Modeling lacaziosis lesion progression in common bottlenose dolphins *Tursiops truncatus* using long-term photographic records

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ABSTRACT: Lacaziosis (lobomycosis) is a skin disease caused by *Lacazia loboi*, occurring naturally only in humans and dolphins. Attempts to culture the pathogen *in vitro* have been unsuccessful, and inoculation studies of lacaziosis development in mice have provided only limited, short-term data on the progression and propagation of *L. loboi*. The present study used photographic data from long-term photo-identification and health assessment projects to model and quantify the progression of lacaziosis lesions in 3 common bottlenose dolphins *Tursiops truncatus* from Sarasota Bay, Florida, USA. Dorsal fin images throughout each animal’s sighting history were examined for lesion growth, and the proportion of lesion coverage in each photograph was estimated using image analysis tools in Adobe Photoshop®. The progression of lacaziosis lesions and lesion growth rates were modeled using a non-linear monomolecular growth model. As data on lacaziosis development and advancement are limited in humans and laboratory animals, dolphins with a long-term case history of the disease may serve as a good animal model to better understand lacaziosis progression. Furthermore, this study demonstrates the utility of long-term population monitoring data for tracking the progression of a poorly understood disease that is relevant to both dolphin and human health.

KEY WORDS: Lacaziosis · *Lacazia loboi* · Bottlenose dolphin · Monomolecular growth model · Skin disease · Sarasota Bay · Lobomycosis

INTRODUCTION

Lacaziosis (*Lacazia loboi*) is a skin disease with fungal etiology that occurs naturally only in humans and dolphins (Taborda et al. 1999). In humans, the disease is considered endemic to people inhabiting Central and South America, as most human cases have been reported in this region (Wiersema & Niemel 1965, Fuchs et al. 1990, Rodrigue-Toro 1993, Brun 1999, Pang et al. 2004, Paniz-Mondolfi et al. 2007). In dolphins, lacaziosis or a lacaziosis-like disease has been reported in 3 species (*Tursiops truncatus*, Migaki et al. 1971, *Sotalia guianensis*, de Vries & Laarman 1973; *T. aduncus*, Kiszka et al. 2009) from waters surrounding South America (de Vries & Laarman 1973, Simose-Lopes & Paula 1993, Van Bressem et al. 2009), Madagascar (Kiszka et al. 2009), France (Symmers 1983) and the United States including the Gulf of Mexico (Migaki et al. 1971, Cowan 1993) and Atlantic Ocean (Caldwell et al. 1975, Bossart 1984, 2003, Reif et al. 2006, Murdoch et al. 2008, Rotstein et al. 2009). The first reported case of lacaziosis in a dolphin was in a bottlenose dolphin *T. truncatus* from Sarasota Bay, on the west coast of Florida (Migaki et al. 1971), and historic reports sug-
gest that the disease is likely endemic to the bottlenose dolphin communities on the east coast of Florida (Caldwell et al. 1975, Murdoch et al. 2008).

