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Publication Information

Mele, Stephen; Picardo, Kristin F.; Cunningham, Gregory B.; and Hurd, Daryl D., "The Isolation and Identification of a Causative Agent of the Feather Disorder Found in African Penguins (Spheniscus demersus)" (2012). *Biology Faculty Publications.* Paper 15.
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The Isolation and Identification of a Causative Agent of the Feather Disorder Found in African Penguins (Spheniscus demersus)

Abstract

Beginning in 2006, wild juvenile African Penguins (Spheniscus demersus) began to prematurely lose their juvenile feathers without immediate regrowth and were brought to the South African Foundation for the Conservation of Coastal Birds (SANCCOB) for rehabilitation5. Without immediate regrowth of feathers, energy is shunted away from growth and used for thermoregulation and metabolism. It has previously been hypothesized that potential viral and bacterial infections may be causing this disorder3,4. To test for this, Avian Polyomavirus (APV) nucleic acids, Budrigars Beak and Feather Disease Virus (BFDV) nucleic acids, and any bacterial nucleic acids were attempted to be isolated from the blood of affected penguins. Blood was drawn from affected and non-affected African Penguins at SANCCOB and stored in 70% ethanol. These samples were collected in 2008 and 2010. The samples were shipped to St. John Fisher College in Rochester, NY during the winter of 2011. Nucleic acids were then extracted from the blood using a QIAamp Blood DNA Mini. After confirmation of DNA via gel electrophoresis, PCR was performed using 2X OneTaq Megamix, water, and primers specific to the targeted viral and bacterial DNA. Gel electrophoresis was run on the PCR products. If DNA was observed at an expected range, then the PCR product was purified using a QIAquick PCR Purification Kit using the protocol included. The purified samples were sent to ATCG, Inc. for sequencing. The results were analyzed using NCBI BLAST. To date, six sequencing samples have shown the prevalence of APV, BFDV, and/or bacteria in the blood of affected penguins.

Disciplines

Biology

Comments

Poster presented at the Rochester Academy of Science Fall Scientific Paper Session, St. John Fisher College, November 10, 2012.

This poster presentation is available at Fisher Digital Publications: http://fisherpub.sjfc.edu/biology_facpub/15

The Isolation and Identification of a Causative Agent of the Feather Disorder Found in African Penguins (*Spheniscus demersus*)

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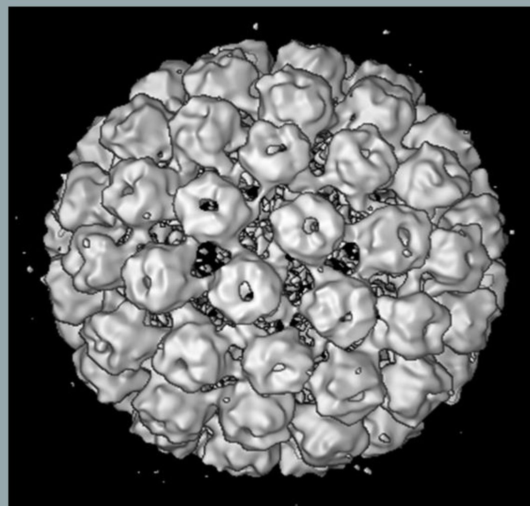
ABSTRACT

Beginning in 2006, wild juvenile African Penguins (*Spheniscus demersus*) began to prematurely lose their juvenile feathers without immediate regrowth and were brought to the South African Foundation for the Conservation of Coastal Birds (SANCCOB) for rehabilitation[5]. Without immediate regrowth of feathers, energy is shunted away from growth and used for thermoregulation and metabolism. It has previously been hypothesized that potential viral and bacterial infections may be causing this disorder[3,4]. To test for this, Avian Polyomavirus (APV) nucleic acids, Budrigars Beak and Feather Disease Virus (BFDV) nucleic acids, and any bacterial nucleic acids were attempted to be isolated from the blood of affected penguins. Blood was drawn from affected and non-affected African Penguins at SANCCOB and stored in 70% ethanol. These samples were collected in 2008 and 2010. The samples were shipped to St. John Fisher College in Rochester, NY during the winter of 2011. Nucleic acids were then extracted from the blood using a QIAamp Blood DNA Mini. After confirmation of DNA via gel electrophoresis, PCR was performed using 2X OneTaq Megamix, water, and primers specific to the targeted viral and bacterial DNA. Gel electrophoresis was run on the PCR products. If DNA was observed at an expected range, then the PCR product was purified using a QIAquick PCR Purification Kit using the protocol included. The purified samples were sent to ATCG, Inc. for sequencing. The results were analyzed using NCBI BLAST. To date, six sequencing samples have shown the prevalence of APV, BFDV, and/or bacteria in the blood of affected penguins.

