ATTACHMENT 6

Sentinel Animal Methods

Overview

This methods section reviews two sentinel animal methods that have been used in avian disease surveillance programs and that may be used for the early detection of avian influenza (AI) virus infection along migratory flyways in the U.S.

Non-commercial Backyard Poultry Flocks

Backyard poultry are defined as domesticated fowl, including chickens, turkeys, waterfowl, and game birds (except doves and pigeons) maintained for hobby or noncommercial egg and meat production (NAHRS FAQ 2005). Backyard poultry are typically allowed to forage freely or may be confined in partially enclosed fenced areas. The evaluation of poultry flocks reared on backyard premises for diseases of interest to the commercial poultry industry has been used as a surveillance method to estimate seroprevalence of selected disease agents as part of health surveys in backyard flocks adjacent to commercial operations. (McBride; Hird; Carpenter; Snipes; Danaye-Elmi, and Utterback 1991;Johnson; Colby; Tablante; Hegngi; Salem; Gedamu, and Pope C. 2004)

In 2005, State animal health officials in Alaska sampled birds at fairs and exhibitions (concentration points). Most exhibitors were 4H or hobby farmers. Fairs and exhibitions are social events and are attended by large numbers of game bird fanciers from remote regions of Alaska. State animal health officials offered testing to exhibitors at three agricultural fairs with the goal of testing every entry to the fair (600 samples representing 100 flocks). This was a voluntary sampling program, but nearly 100% of owners of exhibition game agreed to test in 2005.

- 150 birds were sampled at the Fairbanks fair. Tanana Fair entries represent flocks from a 40 mile radius around the city of Fairbanks (Healy, Tanana, and the North Pole).
- 100 birds were sampled at the Kenai fair. Kenai Fair entries include flocks from Homer to Anchorage
- 300 samples were collected at the Alaskan State Fair in Palmer. Palmer Fair entries include Anchorage, Matanuska Valley (includes flocks as far north as Fairbanks)

In Alaska, poultry chicks are either purchased through mail order or from a few local breeders and may be reared in suburban areas or in remote villages throughout the State. Most backyard birds are reared for egg production and slaughtered for meat prior to the winter season, although there are a growing number of hobbyists that raise show birds. Birds

are often reared on open range or in outside enclosures and sometimes have an opportunity to intermingle with wild waterfowl. Limited resources prohibited widespread backyard bird surveillance testing over the large expanse of the state. Backyard flock surveillance is presently passive and owners request testing after morbidity or mortality events occur in their flock or after noting dead waterfowl or sick waterfowl on their premises. There is currently no census available to estimate the number of backyard flocks in Alaska.

Cloacal swab samples are placed in ethanol and evaluated at the University of Alaska, at Fairbanks by RT-PCR. If surveillance screening samples are positive by PCR, the premise is placed under quarantine and additional cloacal samples taken during the epidemiological investigation are placed in viral transport media and submitted for virus isolation to NVSL in Ames, Iowa.

In 2006, the Alaska Department of Environmental Conservation, Office of the State Veterinarian will sample backyard flocks, near summer water systems where wild and domestic waterfowl congregate and collect environmental samples (bird droppings, water samples) in six general areas:

- Southeast- 2 cities (Juneau, Ketchikan)
- Southcentral- 4 cities (Homer, Soldotna, Anchorage, Matanuska Valley
- Interior- 3 cities (Fairbanks, Healy, Talkeetna)
- Southwest- 2 cities (Bethel, King Salmon)
- Northwest- 2 cities (Nome, Kotezebue)
- Aleutians/Bering Sea- 4 cities (Kodiak, Dutch Harbor, Cold Bay, Pribilof Islands)

The areas listed in bold have the highest priority and cover a majority of the population where domestic poultry is kept. The other 3 areas have substantial populations of wild birds but few domestic backyard flocks. A sample size of 11 is needed to detect avian influenza at a prevalence rate of 25% at a 95% confidence interval in flocks ranging from 10 to 10,000 or more birds.

