THE BIRDSTRIKE IDENTIFICATION PROGRAM AT THE SMITHSONIAN INSTITUTION AND NEW RECOMMENDATIONS FOR DNA SAMPLING

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Abstract. The U.S. Air Force (USAF) and the Federal Aviation Administration (FAA) have been supporting a free-of-charge birdstirke identification program at the Smithsonian Institution for many years. Approximately 50% of the birdstrike cases received are identified to species level using whole feathers, or feather fragments in comparison with museum specimens. The remaining cases are identified using microscopic analysis and/or DNA "barcoding". DNA barcoding is the newest tool in the birdstrike identification toolbox and involves extracting mtDNA (*cytochrome c oxides subunit 1*, COI, *cox1*, "barcode gene") from birdstrike samples that consist of blood and tissue and then matching the unknown sequence to a DNA library available on the Barcode of Life Database (BoLD). We analyzed birdstrike remains during Fall migration 2006 (1 September – 31 December) to evaluate current collecting methods for minute birdstrike evidence, and to analyze the condition of the samples submitted for DNA testing. Although the age of the sample (time from birdstrike event to time of identification) was not a factor in DNA extraction success, the condition of the sample received in the lab was critical. More than 77% of the cases that contained moldy paper towel samples did not yield viable DNA. The poor condition of these samples leads us to recommend modifications to current field collecting techniques for all birdstrike evidence consisting of blood and/or tissue. New field collecting recommendations include: 1) using alcohol (ethanol or isopropyl) or alcohol-based toweletts, instead of water and paper towels, to wipe the aircraft of bird evidence, or 2) use a DNA 'fixing' card such as the Whatman FTA® card to prevent DNA degradation as soon as possible. These methods will help preserve the DNA, prevent mold growth, and increase the ability to extract viable DNA from blood and tissue samples.

Key words: birdstrike identification, bird evidence, COI, DNA barcoding, collecting recommendations

INTRODUCTION

According to a fourteen year study published by the FAA (Cleary et al., 2005), civil aviation lost about $500 million per year from 1990-2004 as a result of bird-aircraft collisions (birdstrikes). In addition, the USAF reports more than 5,000 birdstrikes per year that average more than $35 million worth of damage to military aviation (LeBoeuf, 2007, pers. com.). Management programs to reduce wildlife strikes to aircraft depend on accurate species identifications in birdstrike prevention and aircraft safety design yet only about 24% of the civil birdstrikes are identified to the species level (Cleary et al. 2006). Knowing the bird species identity has helped the USAF design bird avoidance models, redesign windscreens, and reduce the risk of birdstrikes on airfields. Improving birdstrike species identifications has been cited as a critical action needed to improve the ranking of wildlife hazards to aviation (Dolbeer et al. 2000) and will add to our knowledge of bird migration heights and migration timing by species (Dolbeer 2006). Therefore, a fundamental step in formulating plans to discourage birds from interfering with aviation safety is to identify the birds that are causing birdstrike problems.

The Smithsonian Institution has been providing bird species identifications to the USAF for more than 40 years and has worked on identifications for civil aviation for nearly as long. The USAF and the FAA have interagency agreements that provide this identification service free-of-charge to all USAF and US civil aviation personnel with websites available for on-line reporting (FAA: http://wildlife-mitigation.tc.faa.gov/public_html/index.html USAF: https://afsas.kirtland.af.mil/).

In 2003, the FAA's William J. Hughes Technical Center, the Smithsonian Institution, and the USAF began a project to investigate the possibilities of using molecular techniques to identify birdstrikes. In mid-May 2004, a research collaboration was established between the Smithsonian Feather Lab and The University of Guelph (Ontario, Canada) to sequence the mtDNA gene (*cytochrome c oxides subunit 1*, COI, *cox1*, "barcode gene") and build a DNA library for most of the birds of North America. The DNA library is now 93% complete for the birds of the U.S. and Canada and the COI gene effectively discriminates 94% of these bird species (Kerr et al. 2007).

OBJECTIVES OF ANALYSES

The primary objective of this analysis was to determine if current field collecting methods for birdstrike evidence are adequate for DNA testing. We tracked the age of the sample (time from birdstrike event to laboratory sampling); the type/condition of the sample (blood, mold, tissue, etc), and the amount of time that it took to complete a DNA identification once it reached the Feather Lab. We conducted this study mainly during Fall migration (1 September – 31 December 2006) following completion of the DNA barcode library. In addition to analyzing current field collecting methods, we initiated a field testing program with Whiteman Air Force Base, MO to test a DNA fixing card (Whatman FTA®) as a possible improvement for DNA collecting methods.

