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A REVIEW OF PSITTACINE BEAK AND FEATHER DISEASE

CHARACTERISTICS OF THE PBFD VIRUS

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Summary

A novel virus isolated from the feather follicles of cockatoos diagnosed as having psittacine beak and feather disease was characterized by electron microscopy, nucleic acid content, and polypeptide composition. Purified virions displayed an icosahedral symmetry, were nonenveloped, and negatively stained particles had a mean diameter of 14 to 16 nm. Three major viral associated proteins were identified, with approximate molecular weights of 26.3, 23.7, and 15.9 kilodaltons. The viral nucleic acid was found to be single-stranded DNA based on acridine orange staining, resistance to alkali and ribonuclease, and sensitivity to both DNase1 and S1 nuclease. The DNA was estimated to be between 1.7 and 2.0 kilobases (kb) by agarose gel electrophoresis and this size and its circular conformation were confirmed by electron microscopy. A preliminary infectivity study using purified virus to infect neonatal budgerigars induced pathological lesions characteristic of those observed in the natural disease. Based on the extremely small size of the virions and the single-stranded circular viral DNA, we propose that the etiologic agent of psittacine beak and feather disease represents a previously undescribed viral pathogen. Based on the extremely small size of the virions and the small single-stranded circular viral DNA, we have proposed the name Dininuviridae to the International Committee on the Taxonomy of Viruses (ICTV) for the viral family to which the PBFD agent and similar viruses may belong. (Reprinted in part with permission from Virology 171:83-88, 1989.)

Branson W. Ritchie received his DVM degree from the University of Georgia in 1985 and participated in an internship in zoo animal medicine at the Riverbanks Zoo in Columbia, South Carolina. He returned to the University of Georgia where he developed the avian and zoological medicine program in the veterinary curriculum. He currently serves as a clinician in that program, is faculty adviser to the Student Chapter of AAV, and is pursuing a PhD degree in virology.

In the mid 1970’s a disease characterized by symmetric feather dystrophy and loss, development of beak deformities, and usually death was described in a number of South Pacific psittacine birds.1-3 In the last several decades, histologic or clinically suggestive le-
sions of psittacine beak and feather disease (PBFD) have been increasingly recognized in a variety of birds and currently some 30 species of psittacine birds have been reported with the disease. While numerous white cockatoo species are in the reported list of susceptible hosts, there has only recently been documentation of PBFD in a black cockatoo genus.4 Prior to recent reports describing histologic lesions and viral isolation from two species of Amazon parrots (New World Psit­ taciformes), the disease was thought to be restricted to Old World and South Pacific psittacine birds.4·6 Investigations continue to indicate that the actual host range of PBFD remains largely unknown.4 With the wide spread global movement of birds for the pet market, the potential for introducing the highly virulent virus responsible for PBFD into wild populations of the world’s more endangered psittacine species deserves serious consideration.

THE CLINICAL SYNDROME
Psittacine beak and feather disease is most commonly reported in young birds during their first feather formation after replacement of the neonatal down, and neonates as young as two months of age have been described with classic lesions.2 PBFD is reported most commonly in birds less than three years of age, however discussions that birds greater than three years old are safe from the disease are incorrect. Ten-, fifteen- and twenty-year-old birds that had previously shown no signs of PBFD have been diagnosed with the disease2·3·6·9 (Fig. 1).

Both acute and chronic clinical syndromes of PBFD have been described and disease progression varies markedly. The type of clinical disease is possibly influenced by the route of viral exposure, the titer of the infecting virus, the virulence of the viral strain, and the age and condition of the bird when exposure occurs. The acute form of PBFD is common in fledgling and immature birds and is characterized by lethargy, depression, diarrhea and often death. Cross feather lesions in the acute form of the disease can be quite subtle with only a few feathers showing dystrophic changes. If a bird survives the initial viral infection it may start to develop the progressive feather changes recognized in the chronic form of PBFD (Fig. 2).

