EASTERN RESEARCH GROUP, INC.

MEMORANDUM

TO: Fred Porter, Mary Johnson and Brian Shrager, U.S. Environmental Protection Agency

FROM: Jason Huckaby, Eastern Research Group, Inc.

DATE: November 29, 2004

SUBJECT: Information on livestock mortality incineration

1.0 INTRODUCTION

The following items were submitted to EPA during the development of the Other Solid Waste Incineration (OSWI) regulations. The items are included as attachments to this cover memorandum.

Attachment A: McQuiston, Jennifer, et al. "Risk Factors for Spread of Low Pathogenicity H7N2 Avian Influenza Virus Among Commercial Poultry Farms in Virginia, 2002." Pre-publication draft sent via e-mail to Fred Porter, U.S. EPA, on July 15, 2004.

Attachment B: Grimes, Jesse, et al. "Impact of incinerator use on the incidence of infectious diseases in commercial turkey farms." Pre-publication draft sent via e-mail to Fred Porter, U.S. EPA, on March 9, 2004.

ATTACHMENT A:

McQuiston, Jennifer, et al. "Risk Factors for Spread of Low Pathogenicity H7N2 Avian Influenza Virus Among Commercial Poultry Farms in Virginia, 2002." Pre-publication draft sent via e-mail to Fred Porter, U.S. EPA, on July 15, 2004.

Risk Factors for Spread of Low Pathogenicity H7N2 Avian Influenza Virus Among Commercial Poultry Farms in Virginia, 2002

Jennifer H. McQuiston, DVM, MS; Lindsey P. Garber, DVM, MS; Barbara A. Porter-Spalding, DVM; John W. Hahn, DVM, MS; F. William Pierson, DVM, PhD; Sherrilyn H. Wainwright, DVM, MPH; Dennis A. Senne, DVM; Thomas J. Brignole, DVM; Bruce L. Akey, DVM; Thomas J. Holt, DVM

United States Public Health Service, Commissioned Corps Readiness Force, Centers for Disease Control and Prevention, 1600 Clifton Road MS G-13, Atlanta, GA 30333 (McQuiston)

United States Department of Agriculture (USDA): Animal and Plant Health Inspection Service (APHIS): Veterinary Services (VS) Centers for Epidemiology and Animal Health, 2150 Centre Ave, Bldg B, Fort Collins, CO 80526 (Garber, Wainwright)

USDA:APHIS:VS, Eastern Regional Office, 920 Main Campus Drive Suite 200, Raleigh, NC 27606 (Porter-Spalding, Brignole, Holt)

USDA:APHIS:VS, Arkansas Area Office, 1200 Cherry Brook Drive Suite 300, Little Rock AR 72211 (Hahn)

Virginia-Maryland Regional College of Veterinary Medicine, Center for Molecular Medicine and Infectious Diseases, Duckpond Drive, Blacksburg, VA 24061 (Pierson)

USDA:APHIS:VS: National Veterinary Services Laboratory, 1800 Dayton Avenue, Ames, IA 50010 (Senne)

Virginia Department of Agriculture and Consumer Services, Office of Laboratory Services, Richmond, VA 23219 (Akey, current address: NYS Department of Agriculture and Markets, Division of Animal Industry, 1 Winners Circle, Albany, NY, 12235)

Corresponding Author: Jennifer H. McQuiston, Centers for Disease Control and Prevention, 1600 Clifton Road MS G-13, Atlanta, GA 30333; phone 404-639-0041; fax 404-639-2778; email fzh7@cdc.gov

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Objective – To identify risk factors associated with the spread of low pathogenic H7N2 avian influenza (AI) virus among commercial poultry flocks in western Virginia during a 2002 outbreak.

Design – Case control study

Procedure – Questionnaires were used to collect information about farm characteristics, biosecurity measures, and husbandry practices. Questionnaires were administered on 151 infected premises (128 turkey and 23 chicken farms) and 199 non-infected premises (167 turkey and 32 chicken farms).

Results – The most significant risk factor for infection was disposal of dead birds by rendering (odds ratio 7.3, $p < 0.0001$). In addition, bird age ≥ 10 weeks (odds ratio birds aged 10-19 weeks 4.9, birds aged ≥ 20 weeks 4.3 , $p<0.0001$) was significant regardless of poultry species involved. Other significant risk factors identified by the study included using non-family caretakers and the presence of mammalian wildlife. Factors that were not significantly associated with infection included use of routine biosecurity measures (such as locked gates, spray stations, use of showers, disinfecting clothes and boots), food and litter sources, types of domestic animal species on the premises, and presence of wild birds on the premises.

Conclusions – Results suggest that an important factor contributing to rapid early spread of AI virus infection among commercial poultry flocks during this outbreak was disposal of dead bird carcasses by rendering. Biosecurity measures employed following the completion of this study included thoroughly cleaning and disinfecting all vehicles leaving the rendering plant. Rendering represents a high biosecurity risk to the poultry industry because rendering plants serve as a source of contact between commercial and

private farms, and transport vehicles moving from the rendering plant to farms may contribute to rapid spread of virus. Because of the highly infectious nature of AI virus and the devastating economic impact of outbreaks, poultry farmers should consider carcass disposal techniques that do not require off-farm movement, such as burial, composting, or incineration.

Avian Influenza (AI) virus outbreaks in the commercial poultry industry carry serious economic consequences due to bird mortality, depopulation costs, and national and international trade restrictions.[1-3] The identification of high pathogenicity AI virus in U.S. poultry flocks constitutes a national emergency, which is issued by the United States Department of Agriculture (USDA), and necessitates immediate quarantine and depopulation measures to control spread of virus.[3] The identification of low pathogenicity strains of AI virus constitutes more of a clinical and practical dilemma. Although not typically treated as a national emergency, low pathogenicity strains of H5 and H7 AI virus carry the threat of mutation to more highly pathogenic forms;[1;4-6] thus, immediate control of these outbreaks is usually a priority of state and national veterinary authorities.

