

Identification of optimal sample collection devices and sampling locations for the detection of environmental viral contamination in wire poultry cages

Jongseo Mo | Erica Spackman  | Christopher B. Stephens

Southeast Poultry Research Laboratory, US National Poultry Research Center, USDA-ARS, Athens, Georgia, USA

Correspondence

Erica Spackman, Southeast Poultry Research Laboratory, US National Poultry Research Center, USDA-ARS, Athens, GA.
Email: Erica.spackman@usda.gov

Present address

Christopher B. Stephens, Boehringer Ingelheim Animal Health, Athens, Georgia, USA

Funding information

US Department of Agriculture, Agricultural Research Service, Grant/Award Number: 6040-32000-066-D; US Department of Agriculture, Animal and Plant Health Inspection Service, Grant/Award Number: 60-6040-6-005

Abstract

Environmental testing of poultry premises after an outbreak of an infectious disease like avian influenza (AI) or Newcastle disease is essential to promptly verify virus-free status and subsequently return to normal operations. In an attempt to establish an optimized sampling protocol, a laboratory study simulating an AI virus-contaminated poultry house with wire layer cages was conducted. Three sample collection devices, pre-moistened cotton gauze, dry cotton gauze and a foam swab, were evaluated with each of four sample locations within a cage and when sampling all four locations with one device. Virus was detected with quantitative real-time RT-PCR utilizing a standard curve of a quantified homologous isolate of AI virus to determine titre equivalents of virus. The pre-moistened gauze detected the most virus RNA (100% positive, geometric mean titre [GMT): 3.2 log₁₀ 50% embryo infectious doses [EID₅₀] equivalents per 25 cm²) in all four sample locations compared to dry gauze (93% positive, GMT: 2.6 EID₅₀ equivalents per 25 cm²) and foam swabs (95% positive, GMT: 2.8 log₁₀ EID₅₀ equivalents per 25 cm²). The highest viral RNA loads were observed from the cage floor, and when the four locations were sampled with the same device. Overall, the pre-moistened gauze performed the best, and sampling multiple locations within a cage with the same device would likely optimize detection of residual virus.

KEYWORDS

avian influenza, environmental sampling, layer cage, Newcastle disease, virus, wire cage

1 | INTRODUCTION

During recovery from an outbreak of avian influenza virus (AIV) or Newcastle disease virus (NDV) in poultry, ensuring that the infected premises are free of residual virus is critical for releasing a quarantine and restocking. Infected birds can shed high amounts of virus into the environment, thus eliminating residual virus on surfaces in poultry houses constitutes a critical factor in infection control (Dent, Kao, Kiss, Hyder, & Arnold, 2008; Tiwari, Patnayak, Chander, Parsad,

& Goyal, 2006). Surface sampling of the environment is also used to evaluate viral contamination and the efficacy of proper disinfection procedures after an outbreak (Rodriguez-Lazaro et al., 2012; Rose, Noble-Wang, & Arduino, 2016) and may also be utilized as an adjunct to testing animals when attempting to identify infected premises.

Currently, there is not a uniform approach to testing wire cages, which are a challenge because of the minimal surface area and the wire tends to damage collection devices that work well on other surfaces. Wire cages are most commonly used for

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Published 2020. This article is a U.S. Government work and is in the public domain in the USA. *Transboundary and Emerging Diseases* published by Blackwell Verlag GmbH

poultry housing worldwide (Leenstra, Ten Napel, Visscher, & Van Sambeek, 2016) and are utilized in wet markets; therefore, optimization of collection procedures can improve the likelihood of detecting the virus in the environment, as more sensitive methods and tests will yield higher viral loads with the same level of residual virus. Using proper and sensitive collection devices is crucial as low viral concentrations paired with heterogeneous distribution of virus in the environment may complicate the detection of the target virus (Scherer et al., 2009). Establishing an optimized and validated sampling protocol to ensure the absence of virus in such environments after an outbreak is essential for reestablishment of normal business operations in an affected region and minimizing economic implications.

The objective of this study was to compare sampling devices and to determine the optimal locations for virus detection on environmental surfaces in wire cages to provide general guidance and standardized procedures for environmental sample collection and testing for residual AIV or NDV after an outbreak. These data should also contribute to models to determining the optimal number of samples to collect within the goals of a given situation, and to provide information on where the virus would likely settle, for facilitating cleaning and disinfection (C&D) processes. For that purpose, a laboratory-based, animal trial with low pathogenic (LP) AIV was conducted to simulate the environment of poultry houses containing infected birds to evaluate sampling devices and sampling locations.

