Susceptibility of Avian Polyomavirus to Inactivation

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Eight commercially available disinfectants, representing several major classes of chemical disinfectants, were evaluated for their ability to inactivate avian polyomavirus (Budgerigar fledgling disease virus). These disinfectants and their sources are listed in Table 1. Avian polyomavirus was considered to be a good test pathogen for a disinfectant because this nonenveloped virus is a frequently encountered pathogen that is considered to be relatively stable in the environment. Disinfectants were diluted according to the manufacturer's recommendations. Infectious virus preparations were placed in contact with each diluted disinfectant for one minute or for five minutes at room temperature. After removal of the disinfectant by gel filtration, each sample was then placed on cultured cells to determine whether the virus was inactivated. Of the eight disinfectants tested, 0.525% sodium hypochlorite (Clorox) was considered the most economical. A stabilized chlorine dioxide (Dent-A-Gene) was effective and would be expected to be the safest. Chlorohexadine (Nolvasan) reduced but did not eliminate the infectivity of avian polyomavirus, which may explain why aviaries that use this disinfectant in the nursery frequently experience polyomavirus outbreaks. Heating the virus to 60°C for 5 minutes or 30 minutes reduced the titer of the virus, but did not eliminate infectivity.

An Overview of Avian Polyomavirus

The first acute, generalized infection associated with a polyomavirus was described in young psittacine birds and was called Budgerigar fledgling disease (BFD).[1-5] A virus that is similar to the one that causes Budgerigar fledgling disease has been shown to be associated with high levels of sickness, and in some cases death, in

finches and a number of different genera of psittacine birds. It should be noted that while the polyomaviruses that infect Budgerigars, finches and larger psittacine birds have similarities, the clinical presentation, distribution of lesions and problems that the viruses cause within a flock are dramatically different among susceptible species.[1-11]

Infected Budgerigar neonates typically develop abdominal distention, subcutaneous hemorrhage, tremors of the head and neck and ataxia. Survivors may exhibit symmetrical feather abnormalities characterized by abnormally formed primary and tail feathers, lack of down feathers on the back and abdomen and lack of filoplumes on the head and neck. When feather lesions are present, the disease is often referred to as French Molt.

In larger psittacine birds, polyomavirus infections may cause a peracute death with no premonitory signs or death 24 to 48 hours after developing clinical signs including depression and anorexia, delayed crop emptying, regurgitation, weight loss, subcutaneous hemorrhages and diarrhea. Infections may occur in both parent and handraised babies and clinical signs are most common at the time of weaning.[6-8, 12-14] The feather abnormalities that are relatively common with polyomavirus infections in Budgerigars are described less frequently in other psittacine species.

In addition to clinical changes in neonates, polyomavirus infections have also been documented in an eight-month-old Splendid Parakeet *Neophema splendida* and as the cause of sporadic, acute deaths in fully fledged lovebirds less than one year old.[13,15] An adult Moluccan Cockatoo *Cacatua moluccensis* with neurologic signs was diagnosed as having polyomavirus.[14] An outbreak of polyomavirus in an aviary with numerous psittacine species resulted in the deaths of an adult Eclectus Parrot *Eclectus roratus,* a Painted Conure *Pyrrhura picta* and three of eleven adult White-bellied Caiques *Pionites leucogaster* in the collection. The affected birds were two to two and a half years old and had lesions similar to those seen with polyomavirus infections in psittacine fledglings.[16]

Avian polyomavirus infections have been described throughout the world. Characteristic lesions associated with the virus have been demonstrated in companion birds from the United States,[6-8] Canada,[17,18] Japan,[19] Italy,[20] Hungary,[21] Germany[22] and Australia.[13,15] Avian polyomavirus has been associated with disease in a number of different species of companion and aviary birds including Budgerigars, caiques, macaws, Amazon parrots, conures, cockatoos, lovebirds, Splendid Parakeets, Pionus Parrots, African Grey Parrots, Eclectus Parrots, Cockatiels, Crimson Seed-crackers, finches and lories.[1-3, 5-8, 12-15, 23, 24]

The factors involved in polyomavirus infections are not fully understood. One of the formidable problems that occurs with polyomavirus is that normal adults and neonates from infected parents are thought to act as polyomavirus carriers. These birds may intermittently shed the virus into the environment and are thought to be