In vitro attempts to culture the lacaziosis pathogen have not been successful. As a result, very little is understood about the conditions under which the pathogen grows and thrives, as well as its source and transmission route (Wiersema & Niemel 1965, Pang et al. 2004, Lupi et al. 2005, Paniz-Mondolfi et al. 2007). Experimental inoculation attempts have been made using guinea pigs (Wiersema & Niemel 1965), hamsters (Wiersema & Niemel 1965, Opromolla & Noguiera 2000), tortoises (Geochelone denticulate, G. carbonaria and Kinosternon scorpioides; Sampaio et al. 1971), monkeys (Macaca mulatta, M. nemestrina and M. fascicularis; Caldwell et al. 1975), armadillos Euphractus sexcinctus (Sampaio & Braqa-Dias 1977) and mice (Wiersema & Niemel 1965, Caldwell et al. 1975, Opromolla et al. 1999, Madeira et al. 2000, 2003, Belone et al. 2003), but many of those attempts yielded only short-term data on the development of the disease. Opromolla et al. (1999) were able to successfully inoculate a Swiss strain of mice with lacaziosis and follow disease progression up to 18 mo post-inoculation; however, clinical presentation of the disease did not occur, and very few fungal cells were active by the end of the study. Other studies have demonstrated that BALB/c mice may be a suitable animal model for studying the infectivity and aggression of the lacaziosis pathogen, as infection was sustained and lesions developed post-inoculation; however, all of the BALB/c inoculation studies maintained infection for a maximum of 18 mo before the animals were sacrificed, thereby supplying limited short-term data on the progression of lacaziosis (Madeira et al. 2000, 2003, Belone et al. 2003). To our knowledge, there are no published reports of long-term studies that calculate a maximum of 18 mo before the animals were sacrificed, thereby supplying limited short-term data on the progression of lacaziosis amongst diseased individuals. Therefore, very little is known about the quantitative proliferation of *Lacazia lobo*. The objective of the present study was to develop a method using long-term photographic data to quantify the progression of observable lacaziosis lesions amongst diseased individuals from a population of free-ranging bottlenose dolphins.

**MATERIALS AND METHODS**

**Case series.** The Sarasota bottlenose dolphin community has been monitored and studied since 1970. Routine photo-identification (photo-id) surveys began in 1980 (Wells 1991, 2003), in which individual animals with unique dorsal fin profiles are photographed for individual identification (Scott et al. 1990), abundance estimation (Wells & Scott 1990), as well as group composition, behavior (Wells et al. 1987, Wells 1991) and movement monitoring (Irvine et al. 1981, Wells et al. 1987, Wells 1991, 2003). In addition to photo-id surveys, the population has been studied using tagging, citizen’s band, VHF and satellite-linked telemetry, dart biopsies, behavioral and acoustic studies, as well as health assessments (Wells 1991, 2003, Wells et al. 2004). For this project, photographs taken during photo-id surveys and health assessment projects were used for analysis. Since bottlenose dolphin photo-id studies rely on images of the dorsal fin, the dolphins for the present study were selected because lacaziosis lesions were evident on their dorsal fins (Fig. 1).

**Photograph acquisition and selection.** The Sarasota Dolphin Research Program photo-id database was queried to obtain a complete sighting history for all 3 cases. Prior to 2004, photographs were taken with 35 mm film and maintained as slides. For the sightings prior to 2004, slides of each animal were examined and scanned into digital format with the exception of blurry images or photographs where most of the dorsal fin was obstructed. For photographs taken after the digital camera transition in 2004, the entire digital archive of images for each case was acquired.

Although lesions were observed on body parts other than the dorsal fin, photographic coverage of these areas was not consistent in photo-id images; therefore, images revealing lesions on the left and right sides of the dorsal fin were selected for each year of the animal’s sighting history. Where possible, multiple images from the same year were selected; however, only one photo from an individual sighting was used for the analysis. Photographs qualified for analysis if they (1) were at or close to a 90 degree angle to the animal; (2) provided details of the skin’s surface (i.e. lesions could be detected); (3) presented at least 50% of the dorsal fin (i.e. <50% of the dorsal fin was obstructed by water or glare); and/or (4) were not entirely backlit. Images that did not meet at least 3 of these criteria were excluded from the analysis. The best image from each sighting was used for the analysis, unless all of the images from a particular sighting did not meet the inclusion criteria. Table 1 provides a description of the photographic data used for each case.

**Photo registering.** To compare the proportion of lesion coverage among all dorsal fins in the data set, standardized size and scale were necessary. For each side of the dorsal fin, an image taken during a health assessment project was used as the reference image. It was important that the reference image depicted permanent reference markings (e.g. freeze branding and tagging scars) that were visible in each photograph of the time series (Fig. 2). These reference markings were used for image alignment and scale adjustment.
Adobe Photoshop® software (Adobe Systems) was used for photo-registering. Initially, the reference photograph was cropped to a specific size (700 × 500 pixels) to only include the dorsal fin, and this reference image served as the base layer in the application. Each additional image was imported into the application, and the opacity was reduced to approximately 60% so that the reference photograph layer was slightly visible beneath the experimental layer (i.e., field photograph). The opacity adjustment was not standardized because each photograph differed in brightness, thereby requiring individualized opacity reductions to be able to view the underlying reference image. Using the reference points as guides, the experimental layer was resized and rotated to match the reference base layer. This process was repeated for each suitable image in the data set.