HYPOTHESES

The observed feather disorder is being caused by:

1. **A bacterial pathogen**
2. **The Avian Polyomavirus**
3. **The Beak and Feather Disease Virus**



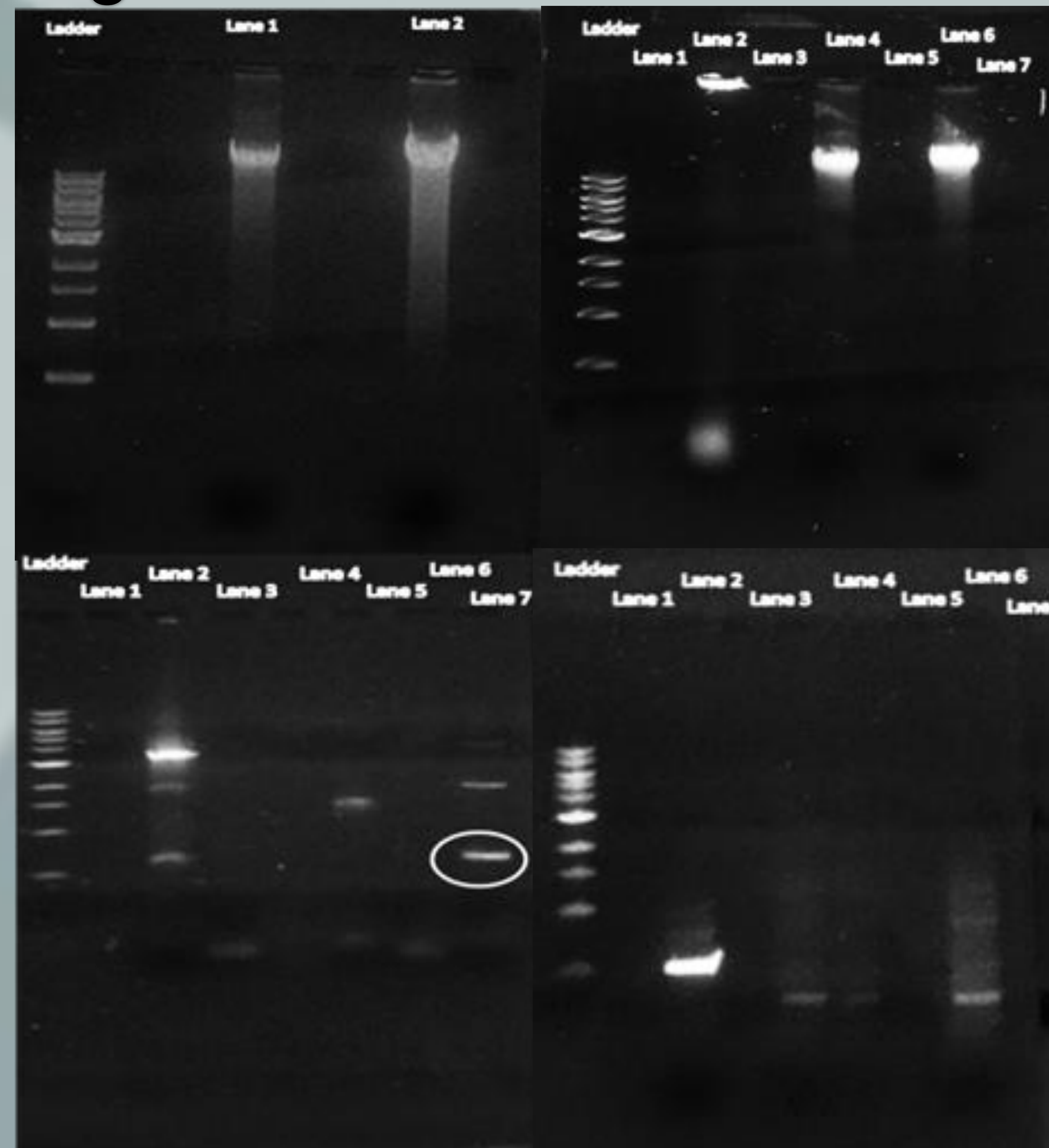
Surface of Avian Polyomavirus

METHODS

Blood samples collected in 2008 and 2010 were stored in 70% ethanol sent to St. John Fisher College. DNA was extracted from blood samples using QIAGEN's DNA Blood Mini Prep and confirmed by gel electrophoresis. Primers that were developed to the genomes of APV and BFDV were used for PCR. Universal bacterial primers were used to amplify bacterial DNA. Please see below for the specific primers used. PCR products were purified and sent to ATGC, Inc. for sequencing.

