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Short communication

Incidence and detection of beak and feather disease virus in psittacine birds in the UAE

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ABSTRACT

Beak and feather disease is caused by Circovirus, which affects actively growing beak and feather cells of avian species. The disease affects mainly young birds while older birds may overcome the disease with few lasting effects. Due to lack of treatment, the only way to control the disease is through hygiene and early diagnosis. As a diagnostic tool, we have established a Taqman probe based real-time PCR assay to detect the presence of the viral genome in psittacine birds in UAE and reported the incidence of circovirus in different species of psittacine birds. The sensitivity of our assay was found to be very high with detection limit of up to 3.5 fg of DNA in the sample. The mean prevalence of circovirus was found to be 58.33% in African Grey Parrots, 34.42% in Cockatoos, 31.8% in amazon parrots and 25.53% in Macaws.

The Taqman assay is a quick, reliable and sensitive detection method that has been instrumental in identifying this disease that was not previously reported in the region.

1. Introduction

Psittacine beak and feather disease (PBFD) is mostly observed in Australian and South African avian species [5]. PBFD was first recognized and described thoroughly in 1975 by Dr. Ross Perry, a veterinary practitioner in Sydney. A number of birds showing feather and beak abnormalities were identified, initially in private collections and in wild flocks in Australia, but subsequently spreading rapidly around different parts of the world (http://www.environment.gov.au).

PBFD has since then been recognized as one of the most significant diseases of psittacine birds worldwide. Initial theories as to its cause included genetic or deficiency problems. Research at Murdoch University, Australia, and the University of Georgia, USA has demonstrated its cause to be an extremely small circovirus, which is a small, non-enveloped DNA virus of the Circoviridae family. It has a single-stranded, circular-DNA genome [12,17] that is 16 nm in diameter, 1993 nucleotides in length, encodes three proteins and is surrounded by a spherical capsid with icosahedral symmetry. The virus specifically infects psittacine birds and is mostly fatal in young birds. Cockatoos, Macaws, African Grey Parrots and Ring-necked parakeets are some of the species known to be susceptible to this virus. Besides Psittaciformes, the virus also infects other avian families like Columbiformes, Passeriformes and Anseriformes [13]. Circovirus, typically, targets actively growing cells in beak, claws and feather follicles causing feather malformation and feather loss. Besides that, it affects the bursa fabric and the thymus, causing immunosuppression, as lymphocyte production becomes limited.

Although, the virus is known to occur naturally in the wild population, the distribution of the disease and factors involved in its spread are not well understood. The virus spreads horizontally – to adjacent birds by direct contact; and in adult carrier birds that reach breeding age, vertically – through the eggs and chicks affecting the next generation. Virus infectivity probably persists in contaminated nests for many months or even years (http://www.theparrotsocietyuk.org).

Young birds usually succumb to the infection while older birds may overcome the disease with few lasting effects. Surviving birds are known to shed the virus and a small percentage of birds acquire lasting immunity [9]. Whilst many attempts have been made to produce a vaccine to combat the infection, to date none has been successful. There is no known treatment and the only way to control the disease is through hygiene, strict isolation or culling of all infected birds. This stipulates the importance of early diagnosis of

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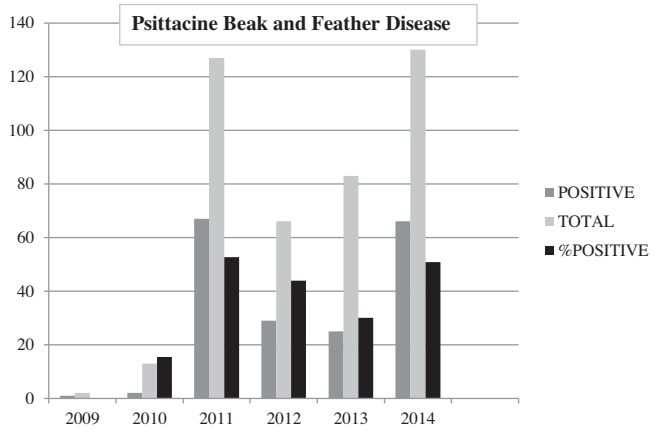

Fig. 1. Year wise (2009–2014) distribution of the total number of samples tested for Circovirus and number of samples identified as positive by real-time PCR.

the disease to control its progression in infected birds and avoid its spread amongst uninfected ones.