Proportion of lesion coverage. Image analysis tools in the latest version of Adobe Photoshop® (version CS4 extended) were used to determine the proportion of lesion coverage of the dorsal fin for each field image. To calculate the area of the dorsal fin in each field photo, the background (i.e., water) was removed using the magnetic lasso tool. The dorsal fin was selected by guiding the magnetic lasso along the outer edge of the dorsal fin profile until the lasso loop was completed (Fig. 3). Once complete, the dorsal fin selection was inverted under the ‘Select’ tab on the menu bar, resulting in the selection of all pixels representing the background. The backspace key was then used to delete the area surrounding the dorsal fin, ultimately leaving only the dorsal fin in the viewing frame (Fig. 4).

The selection tool was then used to select the pixels representing lacaziosis lesions on the isolated dorsal fin image. Contiguous pixels of the lesioned areas were selected with a tolerance ranging from 5 to 20, depending on the level of contrast in each photograph. The shift key was depressed to select multiple lesioned areas, and once all lesions were selected, a new layer was created in the application containing only the pixels that represented lacaziosis lesions (Fig. 4). The magnetic lasso and lesion selection steps were repeated for each photograph in the time series of data, and all layers were cropped to a consistent area of analysis that excluded glare or skin obstruction by water.

To calculate the proportion of lesion coverage, the record measurements function was used to automatically calculate the area of irregular objects. For each layer containing the dorsal fin, the selection tool was used to select all pixels comprising the dorsal fin, and the record measurements function was activated to calculate the area of the selected region. Similarly, the lesion pixels were selected using the selection tool, and the record measurements function calculated the
Table 1. *Tursiops truncatus*. Description of data for all 3 lacaziosis (*Lacazia loboi*) cases including the number of photos used for analysis, time range of the images, number of years where images were not available or suitable for analysis, number of years where there were multiple images to analyze, year (age) when lesions were first detected and year of disease confirmation via biopsy sampling of lesions. Disease progression was analyzed for the right side of the dorsal fin in all 3 cases.

<table>
<thead>
<tr>
<th>Case</th>
<th>No. of images</th>
<th>Time range</th>
<th>No. of years Missing</th>
<th>No. of years Multiple images</th>
<th>Disease presentation</th>
<th>Disease confirmation</th>
</tr>
</thead>
</table>

Fig. 2. *Tursiops truncatus*. Tagging scars on bottleneck dolphin FB28 that were used as permanent reference markings for image registering and alignment. Photos: Sarasota Dolphin Research Program

Fig. 3. *Tursiops truncatus*. The magnetic lasso tool in Adobe Photoshop® was used to outline the profile of the dorsal fin in each image for isolation from the background (i.e. water). Photos: Sarasota Dolphin Research Program

area (in pixels) of all selected lesion areas. The proportion of lesion coverage was determined by:

\[
\frac{\sum \text{Pixels}_{\text{Lesion}}}{\sum \text{Pixels}_{\text{Dorsal Fin}}} \quad (1)
\]

**Growth model developments.**

Using SAS® 9.1 software (SAS), and the Proc GPLOT statement, the proportion of lesioned cells was plotted against the change in time (in 1 yr increments) to assess the overall shape of the data. Gauss-Newton nonlinear iterative methods were used to develop a monomolecular growth model for the lesion progression data (Nutter 1997):

\[
y = [1 - (1 - y_0) \exp(-rt)] \quad (2)
\]