The Office of the State Veterinarian will sample poultry exhibited at the six agricultural fairs (concentration points):

• Deltana Fair	Date to be announced
• Haines Fair	7/26-30/2006
• Tanana Valley Fair	8/4-12/2006
• Kenney Lake Fair	Date to be announced (2^{nd} Week August 2006)
• Kenai Peninsula Fair (Ninilchik)	8/18-20/2006
• Alaska State Fair (Palmer)	8/24 - 9/4/2006

The fair boards have expressed interest in promoting this sampling effort and it is expected that all entries will be tested. The state has just finished construction of a new diagnostic laboratory, Alaska Environmental Health Laboratory in Anchorage, and will develop the capacity to run the diagnostic tests using RT-PCR. If surveillance screening samples are positive by PCR, the premise will be placed under quarantine and additional cloacal samples

taken during the epidemiological investigation are placed in viral transport media and submitted for virus isolation to NVSL in Ames, Iowa.

This approach to sampling non-commercial poultry flocks may be adapted in other areas of the U.S. where there is widespread non-commercial poultry production.

Sentinel Duck Flocks

The second method described is the placement of sentinel duck flocks in wetland environments where they are potentially exposed to and infected with disease agents as they commingle with wild birds. The placement of sentinel flocks of domestic ducks has been used to recover AI and detect influenza epizootics in pelagic bird colonies, and yielded much higher isolation rates compared to isolations from wild birds (Turek; Gresikova, and Tumova 1984;Sinnecker; Sinnecker; Zilske, and Koehler 1982;Sinnecker; Sinnecker, and Zilske 1982). Sentinel ducks have been used to determine the presence of AI and timing of infection associated with the arrival of wild migratory waterfowl in wetland habitats adjacent to market turkey production flocks (Halvorson; Karunakaran; Senne; Kelleher; Bailey; Abraham; Hinshaw, and Newman 1983;Halvorson; Kelleher, and Senne 1985;Kelleher; Halvorson; Newman, and Senne 1985).

In North America, AI isolations from waterfowl have been reported from approximately 30 locations over the past 35 years (Hanson; Stallknecht; Swayne; Lewis, and Senne 2003). Many of these sites are located along each of the four migratory waterfowl flyways (Pacific, Central, Mississippi, and Atlantic) in the continental U.S. Bodies of water with large concentrations of migratory waterfowl and shorebirds might also serve as sentinel sites.

Ideally, surveillance activities should occur at sites at a time when migratory birds are actively nesting and at locations where they marshal and intermingle with other migratory birds transiting the area prior to winter migration. The onset of avian influenza infection in sentinel ducks has been shown to occur in late July and early August in summer breeding areas (infection of range reared turkey flocks was shown to occur about 6 to 8 weeks later) (Halvorson et al. 1985). Avian influenza virus prevalence estimates from published waterfowl surveys indicate that virus can first be detected in naïve juvenile birds in summer breeding areas in July or August (prevalence ranged from 11% to 61% in published surveys) as juveniles emerge from hiding and intermingle with other broods and a subsequent high rate of re-infection as birds marshal for winter migration in October (Hanson et al. 2003; Hinshaw et al. 1985). Avian influenza virus prevalence generally decreases during late fall and winter and may reach a level of 1% or less in over-wintering areas.(Stallknecht ;Webster; Bean; Gorman; Chambers, and Kawaoka 1992) However, virus was isolated from 11% of teals and from 15% of northern pintails in one recent survey of wintering ducks in Texas, suggesting that the avian influenza season may not be a fall season event (Hanson 2003). As a result of early migration, blue winged teal are thought to serve as an immunologically naïve host in wintering areas.

Most virus isolations have occurred in mallards and other species of dabbling ducks, but less commonly in wood ducks and similar species (Stallknecht). Mallards are commonly

associated with habitats located near man, livestock, and poultry and would be more likely to interact with backyard poultry flocks compared with other waterfowl species (Stallknecht and Shane 1988). Although H5, H7, and H9 subtypes have been poorly represented in most waterfowl surveys (H3, H4, or H6 subtypes have been isolated most frequently), pintails and mallards have been shown to be significant reservoirs in one recent survey where H5, H7, and H9 virus subtypes were isolated 21.5% of the time in Minnesota (Hanson and others 2003). The prevalence of AI isolated from blue winged teal on wintering grounds in February in Texas was found to be 22% in 2001 and 15% in 2002 (Hanson 2003). Migration of blue winged teal occurs in late summer and early fall (typically September), prior to the highest period of AI prevalence. Early migration of this species is thought to play a role in maintenance of AI infection on wintering grounds by providing a susceptible population with little or no prior exposure or immunity. However, blue winged teal are less likely to interact with man or livestock, so sites where blue winged teal congregate may not serve as the best sites for surveillance using backyard flocks of domestic waterfowl.