In the United States, AI virus circulates in the northeastern live-bird markets,[6,7] and periodic outbreaks of circulating market strains of virus occur among commercial poultry farms. In 1983-1984, an outbreak of low pathogenicity H5N2 AI occurred in chicken flocks in Pennsylvania; the outbreak boundaries eventually included western Virginia and smaller focal areas of Maryland and New Jersey.[1] Approximately 6 months after its initial detection, the virus mutated to a more highly pathogenic form, causing 80% mortality rates among affected flocks and resulting in the eventual destruction of more than 15 million birds.[1] In 1996-1998, an outbreak of low pathogenicity H7N2 AI virus occurred among commercial poultry flocks in Pennsylvania. Low mortality and production losses occurred with this virus, but the potential economic consequences of allowing the outbreak to continue and risk virus mutation to a highly pathogenic form were unacceptable, and 2.6 million birds were destroyed.[8]

On March 13, 2002, low pathogenicity H7N2 AI virus was confirmed by the USDA National Veterinary Services Laboratory in a northwest Virginia commercial turkey breeder flock.[9] The flock was immediately depopulated by the company and buried on-site in an attempt to minimize virus spread.[9] However, during the next week four additional turkey farms owned by the same company and sharing common truck routes with the index farm were confirmed to have poultry infected with AI virus.[9] On March 21st AI virus was confirmed in a turkey grow-out farm 30 miles from the index farm and owned by a different company,[9] and by April 12th the outbreak encompassed more than 60 flocks and involved five major poultry companies. Although turkey farms were predominantly affected, chicken flocks were confirmed positive for AI as well.

The USDA Animal and Plant Health Inspection Service (APHIS), in conjunction with the Virginia Department of Agriculture and Consumer Services and poultry company representatives, organized an AI Task Force to deal with the expanding outbreak.[9]Although the virus involved in the outbreak was low pathogenicity H7N2 AI, concern over the virus' ability to mutate to a highly pathogenic strain led to a decision to eradicate the virus from the Virginia poultry industry. The AI Task Force used quarantine and depopulation methods in an attempt to control viral spread, and instituted strict dead bird laboratory surveillance by sampling every poultry farm in the region on a weekly basis.[9] Despite these initial efforts, the outbreak continued, and by April 18th, 89 positive flocks had been identified (figure 1). The study reported here was designed by the AI Task Force to rapidly identify risk factors for infection during the outbreak in order to ascertain reasons for the continued spread of AI virus in the region. The study was

implemented in April 2002 near the peak of the outbreak, and results from the study were used by the AI Task Force to establish guidelines for disease control and prevention.

Materials and Methods

Case Definition – Farms were confirmed positive for AI by the presence of compatible clinical symptoms such as decreased food and/or water consumption, decreased egg production, depression, or respiratory symptoms (cough, wheezing, dyspnea) plus at least one positive laboratory test; alternatively, asymptomatic flocks were considered positive if they had at least two positive laboratory tests. Positive laboratory criteria utilized during the outbreak response included: 1) positive Directigen[a] ezyme-linked immunosorbent assay (ELISA) on fresh tracheal swabs,[10,11] 2) positive reverse transcriptase-polymerase chain reaction (RT-PCR) assay on fresh tracheal swabs,[12] 3) positive virus isolation on fresh tracheal swabs with subsequent typing as H7-type AI,[13] and 4) positive AI agar gel immunodiffusion (AGID) with serotyping as H7-type AI,[14,15].

Study Design – A case-control study was conducted to identify risk factors for AI-positive status among commercial poultry farms in the affected region of western Virginia. A questionnaire was developed by AI Task Force epidemiologists and company veterinarians. The questionnaire was designed to collect information about farm characteristics, husbandry practices, biosecurity measures employed on the farm, feed and litter sources, and farm employee activities.

All infected premises completing questionnaires as of May 30, 2002 were included in the study as cases, for a total of 151 case farms (128 turkey farms and 23

chicken farms, table 1). Questionnaires were administered to 199 non-infected farms, including all remaining non-infected turkey farms in the region (n=167) for which questionnaires could be completed and non-infected chicken farms within a one-mile radius to an infected chicken flock (n=32). Overall, 37/55 (67.3%) chicken farms and 247/295 (83.7%) of turkey farms included in the study raised grow-out birds for market, and the remainder were breeder or breeder replacement farms. No table egg layer flocks were included in the study.

Study Implementation - Questionnaires were administered to farm owners or managers by AI Task Force members or company veterinarians. Questionnaires were administered to case farms by on-site visits. Questionnaires were administered to control farms by either on-site visits or by telephone. Data collection began in late April 2002 and continued through the end of May.

Statistical Analyses– Although the initial intent of the study had been to perform separate analyses for turkey and chicken premises, the small number of infected chicken premises in the region prohibited an effective separate analysis. Thus, turkey and chicken farms were grouped for analysis. Data were entered into a database[b] and exported for analysis with commercially available software.[c] Variables were first examined by univariate analysis using a chi-square test, and certain variables having a $p \leq 0.10$ and biological plausibility were selected for backward elimination logistic regression modeling.[d] The Wald test was used to eliminate variables from the multivariate model. A value ≤ 0.05 was required for variables to remain in the final model. Because case and control farms were matched by species (chicken versus turkey), the species variable could not be evaluated statistically. However, the species variable was included in the

model as a covariate to adjust for the potential confounding effect it may have on other variables of interest (e.g., age, flock size). Also, the total number of caretakers and the number of family members working off the farm were included as potential confounding variables for non-family caretakers. Clustering of farms (due to individual owners with multiple premises) was accounted for by use of the Taylor series linearization method.

Results

A total of 151 case farms and 199 control farms were included in the final analysis. All five primary poultry companies in the region were represented, and the number of cases and controls did not differ significantly with respect to company or the number of birds on each farm.

In the univariate analysis (Table 2), several variables met the criteria for selection for multivariate modeling ($p \leq 0.10$). Case farms had a larger number of poultry houses on the farm and were more likely than control farms to use poultry houses with power ventilation. Case farms were also more likely than control farms to have older birds (specifically birds ≥ 10 weeks of age) and to use non-family caretakers for poultry houses. Case farms were less likely to have horses on the farm, but more likely to report the presence of wildlife, such as raccoons, foxes, or opossums. Case farms were also more likely to report disposal of dead birds by rendering; in contrast, non-infected farms were significantly more likely to dispose of bird carcasses by composting.