RESULTS

Birdstrike Identification Cases – 2006 Fall Migration

From 1 September through 31 December 2006, 1,715 birdstrike samples were submitted by USAF and FAA for identification. Traditional morphological identifications were made to species level using whole feathers or a combination of whole feathers and microstructure on 894 (53.1%) samples, while 821 (47.9%) samples were submitted for DNA analysis (including 28 Whatman® FTA DNA sampling cards from Whiteman Air Force Base, MO). Of the 821 cases submitted for DNA analysis, 267 (32.5%) did not contain viable DNA on the first extraction attempt (Dove et al. MS in prep). These samples were subsequently identified using morphological techniques (microscopy) mainly to Family or Order level.

Age of DNA Sample

The amount of time from the birdstrike event to DNA sampling ranged from 1 -116 days (mode = 7 days). To analyze the age of the DNA samples we grouped them by week for 1-28 days; samples more than 4 weeks (29-116 days) were combined into one group (Table 1). Samples that were received one week or less after the strike event yielded viable DNA in 52.6% the cases, whereas those cases that were received more than 29 days after the event resulted in viable DNA in 72.29% of the cases. The highest success rate was found in samples that were received from 22-28 days after the strike (76.9%).

Type of DNA Sample

The type and condition (dry, moldy, tissue, blood, FTA, etc.) of the birdstrike samples that were submitted for DNA sampling was recorded in 527 cases as they were received in the lab. The seven categories of samples included:

1. moldy/wet paper towel
2. wet paper towel
3. dry paper towel
4. dry tissue and/or feather
5. blood on cotton swab
6. irradiated
7. Whatman® FTA card

Dry tissue (or feathers with tissue attached), or blood samples collected on a cotton swab resulted in successful DNA extractions in 135 (69%) and 52 (72%) of those cases respectively (Figure 1). Wet, moldy paper towel samples failed to produce viable DNA in 29 of the 35 (77%) cases submitted in that condition. Surprisingly, samples that were sent to the previous Smithsonian address and were irradiated by the U.S. postal service produced viable DNA in 15 of the 38 cases.

Whatman® FTA cards were used for DNA sampling by USDA biologists and USAF personnel at Whiteman Air Force Base, MO. During this study, 28 cards (19 different birdstrike cases) were received. FTA cards produced viable DNA in 93% of the cases (26 cases). Airfield personnel found these cards to be user-friendly and efficient at sampling blood and tissue.

DNA Identification Time

The amount of time to obtain a DNA identification once the sample reached the Feather Lab ranged from 1-22 days (average time = 5.6 days, mode = 3 days).

CONCLUSIONS

For many years, the birdstrike community has expressed desires to have the expertise to identify birdstrikes but lacked the vast museum research collections and trained professionals dedicated to this one task. DNA identifications of birdstrike samples are now highly practical providing a library of sequences such as the *Barcode of Life Database* (BoLD) is available for comparisons (http://www.barcodinglife.org). Although these databases are quickly becoming populated with numbers of species and multiple samples per species, caution is needed when interpreting the results, especially for the species that possess overlapping 'barcode' clusters, and in regions of the world that lack sufficient species representation in the database.

In this study, the amount of time that lapsed from the collection of the birdstrike evidence to the identification of the sample was not critical. One very old case (116 days) produced viable DNA for species identification and many cases that took weeks to arrive in the lab contained viable DNA in this study. While we do not have an explanation for this result, it encourages sampling and submission of remains even if the birdstrike event occurred far in the past.

The most critical aspect of field sampling in this analysis was the condition or type of sample that was received by the Feather Lab and subsequently submitted for DNA analysis. While there is no way to control the initial type of birdstrike sample that is present on the aircraft, there are ways to improve the way in which those samples are collected and preserved in the field. We found that moldy paper towel swipes of birdstrike remains were unsuccessful for DNA analysis most of the time. Further, any type of sample that was obtained by using the current method of spraying the aircraft with water and wiping with a paper towel (wet or dry paper towel) was not highly successful when compared to other types of samples (dried tissue, feathers with tissue, FTA card). Early results of using the Whatman® FTA DNA 'fixing' cards are promising but more study with various types of birdstrike samples is needed before wide-scale recommendation of this product. Alcohol-based products (e.g. moist toweletts) are an economical alternative for DNA sampling. If the alcohol product provides more than

70% alcohol ingredient, it may also be used as an approved 'treatment' for H5N1 avian influenza virus in overseas shipments.