Feather loss associated with the chronic form of PBFD is basically symmetrical and normal plumage is progressively replaced with dystrophic feathers during a molt. Typically, the first sign of PBFD is the replacement of normal down and contour feathers with dystrophic, necrotic, non-viable feathers that stop growing shortly after emerging from the follicle. It has been assumed that the increased frequency of initial lesions in the powder-down feathers is based on their consistent molt pattern compared to the seasonal loss found in other feather tracts. The disease progresses during the ensuing molts to a point where the flight and tail feathers may also be dystrophic and, depending on the species, the bird may appear bald. In contrast to the classic presentation, some birds have substantial involvement of the flight, tail, and crest feathers with only minimal changes in the powder-down feathers.1·6·9·11 Gross feather lesions associated with PBFD include retained feather sheaths, blood within shafts, short clubbed feathers, deformed curled feathers, stress lines in vanes, and circumferen-

Fig. 1. A producing Major Mitchell hen that was over 20 years old when she first developed clinical signs consistent with PBFD.
tial constrictions\textsuperscript{1,5,6,10,11} (Fig. 3).

Clinical changes in the beak and oral mucosa are characterized by progressive elongation, transverse or longitudinal fractures, palatine necrosis, and oral ulceration\textsuperscript{1,6,11} (Fig. 4).

Depending on the species involved and other factors that remain unresolved, beak pathology may or may not be present. Beak pathology usually develops with the chronic form of the disease following substantial feather loss. However, some individuals develop severe beak lesions with relatively minor feather pathology. Beak pathology does not routinely occur with some species while with others, such as the Sulphur-crested Cockatoos, galahs, little corellas, and Moluccan Cockatoos, beak lesions are relatively common\textsuperscript{1,5,7,11}

Birds can live months to years following the development of clinical changes consistent with PBFD and usually die from pathologic changes induced by secondary bacterial, fungal, or other viral agents. The predilection of birds to die from secondary or opportunistic pathogens has been interpreted to indicate an immunosuppression that is thought to be caused by damage to the thymus and bursa\textsuperscript{7,12-14} While only limited work has been performed to document the proposed immunosuppression, PBFD patients were found to have low concentrations of pre-albumin and gammaglobulin as indicated by serum electrophoresis\textsuperscript{7}.

Several reports indicate the possibility of asymptomatic adults producing neonates with clinical signs of PBFD in successive breeding seasons. This finding suggests a carrier state may exist with vertical or horizontal transmission of PBFD virus from parent to offspring and/or a genetic predisposition to the disease\textsuperscript{3,7,8,15}. In most cases of parent to offspring transmission, epidemiologic investigations indicate probable exposure to the PBFD virus occurring through sources other than the parents.

**PATHOLOGY**

The pathology associated with PBFD has been thoroughly characterized by a number of investigators both at the light and electron microscopic levels. Feather, beak, and claw dystrophy are basically the result of multifocal necrosis of epidermal cells, epidermal hyperplasia, and epidermal hyperkeratosis\textsuperscript{1,6,6,11}. The primary feather pulp lesion of PBFD is characterized by non-suppurative inflammation, including perivascular accumulations of plasma cells and lymphocytes. In chronically infected birds the feather pulp is typically edematous with accumulations of heterophils and macrophages\textsuperscript{1,6,8}. There is commonly a diffuse necrosis of epithelial cells throughout the epidermal collar and in the basal and intermediate layers of the developing feather\textsuperscript{1,6,8}. Basophilic intranuclear and intracytoplasmic inclusion bodies have been consistently demonstrated in sections of the feathers, beak, thymus, and bursa of Fabricius taken from birds with clinical signs of PBFD. The presence of these inclusion bodies is considered diagnostic\textsuperscript{1,6,9}.

The basophilic intracytoplasmic inclusion bodies are variable in size, may be membrane-bound or free in the cytoplasm, and are commonly found in macrophages within the basal and intermediate layers of the epidermal collar and the pulp\textsuperscript{1,5,7,11}. Electron microscopic examination of the intracytoplasmic inclusion bodies have consistently demonstrated 17-22 nm electron dense
Beak and Feather Disease

Fig. 5. Cesium chloride equilibrium density gradient for purification of PBFD virus. An opalescent band was visible (inset; arrow) which corresponds to a protein peak at density 1.378 g/cc (Fraction 29).

Fig. 6. Electron micrograph of negatively stained beak and feather disease virus particles. Virus suspensions were negatively stained with 1% phosphotungstic acid and photographed at 80 kV. Tobacco mosaic virus (arrow: 15 nm mean diameter) is the internal standard.

Fig. 7. Identification of the major viral proteins. (Lane 1) Molecular weight markers: bovine albumin (60,000), carbonic anhydrase (29,000), alpha-lactalbumin (14,200); (Lane 2) Purified virus suspension (25 μg total protein).