Information on the number of recent feed truck visits was available for a subset of farms (109 [72%] case farms and 147 [74%] control farms). Compared with non-infected farms, case farms reported significantly higher numbers of feed truck visits in the 2

weeks prior to interview (p=0.05). Although significant, this variable could not be included in the logistic regression model because information was available for only a subset of farms.

In the univariate analysis, evaluation of general security measures did not reveal any apparent biosecurity breaches that could account for a majority of farm infections. There was no statistically significant difference between use of fencing around poultry houses, whether premise gates were kept locked or not, or whether a spray station was or was not used to clean and disinfect vehicles at the farm entrance. In addition, there was no significant difference between case and control farms in the use of showers, changing of clothes or boots prior to working in poultry houses, regular use of foothbaths, regular use of coveralls and/or rubber boots for poultry house work, or routine washing and disinfection of boots and clothing. The presence of wild birds and various domestic animals including dogs, cats, ruminants, and pigs were similar between cases and controls. The presence of other poultry was uncommon on both case and control farms. Cases and controls reported similar use of rodent and fly control and reported similar attempts to "bird-proof" poultry houses. There was no observable difference between movement of machinery or equipment between premises, litter or food source, or litter management practices.

The following twelve variables were selected for inclusion in a backward elimination logistic regression model: poultry species; number of poultry houses on a farm; bird age; house type; use of non-family caretakers; use of same caretakers for various bird ages; presence of horses on the farm; having a family member work off-site; total number of caretakers; reported presence of raccoons, opossums, or foxes on the

farm; disposal of dead birds by composting; and disposal of dead birds by rendering. Following the backward elimination logistic regression, five variables in the model remained statistically significant risk factors (table 3): use of non-family caretakers; family members working off-farm; bird age ≥ 10 weeks; reported presence of wild mammals; and disposal of bird carcasses by rendering. Although not meeting the p=0.05 criteria for significance in the final model, poultry houses with power ventilation were more likely to be infected than other farms (p=0.06, odds ratio 2.5, 95% confidence interval 1.2-5.3).

Discussion

In this study, disposal of bird carcasses by rendering was the most significant risk factor identified for AI infection. Rendering was likely a prominent feature in the early propagation of this outbreak. The initial five farms infected in this outbreak all disposed of daily bird mortality by rendering, and they shared a common vehicle for transport of birds to a single rendering plant. Thus, early virus spread may have been potentiated by this management practice. The affected region in western Virginia was served primarily by one privately owned rendering plant, which served as a focal mixing point for vehicles and personnel from private and commercial farms across the region. Although rendering was identified as the most significant variable in this study, it was used by only 31% of case farms, and thus is not the only explanation for virus spread. Vehicle traffic to and from the renderer may have played a role in carrying virus across the region exposing farms that did not routinely render dead birds.

Early in the outbreak, one company's farms seemed to be excluded from infection; rendering was generally prohibited by that company's management policy. Although some farms belonging to that company were eventually confirmed with infection, the company's routine policy prohibiting rendering may have resulted in a substantially lower number of infected company farms than might otherwise have occurred. In response to the study results, all commercial poultry companies in the region issued guidelines discouraging rendering, a decision that may have helped limit further spread of virus. Preliminary results from this study, released on June 20, 2002, prompted the Task Force to institute a cleaning and disinfection station at the privately owned rendering plant to assure more adequate cleaning and disinfection of all vehicles exiting the plant.

Another significant risk factor in this outbreak was farms with older birds, particularly birds ≥ 10 weeks of age. This risk factor remained significant in the multivariable model while taking into account confounding by species and rendering. Possible explanations for older bird age being associated with infection include increased susceptibility (i.e., increased housing stresses and effects on immune status) and increased opportunity for virus exposure among older birds (i.e. more frequent visits by feed trucks). The latter explanation is supported by the univariate analysis of the number of feed truck visits in the 2 weeks prior to diagnosis, which showed that increased feed truck activity was a risk factor for AI virus infection.

Several variables were more moderately associated with AI virus infection. Having non-family caretakers work in poultry houses and having owners or family members work at other jobs off-site were significant risk factors and were each approximately twice as likely to result in AI virus infection. This may be related to increased vehicle

traffic on the farm, with vehicles serving as fomites for virus spread from other areas. Caretakers may also be exposed to birds off the farm and bring infection to the farm on their clothes and hands. Reported observation of mammalian wildlife, such as raccoons, opossums, or foxes, near the poultry houses was also significantly associated with infection. These animals may have served as mechanical vectors for transmission from neighboring affected areas. Although not meeting the criteria for significance in the final model, poultry houses with power ventilation showed a trend toward increased AI virus infection; this may be related to greater potential to introduce dust or windborne litter contaminated with AI virus.

This study is subject to several important limitations. The study was employed in an emergency fashion to rapidly assess risk factors mid-way through the outbreak, and the study was designed to assess factors for which interventions could be directed to prevent further virus spread. The rapid development and administration of the questionnaire may have resulted in it being applied in an inconsistent manner in some circumstances, thus biasing responses. Some questions may have been interpreted subjectively by persons completing the questionnaire; for example, the question asking about presence of wildlife on farms may have been interpreted as visual confirmation of wildlife in some cases, and as indicators of wildlife presence (such as tracks, feces, etc) in some cases. In addition, some variables, especially regarding temporal relationships, could not be analyzed due to inconsistent administration of questions. One issue that requires mention is the fact that the nature of the study (i.e., was conducted during an outbreak) meant that farm status (i.e., infected or noninfected) could change during the course of the outbreak. Because the intent of the study was to assess early and current

risk factors for infection, farms that remained negative for AI through May 30, 2002 were considered negative for the purposes of the study, even if they were later diagnosed as infected premises. Last, because this study was designed to assess early risk factors for infection, factors contributing to virus spread later in the outbreak may have been different than factors contributing to early virus spread across the region.