Table 1
Disinfectants, Manufacturers and Tested Dilutions

Agent	Active Ingredient	Manufacturer	Dilution
Avinol-3	Synthetic phenol	Veterinary Products Laboratory P.O. Box 34820 Phoenix, AZ 85067-4820	1:256
Clorox	Sodium hypochlorite	Clorox Company	1:10
Dent-A-Gene	Stabilized chlorine dioxide	Oxyfresh Independent Distributors 156 Wakefield Trace Athens, GA 30605 1-800-999-9551, ext. 105270	1:400
Ethanol	Ethanol 70%	Many	Undiluted
Mikroklene	Iodine	Ecomed Ecolab Center St. Paul, MN 55102 1-800-247-5362	1:192
Nolvasan solution	Chlorohexidine	Fort Dodge P.O. Box 518 Fort Dodge, IA 50501 515-955-4600	1:40
Orange Powder	Citronella	Red-bell, Inc. 116 West Horton St. Zebulon, NC 27597 1-800-334-3528	1:100
Roccal-D	Quaternary ammonium	The Upjohn Company 7000 Portage Road Kalamazoo, MI 49001 616-385-6736	1:400

responsible for the persistence, transmission and spread of the virus through various avian populations.[2, 3, 10, 12, 16-18] A carrier can shed the virus while showing no signs of disease, fatally infecting any susceptible birds that it encounters. Interestingly, is is rarely the birds that die from polyomavirus that are the source of the virus, but rather it is the birds that remain normal that are the likely carriers of the virus and are responsible for introducing it into a nursery or pet shop.

Experimental data and observation in field cases of the disease suggest that viral transmission may occur through feces, urine, respiratory secretions and in feather dust. The virus may also be transmitted to young directly in the egg. Thus, attempts to prevent infections through artificial incubation are of limited value.[1, 5-7, 10, 16, 18, 25, 26]

Avian polyomavirus has previously been shown to be resistant to organic solvents, freezing and thawing and to heating at 56°C for two hours.[2] A polyomavirus that infects primates, SV-40, has been shown to be susceptible to some products containing ethyl alcohol and resistant to others containing the same active ingredient.[1, 27, 28] The environmental stability of avian polyomavirus causes a considerable problem in the aviary because persistently infected birds can shed virus in their feather dust or excrement. Manual removal of any contaminated organic material followed by the application of an appropriate disinfectant is required to prevent or interrupt a disease outbreak.

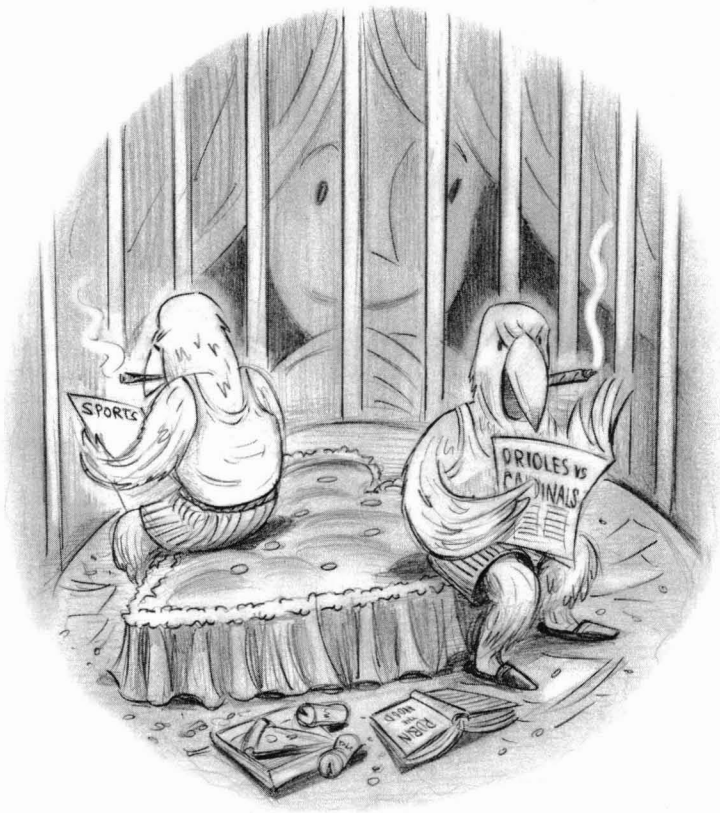
Disinfectants

To be spread from bird to bird, a virus must be capable of surviving a sufficient time outside the originally infected bird for direct or indirect contact with a susceptible host to occur. It is when a virus is in the environment that it is susceptible to inactivation. Any organic matter (i.e., food, feces, feathers, soil) that is in contact with the virus can serve as a protective matrix that increases a virus' survival time outside the host.

A number of disinfectants are available and widely used in veterinary hospitals, pet shops and avicultural settings to control pathogens of concern to companion birds. In general, the efficacy of these disinfectants for viruses that infect companion birds remains unreported. The use of a disinfectant that is ineffective against a particular pathogen can result in the spread of an infectious disease to other susceptible animals within a contaminated area.