The role of shorebirds in avian influenza ecology should be considered separately from that of migratory waterfowl. The highest prevalence of avian influenza virus in shorebirds has been shown to occur in May and in September, which coincides with the times of peak shorebird migration in the northeastern U.S. (Kawaoka; Chambers; Sladen, and Webster 1988). Shorebirds migrating through the Delaware Bay have been shown to have the highest prevalence of AI virus compared with other shorebird populations surveyed at four other locations along the Atlantic flyway (Hanson 2003). Although most isolates reported from shorebirds in this survey were H10 and H12 (H9 and H13 in previous studies), H5 and H7 subtypes were isolated from a small percentage of shorebirds. During May, virus was isolated mostly from ruddy turnstones (9.1%).

The approach to the design of a targeted surveillance method for the detection of avian influenza using either of these two sentinel animal methods should incorporate what is presently known about the ecology and natural history of avian influenza infection in wild waterfowl reservoir species. Sentinel animals are most likely to become infected with AI if exposed to reservoirs in nature during periods of highest viral shedding. As described above, prevalence of infection as measured by virus isolations in published waterfowl surveys has been shown to vary temporally by location, age, season, and species. A targeted approach to sentinel animal surveillance should be designed to:

- Target specific locations where AI has been isolated from wild waterfowl historically;
- Target locations where known primary reservoir species (mallards, blue winged teal, ruddy turnstones) congregate for breeding (resulting in higher concentrations of juveniles susceptible to infection) or wintering (higher concentrations of species with little or no previous exposure) resulting in a higher prevalence of infection;
- Be timed to coincide with periods (seasons) of highest prevalence in the reservoir species, in particular migratory species that originate from an area having high incidence of AI (Southeast Asia).

Methods

Backyard Poultry Surveillance Method

Flock Selection

- Targeted flocks should consist of free range domestic waterfowl or poultry flocks located near marshlands or wetlands.
- Marshlands should contain high density populations of waterfowl or shorebirds.
- Flocks should have an opportunity to directly intermingle with waterfowl (especially mallards) at or near the common watershed via open range or open enclosure or by sharing a common source of water.
- Chose sites adjacent to wetlands where AI virus has been isolated historically.

Timing of Surveillance

- Surveillance should begin in late July and continue through October at sites near northern breeding areas.
- Although, prevalence rates in wild waterfowl were shown to decrease significantly in wintering areas in Louisiana (1%), prevalence in blue winged teal in wintering areas in Texas during February of >10% indicates that some wintering sites may be useful for sentinel surveillance.
- The seasonal peak of AI prevalence in shorebirds occurs in May rather than late summer, so surveillance of backyard flocks in the Delmarva (Delaware Bay) area should be planned for May to coincide with the time of highest prevalence.

Sample Size Estimates

- The average size of backyard poultry flocks in the U.S. is 35 birds (varies from 28 to 49 birds per flock by region). A prevalence estimate for avian influenza of 25% is assumed (NAHMS Poultry '04 Part I 2004).
- A sample size of 11 is needed to detect avian influenza at a prevalence rate of 25% at a 95% confidence interval in flocks ranging from 10 to 10,000 or more birds.(Cannon and Roe 1982)
- Cloacal and tracheal swab samples would be submitted to the appropriate diagnostic labs for RT-PCR testing and to a reference lab for virus isolation.

Sentinel Duck Method

Flock Preparation and Placement

- Construct pens or plan for open fenced enclosures that will hold 10 to 20 ducks and allow contact with released "messenger" ducks and wild ducks. Pens should allow exposure to water contaminated with wild duck feces.
- Deploy pens to selected wetlands (or construct fenced enclosures).
- Arrange to provide basic husbandry.
- Rear one day old ducks in isolation facilities for 6 to 7 weeks.
- Establish AI free status by cloacal swabbing and serologic testing.
- Release 10 to 20 isolation reared "messenger" pinioned mallard or white Peking ducks on selected body of water.
- Place 10 to 20 ducks in pens on selected body of water to intermingle with "messenger" ducks and wild ducks.
- Periodically bleed ducks to determine serologic status and replace H5 seropositive ducks with immunologically naïve ducks.

