moved birds on other broiler and roaster flocks that were not clinically affected by ILT.

The investigators visited 2 broiler-breeder flocks, 2 backyard flocks and one game bird facility, located within

5 miles of the epicenter of the outbreak. Broiler-breeder flock 1 had birds that were 20 weeks of age. There was no evidence of respiratory disease among the birds in the flock. Biosecurity breaches included the presence of cats within the poultry house and loose dogs within the area of the poultry house. Broiler-breeder flock 2 had birds that were 60 weeks of age. There was no evidence of respiratory disease among the birds within the house. Biosecurity breaches included the presence of starlings within the poultry house, poor carcass disposal and poor litter management.

Backyard flock 1 was a mixed species flock that produced eggs for sale. Four birds were sampled for ILT ELISA testing. Two of the four birds tested had titers above 500. Backyard flock 2 was also a mixed species flock. The birds were of advanced age and kept as pets. They were generally unthrifty in appearance. Five birds were sampled for titers to ILT from this flock. All five birds had titers in excess of 1000. When these birds were later challenged with a field strain of the ILT virus, they were very susceptible to the virus exhibiting 100% mortality.

The game bird facility housed several species of game birds in both indoor and outdoor facilities. No species currently thought to be susceptible to ILT were observed at the facility during the visit.

**Case-Control Study:** Case flocks were not located in closer proximity to broiler-breeder operations or the local game bird facility (Table 1). There was no significant difference in farm size (number of poultry houses or total farm capacity) between case and control flocks (Table 1). Proximity to a backyard flock and the game bird facility were significantly associated with clinical cases of ILT (Table 1). The Fisher's Exact Test resulted in a table probability of 0.0031 indicating a significant association between being a case flock and being located within one mile of a backyard flock. Case flocks were 36.00 (95% C.I. 2.69, 481.21) times more likely to be located within 1 mile of a backyard than control flocks. There was no significant association between being a case flock and being within 1 mile of the game bird facility.

**Sero-prevalence Study:** A total of 168 commercial poultry flocks were tested for antibodies to ILT (see Table 2). In accordance with local industry standards, a sero-positive flock was one in which the maximum ILT titer > 500 for one or more birds tested. The prevalence of sero-positive flocks was 57.1%. There was no significant difference between ILT sero-positive flocks and sero-negative flocks in flock age and geometric mean titers for Infectious Bursal Disease, Infectious Bronchitis, or Newcastle Disease. Flocks from the affected integrator were significantly older than those from other integrators. Increased flock age was also significantly associated with negative titers for

Newcastle Disease, Infectious Bronchitis, and Infectious Bursal Disease. There was no significant difference in the distance between backyard flocks and seropositive or seronegative flocks.

There was no significant difference in titers between flocks from the affected integrator and the other companies for ILT and Infectious Bronchitis. Flocks from the affected integrator had significantly lower titers for Infectious Bursal Disease and Newcastle Disease. The flock age was significantly greater for lots from the affected integrator. Newcastle Disease titers < one were significantly associated with increased flock age and lower titers for Infectious Bursal Disease and Infectious Bronchitis. There was no significant association between Newcastle Disease titer and ILT titer.

## Discussion

The 57% prevalence of seropositive flocks was higher than anticipated. This is the first reported ILT seroprevalence survey of clinically normal flocks from different companies in the region. Previous studies in New Zealand and South Australia have reported prevalences in excess of 25% (Lohr and Saywell, 1976; Pulsford and Stokes, 1953). There are two possible explanations for this high seroprevalence rate. The first is that there is a local reservoir for the virus and commercial flocks are routinely exposed. The second possibility is that the test is cross-reacting with some other agent and the seroprevalence is over estimated. Given that there are periodic outbreaks within the region affecting both backyard and commercial poultry operations, it is plausible that there is a local reservoir for the virus.

We have not been able to determine from this outbreak investigation whether the strain of ILT virus responsible for this cluster of cases was a field or a vaccine strain. ILT virus was not isolated from the backyard flocks that were identified as seropositive, rendering strain identification impossible. While some backyard flocks may have served as reservoirs or foci of infection during the 1994-1995 outbreaks, subsequent ILT vaccinations may have spread the vaccine virus strain to other farms, including backyard flocks that may have "adopted" culls or leftover ILT-vaccinated broilers from commercial farms. In fact a more recent case of ILT diagnosed in a backyard peafowl flock on the Delmarva Peninsula was identified by PCR as a vaccine strain that was most likely transmitted from a nearby vaccinated commercial broiler flock.

