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Comparative study between photodynamic and antibiotic therapies for treatment of footpad dermatitis (*bumblefoot*) in Magellanic penguins (*Spheniscus magellanicus*)

Cristiane Lassálvia Nascimento MSc[a,*], Martha Simões Ribeiro[b], Fábio Parra Sellera[c], Gustavo Henrique Pereira Dutra[a], Alyne Simões[d], Carlos Roberto Teixeira[e]

[a] *Veterinary Unit of Santos Aquarium, Santos Aquarium, Av. Bartolomeu de Gusmão S/N, Ponta da Praia, Santos 11035-500, SP, Brazil*
[b] *Center for Lasers and Applications, IPEN-CNEN/SP, Av. Lineu Prestes 2242, Cidade Universitária, São Paulo 05508-000, SP, Brazil*
[c] *Veterinary Medicine and Animal Science School, University of São Paulo (FMVZ/USP), Av. Prof. Orlando Marquês Paiva, 87, Butantã, São Paulo 13635-900, Brazil*
[d] *Laboratory of Oral Biology, Department of Biomaterials and Oral Biology, School of Dentistry, University of São Paulo, Av. Professor Lineu Prestes, 2227, São Paulo 05508-000, Brazil*
[e] *Department of Veterinary Surgery and Anesthesiology, School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (UNESP), Distrito de Rubião Junior, s/n Caixa Postal 560, Botucatu 18618-000, SP, Brazil*

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KEYWORDS
Captivity;
Bed sore;
Photodynamic inactivation;
Pododermatitis;
Rehabilitation

Summary
Background: Bumblefoot, referring to bed-sore-like foot lesions, is one of the most important clinical complications in captive birds and has a multifactorial etiology. Photodynamic therapy has been proposed as an alternative treatment for localized infections in response to the escalating problem of antibiotic resistance. The aim of this study was to compare outcomes in a group of captive *Spheniscus magellanicus* with bumblefoot lesions treated with photodynamic therapy (PDT) or antibiotics (ATB).
Methods: Ten captive Magellanic penguins with preexisting stage III bumblefoot lesions were selected and randomly divided into one PDT and one ATB group, each including 11 pelvic-limb lesions. All animals underwent surgical debridement of lesions. In the ATB group, antibiotic

* Corresponding author at: Av. Francisco Glicério, 121, apto 75, Santos 11065-401, SP, Brazil. Tel.: +55 13991762550; fax: +55 1332787831.
E-mail addresses: cristianelassalvia@hotmail.com (C.L. Nascimento), marthasr@usp.br (M.S. Ribeiro), fsellera@usp.br (F.P. Sellera), dutra@kingnet.com.br (G.H.P. Dutra), lysimoes@usp.br (A. Simões), teixeiracr@fmvz.unesp.br (C.R. Teixeira).

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ointment was applied topically three times a week, and systemic antibiotic and anti-inflammatory drugs were administered daily. In the PDT group, photodynamic therapy was applied three times a week without the use of topical or systemic medication. Lesion areas were photographed, and swabs were collected for culture and sensitivity, on the first day and every 14 days for a total of 84 days. The four species of bacteria showing the most resistance to the antibiotics screened on the antibiogram were used to determine resistance to PDT with an *in vitro* test.
Results: There were significant differences in healing rate and average healing time between the PDT and ATB groups (63.62% vs. 9.09% and 42 vs. 70 days, respectively).
Conclusion: The findings of this study attest to the effectiveness of photodynamic therapy for the treatment of stage III bumblefoot in *Spheniscus magellanicus*.

Background

Magellanic penguins (*Spheniscus magellanicus*) are seasonal visitors in Brazil [1]; thus, occurrence of stranded penguins is common and these animals are frequently taken to rehabilitation centers. Certain diseases can hinder the rehabilitation process [2], including pododermatitis, also known as bumblefoot [3].

The cause of bumblefoot is multifactorial [4]. The primary lesion is ischemic necrosis of the plantar surface of the foot, similar to bed sores, due to standing for long periods on the same portion of the foot on wet and contaminated floors [5]. Consequently, perfusion of the dermal tissues becomes compromised, which allows microorganisms to damage the dermis [6], leading to erosion and ulcer formation. Inappropriate environment and sedentary habits are therefore factors that may explain why this condition is one of the most frequent and important clinical complications in captive birds [7].

The microbial culture and antibiogram are important in the diagnosis and treatment of bumblefoot [8]. However, because of a lack of knowledge about the pharmacodynamics of certain drugs, the results cannot always be reliably extrapolated to the treatment [9]. Important advances have been made in understanding the pathogenesis of bumblefoot, but treatment methods have remained unchanged for a long time [8] and generally involve surgical debridement and long-term antibiotic therapy [10].

The rise of multi-drug-resistant bacteria has led to research efforts to devise alternative forms of treatment to which, hypothetically, bacteria are unable to develop resistance easily [11]. Antimicrobial photodynamic therapy (PDT) has been proposed as an alternative treatment for localized infections (both experimentally and clinically) in response to the escalating problem of antibiotic resistance [12]. PDT is based on the photooxidation of biological material. A photosensitizer, usually a non-toxic dye, is administered topically or systemically and exposed to light of a wavelength appropriate for absorption by the photosensitizer, in the presence of molecular oxygen. The major advantage of PDT mediated by methylene blue is the possibility of inactivating microorganisms without damage to host tissue, because there is effective selectivity of the pathogenic microorganisms for the photosensitizer, excitement of the photosensitizer results in production of reactive oxygen species, with resultant damage due to oxidative stress leading to cell death [13,14]. Photodynamic inactivation has been as effective in multiresistant bacteria as in naïve bacteria [14], and it has not been possible thus far to artificially induce resistance in any tested microorganism [11].