Timing of Surveillance

- Placement of sentinel duck flocks should coincide with backyard flock surveillance seasonally.

Sampling

- Retrieve fecal samples via cloacal swabs from 10 to 20 penned ducks to detect virus weekly and periodically trap messenger ducks for cloacal sampling.

Data Collection

For backyard flocks, a database similar to the one used for Exotic Newcastle Disease (END) surveillance would provide the ability to trace positive samples back to their flock of origin (Accession number; sending facility premises ID; submitter name, address, and contact information; location of animals including premises ID, latitude, and longitude; owner name; flock information including size, number affected, number dead; purpose of submission and relevant clinical information).

Data needed to create predictive geospatial models to evaluate spatial and temporal risk for sentinel duck flocks include: (1) lat/long (in unprojected decimal degrees with a WGS-84 or NAD-83 datum) of the sentinel cage's location; (2) front gate coordinates for the premises; (3) name, address, county, zip code, contact information for the land owner/manager, and occupations of all residents; (4) age, sex, and breed of birds; (5) number of sentinel birds and each bird must have a unique ID (e.g., numbered aluminum leg or wing bands work well); (6) environmental description of area where cage containing sentinel birds is placed; (7) AI

virus test status (birds are bled periodically to evaluate immune status and need for replacement); (8) presence and approximate distance to other birds and mammals; (9) exposure to wild birds and free ranging domestic birds; (10) estimated density of birds and mammals on premises and in the vicinity of the sentinels; (11) exposure of sentinels to human contact other than the avian phlebotomist; and (12) an environmental assessment of the vicinity (e.g., within 100 meters, within 500 meters, and within 1000 meters). These data should be captured on a site survey form. However, a separate form should be used to record: date and time blood samples were collected, the birds' ID number, and the vial number for the blood specimen. With this basic information, other data sources can be used to evaluate proximity to wetlands, bird roosts, position with in normal flyways, terrain features, and more. Access to extensive datasets (e.g. the National Wetlands Inventory and the National Landcover Dataset) and hydrologic models could be used to identify wetlands.

Discussion

Major advantages of the use of sentinel animals to detect AI:

- Backyard bird surveillance programs are already in existence in most states.
- State animal health officials are familiar with a targeted surveillance approach (i.e. surveillance of backyard flocks within a designated radius adjacent to commercial poultry operations).
- The placement of sentinel ducks has been used successfully to isolate AI from wild waterfowl in previous published surveys.
- Mortality in backyard poultry from H5N1 has occurred in other countries.
- Could be done in conjunction with other surveillance methods at the same location for comparison.

Major disadvantages of the use of sentinel animals:

- Locating suitable surveillance sites will require field surveillance or input from wildlife biologists.
- Expense of rearing AI free birds.
- Pen construction and husbandry costs.
- Sentinel flocks are subject to predation.

Recommendations

In order to implement an efficient active sentinel animal surveillance system, sentinel flock locations should be purposefully chosen. Appropriately allocating limited resources to achieve targeted sampling and reduce costs is an important objective of animal disease surveillance programs (McCluskey 2003). Knowledge of disease distribution allows us to focus surveillance activities. In this case, we can use our knowledge of the most likely entry points for H5N1 through migratory waterfowl to locate sentinel animal flocks. In order to target areas for sentinel surveillance with a higher probability of disease, flyway information

should be plotted over waterfowl management areas in order to select sites most likely to have migratory birds from areas where commingling with Eurasian species is most likely to occur. Specific locations in areas where migratory birds from possible northern exposure sites are most likely to be in highest concentration have been identified in other methods sections of this plan. National information on the health and management practices of backyard and small production flocks adjacent to commercial poultry operations in 18 states is available. All of this information should be combined with information on the geographic distribution of poultry producers including sizes and densities of operations in order to produce a risk map. Local animal health officials could then locate sentinel backyard flocks adjacent to waterfowl management areas in poultry dense regions where there is the highest probability of disease transmission. The health status of sentinel backyard flocks could be evaluated on a recurring basis (quarterly, or more often during seasons of the year that pose the highest probability of disease transmission due to higher prevalences) for an active disease surveillance program.

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