2 | MATERIAL AND METHODS

2.1 | Viruses

The A/chicken/Egypt/F12170A/2016 H9N2 LPAIV and A/chicken/Coahuila (Mexico)/IA20/2011 H5N2 LPAIV isolates were obtained from the repository at the Southeast Poultry Research Laboratory (SEPR), US National Poultry Research Center (USNPRC), USDA-Agricultural Research Service. Samples were collected during the course of other scheduled pathobiology studies with each of these viruses. Both viruses were propagated and titrated in embryonating chicken eggs as per standard methods (Spackman & Stephens, 2016).

2.2 | Chickens

All procedures involving animals were reviewed and approved by the US National Poultry Research Center institutional animal care and use committee. Specific pathogen-free White Plymouth Rock hens in production ($n = 32$) were housed in a group of 2 per metal wire 3-tier layer cage (Murray McMurray Hatchery, model 5PLC). Cages were equipped with a common nipple drinker line for each tier (Ziggity Systems Inc.). The hens were 22 weeks old at the start of the experiment with the H9N2 LPAIV and 29 weeks old at the start of the experiment with the H5N2 LPAIV. A dose of LPAIV containing 10^6 50 per cent embryo infectious doses (EID₅₀) per bird in 0.1 ml was

inoculated by the intratracheal route using an oral gavage needle. The inoculum was diluted with brain heart infusion (BHI) media. Hens had ad libitum access to feed and water for the duration of the experiment. The same lighting programme to which the hens were already acclimated to was used. Each hen was tagged for identification.

2.3 | Sampling devices and location

Samples were collected from each of 15 cages containing infected birds at 48, 72 and 96 hr post-challenge, which is the peak of virus shedding. Prior to challenge, a set of negative samples were collected from each hen occupied cage ($n = 15$ per collection device/location combination) to confirm specificity.

The following sample collection devices were used: (a) foam swabs (Puritan 25-1607-1PF); (b) pre-moistened 10.2 cm × 10.2 cm (4" × 4") cotton gauze pads; and (c) dry 10.2 cm × 10.2 cm cotton gauze pads. Four locations around the cage were defined as sampling areas: (a) the floor of each cage; (b) the wall at chicken head and neck level; (c) the egg trough; and (d) the top of the nipple drinker line. During the first experiment with the H9N2, each location was sampled separately with each device (45 samples per device/location combination; each of 15 cages sampled on each of 3 days). During the second experiment with the H5N2, all four sample locations were tested with the same device (45 total samples per device: 3 total samples per each of the 15 cages for each of the three sample days). All collection devices were firmly rubbed multiple times across the length and breadth of the target area (approximately 25 cm² which was about 3 squares × 5 squares in the cage wire grid) in an attempt to collect as much material as possible from a uniformly sized area. The top of the drinker line, which was outside the cage, was swabbed in about a 25-cm² area. Sterile BHI media, which has been documented to preserve AIV and NDV live virus and RNA for diagnostic testing (Erickson et al., 1978; Spackman, Pedersen, McKinley, & Gelb, 2013), were used for eluting and transporting the samples to maximize virus recovery. Dry gauze was directly applied to target areas, and contents were eluted in BHI media after sampling. The material on the gauze was eluted by putting the gauze in a sealable plastic bag and soaking it with 5 ml of BHI media. The gauze was then manually squeezed (similar to stomaching) inside the bag for 5 s, the corner of the bag was then cut-off, and the media were decanted into sterile tubes. A new plastic bag was used for each sample, and the scissors were decontaminated by wiping with 70% ethanol between samples. Gloves were only changed if gross contamination occurred. Pre-moistened gauze was wet with 5 ml BHI media before sampling and went through the same elution process in the plastic bag after sample collection without adding additional media. A volume of 1-2 ml could routinely be recovered from the cotton gauze. The foam swabs were directly applied for sampling in a dry state. After sampling, swabs were swirled in tubes filled with 3 ml of BHI media to release the material. The amount of collection media used for gauze (5 ml) was greater than swabs (3 ml) to account for the media loss from absorption by the gauze and hold-up in the bag. Because the total volume which could be recovered from gauze

varied, which is inherent to this procedure where the aim is to maximize sensitivity by using small volumes of the medium, and because RNA is extracted from a portion of that material, the concentration of analyte is the critical factor rather than the final volume; therefore, we report results by approximate sample collection areas (25 cm²). The swabs were removed by squeezing the swab against the side of the tube to express as much media as possible. Samples were transported on wet-ice and were stored at -80°C until they were processed for real-time reverse transcriptase-polymerase chain reaction (qRT-PCR).