The last infected farm during this outbreak was identified on July 2, 2002, and the final quarantine was lifted on October 9, 2002. In total, 196 positive farms in western Virginia and a single positive farm in West Virginia near the Virginia border were identified in this outbreak, and 4.7 million birds were destroyed.[9] The region of western Virginia where the 2002 outbreak occurred is the same area that was affected by the 1983-1984 outbreak of H5N2 AI virus. Although the source of virus for the 2002 outbreak in Virginia was never identified, the virus appears identical to the low pathogenicity H7N2 strain responsible for recent outbreaks in Pennsylvania and to currently circulating strains in live bird markets in the northeastern United States.[6]

Although a major effort was undertaken at both the state and federal level to control viral spread, a "state of emergency" was never declared during the Virginia outbreak because the virus remained classified as a low pathogenicity strain. All activities of the Task Force were carried out under the authority of Virginia state officials to quarantine and depopulate without promise of indemnity to farmers. Federal operational costs for this outbreak were approximately $14 million, state costs were approximately $1 million, and federal compensation of approximately $67 million was approved for producers and companies involved in the outbreak.9 Total economic costs for the

outbreak, however, are certainly higher due to industry losses associated with production downtime and trade implications.

This outbreak demonstrates the highly infectious nature of AI virus and the need for vigilant biosecurity in the commercial poultry industry. Rendering of bird carcasses represents a significant biosecurity risk, and poultry companies may want to consider using on-farm disposal methods, such as composting, burial, or incineration, to handle daily bird mortality if adequate biosecurity measures for rendering cannot be implemented.

Footnotes

[a] Directigen ELISA, Becton Dickinson, Sparks, MD.

[b] Emergency Management Reporting System (EMRS), USDA-APHIS

[c] SAS/STAT version 8.2, SAS Institute Inc, Cary, NC.

[d] SUDAAN release 5.50, Research Triangle Institute, Research Triangle Park, NC.

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Table 1: Farm type for farms included in the study, Avian Influenza virus outbreak in Virginia, 2002

Farm Type	Number of Case Farms	Number of Control Farms
Chicken – broilers	8	29
Chicken – broiler breeder	14	3
Chicken – broiler breeder replacements	1	0
Turkey – grow-out hens	66	94
Turkey – grow-out toms	38	49
Turkey – breeder hens	16	12
Turkey – breeder hens replacements	6	11
Turkey – breeder toms	2	1
Total Farms	151	199

Table 2. Assessment of risk factors for Avian Influenza virus infection in Virginia, 2002; Univariate analysis

Percent of case and control farms with the following attributes (chi square test)

Variable	Case Farms		Control Farms		p value
	n	(pct)	n	(pct)	
Number of poultry houses on farm					.06
1 house	19/151	(12.6)	42/199	(21.1)	
2 houses	68/151	(45.0)	96/199	(48.2)	
3+ houses	64/151	(42.4)	61/199	(30.7)	
Number of birds on farm					.98
< 20,000	59/136	(43.4)	77/177	(43.5)	
≥ 20,000	77/136	(56.6)	100/177	(56.5)	
Bird age					<.001
< 10 weeks	32/125	(25.6)	101/173	(58.4)	
10-19 weeks	66/125	(52.8)	57/173	(32.9)	

≥ 20 weeks	27/125 (21.6)	15/173 (8.7)	
House type			.02
Power ventilation or double deck	66/151 (43.7)	77/199 (38.7)	
2 or 3 stage	57/151 (37.8)	57/199 (28.6)	
Curtain	28/151 (18.5)	65/199 (32.7)	
Use of perimeter fencing	57/150 (38.0)	76/199 (38.2)	.97
Gates are locked	14/135 (10.4)	14/191 (7.3)	.34
Use a spray station to clean and disinfect vehicles	76/146 (52.1)	92/196 (46.9)	.38
Logbook regularly used to track visitors	125/149 (83.9)	156/198 (78.8)	.27
Park vehicles away from poultry houses	125/150 (83.3)	156/198 (78.8)	.30
Shower available for workers	41/149 (27.5)	43/196 (21.9)	.23
Doors to poultry houses kept locked	41/149 (27.5)	57/197 (28.9)	.78
Footbaths regularly used	141/150 (94.0)	186/199 (93.5)	.84
Nearby body of water			.88
< 0.25 miles	22/115 (19.1)	34/157 (21.7)	

0.25-0.5 miles	26/115 (22.6)	34/157 (21.7)	
> 0.5 miles or none	67/115 (58.3)	89/157 (56.7)	
Litter source			.43
Company A	18/151 (11.9)	13/199 (6.5)	
Company B	39/151 (25.8)	58/199 (29.2)	
Other company	94/151 (62.3)	128/199 (64.3)	
Litter stored in a shed on premises	119/150 (79.3)	151/196 (77.0)	.63
Used litter spread on the ground	94/148 (63.5)	135/196 (68.9)	.29
Used litter shipped off-site	112/148 (75.7)	141/196 (71.9)	.46
backyard poultry within 1 mile	34/136 (25.0)	36/177 (20.3)	.35
Borrow or lend farm equipment	26/150 (17.3)	33/198 (16.7)	.88
Use of family caretakers in poultry houses	136/151 (90.1)	185/196 (94.4)	.30
Use of non-family caretakers in poultry houses	69/151 (45.7)	59/194 (30.4)	.01
Total number of caretakers for poultry houses			.28
1	34/147 (23.1)	59/192 (30.7)	