In this study, we aimed to test a PDT treatment protocol for bumblefoot in captive *S. magellanicus* and compare the results with a group treated with conventional antibiotic therapy.

Materials and methods

Ethics statement

This study was conducted with the approval of the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista (UNESP), Botucatu, SP, Brazil (protocol number 171/2012) and was authorized by the Brazilian wildlife authority (SISBIO no. 44274-1).

Study population, sample collection, and treatment

Ten captive Magellanic penguins were selected. The penguins wore numbered rings and had 1—4 preexisting pelvic-limb bumblefoot lesions classified as stage III according to the staging system of Oaks [15]. The selected animals were randomly divided into two groups: the photodynamic therapy (PDT) and antibiotics (ATB) groups. Each group comprised 11 lesions and each animal belonged only to one of the groups, regardless of the number of lesions present. Each lesion was considered individually even though the host animal might have had multiple hind-limb lesions, usually located on the footpad or tarsometatarsus.

A maximum of seven samples per wound were collected, one every 14 days, over a possible total observation period of 84 days. The observation period was reduced if wound healing was complete before 84 days.

The penguins were sedated with midazolam (2 mg/kg, Dormonid, Roche, São Paulo/SP) and S ketamine (5 mg/kg, Clortamina, Biochimico, Penedo/RJ) and then anesthetized with isoflurane (1—2.5%, Isoforine, Cristalia, Itapira/SP) for surgical debridement of the lesions, and photographic images of the lesions were obtained before and after surgery, as well as every 14 days during the observation period.

In the PDT group, secretions from lesion tissue remaining after surgery were collected on swabs and stored in Stuart medium intended for bacteriological culture and antibiogram. Each sample was labeled with the number of the penguin's ring, left (L) or right (R) foot, and PDT pre-treatment. Following this, PDT was applied to the wound. Then, another sample of the wound secretions was collected and labeled as before, except for PDT post-treatment. The affected limb was then protected with gauze and adhesive bandage. Throughout the observation period, each penguin underwent washing of the feet with a 4% aqueous solution of chlorhexidine, application of PDT to the wound, and application of a fresh protective dressing three times a week.

In the ATB group, collection of wound secretions and labeling of samples were carried out as described above, except for ATB. Neomycin/bacitracin ointment (Nebacetin, Nycomed Pharma, São Paulo/SP) was applied on the wound and enrofloxacin (15 mg/kg; Baytril, Bayer, São Paulo/SP) was administered by intramuscular (IM) injection. The affected limb was then protected with gauze and adhesive bandage. Carprofen (4 mg/kg; Carproflan, Agener Union, Embu-Guaçu/SP) was administered in the food and enrofloxacin (15 mg/kg, IM; Baytril, Bayer, São Paulo/SP) was injected every day. Throughout the observation period, each penguin underwent washing of the feet with a 4% aqueous solution of chlorhexidine, application of antibiotic ointment on the lesion, and application of a fresh protective dressing three times a week. Antibiotics were changed if necessary according to the antibiogram results.

Every 14 days (Days 14, 28, 42, 56, 70, and 84), all the penguins were anesthetized to obtain photographic records of the lesion areas, and samples of wound secretions were collected as described above.

Procedure for PDT: The wound was instilled with an aqueous solution of methylene blue (MB) (concentration of 300 μM; AUDAZ, Brazil) for a pre-irradiation period of 5 min. For irradiation, we used a Laser RECOVER (MM Optics) applied at 1 cm equidistant points perpendicular and in contact to the lesion, as many as were needed to cover the wound, with the following parameters: wavelength, 660 nm; energy/point, 4 J; power, 100 mW; irradiance/point, 3.3 W/cm^2; exposure time/point, 40 s (fluence/point, 133.3 J/cm^2).

Evaluation of lesion area

To obtain the photographic records of the lesions, penguins were anesthetized and placed on a table equipped with a camera mounting device graded in centimeters (Tokina CS 1070). A digital camera (Canon EOS T3i with 18/55 mm IS) with macro lens (100 mm IS Lens, Canon L USM 2.8) was coupled at a constant distance of 30 cm from the table. A label on graph paper was used as a reference scale, and marked with the number of the penguin's ring and the letter D (right) or E (left) indicating the side of the pelvic limb. Subsequently, the images were analyzed using ImageJ software (Java software, Wayne Rasband, USA) to evaluate the lesion area in square centimeters.

Bacterial susceptibility to PDT *in vitro*

Four microorganisms isolated from bumblefoot encountered during the experiment were selected according to Table 1.

The selection criterion was resistance to the greatest number of antibiotics tested by antibiogram. We used this criterion because after long-term treatment with antibiotics, such as for bumblefoot, it is common not to find drugs available for use in penguins for continued care. Thus, the goal was to test quantitatively the efficiency of PDT as an alternative for those cases.

The light source used was a red LED with a wavelength of 660 nm (±15), power of 320 mW, irradiation diameter of 2 cm, irradiance of 100 mW/cm^2, voltage of 2.5/2.6 V, and current of 600 mA. The pre-irradiation time was 5 min for MB and PDT test tubes. The irradiation time was 5 s (0.5 J/cm^2), 10 s (1.0 J/cm^2), 30 s (3.0 J/cm^2), 1 min (6 J/cm^2), or 2 min (12 J/cm^2). The photosensitizer was MB (concentration 1 mM; Sigma–Aldrich, USA).

