

Environmental air sampling to detect exotic Newcastle disease virus in two California commercial poultry flocks

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Abstract. The 2002–2003 Exotic Newcastle Disease (END) outbreak in Southern California poultry provided an opportunity to evaluate environmental air sampling as an efficient and cost-effective means of sampling flocks for detection of a circulating virus. Exotic Newcastle Disease virus was detected by real-time reverse transcriptase PCR from air samples collected using a wetted-wall cyclone-style air sampler placed within 2 m of birds in 2 commercial flocks suspected of being naturally exposed to END virus during the outbreak. Exotic Newcastle Disease virus was detected after 2 hours of air sampling the poultry-house environments of the 2 naturally infected flocks.

Key words: Air sampler; environmental sampling; Exotic Newcastle Disease Virus; RT-PCR.

Avian paramyxovirus is an approximately 150–300 nm, enveloped, single-stranded, negative-sense RNA virus. The avian paramyxovirus group includes Exotic Newcastle Disease (END) virus, the highly contagious virus affecting multiple avian species and associated with a >\$160 million federal emergency response in Southern California and 2 adjoining states from September 2002 to August 2003. During the 2002–2003 END control campaign, more than 80,000 samples were tested by real-time reverse transcriptase PCR (RRT-PCR) using primarily oropharyngeal, tracheal, and cloacal swabs, plus a smaller number of tissue and environmental swab samples.⁴ Swab samples were collected from individual birds obtained through custom slaughter surveillance, routine flock-mortality surveillance, and live-bird testing. Live-bird testing in both commercial and noncommercial flocks used swab specimens collected from a statistically determined representative sample of the population, typically between 10 and 79 birds depending on the flock size.^{1,2}

Concerns associated with live-bird testing programs included breach of flock biosecurity, time and labor requirements for flock or task force employees, and increased stress on handled birds. On the basis of recognition of humans as potential mechanical vectors in the spread of END and other poultry pathogens, minimizing human contact with birds also was considered one of the most critical measures in maintaining high flock biosecurity. In general, custom slaughter surveillance and sampling from routine flock mortality (“barrel”) surveillance had the advantage of not altering flock management practices or requiring contact

with live birds. However, because of increased vaccine use during the END outbreak, the slaughter and barrel surveillance programs were at risk of delaying virus detection due to the potential for reduced or delayed mortality among Newcastle disease–vaccinated END-infected poultry. This brief communication describes the preliminary evaluation of an alternate method of flock surveillance, using environmental air sampling paired with RRT-PCR or virus isolation to detect END virus in commercial poultry flock environments.

Environmental air samples were collected from 2 commercial flocks during the END outbreak in a 2-week period before the detection of the last infected commercial birds in March 2003. A wetted-wall cyclone-style air sampler^a was placed within 2 m but not in direct contact with housed commercial chickens. In brief, air was pulled into the device through a water curtain designed to trap 1–10 μ m particulates in collection fluid. Air was sampled continuously for 8 hours at an approximate collection rate of 265 liters/minute into 5 ml of collection fluid consisting of triple-distilled water with no additives. The collection fluid was recycled in the system 6–20 times/minute for an estimated 50,000/minute concentration ratio and evaporated fluid was replaced automatically from a 150-ml reservoir. Subsamples (2 ml) were removed from the collector at 0, 2, and 8 hours and transported on ice to the California Animal Health and Food Safety Laboratory System (CAHFS) for standard egg-inoculation virus isolation, RRT-PCR, and PCR-amplicon sequence analysis, as described previously,⁴ with modification to a single-tube format using a Smartcycler^b PCR instrument. The RRT-PCR assay targeted the END fusion protein cleavage site and has a reported sensitivity of 99.67% and specificity 99.997% for swab samples and an analytical detection limit between 10 and 100 egg infective doses (EID)₅₀ for END virus.⁴ Oropharyngeal swabs and tissue samples from

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5 randomly selected apparently healthy chickens were also obtained from each flock within 24 hours of air-sample collection.

Flock A was a 3,000-bird breeder chicken operation with cage-free housing and nest boxes on the floor. A drop in egg production with no associated increase in mortality had been reported approximately 48 hours before air sampling was initiated in flock A. The air sampler was placed approximately 0.6 m off the floor and approximately 25 cm outside of the wire-mesh bird enclosure. Flock B was a 7-house layer chicken operation containing 60,000 birds. The house that was air sampled contained approximately 8,600 layer chickens in early production, with birds in single-tier suspended cages. The flock had reported no clinical disease or increased mortality; however, at the time of air sampling, depressed birds and green diarrhea were noted in a cage-row adjacent to the sampling site. The sampler was suspended approximately 1.6 m above the ground, level with the suspended cages, and approximately 2 m from the nearest cage. Both farms were open to the outside environment through windows, curtains, or roof openings and both had ventilators at one end of the house in which the air sampler was located.