Fig. 8. Acridine orange stained glyoxalated treated nucleic acid samples run by agarose mini-gel electrophoresis. Acridine orange fluoresces orange when bound to single-stranded nucleic acids and green when bound to double-stranded nucleic acids. (Lane 1) Glyoxalated molecular weight markers (all bands fluoresces orange); (Lane 2) glyoxalated viral DNA (band fluoresces orange); (Lane 4) glyoxalated phi-X-174 (+) DNA (fluoresces orange). Viral DNA mobility corresponds to 1.8 kb linear molecule and phi-X-174 mobility corresponds to a 5 kb linear molecule.

granules in non-membrane-bound paracrystalline arrays and semicircles. Viral-like particles in the 20-26 nm range have been reported from feather homogenates made from diseased feathers taken from Sulphur-crested Cockatoos, galahs, lovebirds, budgerigars, and Western Rosellas. Based on the estimated size of the virus-like particles and the pathology associated with the disease, the suspected viral etiology had been suggested as being within the Parvoviridae or Picornaviridae families.

RECOVERY AND CHARACTERIZATION OF THE VIRUS

Repeated attempts by various laboratories to adapt the suspected viral agent of psittacine beak and feather disease to in vitro cell culture have been consistently disappointing. Because it is likely that this virus has high in vivo tissue specificity or growth requirements, current studies have been conducted on virus purified from diseased tissue.

Virus was purified by differential and isopycnic centrifugation from affected feather follicles of birds that were diagnosed with PBFD. A single visible band was present in cesium chloride gradients following centrifugation to equilibrium (Fig. 5 inset; arrow). Analysis of the protein content of fractions collected from these gradients revealed a single peak at a density of 1.378 g/cc (Fig. 5).

Ultrastructural analysis of negatively stained virus preparations collected from these fractions revealed a highly concentrated homogenous preparation of 14-16 nm diameter icosahedral, nonenveloped virus particles (Fig. 6). The viral preparation consisted mainly of intact virions at an estimated concentration of 3 X 10^14 particles/ml which was determined based on estimating the number of viral particles observed per grid square on a 400 m grid.

Virus representing an estimated concentration of
$10^6$ virions/feather shaft could be consistently demonstrated by making extracts from individual diseased feathers collected from PBFD positive birds. Virus was not identified in follicle preparations produced from birds that did not have clinical signs of PBFD.

To identify and characterize the proteins associated with purified virus, samples were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 7). Three major protein bands were identified with molecular weights of 26,300$^a$, 23,700$^b$, and 15,900$^c$. The proteins found in the greatest proportions (A and B) were present in approximately equimolar amounts and constituted 88% of the total protein as determined using Beckman DU-64 Spectrophotometer gel analysis software. Trace quantities of proteins in the 60,000 molecular weight range are also present following SDS-polyacrylamide gel electrophoresis in some viral samples evaluated.

Identification and characterization of viral encoded polypeptides will await the results of in vitro translation studies.

Nucleic acid was extracted from the purified virus preparation with SDS-phenol-chloroform and was recovered by ethanol precipitation. The viral nucleic acid was initially identified as single-stranded and of low molecular weight based on its differential staining with acridine orange and by its electrophoretic mobility on agarose gels. Purified viral nucleic acid was stable to base hydrolysis and to digestion with RNase A in low salt buffer. The nucleic acid was confirmed to be single stranded DNA by its susceptibility to digestion by both pancreatic DNase and S1 nuclease.

The size of the viral DNA was estimated to be approximately 1.7-2.0 kb by electrophoresis in alkaline agarose and by glyoxal denaturation followed by agarose gel electrophoresis (Fig. 8). Gels were stained with acridine orange to differentiate between single-stranded (orange fluorescence) and double-stranded (green fluorescence) molecules. Glyoxalated linear DNA fragments (Lane 1) were included as size markers. The glyoxalated viral DNA (Lane 2) migrated to a position between those of the glyoxalated (single-stranded) 1.6 and 2.0 kb linear fragments (Lane 1). Since the conformation of the viral DNA was unknown, a single stranded circular DNA molecule (phi-X-174 (+) strand DNA) was also included as a size marker. Glyoxalated phi-X-174 (+) DNA (ca. 5kb) (Lane 4) comigrated with the glyoxalated (single-stranded) 5 kb linear DNA in this gel system. Based on the electrophoresis data, the size of the viral DNA molecule was estimated to be between 1.7 and 1.9 kilobases.