The microorganisms were cultivated in BHI medium at 37 °C for 12 h, and suspended and homogenized in sterile phosphate buffered solution (PBS; 137 mM final NaCl concentration, 10 mM phosphate, 2.7 mM KCl, pH 7.4) for preparation of inoculum. To estimate the concentration of the inoculum at 10^7 CFU/mL, we used the technique described by Pfaller et al. [16].

Within a flow chamber, eight Eppendorf tubes labeled C (control), L (red LED), MB (methylene blue), 5″ (5 s PDT), 10″ (10 s PDT), 30″ (30 s PDT), 1′ (1 min PDT), and 2′ (2 min PDT) were prepared for the four bacteria, each filled with 720 μL sterile PBS + 40 μL inoculum. In the tubes labeled C and L, 40 μL PBS was added; in the MB tube and all other PDT groups (5″, 10″, 30″, 1′, and 2′), 40 μL MB 1 mM was added. Thus, all tubes contained a final volume of 800 μL.

A 96-well plate was used. Columns one through seven were labeled C, L, MB, 5″, 10″, 30″, 1′, and 2′. The rows were labeled 0–7 according to dilution; 180 μL sterile PBS was placed in all groups from row one. In row 0, 180 μL sterile PBS was placed only in the C, L, and MB groups.

C, L, and MB groups: An aliquot of 20 μL was deposited in row 0 of the groups C, L, and MB. Thus, the final volume in each well was 200 μL.

5″, 10″, 30″, 1′, and 2′ groups: Aliquots of 400 μL were pipetted from each group, deposited into a 24-well cell culture plate, and irradiated according to respective times. Subsequently, an aliquot of 200 μL from each group was added to its corresponding well in row 0 of the 96-well plate. These samples were diluted and subsequently seeded according to the method of Jett et al. [17].

Statistical analysis

The results were evaluated using the following parameters: effectiveness in eliminating the infectious focus, effectiveness of PDT in eliminating resistant bacteria (demonstrated on antibiogram) *in vitro*, and time for tissue repair.

Evaluation of the lesion area: Because of the presence of deviations from a standard Gaussian distribution, the Wilcoxon test [17] was used to compare the circumference of lesions between experimental groups. The comparison between groups was performed for each time point. Survival

Table 1 Antibiotic-resistant microorganisms isolated from footpad dermatitis in Magellanic penguins (*Spheniscus magellanicus*).

Microorganism	Amikacin	Ceftazidime	Ciprofloxacin	Clindamycin	Gentamicin	Nitrofurantoin	Norfloxacin	Penicillin	Bacitracin	Neomycin	Enrofloxacin	Streptomycin
E. coli	S	R	R	–	R	R	R	–	R	R	R	R
P. aeruginosa	R	–	S	–	S	R	R	–	R	R	R	–
P. mirabilis	R	S	S	–	R	S	R	–	R	R	R	R
S. aureus	S	–	R	R	R	S	R	R	R	R	R	–

S: susceptible; R: resistant.

Table 2 Penguin identification numbers, number of lesions, and number and percentage of samples that were collected.

	Penguin	Number of lesions	Number of samples	Percentage
ATB	23	1	7	5.47
	37	2	14	10.94
	67	1	6	4.69
	68	3	21	16.41
	80	2	14	10.94
	235	2	10	7.81
PDT	49	2	10	7.81
	94	4	20	15.63
	257	3	12	9.38
	264	2	14	10.94

curves [17] were produced to show the rate of healing of lesions between groups. Healing rates were compared using the Kaplan—Meier test.

Microbiota and antibiogram: Frequency distributions were produced to estimate the prevalence of microorganisms isolated from the lesions of interest and percentage of resistance to a number of antimicrobial isolates. Statistical significance was defined as $P < 0.05$.

Results and discussion

Samples: Clinical staging for pododermatitis in penguins is not standardized [6]. In this study, we used the five-stage classification system suggested by Oaks [15], in which stage III lesions are characterized by localized infection, being within the scope of PDT. The number of samples collected per animal ranged between 6 and 21, and the individual percentage ranged from 4.69% to 16.41% according to the number of lesions and healing time (Table 2). Lesions were located on the footpad (83.59%) or tarsometatarsus (16.41%).

Lesion area: The median area of all lesions was calculated at each time of treatment. In statistical comparison between groups at each time point, there was no significant difference ($P > 0.05$) in lesion area at the start (Day 0, $P = 0.43$) and at Days 14 ($P = 1.0$), 42 ($P = 0.38$), 56 ($P = 1.0$), 70 ($P = 0.55$), and 84 ($P = 0.44$). However, at Day 28 ($P = 0.06$) there was a statistical trend in favor of the group treated with photodynamic therapy. The ATB group showed little variation in both the size and the number of lesions unhealed over time. The PDT group showed pronounced decrease in lesion area in the first 28 days and healing of more than half of the lesions up to 56 days of treatment. The size of the lesion had a tendency to increase influenced by remaining unhealed wounds (Fig. 1).