2.4 | Sample processing and qRT-PCR

RNA was extracted from a total of 45 samples for each combination of collection device and sample location using the MagMax96 Viral RNA Isolation Kit System (Thermo Fisher Scientific) and the KingFisher Flex Magnetic Particle Processing System (Thermo Fisher Scientific). The manufacturer's instruction was followed with the addition of an additional wash step to remove inhibitors (Das, Spackman, Pantin-Jackwood, & Suarez, 2009). qRT-PCR was performed based on the standard USDA M gene AIV qRT-PCR procedure (Spackman et al., 2002) using an Applied Biosystems® 7,500 Fast Real-Time PCR system (Thermo Fisher Scientific). Cycle threshold (C_t) values were calculated by the 7,500 Fast Software v2.3 (Applied Biosystems). For relative quantification, cycle threshold (C_t) values obtained from qRT-PCR were converted to titre equivalents based on the titre of the viruses, each of which was used as qRT-PCR standards for the appropriate samples. The limit of detection (LOD) or the cut-off value was determined based on the endpoint when the qRT-PCR assay did not detect any signal in the RNA from serially diluted standard viruses.

2.5 | Statistical analysis

The Mann-Whitney test was used to compare the viral RNA loads that were detected in the samples, and the proportion of positive samples was compared with the Fisher exact test (Prism 8 version 8.2.1, GraphPad Software). The qRT-PCR detection limit was 0.7 log₁₀ EID₅₀ equivalents per 25 cm²; therefore, all samples where virus was not detected were given an imputed titre of 50% of the LOD (0.4 log₁₀ EID₅₀ equivalents per 25 cm²) (Cohen, 1959).

3 | RESULTS

3.1 | Comparison of collection devices

Quantitative RT-PCR assay was used to detect the presence of viral RNA recovered by each device to compare the sensitivity among sampling devices (Table 1, Figure 1). Data from all 3 days were compiled for each sampling device. No viral RNA was detected from samples collected prior to challenge (data not shown). Overall, the pre-moistened gauze had the highest positivity rate (99%) and detected the most virus RNA (geometric mean titre [GMT]: 3.2 log₁₀ EID₅₀ equivalents per 25 cm²) from each of the four sample locations compared to dry gauze (93% positive, GMT of 2.6 EID₅₀ equivalents per 25 cm²) and foam swabs (95% positive, GMT of 2.8 log₁₀ EID₅₀ equivalents per 25 cm²). Pairwise comparisons between the collection devices showed that the pre-moistened gauze recovered significantly more viral RNA than the dry gauze in each of the sample locations, and for the area on top of the drinker line compared to the foam swab (*p* = .0079) (Table 1). Viral RNA recovery was not significantly different between sampling devices for other comparisons. There was only one situation where the proportion of positive samples was significantly different between two devices; significantly more (*p* = .0262) samples collected with pre-moistened gauze were positive than samples collected with the foam swab from the drinker line.

3.2 | Comparison of viral RNA loads between sample locations

To identify the target area where the virus is most likely to be detected in layer cages, viral RNA loads detected on top of the drinker line, cage head level, cage floor and egg tray were compared (Figure 2). Higher viral RNA loads were observed on the cage floor, along with a higher proportion of positive samples (0.99) compared to other areas; however, the pairwise comparison of viral RNA loads between locations was not significant (data not shown). The proportion of samples that were positive in each area was not significantly different.

Environmental samples from a second experiment collected with the same device for all four sample locations were statistically

TABLE 1 Proportion of positive samples and geometric mean of viral RNA (expressed as titre equivalents) recovered by sample collection devices and location

Collection device	Location of sample collection				All locations with one device
	Drinker line	Cage wall head level	Cage floor	Egg trough	
Dry cotton gauze	42/45 ^a (2.5) ^b	41/45 (2.4)	44/45 (2.7)	40/45 (2.3)	44/45 (2.0)
Pre-moistened cotton gauze	45/45 (3.0)	45/45 (3.0)	45/45 (3.5)	43/45 (2.8)	45/45 (3.0)
Foam swab	39/45 (2.3)	43/45 (2.6)	44/45 (3.1)	44/45 (2.6)	42/45 (2.0)

^a# of positive samples/# of total samples.

^bGeometric mean of titre equivalents detected (log₁₀ 50% embryo infectious dose) per 25 cm².