In order to rule out the possibility that DNA purification steps had produced the observed 1.7-1.9 kb species, purified virus suspension was adjusted to 0.05% in SDS and loaded onto an agarose minigel in Tris-borate-EDTA buffer containing SDS. Following this gentle dissociation of viral components, a single orange staining band was observed with the same mobility as that of purified viral DNA.

To confirm the size of the viral DNA and to determine its molecular conformation, the nucleic acid was visualized directly by electron microscopy (Fig. 9a & 9b). The viral genome consisted of predominately circular
Fig. 10. Twenty-five-day-old neonatal budgerigar inoculated with purified PBFD virus at five days of age by oral and intracloacal routes. The bird exhibited slowed maturation and poor feather formation.

molecules, (72% circular, 28% linear). Using phi-X-174 (+) strand DNA as an internal size standard (5,386 bases, 1.77 microns – Bethesda Research Laboratories), image analysis of the nucleic acid indicated that the circular viral genome was approximately 2.0 kb.

**INFECTIVITY**

To confirm that this virus is the etiologic agent of PBFD, preliminary infectivity studies using neonatal budgerigars have been performed. This avian species had been previously reported as an acceptable host for PBFD infectivity studies.16 Inoculation of birds with the purified virus through the combined oral, and intracloacal routes produced clinical signs of stunting and poor feather formation (Fig. 10). Histologic lesions consistent with PBFD were demonstrated in follicular epithelium, the thymus, and the bursa of Fabricius of the infected birds. The lesions were confirmed to be viral induced by demonstrating specific binding with FITC-labeled rabbit anti-PBFD virus antibody (Figs. 11, 12).

**COMPARATIVE CHARACTERISTICS OF PBFD VIRUS**

Based on previous ultrastructural analysis of cytoplasmic inclusion bodies from birds diagnosed with PBFD, the viral-like particles identified in these inclusions have been described as being most likely within the family Paroviridae.1,2,4,8,15 Paroviruses have been considered to be the smallest DNA viruses producing disease in animals and are characterized as icosahedral nonenveloped viral particles 19-22 nm in diameter that contain a single-stranded linear DNA genome of approximately 5 kb.18 Polypeptides associated with mammalian parovirus have molecular weights of 73,000-92,000; 63,500-64,000; 61,000-63,000; and 40,000-56,000. The largest protein has been consistently demonstrated for all autonomous paroviruses.19

In contrast, the PBFD virus is a 14-16 nm diameter, icosahedral, nonenveloped virion. The size of these particles is significantly smaller than that reported for any previously described pathogenic virus isolated from animals (3-8 nm smaller than parovirus) and the genome consists of single-stranded circular DNA of an estimated size of 1.7-2.0 kb. The protein composition of PBFD virus is also different from that described for paroviruses with the three major viral associated proteins having molecular weights of 26,300, 23,700, and 15,900.

The virion size and nucleic acid characteristics described for the PBFD virus are similar to those found for a nonpathogenic virus that chronically infected a porcine (PK-15) cell line.20 This virus, however, exhibited no evidence of pathogenicity when susceptible swine were infected.21 The porcine circovirus has been reported to have a single protein of MW 34,000 and a single stranded circular DNA of 1.7 kb. Further comparisons between the porcine circovirus and the PBFD virus are being made.

Based on the radical differences in virion dimension,
polypeptide composition, and nucleic acid size and configuration, we suggest that the etiologic agent of psittacine beak and feather disease is a prototype for a new family of pathogenic animal viruses. We have suggested to the ICTV the name Diminuviridae, which means smaller than average, for the family to which the PBFD virus and related agents may belong.

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Key words: Psittacine beak and feather disease, PBFD, Diminuviridae, Virus, Viral characteristics.