Comparing the healing rates between the groups, there was a significant difference ($P = 0.02$) for PDT with 63.64% ($f = 7$) of healed lesions compared with 9.09% ($f = 1$) for ATB. Regarding the duration of treatment until complete healing, there was a significant difference between groups ($P = 0.01$). The PDT group showed a variation between 28 and 70 days,

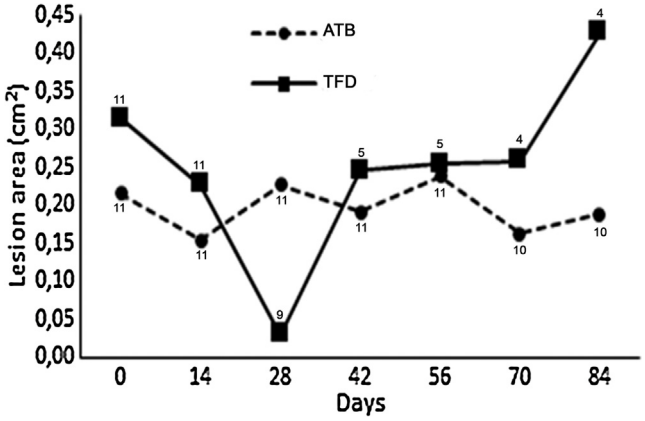

Figure 1 Change in lesion area. Relationship between time of treatment in days and the median of lesion areas in cm^2 in PDT and ATB groups. The number of remaining unhealed lesions per group at each time is highlighted.

the average being 42 days. In the ATB group, the one cured lesion took 70 days to complete healing (Fig. 2).

Fig. 3 shows the change in the area of one lesion treated with PDT (Fig. 3A) and one with ATB (Fig. 3B) at different time points.

Microbiota: Fig. 4 shows the prevalence of all organisms found. *Escherichia coli* was the most frequent microorganism found with a prevalence of 16.55% ($f = 23$). Comparing the prevalence of microorganisms between the groups, no statistically significant differences were observed (Fig. 5).

We selected the four most commonly found bacteria and observed the prevalence of each pathogen by sampling time within each group. None of the microorganisms were prevalent at all of the different sampling points in both the PDT and ATB groups (Fig. 6). In fact, individual microorganisms disappeared, were replaced, or recurred throughout the treatment.

Susceptibility tests: Of the antibiotics tested, drugs like bacitracin and neomycin, commonly found in formulations for topical use, showed more than 90% resistance in this study. Of the broad-spectrum drugs challenged, only gentamicin, amikacin, ceftazidime, and nitrofurantoin showed less than 30% susceptibility among microorganisms found (Table 3). There was neither significant difference

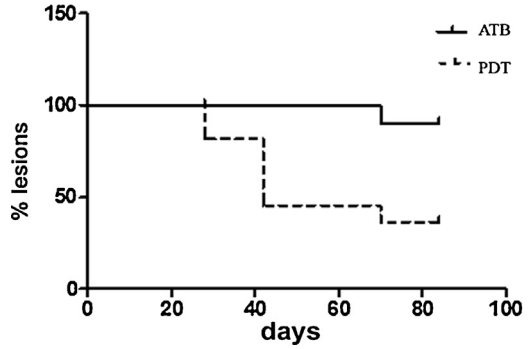

Figure 2 Healing time. Percentage of number of lesions at various time points in PDT and ATB groups (time to complete healing).

Table 3 Prevalence of antimicrobial resistance of total bacterial samples isolated from bumblefoot in Magellanic penguins.

	Neomycin	Bacitracin	Enrofloxacin	Clindamycin	Norfloxacin	Penicillin	Ciprofloxacin	Gentamicin	Amikacin	Nitrofurantoin	Ceftazidime
C	95	98	98	24	95	36	96	80	81	88	46
F	91	93	71	15	36	13	31	22	19	19	5
%	95.8	94.9	72.5	62.5	37.9	36.1	32.3	27.5	23.5	21.6	10.9

C: cumulative frequency; F: frequency of resistant microrganisms; %: percentage of resistant microrganisms.