The ideal disinfectant would rapidly inactivate a wide variety of bacteria, viruses and fungi, would be safe to use on inanimate objects, and would be safe and non-toxic to humans or animals. The health hazards associated with frequent exposure of companion birds (particularly neonates) as well as hospital or aviary personnel to harsh disinfectants or their fumes rarely are considered when choosing a disinfectant.

Disinfectants are not uniformly effective against all organisms. Therefore, a product should be chosen based on its safety for exposed individuals and animals and its specific ability to inactivate important pathogens. Viruses that have a lipoprotein envelope are typically labile and are inactivated by most disinfectants. In comparison, viruses that do not have a lipoprotein envelope are typically resistant to harsh environmental conditions and many disinfectants. Avian



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polyomavirus does not have a lipoprotein envelope and is considered relatively stable under adverse conditions. It could be hypothesized that the products used in this study that inactivated avian polyomavirus also might be effective against enveloped viruses of importance to companion birds (i.e., Herpesviridae, Paramyxoviridae and Togaviridae). Given the variety of disinfectants that will inactivate polyomavirus under experimental conditions, the choice of which disinfectant to use should be based on such factors as personal safety, the safety of any exposed animals, environmental impact and economics.

A summary of the activity of each disinfectant tested against avian polyomavirus is listed in Table 2. The results of the present study suggest that sodium hypochlorite is the most inexpensive disinfectant tested that would inactivate avian polyomavirus. However, this compound does produce fumes that can be irritating to mucus membranes and must be used in areas with sufficient ventilation. In addition, sodium hypochlorite is irritating to the skin, is corrosive to metals and produces carcinogenic by-products.[29]

Table 2
Activity of Disinfectants at Recommended Dilutions Against Avian Polyomavirus

Agent	1 Minute Exposure	5 Minute Exposure
Avinol-3	Excellent	Excellent
Clorox	Excellent	Excellent
Dent-A-Gene	Excellent	Excellent
Ethanol	Excellent	Excellent
Mikroklene	Good	Good
Nolvasan solution	Poor	Poor
Orange Powder	Poor	Poor
Roccal-D	Good	Good

Stabilized chlorine dioxide was found to inactivate polyomavirus. Some studies suggest that in many applications chlorine dioxide may be a superior disinfectant to sodium hypochlorite.[30] At working dilutions, stabilized chlorine dioxide is considered safe for humans and animals and is used by many municipalities as the principle agent to eliminate potential pathogens from drinking water. In Europe, chlorine dioxide is used to treat drinking water because, unlike chlorine, it does not form carcinogenic trihalomethanes, chlorophenols or chloramines.[31, 32]

Chlorohexidine did not completely inactivate avian polyomavirus which may explain why nurseries that use this product to soak syringes between feedings can still experience polyomavirus outbreaks.

The present study was designed to evaluate the effectiveness of various disinfectants on avian polyomavirus under ideal conditions. It should be noted that the presence of organic debris (i.e., food, feces, feathers, soil) will reduce the efficacy of most disinfectants, increasing the contact time needed for a disinfectant to inactivate a pathogen. Disinfectants that contain a detergent may be more effective in removing organic debris and increase the effectiveness of a disinfectant under these conditions. To maximize the effects of a disinfectant, contaminated surfaces should be thoroughly cleaned, and the disinfectant should be allowed to remain in contact with the surface for a sufficient period of time. For this study, one minute was used as the minimum exposure time for avian polyomavirus and a particular disinfectant.

Preventing Polyomavirus Infections

The environmental stability of avian polyomavirus and predisposition to infection in susceptible birds underscores the importance of choosing an effective disinfectant. This is particularly true in a veterinary hospital, pet shop or aviary where clinically affected birds can contaminate the environment, creating the potential for transmission of the virus.

With the highly infectious nature of the virus, particularly to young psittacine birds, closed breeding operations that do not allow visitors should be encouraged. During an outbreak, birds that are actively shedding the virus can be identified by using a DNA probe.[10, 16, 26] A cloacal swab of any bird that is being added to a collection should be analyzed during the quarantine period to determine whether a bird is shedding polyomavirus (Avian Research Associates, 100 Techne Center, Suite 101, Milford, OH 45150).

Birds that are subclinically infected with avian polyomavirus can be managed by maintaining them in restricted environments in which they do not directly or indirectly (i.e., through contaminated excrement, secretions, bedding or enclosures) expose susceptible birds. These birds should be isolated, not euthanized, because they are likely to be of no further concern when an effective vaccine becomes available for widespread use.

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