The primary assumptions of a monomolecular model are that disease or lesion growth is most rapid at the beginning of an epidemic or disease onset, and the rate of growth decreases over time relative to the remaining area or tissue that is not consumed by lesion or disease (Nutter 1997). Furthermore, the total proportion of diseased area cannot exceed 1; therefore, the first parameter term is set equal to 1. In the growth model equation, \(y_0\) is the parameter estimating the starting value for diseased tissue, and the lesion growth rate parameter \(r\) is calculated by an iterative method. The rate parameter can be estimated using the Proc NLIN command in SAS®; however, the starting values for iteration must be established by the programmer (Fekedulegn et al. 1999). In the present study, the starting values were determined by calculating the total rate of change in lesion coverage when \(t = 0\) to the last time point in the study. The expression used to calculate the total rate was:

\[
(y_x - y_0)/(t_x - t_0) \quad (3)
\]

where \(y_x\) is the amount of lesion coverage calculated for the last time point, \(y_0\) is the initial proportion of lesion coverage at the beginning of the study, \(t_x\) is the year of the last time point and \(t_0\) is the year of the first time point. The statistical significance esti-
mated by the F-test is not appropriate for the examination of model fit for nonlinear models (Fekedulegn et al. 1999), therefore an adjusted $R^2$ value, adjusted for a no-intercept model (Chatterjee & Hadi 2006), was used to examine model fit.

Lesion growth rates for each time point were calculated using the equation:

$$\frac{dy_i}{dt} = r \times (1 - \text{predicted}_i)$$

(4)

where predicted, was equal to the predicted value of $y$ from Eq. (2), based on the growth model parameters, at time $t_i$. For all dolphin cases, these growth rates were plotted for each year of the time series to reveal any changes or patterns in growth rates over time.

RESULTS

Growth modeling

For each case, the quantity and quality of the photos for the right side of the dorsal fin were better than the left side of the fin, and the right side data set had fewer missing years and more photos available per year. For these reasons the growth modeling was conducted using photographs of the right side of the dorsal fin. Non-linear monomolecular modeling of the proportion of lesion coverage over time for dolphin cases FB28, FB98 and FB96 produced the growth rate parameters reported in Table 2 and curves in Fig. 5. The adjusted $R^2$ values (Table 2) revealed that the model provided a good fit for the data, as all $R^2$ values indicated that the model explained greater than 90% of the variation in the data. The growth rate curves (Fig. 6) for 2 of the cases (FB28 and FB98) indicated a gradual decrease over time, whereas the growth rate for the third case, FB96, reflected a relatively steady rate. Because the sample size for these models and growth rate curves is small, general conclusions regarding the progression of lacaziosis cannot be established.

These modeling methods rely on long-term photographic data to quantify the progression of lesions over time. For the 3 cases presented in the present study,

Table 2. Lesion growth parameter estimates ($r$), standard error (SE), 95% confidence intervals (CI) and adjusted $R^2$ for 3 bottlenose dolphin *Tursiops truncatus* lacaziosis (*Lacazia loboii*) cases

<table>
<thead>
<tr>
<th>Case</th>
<th>$r$</th>
<th>SE</th>
<th>95% CI</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB28</td>
<td>0.052</td>
<td>0.00272</td>
<td>0.0468, 0.0578</td>
<td>0.985</td>
</tr>
<tr>
<td>FB98</td>
<td>0.042</td>
<td>0.00257</td>
<td>0.0370, 0.0477</td>
<td>0.995</td>
</tr>
<tr>
<td>FB96</td>
<td>0.006</td>
<td>0.00362</td>
<td>-0.00231, 0.00605</td>
<td>0.914</td>
</tr>
</tbody>
</table>
the longitudinal data available for growth modeling comprised more than a decade; however, decades of photographic data may not be available for many populations that are monitored using photo-id methods. Data reduction analyses were conducted to identify potential data cut-points, or the amount of data that would produce a growth curve statistically comparable to the original model. The data sets for FB28 and FB98 were more robust, so the reduction analyses were performed using the lesion proportion data from these cases. The lesion proportion calculations for the initial 5 and 10 yr for each case were modeled using the same methods described previously. The parameter estimates for the full-data and reduced-data models are presented in Table 3, and the model curves are presented in Fig. 7. The results of the data reduction analyses provide preliminary evidence that 5 or 10 yr of images depicting the earliest periods of lesion progression may provide sufficient data to model lesion growth in the selected individuals, when compared to the curves and confidence intervals estimated by the full-data models. However, because these data reduction analyses were conducted for only 2 cases, we cannot definitively state that 5 or 10 yr of data will accurately predict the progression of lacaziosis in all dolphins infected with the disease.