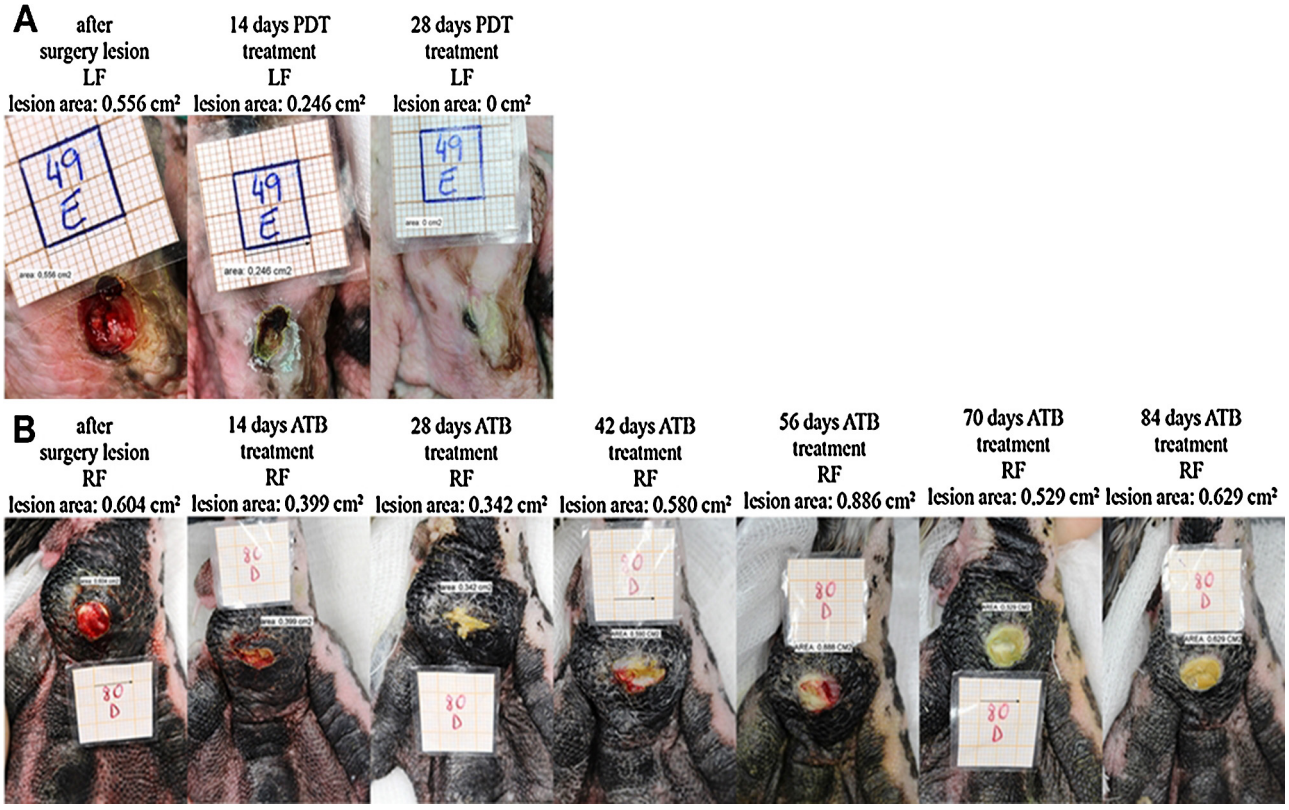

Figure 3 Photographic record of lesion area. Changes throughout the observation period in bumblefoot lesions on the left footpad (LF) of Magellanic penguin no. 49 (A) treated with PDT and on the right footpad (RF) of Magellanic penguin no. 80 (B) treated with ATB.

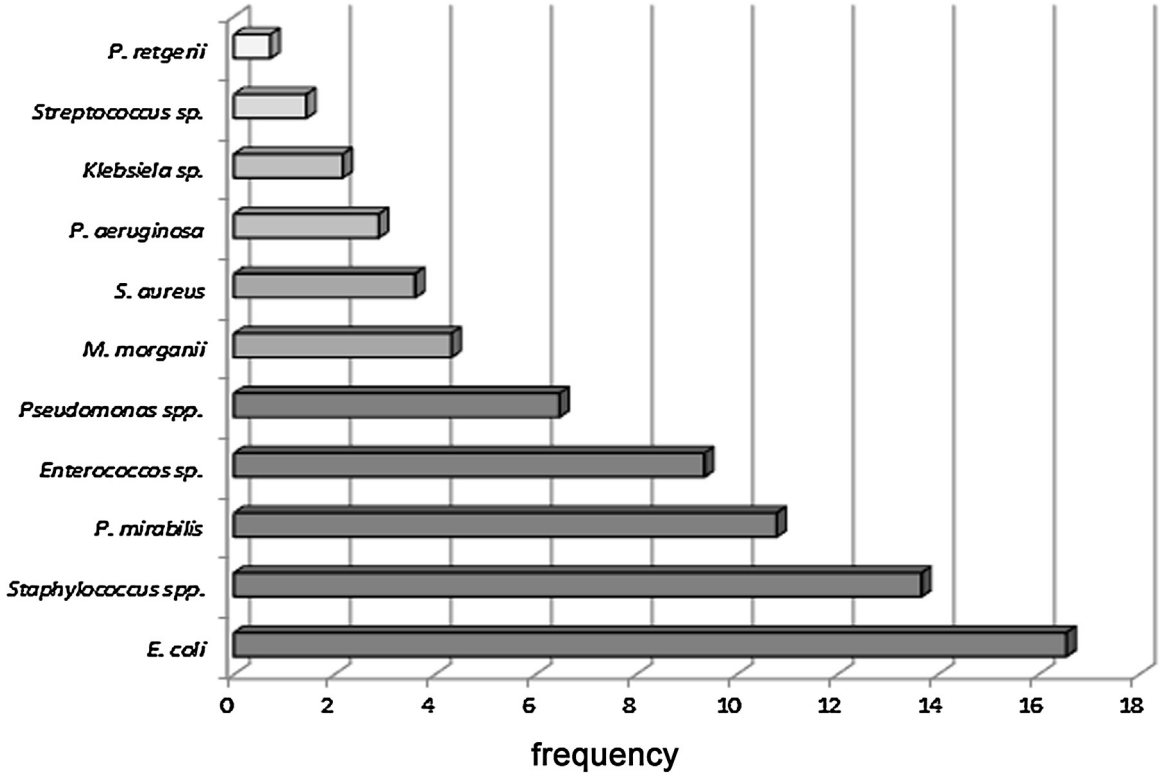

Figure 4 Prevalence of microorganisms. Prevalence of microorganisms in the total samples of footpad dermatitis in Magellanic penguins (*S. magellanicus*) during the experimental period in both groups.