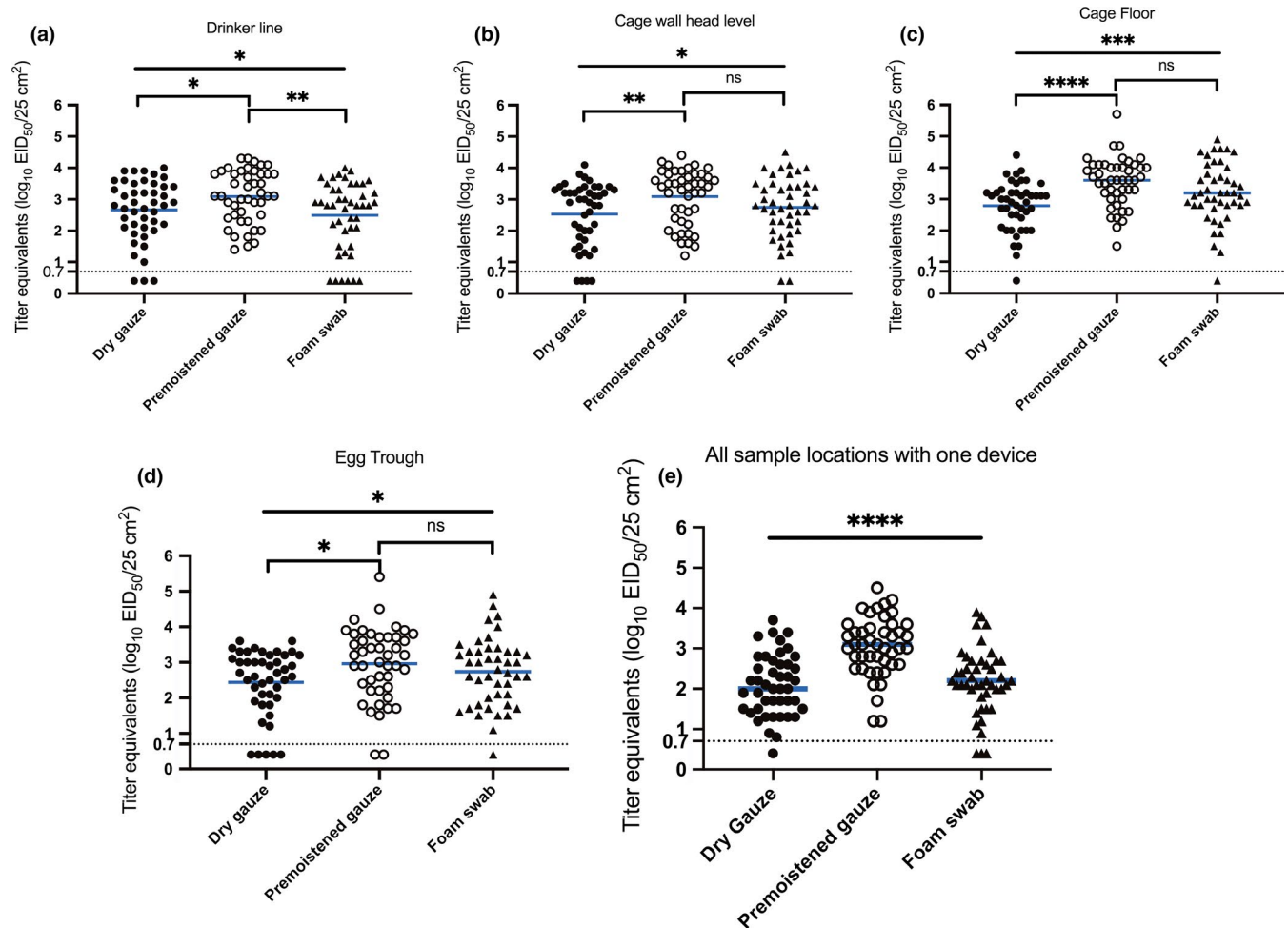


FIGURE 1 Detection of virus RNA by collection device. The titre equivalent for each sample is shown; the blue bar shows the mean of the titre equivalent. The limit of detection was $0.7 \log_{10}$ 50% embryo infectious dose (EID₅₀) equivalents per 25 cm²; therefore, the titre for the samples, which were not detected, was set to $0.4 \log_{10}$ EID₅₀ equivalents per 25 cm² as per the method of Cohen (1959). *p*-values for pairwise comparisons with the Mann–Whitney test are as follows: ns = not significant, * *p* < .05, ** *p* < .01, *** *p* < .001, **** *p* < .0001

analysed, and viral RNA loads were compared (Table 1, Figure 2). Similar to the results with the first experiment where cage areas were sampled separately, the pre-moistened gauze recovered the most viral RNA ($3.1 \log_{10}$ EID₅₀ equivalents per 25 cm²) and 100% of the samples were positive compared to dry gauze ($2.1 \log_{10}$ EID₅₀ equivalents per 25 cm², 98% positive) and foam swabs ($2.2 \log_{10}$ EID₅₀ equivalents per 25 cm², 94% positive). While the viral RNA loads in the samples collected from the pre-moistened gauze were significantly higher than other devices, the statistical difference in viral RNA loads detected between samples collected with dry gauze and foam swab was non-significant (Table 1). The proportion of samples that were positive did not differ significantly among the sample collection devices.