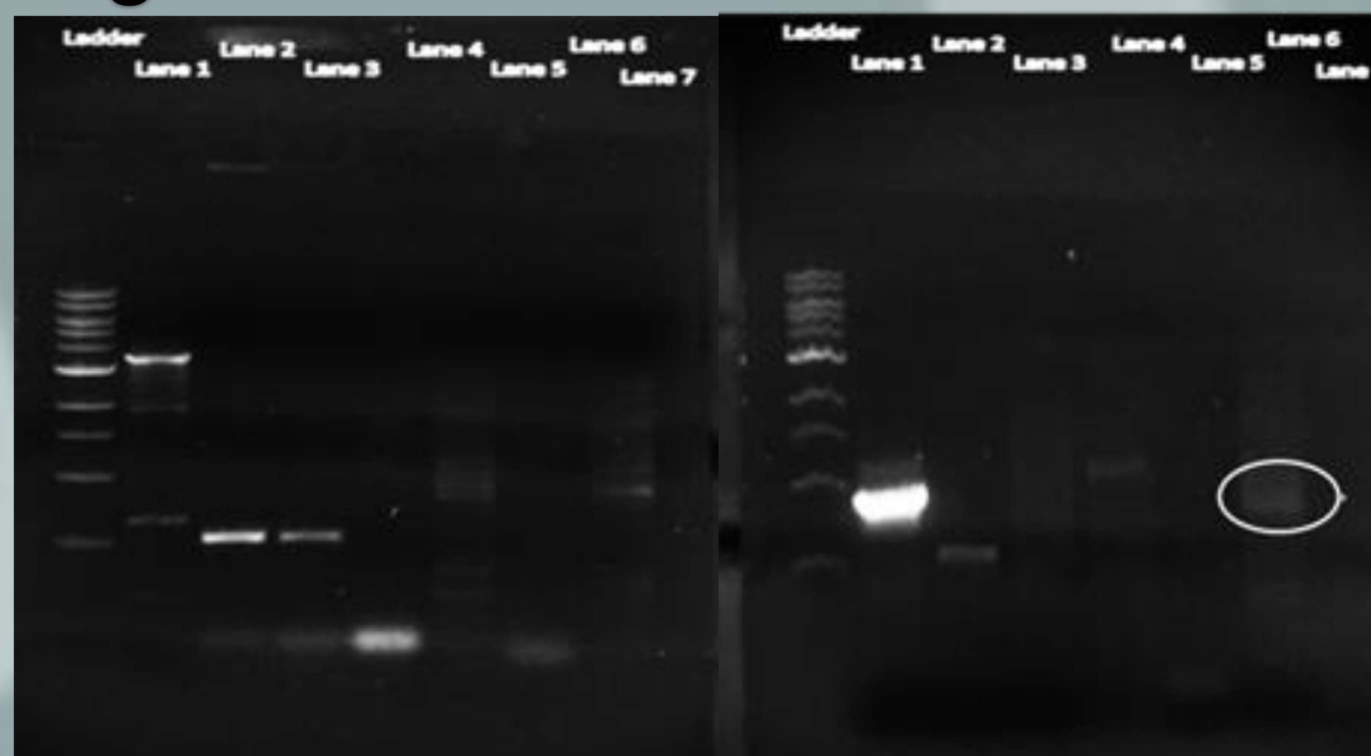
RESULTS

Figure 1



Top Left: Blood from healthy Seneca Park Zoo penguins were spiked with diluted APV virions (1:50 in a CsCl solution). Lane 1 was stored in 70% ethanol while Lane 2 was stored as is. *Top Right*: Blood from healthy Seneca Park Zoo penguins were spiked with *Staphylococcus epidermidis*. Lane 1 was stored in 70% ethanol while Lane 2 was stored as is. *Bottom Left*: PCR amplification of the APV spiked blood using primer combinations 4 (Lane 2), 7 (Lane 3), 9 (Lane 5), 10 (Lane 6), and 11 (Lane 7). *Bottom Right*: PCR amplification of the *S. epidermidis* spiked blood using primer combination 1 (Lanes 2, 3, 4, and 6).

Figure 2



Left: PCR of extracted DNA from affected South African penguin 579. Lane 1 amplified a control APV experiment using primer combination 4 while Lane 2 amplified a known bacterial control using primer combination 1. Experimental bacterial DNA was amplified around 500 bp using primer combination 1 (Lane 3). *Right*: PCR of extracted DNA from affected South African penguin 757. An APV control experiment amplified DNA around 800 bp using primer combination 10 (Lane 1) while a bacterial control experiment amplified a 500 bp region of DNA using primer combination 1. An experimental DNA region was amplified around 800 bp using primer combination 10).

Table 1

Primer Combination Number	Primer Combination
1	*SSU-bact-27f* and *SSU-bact-519r*
2	$BFDV_F/BFDV_R$
3	APV A_F/A_R
4	APV B_F/B_R
5	APV F_2/R_2
6	APV A_F/B_R
7	APV A_F/R_2
8	APV A_R/B_F
9	APV A_R/F_2
10	APV B_F/R_2
11	APV B_R/F_2

*F = forward primer, R = reverse primer

**Primers F_2 and R_2 were designed and tested by Dr. Daryl Hurd

DISCUSSION AND FUTURE DIRECTION