The current method of spraying water on the aircraft and wiping the area with a paper towel is not ideal for DNA extraction success (Figure 1). While this method is usually successful for obtaining tiny downy feather barbs, it failed to produce viable DNA in most of the samples examined here. Based on the results of this analysis, we are updating the collecting methods for minute birdstrike evidence that consist of only blood or tissue to the following:

Recommendations for DNA Collecting

- Wear protective gloves
- Use only alcohol wipes, ethanol/isoproply in a spray bottle, or Whatman® FTA cards for DNA samples (blood/tissue/snarge).
- Sample as soon as possible after the birdstrike event
- Send the sample for identification immediately, especially if the sample is moist
- Do *not* use water to collect DNA (blood/tissue) samples
- Wash hands after sampling

Ship DNA samples to:

Feather Identification Lab
Smithsonian Institution
NHB E-600, MRC 116
PO Box 37012
Washington, DC 20013-7012
(202) 633-0801
dovec@si.edu (email or call for overnight shipping instructions)

NOTE: CONTINUE TO COLLECT WHOLE FEATHERS AND FEATHER FRAGMENTS. DNA analysis is only used in birdstrike cases that have blood, tissue or if the identification cannot be made based on feather morphology. Furthermore, viable DNA is not found in approximately 32% of the cases (Dove et al., MS in prep, 2007) and the identifications are made mainly to the Order and Family level using microscopic and morphological methods.

We predict that the amount of time to complete the DNA identifications will decrease from the average 5.6 days to 3-4 days now that laboratory procedures and organization of data are well established. Field sampling improvements will better preserve the DNA and ultimately result in increased species-level identifications using molecular methods.

Knowing that species-level identifications are possible with minute blood and tissue samples will improve birdstrike reporting on a global scale, provide more accurate data for birdstrike models, and ultimately aid in the improvement of aviation safety. Because

32% of the blood and tissue samples that were submitted for DNA identification do not produce viable DNA on the first past extraction attempt, traditional identification methods (morphology, microscopy) are still necessary for birdstrike identification of blood and tissue samples and for the remaining 50% of the samples that contain whole feather samples.

Table 1.

Figure 1. DNA identification success of 499 birdstrike cases based on the type of sample as it arrived in the Feather Lab. Numbers on the bar graph indicate the total number of samples that resulted in DNA identifications (light bar) versus the number of samples that did not contain viable DNA for identifications (dark bar) and are grouped according to the type of the sample.

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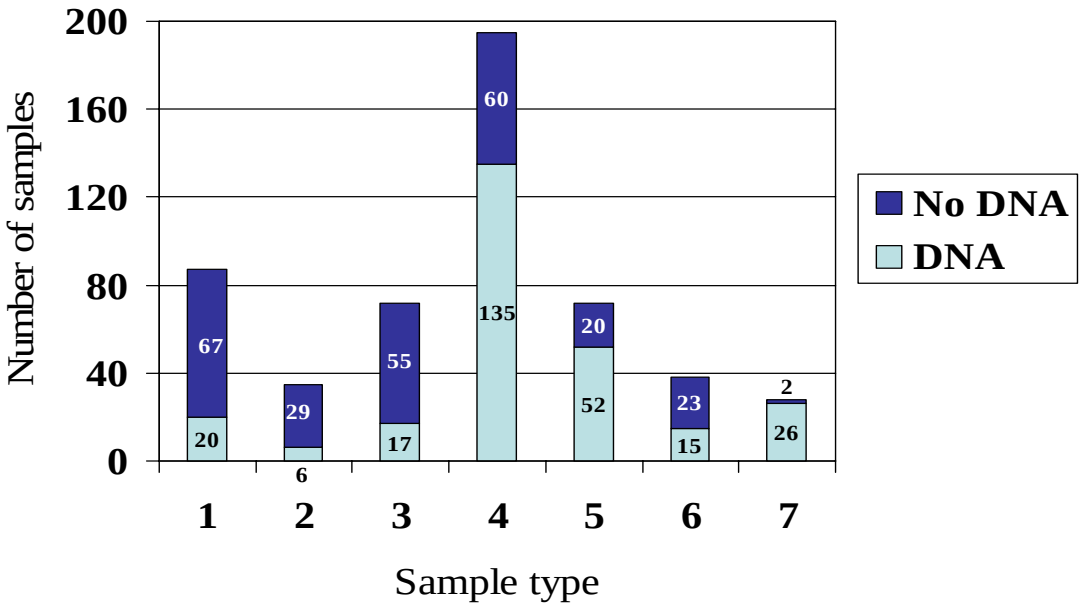
Table 1. DNA samples we grouped by week for 1-28 days; samples more than 4 weeks (29-116 days) were combined into one group (+). The age of the sample was not critical to DNA extraction success indicating that birdstrike evidence from events far in the past can be identified using DNA methods.

Week	Days	% DNA Success	n
1	1 – 7	52.6	152
2	8 – 14	67.1	422
3	15 – 21	66.1	147
4	22 – 28	76.9	47
+	29 – 116	72.3	36

Figure 1.



Sample Condition Key:

1. Moldy/Wet paper towel
2. Wet paper towel
3. Dry paper towel
4. Dry tissue and/or feather
5. Blood on cotton swab
6. Irradiated
7. Whatman® FTA card