4 | DISCUSSION

Environmental testing of poultry premises, which utilize wire caging, after an outbreak, whether post-C&D or after a fallow period, is essential to promptly verify virus-free status to help release quarantines in a timely manner to minimize the economic impact by re-establishing product

movements and restocking. Premises, including caging, may also be tested as an adjunct to infection surveillance and monitoring programmes. Thus, establishing a reliable testing method that provides a high level of confidence of virus detection on the infected premises is important.

The type of device and media used for sample collection can influence the sensitivity and proportion of positive samples of sampling methods (Julian, Tamayo, Leckie, & Boehm, 2011). In this study, we simulated an AI-infected poultry house, and three devices and four sampling locations in wire cages were directly compared pairwise to determine the sensitivity of viral RNA detection. Overall, the pre-moistened gauze method performed the best regarding detection sensitivity (i.e. the amount of virus RNA recovered), which correlated with producing the highest proportion of positives. Importantly, although we used BHI media, numerous other viral transport media are available and have been verified to work well with viruses, including influenza and NDV.

The high overall sensitivity of the pre-moistened gauze method seems likely to be due to the pre-moistening process, in which the BHI media may have worked as an eluent that helped detach adhered viruses from the surface, resulting in higher yield. This is

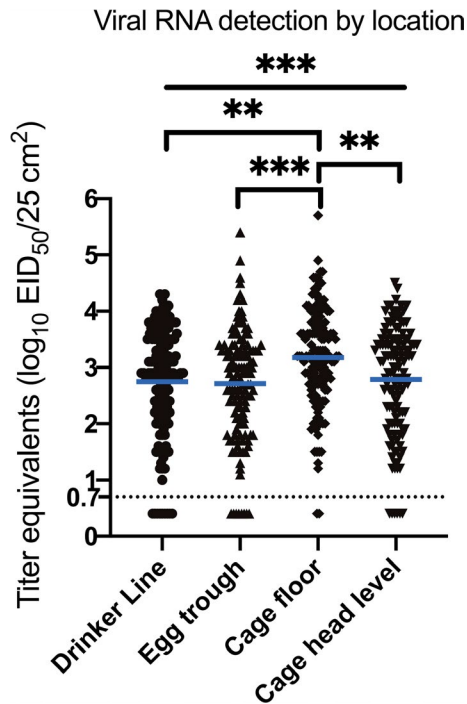


FIGURE 2 Detection of virus RNA by collection location. The titre equivalent for each sample is shown; the blue bar shows the mean of the titre equivalent. The limit of detection was $0.7 \log_{10}$ 50% embryo infectious dose (EID₅₀) equivalents per 25 cm²; therefore, the titre for the samples, which were not detected, was set to $0.4 \log_{10}$ EID₅₀ equivalents per 25 cm² as per the method of Cohen (1959). *p*-values for pairwise comparisons with the Mann–Whitney test are as follows: ***p* < .01, ****p* < .001

not surprising as some studies demonstrated that transfer of viruses from contaminated surfaces was higher under wet conditions (Ansari, Sattar, Springthorpe, Wells, & Tostowaryk, 1988; D'Souza et al., 2006; Hall, Douglas Jr, & Geiman, 1980). In contrast, pre-moistening can saturate the collection device, which can reduce detection, which is why testing-specific conditions are important. Foam swab sampling, which was evaluated as a collection method due to its wide usage in the field and ease of application, was not as efficient as the pre-moistened gauze in yielding virus recovery. One of the reasons behind this may be owing to the smaller contact area of the tip. Contributions to differences in virus recovery might also include the efficiency of the different elution methods between the gauze and foam swabs, in which the eluted virus was more readily released when they were 'squeezed out' of the gauze, compared to post-collection dipping and stirring to rinse the swabs.

When comparing viral RNA loads detected at each sampling location, higher virus recovery from the cage floor suggests that it may be the primary site of deposition/settling of the virus within the wire cage. One possibility is that the cage floor is the main site of deposition for large airborne virus droplets (Morawska, 2006), and especially for viruses shedding from the cloaca through defecation and egg lay. This is not surprising as it has been reported in various studies that AIV and NDV are consistently excreted by both faecal and respiratory secretions (Kapczynski & King, 2005;

Lu & Castro, 2004; Miller, King, Afonso, & Suarez, 2007; Okamatsu et al., 2007; Swayne, Perdue, Garcia, Rivera-Cruz, & Brugh, 1997), in which viral loads were higher in the faeces in some occasions (Slemons & Swayne, 1990; Swayne et al., 1997).