While it cannot be conclusively established from this single cluster of clinical cases of ILT, the very strong association between proximity to backyard flocks and risk of being a clinical case of ILT is an important finding. The database of backyard flocks was developed in 2002-2003, some time after the actual outbreak used for

Table 1: Univariate analysis of continuous risk factors for clinical ILT

	Controls N=14	Cases N=7	t-statistic	p
Distance from a Backyard Flock (m)	2882.14	1324.29	3.51	0.001
Distance from a Broil-Breeder Flock (m)	6332.86	5452.86	0.45	0.66
Distance from the game-bird facility (m)	16530.71	8208.57	2.62	0.03
Number of houses	2.43	2.29	0.26	0.8
Total farm capacity	41436.64	53428.57	-0.79	0.45

Table 2: Univariate analysis of categorical risk factors for clinical ILT

	Risk Factor + Mean Flock Age (in days)	Risk Factor - Mean Flock Age (in days)	p
Flock contracted by affected company	48.4	45.0	0.02
Newcastle Disease titer >1	47.0	49.0	0.04
Infectious Bronchitis titer > 1000	46.2	49.5	0.01
Infectious Bursal Disease titer > 1000	47.0	50.0	0.03

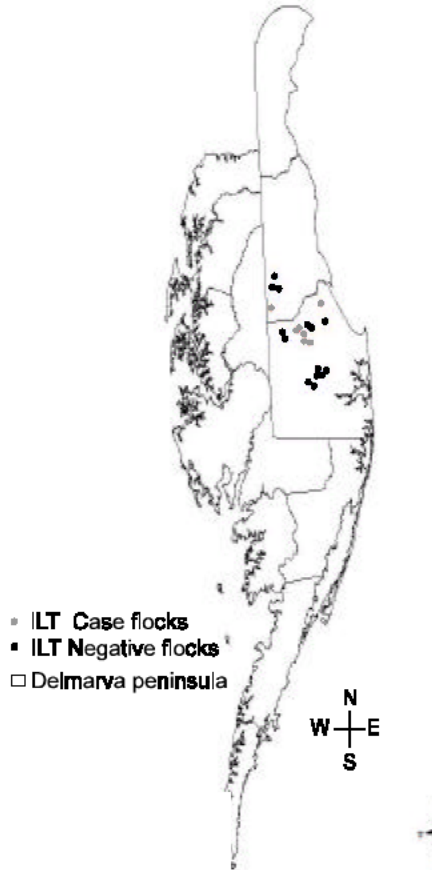


Fig. 1: Geographic distribution of 7 ILT case flocks and 14 randomly selected control flocks matched on company and geographic region from a 1998 cluster on the Delmarva Peninsula, USA.

the case-control study. As a result there is the potential for some misclassification error if backyard flocks that were present at the time of the outbreak are no longer

present and if new backyard flocks have entered the study area since the outbreak. This misclassification is expected to be randomly allocated across case and control flocks and should not bias the findings. Previous reports in the literature have indicated an association between backyard poultry flocks and outbreaks of ILT in commercial poultry operations (Adair *et al.*, 1985; Curtis and Wallis, 1983; Mallinson *et al.*, 1981). The strength of the association and the coherence with the current literature support the conclusion that proximity to a backyard flock is an important risk factor for ILT case status. This is the first study in which the risk associated with proximity to a backyard flock was quantified with the aid of GIS databases of commercial and backyard flocks.

One unique feature of this outbreak is that it was clustered within a single poultry integrator and with the exception of the index case was also clustered within a single production line, roasters. This system does not follow the typical “all-in-all-out” system. The roasters have been exposed to vehicles, personnel, and equipment associated with removal of the fillers, they then remain on the farm for an additional 3 weeks before being sent for processing.

Each of the roaster flocks that became clinically affected were diagnosed after removal of the filler birds and within a time period consistent with the incubation period of the virus. There was also an apparent association between the vehicles used for filler removal in several of the case flocks. Attempts at isolation of ILT virus from vehicles used in filler removal were not successful.

Thus far, this outbreak investigation has identified a presumptive local source for the agent (backyard flocks) and a presumptive means of introduction into commercial roaster flocks (filler removal). The next issue is what risk factors are causing some flocks to be at increased risk for clinical disease while apparently many other flocks are exposed to the virus but fail to develop

clinical disease. The explanation for this may be found in the results of the serological testing for other viral poultry diseases. Birds from the affected integrator were significantly older than the other integrators. Birds from the affected integrator had significantly lower titers for both Newcastle Disease and Infectious Bronchitis. The increased flock age observed from the affected integrator is due to the roaster production system. These older birds may also be experiencing a decline in immunity to diseases against which they are usually vaccinated. The lack of paired serological samples prevents the establishment that there is indeed an age associated decline in immunity to viral poultry diseases that may render roasters at increased risk for clinical illness during the final phase of their grow-out period. However, this is an entirely plausible explanation for this outbreak and it warrants additional study. The association between negative titers for Newcastle Disease and IBD titers, IBV titers, and increased flock age also supports this hypothesis.

Overall the findings for this outbreak investigation are consistent with other studies. Previously identified risk factors for clinical ILT include people, vehicles and equipment, wildlife and pets, backyard flocks, vaccinated flocks, and wind-borne spread. The development of commercial and backyard poultry flock databases facilitated the quantification of the risk imposed by backyard flocks to local commercial flocks in a unique manner. The sero-prevalence survey revealed three important findings. The first is the scope of the problem is much greater than previously thought. This is reflected by the high prevalence of sero-positive flocks. The second is that immunological status of the bird may be an important risk factor for development of clinical disease. Lastly production systems with older birds that are not managed as "all-in-all-out" may be at much greater risk of clinical ILT and perhaps other infectious diseases.

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