Researcher

Avipoxviruses

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Abstract: Avipoxviruses (APVs) belong to the *Chordopoxvirinae* subfamily of the *Poxviridae* family. APVs are distributed worldwide and cause disease in domestic, pet and wild birds of many species. APVs are transmitted by aerosols and biting insects, particularly mosquitoes and arthropods and are usually named after the bird species from which they were originally isolated. The virus species Fowlpox virus (FWPV) causes disease in poultry and associated mortality is usually low, but in flocks under stress (other diseases, high production) mortality can reach up to 50%. APVs are also major players in viral vaccine vector development for diseases in human and veterinary medicine. Abortive infection in mammalian cells (no production of progeny viruses) and their ability to accommodate multiple gene inserts are some of the characteristics that make APVs promising vaccine vectors.

Keywords: Avipoxviruses (APVs); *Chordopoxvirinae; Poxviridae;* mosquitoes; vaccine vectors

Introduction

Avipoxviruses (APVs) are among the largest and most complex viruses known. APVs belong to the Chordopoxvirinae subfamily of the Poxviridae family **(Fauquet et al.,2005).** They infect and cause diseases in poultry, pet and wild birds of many species which result in economic losses to the poultry industry. Infections have also been reported in a number of endangered species or species in captive-breeding recovery programs **(Bohls et al., 2006).** APVs are transmitted via biting insects and aerosols and are usually named on the basis of the bird species from which the virus was first isolated and characterized (**Tripathy et al.,2000)** The disease, which is characterized by proliferative lesions of the skin and diphtheric membranes of the respiratory tract, mouth and oesophagus has been described in avian species **(Bollinger, 1873)**. Although APV infections have been reported to affect over 232 species in 23 orders of birds **(Bolte et al., 1999),** our knowledge of the molecular and biological characteristics of APV is largely restricted to fowlpox virus (FWPV) and canarypox virus (CNPV) for which full-genome sequences are available **(Tulman et al., 2004).** Currently, only ten avipoxvirus species are listed under the genus by the International Committee on Taxonomy of Viruses (ICTV) **(Fauquet et al., 2005)**; thus, it is safe to assume that many APVs have yet to be characterized. Recombinant APVs have been evaluated for use as vaccine vector candidates against infectious diseases **(Boyle, 2007)**. APV-vectored vaccines are already in use in veterinary medicine **(Beard et al., 1991)**

Definition

Avipoxviruses are large, oval-shaped enveloped viruses whose genome consists of double stranded DNA ranging in size from 260 to 365 kb **(Tulman et al.,2004)**. Unlike most other DNA viruses, APVs replicate easily in the cytoplasm of infected avian cells which results in a characteristic cytopathic effect (CPE) 4 to 6 days post infection depending on the virus isolate **(Tripathy et al.,2000)**. APVs also multiply on the chorioallantoic membrane (CAM) of embryonated eggs, resulting in the formation of compact, proliferative pock lesions that are sometimes focal or diffuse **(Cox, 1980)**. However, some isolates, especially from the host species great tit (*Parus major*), have failed to multiply on CAM of chicken embryos **(Holt and Krogsrud, 1973).** APVs are the etiologic agent of disease characterized by skin lesions in both wild and domestic birds **(Tripathy et al., 2000).** Histologically and ultrastructurally, APVs undergo morphologic stages that are similar to other chordopoxviruses, including the formation of intracytoplasmic inclusions bodies, a characteristic which has been observed in some epithelial and mononuclear cells of permissive hosts. APV particles can be detected and further characterized by use of transmission electron microscopy (TEM) **(Weli et al., 2004)**.

Classification

Great discoveries made in the mid-nineteenth century facilitated major advances in pox virology. Based on the report by Bollinger **(Bollinger, 1873)** on poxvirus infected cells in chickens, and subsequent work by Fenner and Burnet **(Fenner and Burnet, 1957)**, APVs and other poxviruses were classified on the basis of original host, growth and morphological characteristics in the CAM of embryonated eggs or cell cultures and on clinical manifestations in different diseases of humans, birds and animals **(Fenner,2000)** rather than on genetic identity, which may provide both rapid and reliable virus identification **(Weli et al., 2004)**. These criteria have remained the basis for subsequent classification of APVs despite development of new molecular tools that have the capability of resolving the issue of species specificity of APV.