In flock A, between 100 and 1,000 virus particles, as determined by END RRT-PCR cycle threshold and estimated EID_{50} standard curves, were detected in both 2- and 8- hour air samples. Virus was also detected by RRT-PCR and virus isolation in oropharyngeal swabs and from pooled tissue. The virus detected was confirmed as END virus by direct sequence analysis of RRT-PCR amplicons^{3,4} and represented the first time during the outbreak that the fusion protein cleavage site consisting of the amino acid pattern ¹¹⁰GGRRQRRFVG¹¹⁹ (GenBank AY266476) was detected. The fusion protein cleavage site sequence varied from the index case sequence of ¹¹⁰GGRRQKR FVG¹¹⁹ (GenBank AY216490) by 1 amino acid and was subsequently detected from commercial chickens, noncommercial chickens, and domestic pigeons. The nucleic acid sequences of the RRT-PCR amplicons for flock A were identical for air samples, oropharyngeal swabs, tissue swabs, and virus isolates recovered by egg inoculation. In flock B, between 10 and 100 EID_{50} of END virus was detected in the 2-hour air sample, oropharyngeal samples, and tissue samples by both RRT-PCR and virus isolation. The 8-hour air sample was negative by RRT-PCR, but positive by virus isolation, suggesting the presence of virus below the RRT-PCR detection limit of 10 viral particles. The finding was not unexpected because virus isolation using egg inoculation was found to be 1–10 EID_{50} more sensitive compared with the RRT-PCR used in this study.⁴ Sequence confirmation for END RRT-PCR am-

plicons from END virus-positive samples from flock B confirmed END virus with the variant fusion protein cleavage site sequence. Time zero for both the air-sampled flocks as well as 0-, 2-, and 4-hour samples collected in a poultry-free environment subsequent to flock sampling were negative for END virus by both RRT-PCR and virus isolation.

The evaluation of environmental air sampling to detect an aerosolized virus, although preliminary and based on a very limited sample of 2 commercial poultry flocks naturally infected with END, indicates that the technique has potential application as an effective sampling tool. Despite the air collector being designed to detect 1–10 μm particulates, preliminary evaluation indicates that the 10-fold smaller paramyxovirus virions were collected with other airborne dust and debris and recovered in concentrations sufficient to detect the virus using the RRT-PCR procedure or by the more sensitive but time-intensive egg-inoculation technique.

Environmental sampling has several advantages for flock surveillance, including minimizing direct human contact with flocks, eliminating individual bird handling, and providing a time- and cost-efficient sampling technique. The associated costs of environmental air sampling, after original purchase of an air sampler, would be limited to the time invested in placing the air sampler in a flock plus the cost of either RRT-PCR or virus isolation to test the collected air sample(s). In comparison, placement of sentinel birds to detect virus during the recent END outbreak was estimated at \$58 per bird placed and an average of 10 birds placed per flock (B. McCluskey, personal communication). In addition, live-bird surveillance efforts after eradication of the disease have been complicated by uneasiness on the part of veterinarians and producers to breach flock biosecurity, as well as to invest the additional time and human resources required to handle live birds for collection of oropharyngeal and cloacal samples.

Air sampling of poultry environments to detect viral pathogens, although never applied as a routine surveillance or monitoring tool, is not a new concept. Delay et al. originally documented recovery of Newcastle disease virus from poultry environment air samples in 1948,⁵ and Brugh and Johnson reported the recovery of avian influenza virus from poultry environment air samples in 1986 (M. Brugh, personal communication). As demonstrated during the 2002–2003 END outbreak, use of a commercially available air sampler, paired with virus detection using RRT-PCR, allows environmental air sampling to be a more accessible, time-efficient, and practical surveillance tool than described previously. Before routine application, the current protocol for air sampling requires significant refinement and optimization for detection sensi-

tivity over a range of preclinical and clinical disease events, as well as optimization for different humidity, collection times, collection temperatures, and proximity placement under different management scenarios. Further development efforts are warranted by the preliminary findings, which are also encouraging for routine surveillance or targeted detection of other viral pathogens in environments where animal density is high, such as sale yards, confinement barns, or enclosed shipping containers.

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Sources and manufacturers

- a. Smart Air Sampler System, Research International, Monroe, WA.
- b. Cepheid Inc., Sunnyvale, CA.

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Fatal mycobacteriosis with hepatosplenomegaly in a young dog due to *Mycobacterium avium*

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Abstract. Cases of disseminated *Mycobacterium avium* infections in dogs are rare because it appears that the species is innately resistant to infection. A 2-year-old, castrated, 5 kg Shih Tzu-Poodle-cross developed anemia, abdominal pain, lethargy, and splenomegaly. Histological examination of surgically removed spleen indicated marked granulomatous splenitis with myriad intracytoplasmic acid-fast bacterial rods. Ultrastructural examination revealed the presence of 3–4- μ m-long mycobacteria in phagolysosomes of epithelioid macrophages. Tissue extract of lightly fixed spleen was positive for *M. avium* 16S ribosomal RNA and negative for *M. tuberculosis* complex IS6110 DNA by polymerase chain reaction testing. Anemia was associated with the presence of mycobacteria-infected macrophages in bone marrow. The animal's condition deteriorated, and euthanasia was performed after a clinical course of 2 months. The principal morphological findings at necropsy were severe diffuse granulomatous hepatitis, enteric lymphadenomegaly, and segmental granulomatous enteritis with intralésional mycobacteria present. *Mycobacterium avium* was cultured from enteric lymph nodes sampled at necropsy. The source of infection was not established but was presumed to be environmental with an enteric portal of entry.

Key words: Anemia; chronic disease; dogs; hepatitis; *Mycobacterium avium*; pathology; polymerase chain reaction.

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Mycobacterial infections with *Mycobacterium avium* complex (MAC), which includes *M. avium* and *M. intracellulare*, as well as *M. tuberculosis* complex (which includes *M. tuberculosis* and *M. bovis*) are rare in dogs.^{6,7} Dogs are relatively less susceptible to infections with MAC organisms compared with *M. tuberculosis* complex.¹⁵ In a recent report, which summarized the clinical, microbiological, and morpholog-