The control experiments produced a PCR product that, when sequenced and entered into NCBI BLAST, were 99% similar to their parent NDA (bacteria to *S. epidermidis* and viral to APV; Figure 1). Two affected penguins out of 10 were able to be PCR amplified and sequenced. Sample 579 that tested for bacteria (Figure 2, Lane 3) amplified a region of DNA that was 95% similar to various *Staphylococcus* species. Samples 579 and 757 testing for APV and BFDV (Figure 2, Lanes 4 and 6) amplified a DNA region which all were sequenced to be Budgerigars Fledgling Disease Polyomavirus around the similarity of 98%.

The data does not fully support a definitive cause of the fledgling disease. However, the fact that only affected South African penguins were able to have certain regions of DNA PCR amplified using very specific viral primers and universal bacterial primers has something to be said. The control experiments provided a basis that APV primers could only amplify regions of DNA for APV. Likewise, BFDV primers were not able to amplify any region of APV DNA. Therefore, there is very little possibility of primers targeting non-specific regions of DNA.

The samples sent were extracted in 2008 and 2010 and stored in 70% ethanol to inactivate any live viruses and bacteria. This was required by the United States in order to have the samples shipped in. Ethanol can deteriorate membranous cells. Once DNA is exposed to ethanol, it can deteriorate which may lead to a false negative result for APV, BFDV, or bacterial screens[2].

Future experimentation may entitle a student to travel to South Africa in order to be able to work on fresh samples. Also, future research can utilized products from QIAGEN that are meant to effectively preserve DNA in order to have unaffected samples to research with.

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