Case Report

Poxvirus Infection in a Colony of Laboratory Pigeons (*Columba livia*)

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Pigeons (*Columba livia*) are used in biomedical research for studies of vision, cognition, neuronal pathways, and spatial orientation. Because there are few commercial laboratory sources, research pigeons are typically acquired from local fancier breeders or bred onsite. For acquired pigeons, the health and vaccine status is often unknown. A juvenile pigeon, born onsite and living in an enclosed outdoor loft, presented with small, bleeding, wart-like lesions on the medial aspects of digits 1 and 4. Topical treatment was initiated. Within a week, 4 fledglings were reported for small, dark papular lesions on the face, head, neck, and beak, and shortly thereafter, 2 additional juvenile pigeons developed similar lesions. The fledglings were euthanized, and histologic examination revealed numerous intralesional eosinophilic cytoplasmic viral inclusions (Bollinger bodies) confirming a diagnosis of poxvirus infection, likely pigeon pox. Although usually self-limiting, pigeon pox can cause moderate to severe lesions in fledgling and juvenile birds. Vaccination with a modified live poxvirus labeled for chickens was used to create herd immunity to pigeon poxvirus. Since vaccination of our entire flock and implementation of more stringent health protocols, all lesions have resolved, and no new lesions have been noted.

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Although a less commonly used species, pigeons (*Columba livia*) are used in biomedical research for studies of vision, cognition, behavior, neural science, and spatial orientation.^{1,16,17,20} Research at our institution is focused on neurobiology and homing abilities. Traditionally, research pigeons have been maintained in standard laboratory cages, modified flight rooms, or modified outdoor lofts, with recommended floor space according to the *Guide for the Care and Use of Laboratory Animals* of 0.8 square feet per animal.⁶ Because there are few known laboratory sources for pigeons,¹³ animals used in research are often either acquired from local fancier breeders or bred onsite in breeding or flock rooms. Consequently, the health or vaccine status of juvenile to young adult animals can be unknown, and a variety of infections could occur. Moreover, pigeons in outdoor lofts or released to allow for homing behavior, such as those at our institution, incur the additional risk of being exposed to wildlife and a partially uncontrolled environment.

Common health problems in pigeons include trauma, ectoparasites, and pododermatitis. Like laboratory rodents, primary infectious diseases are rare in completely closed research colonies but can occur sporadically in outdoor-housed birds, with case reports of papillomavirus and poxvirus infection.^{10,14,15} Pigeon pox is a species of poxvirus in the genus *Avipoxvirus* (*Avipox* subgroup poxvirus).^{2,19} This double-stranded enveloped DNA virus causes mild to severe slow-developing disease in infected animals, most commonly chickens, turkeys, and pigeons. Similar to poxviruses in other species, pigeon pox is generally self-limiting but can cause more severe disease in young or immunocompromised animals. This report describes a series of

cases of poxvirus in an outdoor colony of group-housed laboratory pigeons and the management strategies implemented in response to the outbreak to prevent future occurrences.

Case Report

Pigeons used for the study of neurologic behavior and homing are maintained under IACUC-approved protocols at an AAALAC-accredited facility located in Houston, TX. Animals used for active research are either bred at the facility or purchased from a reputable pigeon fancier with known herd health history, because no local commercial laboratory source for pigeons is available. On arrival, all birds undergo a 14-d quarantine, which includes prophylactic topical treatment with 0.2 mg/kg ivermectin diluted 1:10 in propylene glycol (Ivomec, Merial, Lyon, France) and a permethrin-containing emulsified insecticide spray (Permethrin II, Bayer Animal Health, Leverkusen, Germany) to remove ectoparasite burdens, as well as prophylactic antibiotics and antihelminthics (amprolium, Ridzol S, tylosin, and levamisole; 5-in-1, Vita-King Products, Wanatah, IN) in the drinking water for treatment of endoparasites. Serology testing and bacterial culture are not performed on incoming birds as a part of the import or quarantine procedure because the pigeon colony is not considered SPF. After quarantine, birds reside group-housed in either an indoor breeding-flight room or an outdoor enclosed loft specifically designed for the housing of animals on study for navigational research. Animals housed outdoors are provided with daily flying opportunities as part of the research design to study homing behaviors. Within the loft, animals are health-checked daily; water bowls are refilled daily and sanitized weekly. Feed is provided twice daily by an automated feeding device that is refilled as needed and sanitized weekly. Loft nesting boxes bedded with hay are cleaned and changed weekly (or as needed), and sanitization

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of nonremovable structures occurs every other week with a quaternary ammonia disinfectant (Labsan 256-CPQ, Sanitation Strategies, Holt, MI). Care is taken to separate animals from the cleaning so that direct chemical contact does not occur. In terms of a preventative health program, loft pigeons are prophylactically treated annually with topical ivermectin and Permethrin II as previously described to prevent ectoparasite burdens. The area surrounding the loft is treated quarterly by a facility-hired company for the management of pests.