Psittacine beak and feather disease has been found to be widespread in different countries of the world with a reported prevalence of 23% in Australia [5], 40.4% in Germany [10], 8% in Italy [1], 41.2% in Taiwan [3] and 3.5–4% in USA [2]. The disease has been identified previously using traditional serological methods. Raidal et al. [11] have reported anti-PBFD antibodies in Australian psittacine species and Macwhirter [7] has reported the presence of antibodies against the virus in the range of 41–94% in sulfur crested Cockatoos, galahs, little corellas and long billed corellas. However, serological tests are good indicators of immune status and presence of chronic disease in the bird, but are of little value in predicting a clinical diagnosis of the disease. More recently, molecular based tests [4,8,15,18] that use specific primers for the target sequence, have been more successful in making a timely diagnosis of the disease.

In the past few years, there has been an increase in the international trade of live birds across the world [7]. The bird markets in the UAE are also swamped with birds imported from different countries that may be carriers of diseases. These birds are usually bought by owners from these markets and housed as pets or held captive in private farms.

We report here for the first time in the UAE, the prevalence of circovirus causing beak and feather disease, through detection of viral particles in blood and feathers of psittacine birds using a highly sensitive Taqman probe-based real-time PCR assay that is established in our laboratory. As the PCR method detects the virus (antigen), the assay can detect active as well as latent infection.

2. Materials and methods

A total of 421 blood or feather samples from pet shops, veterinary clinics, private owners and wildlife parks were received for testing over a period of five years, from October 2009 to December 2014. Although we recommended and mostly received blood for testing, some clients submitted feather samples instead. Fig. 1 shows the number of samples received each year and the number of positive samples identified. The species tested included African Grey Parrots, Macaws, Cockatoos, Parakeets, Galahs, Amazon and Conure.

2.1. Extraction of DNA from blood

DNA was extracted from EDTA blood using the Qiagen DNeasy blood and tissue kit (Qiagen Ltd., Crawley, United Kingdom) according to the manufacturer's instructions. Briefly, 10 μL of blood was mixed with 190 μL of PBS and 20 μL of proteinase K was added. After addition of 200 μL Buffer AL, the sample was vortexed and incubated at 56 °C for 10 min. Then, 200 μL ethanol (96–100%) was mixed with the sample and it was loaded into a DNeasy Mini Spin Column for purification and DNA was eluted in 50 μL of elution buffer and stored at 4 °C. DNA was quantified using Nanodrop ND-1000 spectrophotometer (USA).

2.2. Extraction of DNA from feathers

Alternatively, DNA from roots of feathers was extracted using alkaline lysis (heating in 20 μL of 0.2 M NaOH for 7 min at 95 °C and neutralizing with 75 μL of 0.1 M Tris–HCl) and purified using phenol–chloroform method.

2.3. PCR amplification

Primers PBFD-F GCCCACGTGACTTCAAGACT and PBFD-R ACGGAGCATTTCGCAATAAG (Metabion, Germany) were designed using Primer 3 express software to amplify a 194-bp region of the replication associated protein gene (V1) (Gene bank accession DQ397817.1) of beak and feather disease virus isolate AFG5-ZA. These were used in conjunction with the Taqman probe Fam-TCGTGGGGACCTCGATCTCACTCG-Tamra. The real-time PCR was performed using Roche Light Cycler 2.0 (Manheim, Germany) under the following conditions: initial denaturation at 95 °C for 8 min, amplification at 95 °C for 10 s, 51 °C for 20 s and 72 °C for 30 s for 45 cycles. A Circovirus positive amplification control and negative control without DNA was included in the PCR. Samples that showed a Ct value of ≤40 and an exponential fluorescence were scored as positive and samples that did not fulfill these criteria were scored as negative.

2.4. Duplex PCR with internal control

To ensure the success of our extraction protocol, we incorporated an internal DNA extraction control (DEC) (Bioline, UK) in the sample. 4 μL of DEC was added to the sample or PBS (negative extraction control) at the lysis stage and extraction was done as above using the Qiagen DNeasy blood and tissue kit. Duplex PCR with internal control primer (control mix) (Bioline, UK) was setup with the same cycling conditions as above.

2.5. Sensitivity

Sensitivity of the test was determined by making 10-fold serial dilutions of DNA obtained from positive blood and feather samples with an initial concentration of 86 and 35 ng/μL, respectively. PCR amplification was performed using at least 8 log dilutions and a standard curve was prepared.

2.6. Sequencing

Circovirus positive DNA from 7 African Grey Parrots, 3 Macaws and 2 Cockatoos were selected for sequencing. Forward and Reverse primers were: PBFD-F and PBFD-R. Sequencing was done using dideoxy Sanger method and run on the ABI 3730 XL (USA). Sequences were analyzed using sequencing analysis software (Version 5.1.1) and aligned using DNAMAN.