Members of the genus *Avipoxvirus* belong to the subfamily *Chordopoxvirinae* which shares several biological features with other poxviruses **(Tulman et al.,2004).** Currently, little is known of the number of species within the genus. While only ten strains have so far been identified and classified Worldwide as APV **(Fauquet et al.,2005)**, avian poxvirus infections have been reported to affect a wide range of bird species **(Bolte et al., 1999)**. These strains vary in virulence and host specificity, demonstrating an urgent need for further analyses and characterization of new isolates.

Pathogenicity

APV infections are associated with significant levels of morbidity and mortality in domestic and wild bird populations **(Tripathy and Reed,2003)**. Most of the investigations and reported cases are based on single APV isolates, which makes it difficult to address the pathogenicity of different APVs in different bird species. Chickens are commonly used to determine the pathogenicity of new isolates, but chickens may not be the ideal host, since APVs from wild birds may not multiply in chickens. In an attempt to identify and characterize the pathogenicity of APVs, Tripathy and others **(Tripathy et al.,2000)**

Diseases

During avipox outbreaks, mortality can reach 80 to 100% in canaries and other finches. This is in contrast to a generally lower mortality seen in chicken and turkey **(Tripathy and Reed,2003)**. Transmission of virus can occur through a break in the skin or, more commonly, when vectored by biting insect such as mosquitoes and mites **(Proctor and Owens,2000)**. Aerosols generated from infected birds, or the ingestion of contaminated food or water have also been implicated as a source of transmission **(Clubb, 1986).** The disease is most commonly characterized by cutaneous proliferative lesions consisting of epithelial hyperplasia of the epidermis that resulting in proliferative, wart-like projections. They are primarily confined to unfeathered parts of the body, such as legs, feet, eyelids and the base of the beak Scars are usually visible after recovery and healing of skin lesions. The mortality in wild birds is usually low, depending on the number and size of the proliferative lesions. However, if infection occurs in feather-free areas of the skin, with secondary bacterial infection, mortality may be high. The other and less common form of APV infections is the diphtheritic or wet form **(Moss, 1992)** which occurs as fibrino-necrotic and proliferative lesions in the mucosa of the digestive and upper-respiratory tracts, and generally has a higher mortality than the cutaneous form **(Tripathy and Reed,2003).** In some instances, birds display both cutaneous and diphtheritic forms and in those cases, mortality rates are often higher compared to the cutaneous form alone. Despite the variety of hosts and virus strains, associated pathology remains the same in infected domestic birds, although clinical signs vary depending on the virulence of the virus, susceptibility of the host, distribution and type of lesions **(Tripathy and Reed,2003).** There exist a relationship between FWPV and the avian retrovirus, reticuloendotheliosis virus (REV) (see section on APVs and REV). However, the possible roles that simultaneous REV infection arising from the provirus integration into the FWPV genome might play in the expression FWPV during disease outbreak remain unresolved. It is well known that REV infection leads to immunosuppresion (**Walker et al., 1983)**

Diagnosis of APV infections

Clinical diagnosis

Clinical features of infected birds show multiple skin lesions varying from papules to nodules. Gross lesions in both the cutaneous and the diphtheritic forms, seen on birds and during necropsy, are usually sufficient to suspect APV infection **(Tripathy and Reed,2003)**. However, these signs are sometimes not sufficient for definitive diagnoses of APV infection as other agents, such as papilloma virus, scaly leg mites **(Pennycott et al.,2003)** and mycotoxins may produce similar lesions in the skin (Tripathy and Reed,2003), and conditions like candidiasis, capillariasis and trichomoniasis may give lesions in the oral cavity similar to the diphtheritic form of APV infection **(Riper and Forrester,2003).**

Laboratory diagnosis

Histopathology and electron microscopy

Suspicion of clinical signs of APV infection can if possible be supported by necropsy, especially if the oral cavities to reveal the diphtheritic form. Further, histopathology on tissue sections using the classic Wright's Giemsa stain may reveal typical large, solid or ring-like, eosinophilic intracytoplasmic inclusions

known as Bollinger bodies **(Bollinger, 1873);**. Transmission electron microscopy (TEM) may also reveal definite proof of APV infection, demonstrating the typical APV particles within inclusion bodies. APV identification may also be carried out by negative staining electron microscopy with 2% phosphotungstic acid (PTA) on infected cells. This method has typically been used by national reference or research laboratories to identify APV **(Weli et al.,2004).**