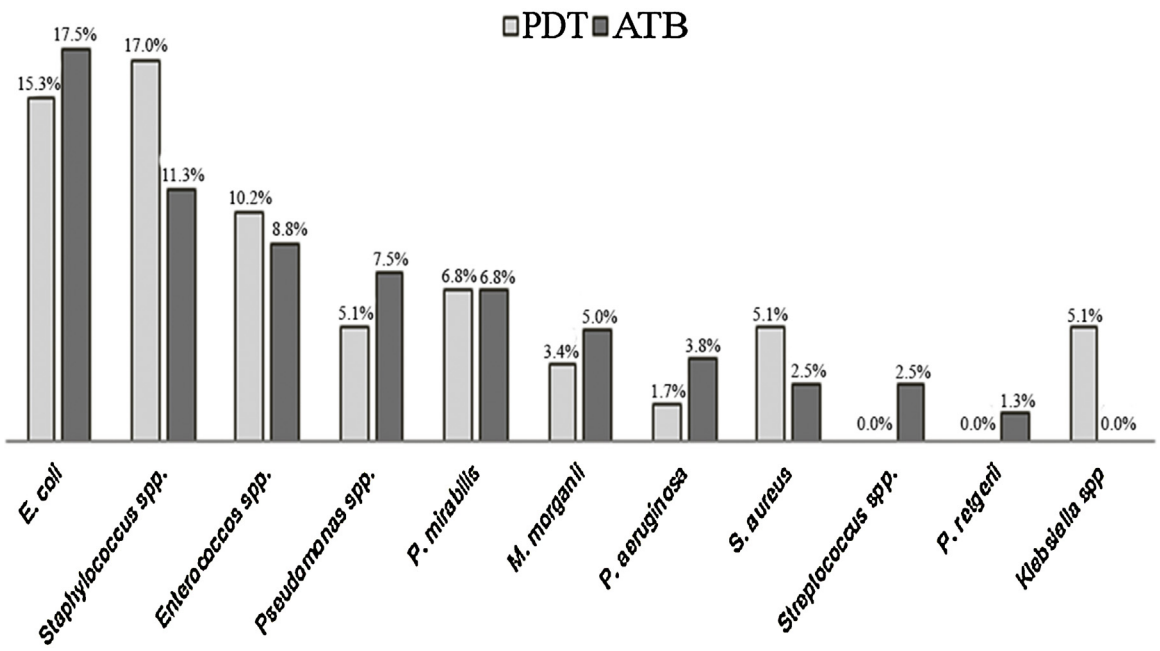

Figure 5 Prevalence of microorganisms per group. Comparison of the prevalence of microorganisms isolated from samples of bumblefoot in Magellanic penguins (*S. magellanicus*) over the whole period of observation in the ATB and PDT groups.

regarding the reliability of challenged antibiotics in Groups ATB and PDT, nor in samples taken immediately after PDT.

Test of resistance to PDT in vitro: As expected, there were no significant differences in the number of colony forming units per milliliter (CFU/mL) among the control groups C, L, and MB [18]. Fig. 7 displays control and PDT groups for all four bacteria tested. *In vitro* tests showed effective bacterial reduction for *Pseudomonas aeruginosa* (Fig. 7A), *Proteus mirabilis* (Fig. 7C), and *Staphylococcus aureus* (Fig. 7D) depending on irradiation time. In fact, 5″, 10″, and 30″ PDT were ineffective overall. For *P. aeruginosa*, we observed that total bacterial killing occurred after 1 min of irradiation, while for *P. mirabilis* and *S. aureus*, a complete reduction of bacterial burden was obtained after 2 min of irradiation. Under tested parameters in this study, PDT was not able to efficiently inactivate *E. coli* (Fig. 7B). Even after 2 min of irradiation, a slight bacterial decrease of about 1 log was obtained.

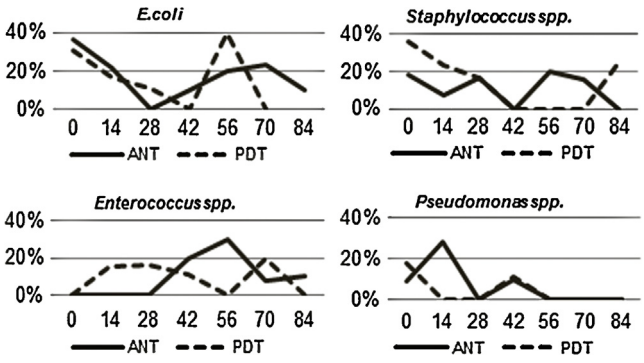

Figure 6 Prevalence of microorganisms at different times. Prevalence in percentage of the four microorganisms most commonly found in Magellanic penguin bumblefoot at various time points in the ATB and PDT groups.

The difficulties in the treatment of bumblefoot reported in the literature [8] are explained by many factors, the main challenges being constant contamination and compression of the injury accentuating the ischemic process. Penguins do not perch, so they are constantly in contact with feces and urine, so it is very difficult to avoid recontamination of wounds even if environmental hygiene is constantly maintained. It is important to note that, in both groups, the protective dressings were used to reduce wound trauma and decrease contamination by contact with the soil. However, the dressings would not have prevented contamination, because they are not impervious and penguins defecate constantly and do not perch. The site of the lesions found in this study corroborates the statement that lesions develop at the point of support in certain areas of the limbs [6].

The literature states that the management of the animals plays an important role in the development of bumblefoot, and that it is necessary to shorten the stand time and increase the swimming time [8]. The management of animals in captivity to decrease the time standing and maintain a standard of swimming similar to that observed in the wild is difficult because of the limited space that is not stimulating for birds, so measures of environmental enrichment and conditioning are recommended [19]. In this study, the routine of penguins was not changed to avoid interference with results.