In Summer 2016, an outdoor-housed pigeon presented to the veterinary staff for small, yellow-to-brown pustules on the dorsoplantar aspect of the feet. Lesions were biopsied, revealing localized severe necrosuppurative epidermitis with intralesional colonies of bacterial cocci and mononuclear dermatitis (Figure 1). These lesions were determined to be of a noninfectious cause, likely related to localized irritation or trauma, with a secondary bacterial infection. Topical antibiotic treatment (Neomycin–Polymixin–Bacitracin Ointment, Butler Animal Health Supply, Dublin, OH) and cleaning of the lesions with dilute povidone–iodine solution resulted in healing with no further issues.

The next year (July 2017) similar lesions on the foot were observed on another young-adult pigeon and consisted of small, yellow-to-brown, bleeding, wart-like growths on the medial aspect of digits 1 and 4. This pigeon had recently been relocated from indoor housing to loft housing, for participation in the study. In light of the history of similar lesions in a loft-housed bird, topical treatment and cleaning were initiated as previously described because the initial presumptive diagnosis was bacterial epidermitis and dermatitis. However, despite topical treatment, the lesions became progressively larger and more distributed throughout the colony. Within 1 wk, 4 loft fledglings were reported to have small, dark papular lesions on the face, head, neck, and beak. Shortly thereafter, 2 more young-adult animals developed lesions on their feet and head (Figure 2). Given the severity of the lesions, all 4 fledglings were euthanized, and treatment as previously described was continued for the 3 young-adult pigeons. Histologic examination revealed numerous intralesional eosinophilic cytoplasmic viral inclusions (Bollinger bodies), confirming a diagnosis of poxvirus (Figure 3). Although speciation was not performed, the histopathologic characteristics and clinical presentation supported the diagnosis of pigeon pox.

To create herd immunity, all pigeons in the colony (indoor and outdoor) were vaccinated on the same day by using a modified live pigeon pox vaccine developed for chickens (Pigeon Pox Vaccine [live virus], Hygieia Biologic Laboratories, Woodland, CA) through the feather pluck method. To this end, feathers were removed from the distal aspect of a leg to create an open feather follicle, and the reconstituted vaccine was brushed into the lesion to achieve vaccination. The vaccine was reconstituted in accordance with manufacturer instructions. A sterile cotton-tipped applicator was used to brush a small quantity of the vaccine into the open follicles. A single stroke was used, and vaccination was confirmed visually through the presence of the colored vaccine diluent within the opened follicles. The vaccine was handled, stored, and disposed of per manufacturer's recommendation. Within 2 wk of vaccine administration, approximately 75% of the colony (both outdoor and indoor) had responded to the vaccine, which created a localized infection characterized by swelling, discoloration, and follicular distension at the site of vaccination (Figure 4). The 25% of the colony that did not respond to the vaccine likely either had natural exposure to poxvirus previously or received vaccination prior to import into the facility. On the slight chance that an animal did

not respond and was not previously exposed, the creation of lesions in the majority of the colony likely would have led to exposure of any naïve animals. Within 1 mo of vaccination, all mild lesions had resolved. The colony is now empirically considered immune to poxvirus, with the implementation of more stringent quarantine and vaccination protocols.

Discussion

Many factors led to the diagnosis of pigeon pox without microbiologic speciation. First was the timeframe in which the disease was seen. Poxvirus is spread primarily through an insect vector (mosquitoes or biting flies), but direct viral contact (contaminated feed or water, direct shedding at lesions) or aerosolization of viral particles (either in urine or saliva) are also described.⁹ Because summer is when many insect vectors, especially mosquitoes, hit peak population densities, the likelihood of transmitting poxvirus during this time is increased. In addition, given that the initial infected pigeons were housed in the outdoor loft with daily flying opportunities, the likelihood of spread through environmental shedding by other native pigeons is high. The location of the presenting lesions on unfeathered skin further aided in the diagnosis of pigeon pox. Although usually a nuisance disease, the dry form of pigeon pox results in small papules that progress to wart-like thick scabs on featherless areas of the body; common locations are the eyelids, around the beak, and legs.^{10,12} Gross internal lesions, as expected with the wet or diphtheric form of poxvirus, are less common but more severe, especially in young or immunocompromised birds. Lesions are characterized by soft, caseous, necrotic lesions to the mucous membranes of the mouth, pharynx, larynx, and trachea.^{10,12} Birds infected with the wet form of poxvirus will appear sick and often demonstrate anorexia, dyspnea, and weight loss. Mortality in these animals typically is secondary to asphyxiation due to necrotic material in the respiratory tract. Our original pigeons presented with lesions on the feet, one of the hairless regions through which mosquitoes or other biting insects could spread the disease. The fledglings demonstrated more severe disease originating on the hairless regions of the head, beak, and face. Although internal lesions were not detected at necropsy, the lesions of the head, especially in the youngest fledgling, were of a more necrotic nature than that of older animals, and animals were beginning to demonstrate a decline that could have been associated with the start of the diphtheric or wet form of poxvirus infection.