One of the goals of this study was to provide data that can be utilized to calculate the optimal numbers of samples to collect to make the process less laborious and to reduce costs. Sampling multiple locations with the same collection device was viable and would likely help reduce the number of individual samples to save time and resources, and yet obtain valid results. The concern with collection large sample areas with one device is that the device could saturate and re-deposit material back onto the sample surface, which results in lower sensitivity.

Although in this study the cage floor seemed to be the primary deposition site for viruses, it may not be the same for some strains that mainly shed through the respiratory route, as reported in some studies (Horimoto et al., 1995; Swayne & Beck, 2005). Also, shedding patterns or routes of AIV and NDV could differ depending on the strain and host immune status (Kapczynski & King, 2005; Spackman, 2009; Spickler, Trampel, & Roth, 2008). Therefore, a protocol for sampling multiple locations with one device can efficiently accommodate different shedding patterns.

Although most of our samples were positive and the levels of virus detected were moderate, wire cages are not expected to be the best location to target in most situations. Lopez et al. (2018) reported a study where commercial table egg layer cages were tested during an outbreak of H5N2 highly pathogenic avian influenza in the United States in 2015 had a relatively poor viral RNA detection rate (4 positive cages out of 18 samples) versus other locations in the facilities tested. The detection rate was likely higher in our study for numerous reasons. First, the sample handling methods differed (i.e. Lopez et al., transported the gauze dry, which has been shown to decrease virus detection by qRT-PCR and virus isolation (Spackman, Pedersen, McKinley, & Gelb, 2013), and a larger volume of medium was used to elute the sample). The use of experimentally inoculated birds was probably also a major contributing factor. Importantly, wire cages are commonly used to house poultry worldwide and there are cases where sampling them is appropriate even when they may not universally be the best sample area (e.g. cages where infected birds were housed).

The cages were sampled without any cleaning treatment for several reasons. First, untreated cages may be targeted for testing in numerous situations, for example on premises which were allowed to lie fallow post-outbreak or with monitoring programmes with vaccinated birds. Also, positive samples are needed to compare the sample collection devices accurately; cleaning the cages could result in too few positive samples for meaningful comparison. The goal was to determine the best procedures to detect virus because the collection devices are the most sensitive and the areas where virus is likely to be because it is where the highest concentrations are deposited can be targeted, regardless of cleaning procedures. There may also be some variation among specific caging and facilities, but virus will likely be found where dust, oral secretions and faecal matter can settle and accumulate.

In this study, we evaluated collection devices combined with specific target sampling areas in wire cages to provide a practical environmental sampling method to improve viral disease detection and define putative virus accumulation sites for efficient testing. For this study, we used qRT-PCR for virus detection. In a field situation, qRT-PCR can be used to screen samples, but positive samples would likely be tested by virus isolation to determine whether the virus was viable. Detecting viruses in a post-C&D environment is crucial for preventing potential distribution of infectious agents through fomites such as contaminated surfaces, as they can be the starting point of viral transmission to non-affected neighbouring premises, which would be densely populated with susceptible hosts (Boone & Gerba, 2007; Springthorpe & Sattar, 1990). Secondary fomite transmission between poultry farms is likely attributed to residue viruses in the environment (Alexander, 1995; Dent et al., 2008; Tiwari et al., 2006).

ACKNOWLEDGMENTS

We gratefully acknowledge Scott Lee, Jesse Gallagher, Roger Brock, Anne Hurley-Bacon and Melinda Vonkumthong. This research was supported by USDA-ARS project 6040-32000-066-00D and USDA-APHIS Agreement #60-6040-6-005. This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). ORISE is managed by ORAU under DOE contract number DE-SC0014664. Mention of trade names or commercial products does not imply recommendation or endorsement by the USDA. All opinions expressed in this paper are the authors and do not necessarily reflect the policies and views of USDA, ARS, DOE or ORAU/ORISE. The USDA is an equal opportunity provider and employer.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received. All research involving animals was reviewed and approved by the Institutional Animal Care and Use Committee of the US National Poultry Research Center and adhered to the Federation of Animal Science Societies 'Guide for the Care and Use of Agricultural Animals in Research and Teaching'.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request to the corresponding author.