The group treated with PDT showed better performance than that treated with antibiotics during the first month of treatment. Remple [10] states that bumblefoot responds better to antibiotics long term because of the ischemic nature of the lesion. PDT is applied directly to the infected area, selectively destroying a large number of microorganisms without causing tissue destruction [14].

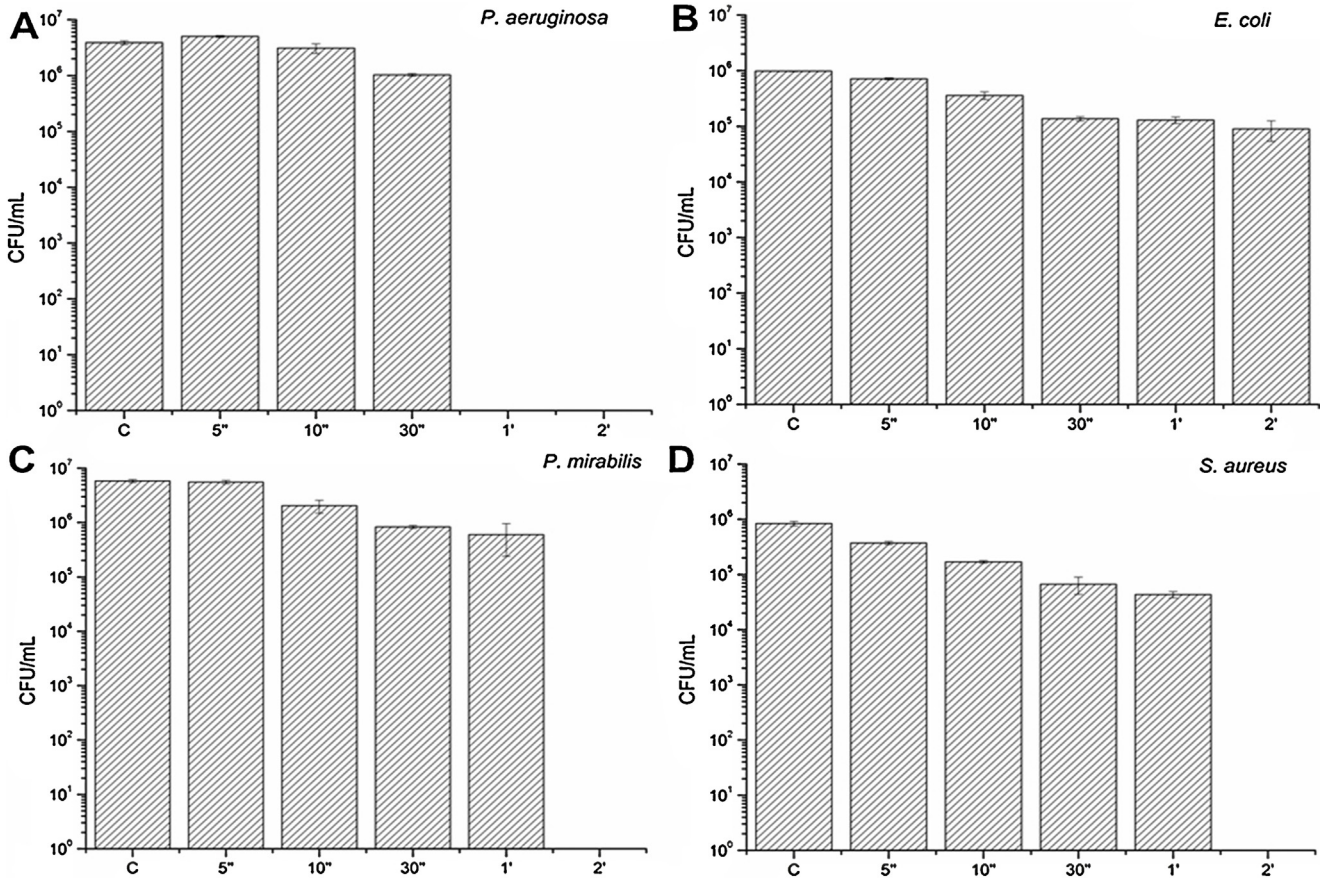

Figure 7 *In vitro* sensitivity for PDT. Average number of CFU/mL in different groups in the PDT sensitivity *in vitro* test. Groups included control groups (C, L, and MB) and PDT groups (5″, 10″, 30″, 1′, and 2′) for *P. aeruginosa*, *E. coli*, *P. mirabilis*, and *S. aureus* from *S. magellanicus* footpad lesions.

Because prevention of contamination of wounds is difficult in penguins, the scheme of repeated PDT at short intervals (in this case three times per week) is important to prevent contamination from developing into infection. The broad spectrum and the localized action of PDT are advantages compared with antibiotics, which depend on bioavailability [9] and effectiveness against bacterial contaminants at the time.

The microbiological findings agree with Cooper [8], who reported that *E. coli* is the microorganism most commonly encountered. Other Gram-negative bacteria such as *P. mirabilis*, *Pseudomonas* spp., *P. aeruginosa*, and *Morganela morganii*, and Gram-positive bacteria such as *Staphylococcus* spp., *S. aureus*, and *Enterococcus* spp., were also frequently found, confirming the findings of Osório [20].

The variation of microorganisms found at different time points of the observation period supports the assertion, made by Reidarson et al. [6], that microbiota present in the skin damage the dermis because of initial injury caused by ischemic necrosis. The constant contamination by new infectious agents imposes the need for constant monitoring of treatment efficacy through antibiogram testing, as recommended by Cooper [8], to ensure the effectiveness of antibiotic therapy. As reported by Osório [20], isolates showed varying degrees of susceptibility in antimicrobial testing. Of 11 drugs tested, only four showed less vulnerability than 30% in the tested microorganisms. This finding confirms that prescribing empirical treatment is undesirable.