2 3+	46/147 (31.3) 67/147 (45.6)	61/192 (31.8) 72/192 (37.5)	
Use of same caretakers for different bird ages	85/145 (58.6)	136/185 (73.5)	.02
Owner/family works off-site	78/149 (52.3)	92/197 (46.7)	.36
Wear coveralls in poultry house	69/150 (46.0)	74/198 (37.4)	.16
Wear rubber boots in poultry house	122/151 (80.8)	152/197 (77.2)	.44
Regularly wash and disinfect clothes/boots	139/150 (92.7)	182/193 (94.3)	.55
Take shower before entering poultry house	85/148 (57.4)	114/198 (57.6)	.98
Take shower upon exiting poultry house	111/150 (74.0)	151/198 (76.3)	.67
Beef cattle on farm	89/150 (59.3)	116/195 (59.5)	.98
Dairy cattle on farm	25/148 (16.9)	26/191 (13.6)	.42
Horses on farm	26/148 (17.6)	55/192 (28.6)	.02
Sheep on farm	10/148 (6.8)	20/192 (10.4)	.27
Goats on farm	7/148 (4.7)	12/192 (6.3)	.55
Pigs on farm	6/146 (4.1)	8/190 (4.2)	.97

Dogs on farm	101/150 (67.3)	139/195 (71.3)	.46
Cats on farm	93/150 (62.0)	107/192 (55.7)	.28
Poultry on farm	4/148 (2.7)	2/193 (1.0)	Too few
Frequency of Rodent control			.78
check every 6 weeks	119/147 (81.0)	162/197 (82.2)	
check less frequently	28/147 (19.0)	35/197 (17.8)	
no rodent control	0/147 (0)	0/197 (0)	
Fly control	117/148 (79.1)	159/197 (80.7)	.72
Attempts to "bird-proof" poultry house	133/149 (89.3)	173/192 (90.1)	.80
Wild birds seen in poultry house	31/148 (20.9)	47/192 (24.5)	.45
Raccoons, opossums, foxes seen around poultry houses	62/150 (41.3)	60/191 (31.4)	.08
Wild turkeys, pheasants, or quail seen around poultry houses	17/150 (11.3)	17/191 (8.9)	.48
Wild water fowl seen around poultry houses	31/151 (20.5)	40/190 (21.1)	.91

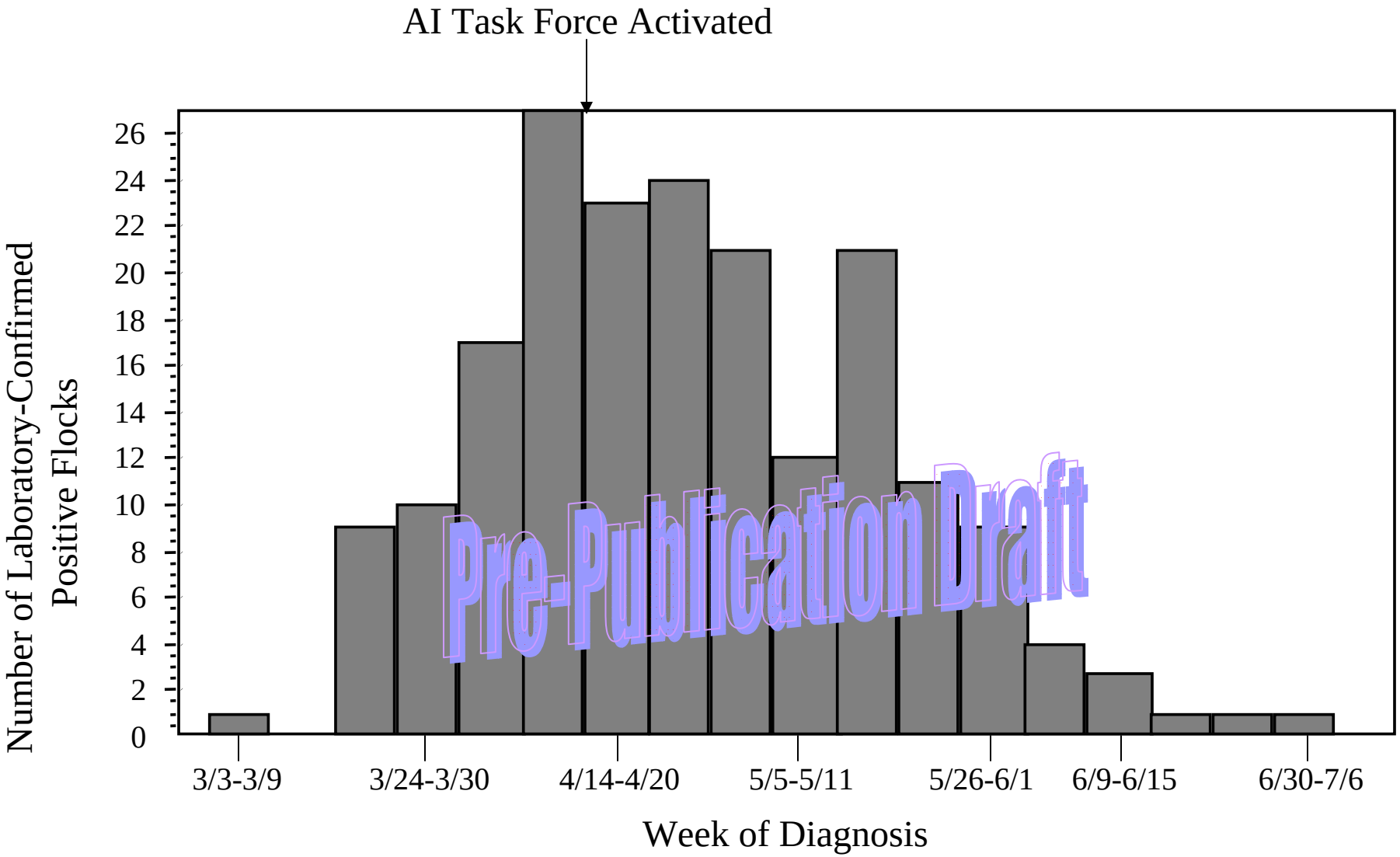
Dead birds disposed of by burial	5/144 (3.5)	2/187 (1.1)	Too few
Dead birds disposed of by incineration	23/145 (15.9)	26/188 (13.8)	.61
Dead birds disposed of by composting	94/147 (63.9)	148/190 (77.9)	.008
Dead birds disposed of by rendering	46/147 (31.3)	17/184 (9.2)	<.001
Frequency of feed truck visit in previous 2 weeks			.05
1 visit	17/106 (16.1)	43/147 (29.2)	
2 visits	49/106 (46.2)	53/147 (36.1)	
3 + visits	40/106 (37.7)	51/147 (34.7)	

Table 3 - Assessment of risk factors for Avian Influenza virus infection in Virginia, 2002; Backward elimination logistic regression model.