In most settings, treatment of poxvirus is not recommended because disruption of the lesions will further disseminate the virus.^{4,11} In addition, avian poxvirus is very persistent within the environment and can survive for years in dry scabs or on dry surfaces,⁹ thus leading to the potential perpetuation of the disease in a colony of otherwise healthy animals. Treatment for birds of prey or of early lesions in doves, cranes, and canaries has been documented^{3,5,8,11,18} but typically involves invasive lesion removal with dermal burns and nonspecific treatment for symptomatic disease, such as supportive care and vitamin supplementation. In high-density colony situations, such as an aviary, the health of the colony is outweighed by the life of an infected bird; consequently a suspected diagnosis of poxvirus often leads to euthanasia of infected animals to protect the remaining population. As active study animals, the health of each individual pigeon is critical for data collection for ongoing studies, and we therefore elected for symptomatic treatment despite the possible complications. To prevent further infection, we created herd immunity in our flock of pigeons as described earlier and increased sanitation practices. All equipment used in the

Figure 1. Histology of initial presentation of lesions on a single pigeon in Summer 2016. (A) Low-magnification view of skin lesion showing extensive serocellular crust and superficial bacterial colonies. Scale bar, 200 μ m. (B) Higher-magnification view of skin lesions with serocellular crusts (white arrow), inflammation, and reactive hyperplasia. Scale bar, 100 μ m.

Figure 2. Gross lesions of the (A) head and beak and (B) foot of 2 young adult pigeons approximately 1 wk after appearance in Summer 2017. At presentation, these lesions were less than 2 mm in diameter (not pictured). Cleaning of the lesions led to the progressive growth that is seen here.

loft during the time of the outbreak was immediately cleaned by using a hydrogen-peroxide-based cleaner (Rescue, Virox Animal Health, Oakville, Ontario), and then brought directly to the dirty side of cagewash, where it was autoclaved. Full decontamination of all pigeon housing areas occurred by using a quaternary ammonia disinfectant (Labsan 256-CPQ, Sanitation Strategies) after the resolution of all clinical signs of poxvirus. In

addition, the colony quarantine procedure was updated to include vaccination against poxvirus prior to release from quarantine to ensure continued flock immunity. Poxvirus vaccination is now administered to all fledglings born onsite once they are older than 6 wk. Fledglings will continue to be the highest-risk group due to their increased susceptibility, however, maternal antibodies to poxvirus due to vaccination of the hens should

Figure 3. Histologic images of poxviral lesions. (A) Irregular epidermal proliferation with inflammation and serocellular crust formation. Scale bar, 1 mm. (B) Lesions contained numerous bacterial colonies (white arrow) and typical poxviral intracytoplasmic inclusion bodies (black arrow) that were as large as 15 μ m in diameter. Scale bar, 100 μ m.

Figure 4. Comparison of pigeons with (A) no vaccine response and (B) a positive vaccine response. Note the swelling and follicular distension, indicating a positive response to vaccination. The residual blue/green coloration of the feather follicles is due to the vaccine diluent. All vaccine reactions appeared within 1 wk of vaccination and resolved within 2 to 4 wks thereafter.

aid in preventing disease transmission prior to vaccination of the fledglings themselves. Although mosquito netting could be added to the outdoor housing area to decrease animal exposure to the mosquito vector, doing so is impractical in our situation because the animals are allowed daily flying opportunities, so the entirety of their environment cannot be controlled. In

addition, the immune status of this colony could be confirmed through antibody titers or revaccination with observation of a low number of new takes, but this effort seems unnecessary at this current point in time. If additional cases arise in the future, these options may be considered to confirm immunity to avian poxvirus.

Many procedures for vaccination, general health, and quarantine have been created for pigeon fanciers to maintain a healthy colony of birds without veterinary oversight. Often the treatment regimens used include off-label products developed for poultry or items available from local farm stores or pigeon-fancier websites. Currently, no pigeon pox vaccines are licensed for pigeons within the United States. In both Australia and Germany, vaccination protocols are described for pigeon fanciers prior to participation in flying competitions, but all of the vaccines used are off-label products designed for chickens for the prevention of poxvirus.^{7,21} Although the use of poultry vaccines for nonpoultry species typically is not recommended, we opted to use the Hygieia vaccine due to its availability in the United States and in light of the recommendation from a poultry veterinarian whom we consulted during this outbreak.

In conclusion, this case report summarizes the clinical signs, gross and histopathology findings, treatment, and preventative measures for an outbreak of pigeon pox in an outdoor-housed research pigeon colony. As of the submission of this report, the colony of pigeons has remained free of poxvirus lesions. The implementation of colony health measures through vaccination and stricter quarantine protocols for incoming animals has thus far succeeded in preventing further infections, and no adverse reactions to vaccination have been observed. If future incident cases do occur, treatment can be initiated without fear of spread to other animals given the colony immunity.

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