**REFERENCES**


**TRANSLATIONS**

**A REVIEW OF PSITTACINE BEAK AND FEATHER DISEASE**

**Zusammenfassung**

Resumen

Se ha aislado un nuevo virus de los folículos de las plumas de cacatúas el cual se diagnosticó como la enfermedad de pico y plumas. Este virus se identifica mediante microscopía electrónica, contenido de ácido nucleico y composición de los polipéptidos. Los viriones purificados muestran una simetría icosaedrónica, son viriones desnudos y con partículas con tinciones negativas con un diámetro promedio de 14 a 16 nm. Se identificaron tres proteínas asociadas a este virus con pesos moleculares de 26.3, 23.7 y 15.9 kilodaltones. El ácido nucleico viral es de una sola hélice demostrado por una coloración de acridina naranja resistente a los alcalis y a la ribonucleasa y sensible a la DNasa 1 y Nucleasa S1. Su contenido de ADN se estimó en un margen de 1.7 a 2.0 kb por electrophoresis en gel de agarosa. Su conformación fue confirmada por microscopía electrónica. Se hizo un estudio de su capacidad infectiva utilizando el virus purificado inyectando periquitos australianos recién eclosionados en los que se indujeron las lesiones micro- y macroscópicas que se observan en la forma natural de la enfermedad. Tomando como base el tamaño extremadamente pequeño del virión, hemos propuesto el nombre de Diminovirus al Comité Internacional para la Taxonomía de los virus (con sus siglas en Inglés ICTV) para la familia hacia la cual el agente de la "enfermedad de pico y plumas" y virus similares pertenecen. (Reproducido en parte con permiso de la revista "Virology" 171:83-88, 1989.)

La Sommaire

Un nouveau virus isolé à partir des follicules des plumas de cacatoe atteint du PBFD, a été identifié par la microscopie électronique, ainsi que par le contenu de son acide nucléique, et la composition de ses polypeptides. Les virions purifiés qui ont révélé une symétrie icosaédrique, étaient non-envelopés, et les particules à coloration negative avaient un diamètre moyen de 14 à 16 nm. Trois protéines virales majeures associées furent identifiées, avec des poids moléculaires d'environ 26.3, 23.7 et 15.9 kilodaltons. L'acide nucléique viral s'est révélé être d'un seul brin, coloré par l'orange d'acridine, résistant à l'alcali, et à la ribonuclease, et sensible à la fois à DNase 1 et S1 nucléase. Le DNA a été estimé entre 1.7 et 2.0 kilobases (kb) par électrophorèse sur gel d'agarose et cette taille, ainsi que sa conformation circulaire, ont été confirmés par la microscopie électronique. Une esquisse préliminaire du pouvoir pathogène du virus purifié par contamination expérimentale de perroquets ouvrière nouveau-nées, a révélé l'induction de lesions pathologiques identiques à celles observées dans la maladie naturelle. En se basant sur la très petite taille des virions, et la conformation en un seul brin circulaire du DNA viral, nous proposons ce virus pathogène initialement non décrit, comme étant l'agent étiologique du PBFD. En se référant à la très petite taille des virions, d'une part, et au petit DNA en un seul brin circulaire d'autre part, nous avons proposé le nom de Diminuviridae comme famille virale à laquelle l'agent du PBFD, et les virus similaires, doivent appartenir, au comité international sur la taxonomie des virus. (reproduit en partie avec l'autorisation de Virology 171:83-88, 1989.)

TREATMENT OF A FALCON FOR LOCALIZED TETANUS

Zusammenfassung

Ein Sakerfalke mit klimischen Symptomen, die auf lokalisierteren Tetanus in Zusammenhang mit "dicken Händen" hinwiesen, wurde vorgestellt. Der erfolgreiche Behandlungsversuch bestand aus lokaler Entfernung von Debris, Applikation von antimikrobiell wirksamen parenterale e imunoterapia tuvo éxito. Las síntomas clínicos fueron desapareciendo gradualmente en un lapso de 30 días.

Resumen

Un Halcón Sacre fue presentado con síntomas clínicos de tétano localizado, aparentemente asociado con una lesión del tipo conocido como pododermatitis o "clavos." El tratamiento con debridación quirúrgica, antibióticos parenterales e inmunoterapia tuvo éxito. Los síntomas clínicos fueron desapareciendo gradualmente en un lapso de 30 días.

La Sommaire

Un faucon Sacre a été présenté avec des signes cliniques consistant en tétanies localisées, apparemment en relation avec une lésion de podagre. La guérison fut obtenue par un curetage local associé à un traitement antimicrobien systémique, et une immunotherapie. La régression des signes cliniques, jusqu'à leur disparition s'effectuait progressivement sur une période de 30 jours.

German translations by Helga Gerlach.
Spanish translations by Jesus Estudillo, Johanna Storm, and Javier Lopez.
French translations by Christian Bougerol and Steve Metz.