Variable	Case Farm (% with factor)	Control Farm (% with factor)	Odds Ratio (95% CI)	p value
Bird age				<.0001
< 10 weeks	25.6	58.4	1	
10-19 weeks	52.8	32.9	4.9 (2.5 – 9.6)	
≥ 20 weeks	21.6	8.7	4.3 (1.7 – 10.9)	
Use of non-family caretakers in poultry houses *	45.7	30.4	2.1 (1.1 – 4.1)	0.04
Owner/family works off-site *	46.7	52.3	2.0 (1.1 – 3.7)	0.03
Raccoons, opossums, foxes seen around poultry houses*	41.3	31.4	1.9	0.04
Disposal of bird carcasses by rendering *	31.3	9.2	7.3 (3.3 – 15.9)	<.0001

* reference level = absence of factor

Figure 1. Outbreak curve for low pathogenic H7N2 avian influenza (AI) virus by week of laboratory confirmation, Virginia, March-October 2002.


AI Task Force Activated
Number of Laboratory-Confirmed Positive Flocks
0
2
4
6
8
10
12
14
16
18
20
22
24
26
3/3-3/9
3/24-3/30
4/14-4/20
5/5-5/11
5/26-6/1
6/9-6/15
6/30-7/6
Week of Diagnosis
Pre-Publication Draft

ATTACHMENT B:

Grimes, Jesse, et al. "Impact of incinerator use on the incidence of infectious diseases in commercial turkey farms." Pre-publication draft sent via e-mail to Fred Porter, U.S. EPA, on March 9, 2004.

Title: Impact of incinerator use on the incidence of infectious diseases in commercial turkey farms.

Authors: Jean-Pierre Vaillancourt

Population Health and Pathobiology, College of Veterinary Medicine

North Carolina State University, 4700 Hillsborough [illegible], Raleigh, NC 27606

David Rives

Prestage Farms, Clinton, North Carolina, **address needed**

Shannon Jennings

Murphy-Browns, **address needed**

Jesse Grimes

Poultry Science, College of Agriculture and Life Sciences

North Carolina State University, 115 Patterson Hall, Raleigh, NC 27695

Key words: Incinerator, poultry mortality, infectious diseases, dead bird disposal

Running title: Incinerators and diseases

Statement of primary audience: flock supervisors and veterinarians

To whom correspondence should be addressed: jesse_grimes@ncsu.edu

SUMMARY

Dead birds can be a source of infectious diseases on commercial poultry farms. The risk is believed to be higher when rendering is used to dispose of the mortality. Rendering trucks may indeed contribute to the spread of pathogens. Therefore, it was hypothesized that using incinerators to destroy dead birds on farms would reduce this risk and would result in a reduction of infectious disease outbreaks. Production and disease data were obtained from two integrated turkey companies located in North Carolina. A total of 3245 flocks were included in this three-year retrospective study (1999-2001). The status of each flock was determined for the presence of turkey coronavirus infection (TCV), mycoplasmosis caused by *Mycoplasma gallisepticum* (MG), and fowl cholera (cholera). Flocks raised on farms without incinerators were about two times more likely to have experienced TCV, MG, or cholera than farms equipped with incinerators ($p<0.01$). This was mainly due to a reduction in the incidence of TCV.

DESCRIPTION OF PROBLEM

Dead birds dying or destroyed on the farm are assumed to be an important source of disease by being a direct source of germs, by attracting insects and wild or domesticated animals that carry disease, or by bringing on the farm potentially contaminated people and trucks hired to dispose of dead birds (CD, Poultry Times paper). However, there is a paucity of publication on this topic in the scientific literature. Renwick et al (1992) in Canada demonstrated that the method of dead bird disposal was associated with water contamination by *Salmonella*. But they compared off-site disposal (rendering and incineration to a common location) to on-site disposal (manure pile, fed to pets, or thrown on field). The on-site disposal was associated with *Salmonella* contamination, but these types of disposal do not correspond to usual methods in commercial poultry production in the United States today. The most frequent forms of dead poultry disposal

on commercial farms are pickup for rendering, incineration, composting, on farm disposal pit, on farm burying , common disposal location. Concerns about the spread of infectious diseases, mainly in regions highly populated with commercial poultry farms, have raised the issue of dead bird disposal, as risk factor potentially associated with the incidence of diseases on farms. The recent series of epidemics in the United States and around the world of reportable diseases, such as avian influenza and Newcastle disease, has heightened these concerns.

The objective of this study was to determine, using field data, whether turkey flocks raised with incinerators as method of dead bird disposal were less likely to be affected by infectious diseases compared to flocks raised on farms not equipped with incinerators. In North Carolina, the alternative method of dead bird disposal was rendering performed by specialized companies collecting birds farm to farm.

MATERIALS AND METHODS

Study population

The study population comprised turkey flocks from two integrated North Carolina companies. Data from 1648 flocks raised on 98 farms were obtained from company A and from 1722 flocks raised on 212 farms from company B. These flocks were processed between January 1999 and December 2001. A flock was defined as a single placement of turkeys, housed on one or more poultry houses.

Outcome variables

Disease and production parameters were considered as outcome variables. Data were available for three diseases of importance in turkey production in North Carolina: turkey coronavirus infection (TCV), fowl cholera (Pasteurellosis), and mycoplasmosis or MG (caused my

Mycoplasma gallisepticum). Each disease was confirmed with diagnostic tests performed by technicians of the North Carolina Department of Agriculture Diagnostic Laboratory Services (TCV ELISA, immunofluorescence; Cholera: bacterial isolation; MG: ELISA, PCR, isolation). Production data included total mortality (percentage difference between birds placed and birds processed); percentage abattoir condemnation (only full carcass condemnation); feed conversion (pounds of feed divided by pounds processed); total medication cost (including vaccine?, antibiotics, what else; presented as cents per pound processed).