Virus isolation

Demonstration of infectious virus by inoculation of homogenates of clinical samples of typical APV skin lesions onto the CAM of embryonated hen's eggs is the gold standard method for diagnosis of APV, although some strains of APV do not grow readily on chicken embryos **(Holt and Krogsrud, 1973).** Eggs are first swabbed with 70% alcohol and a pore is made in an area over the air-cell and another one on the other side of the egg to make a false air sac and lower the CAM by negative pressure using a rubber bulb. Inoculation of infectious samples by the CAM route is performed with sterile disposable 1 mL syringe with approximately 0.1-0.2 mL of inoculum. Eggs are incubated at 37°C for 5 days with daily candling to check for embryo death. Pock lesions measuring in size 0.5-1.5 mm are observed on the membrane 3-5 days after inoculation, depending on the virulence of the virus **(Cox, 1980, Holt and Krogsrud, 1973).** Another method of isolation of APV requires the excision and homogenization of clinical skin lesions and inoculation of a homogenate supernatant onto a permissive cell culture, such as CEF cells. This results in the formation of CPE within 4-6 days post inoculation, depending on the virus isolate and on the multiplicity of infection (MOI) **(Tripathy et al.,2000).**

Molecular techniques for detection and characterization

APV are increasingly being detected and characterized by PCR, Restriction fragment length polymorphism (RFLP), Southern blot hybridization, and cycle sequencing, directed at specific genes such as the 4b core protein gene **(Luschow et al.,2004, Weli et al.,2004)**. PCR allows for sensitive and specific detection of viral nucleic acids and has been shown to increase the diagnostic sensitivity for many viral pathogens when compared to culture. A PCR amplicon sequence allows a rapid search for homologous sequences in gene databases, to verify and identify the virus in question and to address phylogenetic relationships. Detection by real-time PCR has been used to identify recombinant APV from individual plaques **(Boyle et al.,2004).** This method eliminates the need for amplification and hybridization from the transient dominant protocol and results in

significant savings of time at each round of plaque purification **(Boyle et al.,2004).**

Serological assays

The conventional serological techniques of passive neutralization and agar-gel immunodiffusion are in continued global use for surveillance and disease control efforts in domestic poultry species **(Baxi and Oberoi, 1999, Tadese et al.,2003)** despite the availability of modern molecular and immunoassay techniques. The tests are time consuming, especially when carried out with large numbers of sera, and sensitivity appears to be low when compared with other detection method, such as enzyme linked immunosorbent assay (ELISA) **(Smits et al.,2005).** ELISA has been described as a non-species specific test approach for birds **(Buscaglia et al., 1985).** It is a faster and easier method to detect antibodies against APV, particularly when large numbers of sera are to be tested. The technique is also more sensitive than the neutralization test **(Weli et al., 2004, Buscaglia et al., 1985)**. ELISA protocols have also been developed and used to test the efficacy of FWPV vaccines in commercial and wild bird species where agar-gel immunodiffusion is ineffective due to lack of precipitating antibodies **(Kim and Tripathy, 2006, Wang et al.,2006)**.

Prevention and treatment

The challenges of controlling APV disease in poultry are driven by economics, and require strategies that keep cost low while maintaining treatment efficacy. Prophylaxis can be achieved by vaccination **(Boulanger et al., 2000). Doyle, (1930)** reported the use of live FWPV or Pigeonpox virus for vaccination against APV infection. Since then, recombinant and live modified vaccines have been developed and used to prevent APV infections in chickens, pigeons, turkeys and quails **(Wang et al.,2006, Taylor et al., 1994);**. These vaccines are very effective and have undoubtedly contributed immensely to the prevention of the disease in commercial poultry farming **(Paoletti, 1996(.** Since different APVs are isolated from a wide range of bird species and since only a few isolates have been characterized, development of a taxon-specific vaccine, directed to all species, has been difficult. Thus, available vaccines are often applied on the basis of experimentation, and more knowledge of molecular biology, pathology and epidemiology of these viruses is necessary to develop vaccines that effectively can protect a range of bird species. As in most viral infections, there is no specific treatment for avian poxvirus infections in birds **(Boulanger et al., 2000, Redig, 1979).** Available treatments include the use of iodine-glycerin application on proliferating skin lesions to aid healing **(van Heelsbergen et al., 1929),** antibiotics to control

secondary bacterial infections and vitamin A to aid healing **(Cooper, 1985).**

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