### DISCUSSION

The primary objective of the present study was to quantify the progression of lacaziosis in bottlenose dolphins with long-term case histories, and to demonstrate the utility of using photographic data from field studies and health assessment projects. Although our sample size was small, we were able to successfully apply these methods to 3 long-term cases of lacaziosis in wild bottlenose dolphins. Generalized statements about the progression of lacaziosis in infected dolphins cannot be made due to the limited number of cases.
used for analysis; however, the model fit for all cases revealed that these methods can potentially be used to quantify the progression of lacaziosis lesions over time.

We also determined that photo-id and health assessment data can be used to document disease progression, but with a few caveats. First, if disease progression is slow, as in the case of lacaziosis, long-term data are needed to accurately assess disease progression. In all 3 of the cases presented here, documentation of lacaziosis lesions was available for at least 10 yr. The preliminary model reduction analyses indicated that 5 and 10 yr of disease documentation may provide a predictive model of lacaziosis progression. Although multi-decadal photographic records are available for many of the Sarasota bottlenose dolphins, this is generally not the case for many other photo-id programs across the globe. Application of the methods described in the present study to more cases may help to establish a firm cut-point that will maintain model accuracy while accommodating shorter-term data sets.

Consistency in the data is also important in developing a model to quantify disease progression. In all cases, the right side of the dorsal fin provided a better opportunity for robust model development than images of the left side of the fin. We recommend that every effort be made to photograph both sides of the dorsal fin. If sufficient data were available for both sides of the dorsal fin, we could have modeled lesion growth for multiple body areas, and potentially increased the precision of the model. While ensuring that adequate photographic coverage of both sides of the dorsal fin has been made for each dolphin sighting may increase the effort and survey time for photo-id projects, we have shown herein that consistent and long-term photographic data can be used as a non-invasive means to model the progression of disease in a wild animal.

Based on current photo-id practices, if data from photo-id studies are used for tracking disease in a wild population, the disease of interest must be presented on areas that are routinely photographed during types of studies (i.e. dorsal fin), otherwise misclassification of cases can occur, as well as missed opportunities to track the disease. In these cases, only the lesions observed on the right side of the dorsal fins were used to model the progression of the skin disease because the dorsal fin is regularly captured during photo-id surveys. We recognize that the lesion growth patterns observed on the dorsal fin may not be representative of lacaziosis progression on other parts of the body; however, inconsistencies in the coverage of other body parts prevented the assessment of lesion growth in these other areas (i.e. flank, flukes). For future studies using photo-id images to recognize and track overt signs of disease in wild populations, we recommend that photo-id teams, in addition to documenting the identifying features of an individual (i.e. dorsal fin, fluke), make a concerted effort to increase the photographic coverage of more body parts (e.g. peduncle, dorsum).

Although reliance upon photo-id images may prevent the assessment of lesion growth and development on every aspect of the body, photographic data acquired through photo-id projects provide a routine and non-invasive means to document, track and quantify their progression in wild populations. The methods developed in the present study relied on lesions that result from lacaziosis infection; however, we anticipate that any disease that presents with overt characteristics and is adequately documented in photo-id images can be studied using these quantitative techniques. Furthermore, the image analysis methods established by the present study, including the application of the monomolecular model, provide a framework for future studies comparing disease progression rates among individuals in a population.

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LITERATURE CITED