ORCID

Erica Spackman  <https://orcid.org/0000-0002-4935-2360>

REFERENCES

- Alexander, D. (1995). The epidemiology and control of avian influenza and Newcastle disease. *Journal of Comparative Pathology*, 112(2), 105–126. [https://doi.org/10.1016/S0021-9975\(05\)80054-4](https://doi.org/10.1016/S0021-9975(05)80054-4)
- Ansari, S., Sattar, S., Springthorpe, V., Wells, G., & Tostowaryk, W. (1988). Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *Journal of Clinical Microbiology*, 26(8), 1513–1518. <https://doi.org/10.1128/JCM.26.8.1513-1518.1988>
- Boone, S. A., & Gerba, C. P. (2007). Significance of fomites in the spread of respiratory and enteric viral disease. *Applied and Environment Microbiology*, 73(6), 1687–1696. <https://doi.org/10.1128/AEM.02051-06>
- Cohen, A. C. (1959). Simplified estimators for the normal distribution when samples are singly censored or truncated. *Technometrics*, 1(3), 217–237. <https://doi.org/10.1080/00401706.1959.10489859>
- Das, A., Spackman, E., Pantin-Jackwood, M. J., & Suarez, D. L. (2009). Removal of real-time reverse transcription polymerase chain reaction (RT-PCR) inhibitors associated with cloacal swab samples and tissues for improved diagnosis of Avian influenza virus by RT-PCR. *Journal of Veterinary Diagnostic Investigation*, 21(6), 771–778. <https://doi.org/10.1177/104063870902100603>
- Dent, J. E., Kao, R. R., Kiss, I. Z., Hyder, K., & Arnold, M. (2008). Contact structures in the poultry industry in Great Britain: Exploring transmission routes for a potential avian influenza virus epidemic. *BMC Veterinary Research*, 4(1), 27. <https://doi.org/10.1186/1746-6148-4-27>
- D'Souza, D. H., Sair, A., Williams, K., Papafragkou, E., Jean, J., Moore, C., & Jaykus, L. (2006). Persistence of caliciviruses on environmental surfaces and their transfer to food. *International Journal of Food Microbiology*, 108(1), 84–91. <https://doi.org/10.1016/j.ijfoo.2005.10.024>
- Erickson, G. A., Brugh, M., & Beard, C. W. (1978). Newcastle Disease and Avian Influenza Virus Stability Under Simulated Shipping Conditions. Paper presented at the Twenty-first Annual Meeting of American Association of Veterinary Laboratory Diagnosticians. Buffalo, NY: United States Animal Health Association. <https://www.usaha.org/proceedings>
- Hall, C. B., Douglas, R. G. Jr, & Geiman, J. M. (1980). Possible transmission by fomites of respiratory syncytial virus. *Journal of Infectious Diseases*, 141(1), 98–102. <https://doi.org/10.1093/infdis/141.1.98>
- Horimoto, T., Rivera, E., Pearson, J., Senne, D., Krauss, S., Kawaoka, Y., & Webster, R. (1995). Origin and molecular changes associated with emergence of a highly pathogenic H5N2 influenza virus in Mexico. *Virology*, 213(1), 223–230. <https://doi.org/10.1006/viro.1995.1562>
- Julian, T. R., Tamayo, F. J., Leckie, J. O., & Boehm, A. B. (2011). Comparison of surface sampling methods for virus recovery from fomites. *Applied and Environment Microbiology*, 77(19), 6918–6925. <https://doi.org/10.1128/AEM.05709-11>
- Kapczynski, D. R., & King, D. J. (2005). Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine*, 23(26), 3424–3433. <https://doi.org/10.1016/j.vaccine.2005.01.140>
- Leenstra, F., Ten Napel, J., Visscher, J., & Van Sambeek, F. (2016). Layer breeding programmes in changing production environments: A historic perspective. *World's Poultry Science Journal*, 72(1), 21–36. <https://doi.org/10.1017/S0043933915002743>
- Lopez, K. M., Nezworski, J., Rendahl, A., Culhane, M., Flores-Figueroa, C., Muñoz-Aguayo, J., ... Cardona, C. J. (2018). Environmental sampling survey of H5N2 highly pathogenic avian influenza-infected layer chicken farms in Minnesota and Iowa. *Avian Diseases*, 62(4), 373–380. <https://doi.org/10.1637/11891-050418-Reg.1>
- Lu, H., & Castro, A. E. (2004). Evaluation of the infectivity, length of infection, and immune response of a low-pathogenicity H7N2 avian