Predictor variables

For each flock, data was available on the farm of origin, date flock was placed, number of birds placed, type of birds (toms versus hens), type of operation (brooder, brooder and grow-out, only grow-out; single age (all-in all-out) versus multi-age production).

Incinerators were installed on several farms during the study period. Therefore, the date when they were installed was made available. This allowed to determine if a flock was raised when incinerators were present or not. Data was adjusted according to the following criteria: If a flock was placed over 3 weeks before the arrival of the incinerator, the flock was considered as not having the benefit of the incinerator.

Data analysis

Data were initially entered into database files (Excel, Microsoft corporation) and subsequently converted into Statistix (Analytical Software, PO Box 12185,Tallahassee FL 32317) files for statistical analysis.

Univariate analyses were performed according to the outcome variable. Pearson's chi-square analysis was used for categorical disease data (presence or absence of TCV, or MG, or cholera, or all diseases combined). For continuous variables (mortality, feed conversion, cost, and

condemns), a non-parametric method, the Median test, was used to compare two groups at a time. In order to compare three groups at a time (evaluation of production performances for flocks without diseases, with one disease, or with two diseases), the Kruskal-Wallis One-Way Analysis of Variance test was performed.

Finally, the relative risk of being diseases depending on the predictor variable was measured using odds ratios. An odds ratio of 1 indicates that the risk is the same (e.g., the presence or absence of a predictor variable, or risk factor, yields the same risk). When the odds ratio is above 1 and this is statistically significant ($p<0.05$), then the presence of the factor is said to increase the risk accordingly (i.e., an odds ratio of 2 is interpreted as a two fold increase in risk). When the odds ratio is less than 1 and is also statistically significant, one can say that the presence of the factor reduces the risk (i.e., an odds ratio of 0.5 indicates that the presence of the factor reduces the likelihood of the outcome (e.g., disease) by a factor of about 2).

RESULTS AND DISCUSSION

Company A experienced one of the three diseases included in this study in 11.5% of its flocks. However, these flocks were distributed in almost two thirds of the farms. None of the flocks had more than one of these diseases. A similar proportion of farms under contract with company B were also affected, although more flocks were involved (14.5%). This is mainly because company B had more cases of fowl cholera (48 cases, 3.0%) (Table 1). Contrary to company A, 9 company B flocks experienced two diseases (6 had TCV and cholera; 2 had TCV and MG; 1 had MG and cholera) during the study period.

Although single age production (all-in all-out) is known to reduce the risk of infectious diseases and has been associated with improved production performances (ref), the opposite association was observed in this study (Tables 2, 4, and 5). However, this may be explained by the fact that

farms were not converted at random from multi-age operations to single age. Indeed, both companies gave priority to farms with a history of infectious disease outbreaks and poor production results.

The adjustment performed for the predictor variable “incinerator” (categorization as “without incinerator” any flocks that were at least 3 weeks of age before incinerators were installed) was necessary in order to include data from flocks already in production when incinerators were added to the farm. Any bias created by this adjustment would likely go towards the null hypothesis that there is no difference between flocks raised with and without incinerators. Even so, a reduction in disease incidence was associated with the use of incinerators. Indeed, when all diseases were considered for company A, 8.0% of flocks with incinerators were diseased versus 15.2% of flocks without incinerators ($p<0.0001$). Hence flocks without incinerators were 2.1 times more at risk of being diseased than flocks with incinerators. The difference was not as large for company B (11.7% with incinerators versus 16.9% without incinerators), but it was still statistically significant ($p=0.003$). The analysis considering one disease at a time showed that the difference was essentially due to TCV (Table 2), a highly transmissible viral disease. There was no statistically significant difference for MG (Table 3) or cholera (Table 4).

The association between hen flocks and TCV or cholera for company B (Tables 2 and 4) might have biased the result obtained when comparing the presence or absence of incinerators on farms because more hen flocks were raised without the benefit of incinerators for this company (57.4% versus 42.6% hen flocks with incinerators). However, this was not the case for company A and yet the same relationship was observed between the presence of incinerators and the incidence of diseases (i.e., significant reduction).

Based on a univariate analysis, production parameters were not improved by the usage of incinerators (Table 5). However, the reduction in TCV cases associated with incinerators represents a valuable benefit. Turkey coronavirus is known to act as primary agent, increasing the clinical impact of secondary invaders like *Escherichia coli* and *pasteurella multocida* (fowl cholera). This is illustrated in Table 6, where all production parameters were significantly more elevated when two diseases were recorded in the same flock compared to one or none ($p<0.0001$).

CONCLUSIONS AND APPLICATIONS

The field evidence obtained with this retrospective study supports the hypothesis that using incinerators to dispose of dead birds during production reduces the risk of infectious diseases on these farms. Other on-farm mortality disposal methods may produce similar results, but such methods are not widely used in North Carolina and, therefore, were not included in this study. It is noteworthy that the main disease reduction was observed with TCV. This disease is highly contagious and is known to increase the negative impact of secondary pathogens (Guy's paper as reference). Therefore, this should be a consideration when calculating the cost-benefit of incinerator use on farms.

REFERENCES AND NOTES

Table 1: Description of flocks raised between January 1999 and December 2001 on farms under contract with two integrated turkey companies in North Carolina.

	Company A	Company B
Number of farms	98	206
Number of flocks in the study	1648	1597
Number of flocks by sex (%) Toms Hens	 931 (56.5%) 717 (43.5%)	 1098 (68.8%) 499 (31.2%)
Percentage of flocks raised on single age farms (all-in all-out production)	124 (7.5%)	453 (28.4%)
Number (%) of flocks raised with an incinerator on the farm	840 (51%)	912 (57.1%)
Number (%) of farms affected by at least one disease	62 (63.3%)	121 (58.7%)
Number (%) of cases of turkey coronavirus infection	168 (10.2%)	163 (10.2%)
Number (%) of cases of Fowl Cholera	7 (0.4%)	48 (3.0%)
Number (%) of cases of mycoplasmosis (*M. gallisepticum*)	15 (0.9%)	21 (1.3%)
Number (%) of flocks affected by more than one disease (data available only for the 3 diseases reported in this study)	0 (0%)	9 (4.4%)

175 Table 2: incidence of turkey coronavirus infection (TCV) (number of flocks and percentage) depending on type of production, and presence or

176 absence of on-farm incineration as method of dead bird disposal.