- influenza virus in specific-pathogen-free chickens. *Avian Diseases*, 48(2), 263–270. <https://doi.org/10.1637/7064>
- Miller, P. J., King, D. J., Afonso, C. L., & Suarez, D. L. (2007). Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. *Vaccine*, 25(41), 7238–7246. <https://doi.org/10.1016/j.vaccine.2007.07.017>
- Morawska, L. (2006). Droplet fate in indoor environments, or can we prevent the spread of infection? *Indoor Air*, 16(5), 335–347. <https://doi.org/10.1111/j.1600-0668.2006.00432.x>
- Okamatsu, M., Saito, T., Yamamoto, Y. U., Mase, M., Tsuduku, S., Nakamura, K., ... Yamaguchi, S. (2007). Low pathogenicity H5N2 avian influenza outbreak in Japan during the 2005–2006. *Veterinary Microbiology*, 124(1–2), 35–46. <https://doi.org/10.1016/j.vetmic.2007.04.025>
- Rodríguez-Lázaro, D., Cook, N., Ruggeri, F. M., Sellwood, J., Nasser, A., Nascimento, M. S. J., ... van der Poel, W. H. M. (2012). Virus hazards from food, water and other contaminated environments. *FEMS Microbiology Reviews*, 36(4), 786–814. <https://doi.org/10.1111/j.1574-6976.2011.00306.x>
- Rose, L. J., Noble-Wang, J., & Arduino, M. J. (2016). Surface sampling. In Marylynn Yates Cindy Nakatsu Robert Miller & Suresh Pillai (Eds.), *Manual of Environmental Microbiology* (4th ed., pp. 2.6. 2-1-2.6. 2-14). Washington, DC: American Society of Microbiology.
- Scherer, K., Mäde, D., Ellerbroek, L., Schulenburg, J., Johne, R., & Klein, G. (2009). Application of a swab sampling method for the detection of norovirus and rotavirus on artificially contaminated food and environmental surfaces. *Food and Environmental Virology*, 1(1), 42. <https://doi.org/10.1007/s12560-008-9007-0>
- Slemons, R. D., & Swayne, D. E. (1990). Replication of a waterfowl-origin influenza virus in the kidney and intestine of chickens. *Avian Diseases*, 277–284. <https://doi.org/10.2307/1591409>
- Spackman, E. (2009). The ecology of avian influenza virus in wild birds: What does this mean for poultry? *Poultry Science*, 88(4), 847–850. <https://doi.org/10.3382/ps.2008-00336>
- Spackman, E., Pedersen, J. C., McKinley, E. T., & Gelb, J. (2013). Optimal specimen collection and transport methods for the detection of avian influenza virus and Newcastle disease virus. *BMC Veterinary Research*, 9(1), 35. <https://doi.org/10.1186/1746-6148-9-35>
- Spackman, E., Senne, D. A., Myers, T. J., Bulaga, L. L., Garber, L. P., Perdue, M. L., ... Suarez, D. L. (2002). Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *Journal of Clinical Microbiology*, 40(9), 3256–3260. <https://doi.org/10.1128/JCM.40.9.3256-3260.2002>
- Spackman, E., & Stephens, C. (2016). Virus isolation and propagation in embryonated eggs. In S. Williams A *Laboratory Manual for the Isolation and Identification of Avian Pathogens*, 6th ed. Jacksonville, FL: American Association of Avian Pathologists.
- Spickler, A. R., Trampel, D. W., & Roth, J. A. (2008). The onset of virus shedding and clinical signs in chickens infected with high-pathogenicity and low-pathogenicity avian influenza viruses. *Avian Pathology*, 37(6), 555–577. <https://doi.org/10.1080/03079450802499118>
- Springthorpe, V. S., & Sattar, S. A. (1990). Chemical disinfection of virus-contaminated surfaces. *Critical Reviews in Environmental Science and Technology*, 20(3), 169–229.
- Swayne, D. E., & Beck, J. R. (2005). Experimental study to determine if low-pathogenicity and high-pathogenicity avian influenza viruses can be present in chicken breast and thigh meat following intranasal virus inoculation. *Avian Diseases*, 49(1), 81–85. <https://doi.org/10.1637/7260-081104R>
- Swayne, D., Perdue, M., Garcia, M., Rivera-Cruz, E., & Brugh, M. (1997). Pathogenicity and diagnosis of H5N2 Mexican avian influenza viruses in chickens. *Avian Diseases*, 41, 335–346. <https://doi.org/10.2307/1592187>
- Tiwari, A., Patnayak, D. P., Chander, Y., Parsad, M., & Goyal, S. M. (2006). Survival of two avian respiratory viruses on porous and nonporous surfaces. *Avian Diseases*, 50, 284–287. <https://doi.org/10.1637/7453-101205R.1>

How to cite this article: Mo J, Spackman E, Stephens CB. Identification of optimal sample collection devices and sampling locations for the detection environmental viral contamination in wire poultry cages. *Transbound Emerg Dis*. 2021;68:598–604. <https://doi.org/10.1111/tbed.13721>