	Company A				Company B			
	TCV positive	TCV negative	Odds Ratio	p-value	TCV positive	TCV negative	Odds Ratio	p-value
Type of bird								
Toms	93 (10.0)	838	0.95	0.754	97 (8.7)	1002	0.624	0.005
Hens	75 (10.5)	642			67 (13.4)	432		
Type of production								
Brooder/grow-out	164 (10.2)	1448	0.91	0.854	N/A[B]	N/A	N/A	N/A
Grow-out	4 (11.1)	32						
Farm management								
Single age[A]	22 (17.7)	102	2.0	0.0039	53 (11.7)	400	1.25	0.249
Multi-age	146 (9.6)	1378			110 (9.6)	1034		
Incinerator present	57 (6.8)	783			73 (8.0)	839		
Incinerator absent	111 (13.7)	697	0.48	<0.0001	90 (13.1)	595	0.58	0.0008

177 [A] Single age: All-in all-out production

178 [B] N/A = not available

179 Table 3: incidence of mycoplasmosis (MG) (number of flocks and percentage) depending on type of production, and presence or absence of on-

180 farm incineration as method of dead bird disposal.

	Company A				Company B			
	MG positive	MG negative	Odds Ratio	p-value	MG positive	MG negative	Odds Ratio	p-value
Type of bird								
Toms	14 (1.5)	917	10.93	0.0038	19 (1.7)	1079	4.38	0.031
Hens	1 (0.1)	716			2 (0.4)	497		
Type of production								
Brooder/grow-out	15 (0.9)	1597	_[C]	0.561	N/A[B]	N/A	N/A	N/A
Grow-out	0 (0.0)	36						
Farm management								
Single age[A]	1 (0.8)	123	0.88	0.899	5 (1.1)	448	0.79	0.641
Multi-age	14 (0.9)	1510			16 (1.4)	1128		
Incinerator present	6 (0.7)	834			16 (1.8)	896		
Incinerator absent	9 (1.1)	799	0.64	0.393	5 (0.7)	680	2.43	0.075

181 [A] Single age: All-in all-out production

182 [B] N/A = not available; [C] Odds ratio cannot be calculated because one of the cell (MG positive/grow-out) equals 0

Table 4: incidence of fowl cholera (number of flocks and percentage) depending on type of production, and presence or absence of on-farm incineration as method of dead bird disposal.

	Company A				Company B			
	Cholera positive	Cholera negative	Odds Ratio	p-value	Cholera positive	Cholera negative	Odds Ratio	p-value
Type of bird								
Toms	3 (0.3)	928	0.58	0.466	45 (4.1)	1053	7.07	0.0001
Hens	4 (0.6)	713			3 (0.6)	496		
Type of production								
Brooder/grow-out	7 (0.4)	1605	- [C]	0.692	N/A [B]	N/A	N/A	N/A
Grow-out	0 (0.0)	36						
Farm management								
Single age [A]	3 (2.4)	121	9.42	0.0004	24 (5.3)	429	2.61	0.0007
Multi-age	4 (0.3)	1520			24 (2.1)	1120		
Incinerator present	4 (0.5)	836			22 (2.4)	890		
Incinerator absent	3 (0.4)	805	1.28	0.743	26 (3.8)	659	0.63	0.109

[A] Single age: All-in all-out production

[B] N/A = not available; [C] Odds ratio cannot be calculated because one of the cell (MG positive/grow-out) equals 0

187 Table 5: Median[A] production performance depending on type of production, and presence or absence of on-farm incineration as method of dead

188 bird disposal.

	Company A				Company B			
	Mortality %	Condemns %	Feed conversion	Medication cost ($/lb)	Mortality %	Condemns %	Feed conversion	Medication cost (cent/lb)
Type of bird								
Toms	10.92	2.71	1.70	0.19	10.06	2.31	2.55	0.26
Hens	5.29	1.04	2.20	0.25	3.74	1.20	2.11	0.29
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.004
Type of production								
Brooder & grow-out	8.70	1.96	2.00	0.21				
Grow-out	10.39	2.37	1.72	0.19	N/A	N/A	N/A	N/A
p-value	0.0002	0.007	<0.0001	0.768				
Farm management								
Single age	6.91	1.32	2.17	0.25	11.19	2.50	2.65	0.24
Multi-age	8.89	2.07	1.95	0.21	4.50	1.38	2.20	0.29
p-value	0.0015	<0.0001	<0.0001	0.039	<0.0001	<0.0001	<0.0001	<0.0001
Incinerator present	8.62	1.77	2.03	0.21	8.83	1.84	2.43	0.28
Incinerator absent	8.91	2.30	1.93	0.21	8.00	1.85	2.44	0.27
p-value	0.208	<0.0001	0.061	0.826	0.018	0.891	0.800	0.300

189 [A] The median value (50th percentile) is presented because the data are not normally distributed; the Median Test was used for comparisons

190 Table 6: Production performances depending on the number of diseases diagnoses in a turkey flock (company B)[A].

	Mortality (%)		Condemns (%)		Feed conversion		Medication cost (cent/lb)	
	Mean	Median	Mean	Median	Mean	Median	Mean	Median
No disease (1374 flocks)	7.81	7.73 [a]	1.73	1.76 [a]	1.99	2.39 [a]	0.29	0.26 [a]
1 disease (214 flocks)	10.56	10.22 [a]	2.70	2.44 [a]	2.46	2.52 [b]	0.56	0.37 [b]
2 diseases (9 flocks)	15.48	12.64 [b]	3.87	3.26 [b]	2.55	2.60 [c]	1.05	1.31 [c]

191 [A] A difference in lower case letter (a, b, c) indicates a statistically significant difference at $p<0.05$ between the variables being compared