The findings of the *in vitro* resistance test for PDT corroborate other studies in the literature that show that PDT is capable of destroying resistant microorganisms even after prolonged use of antibiotics [21,22]. However, it is well established that susceptibility of microorganisms to PDT is dependent on several factors such as concentration of photosensitizer, pre-irradiation time, kind of microorganism, and irradiation time. Probably, longer exposure times are necessary for complete eradication of *E. coli*.

Thus, PDT is appropriate for bumblefoot treatment because broad spectrum of action makes repetitive antibiogram testing unnecessary, it does not develop resistance, and topical application obviates concerns about bioavailability.

PDT is simple to use and does not induce deleterious side effects [11], besides being painless and well tolerated by penguins [23]. Possible stress due to physical restraint can be minimized through conditioning of animals in permanent captivity [7].

Conclusion

The findings of this study attest to the effectiveness of PDT for the treatment of local infection due to bumblefoot in

S. magellanicus. The results of decreased lesion area in the early phases of treatment create opportunity for new studies of the association of this technique with management measures and the search for new dressings to avoid contamination and improve healing.

Conflict of interest statement

The authors have declared that no conflicts of interest exist.

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References

[1] Silva Fillho RP, Ruoppolo V. Sphenisciformes (Pinguim). In: Cubas ZS, Silva JCR, Catão-Dias JL, editors. Tratado de Animais Selvagens. 1st ed. São Paulo: Roca; 2006. p. 309—23.
[2] Xavier MO, Soares MP, Meinerz ARM, Nobre MO, Osório LG, Silva Filho RP, et al. Aspergillosis: a limiting factor during recovery of captive Magellanic penguins. Braz J Microbiol 2007;38:480—4.
[3] Fowler GS, Fowler ME. Order Sphenisciformes (penguins). In: Fowler ME, Cubas ZS, editors. Biology, medicine, and surgery of South American wild animals. 1st ed. John Wiley & Sons; 2001. p. 53—64.
[4] Hocking PM, Mayne RK, Else RW, French NA, Gatcliffe J. Standard European footpad dermatitis scoring system for use in turkey processing plants. Worlds Poult Sci J 2008;64:339.
[5] AZA (American Zoo and Aquarium Association). Penguin husbandry manual. 3rd ed; 2005. p. 142.
[6] Reidarson TH, McBain J, Burch L. A novel approach to the treatment of bumblefoot in penguins. J Avian Med Surg 1999;13:124—7.
[7] Erlacher-Reid C, Dunn JL, Camp T, Macha L, Mazzaro L, Tuttle AD. Evaluation of potential variables contributing to the development and duration of plantar lesions in a population of aquarium-maintained African penguins (*Spheniscus demersus*). Zoo Biol 2011;31:291—305.
[8] Cooper JE. Birds of prey. 3rd ed. Oxford, UK: Blackwell Science Ltd.; 2002.
[9] Soriano-García F. Pharmacokinetic and pharmacodynamic aspects of interpretive reading of the antibiogram. Enferm Infecc Microbiol Clin 2013;28:461—6.
[10] Remple JD. A multifaceted approach to the treatment of bumblefoot in raptors. J Exot Pet Med 2006;15:49—55.
[11] Dai T, Huang Y-Y, Hamblin MR. Photodynamic therapy for localized infections — state of the art. Photodiagn Photodyn Ther 2009;6:170—88.
[12] Huang L, Xuan Y, Koide Y, Zhiyentayev T, Tanaka M, Hamblin MR. Type I and Type II mechanisms of antimicrobial photodynamic therapy: an in vitro study on Gram-negative and Gram-positive bacteria. Lasers Surg Med 2012;44:490—9.
[13] Henderson BW, Dougherty TJ. How does photodynamic therapy work? Photochem Photobiol 1992;55:145—57.
[14] Hamblin MR, Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci 2004;3:436—50.
[15] Oaks JL. Immune and inflammatory responses in falcon staphylococcal pododermatitis. In: Redig PT, Cooper J, Kemple D, Hunter B, editors. Raptor biomedicine. Minneapolis: University of Minnesota Press; 1993. p. 251.
[16] Pfaller MA, Burmeister L, Bartlett MS, Rinaldi MG. Multicenter evaluation of four methods of yeast inoculum preparation. J Clin Microbiol 1988;26:1437—41.
[17] SAS. SAS/STAT 9.3 user's guide. Cary, NC: SAS Inst Inc.; 2011. p. 8640.
[18] Kashef N, Esmaeeli Djavid G, Siroosy M, Taghi Khani A, Hesami Zokai F, Fateh M. Photodynamic inactivation of drug-resistant bacteria isolated from diabetic foot ulcers. Iran J Microbiol 2011;3:36—41.
[19] Reisfeld L, Barbirato M. Reducing bumblefoot lesions in a group of captive Magellanic penguins (*Spheniscus magellanicus*) with the use of environmental enrichment. Pesqui Vet Bras 2013;33:791—5.
[20] Osório LG, Xavier MO, Ladeira SRL, Silva Filho RP, Faria RO, Vargas GD, et al. Study of bacteria isolated from the foot pad of *Spheniscus magellanicus* with and without bumblefoot. Arq Bras Med Vet Zootec 2013;65:47—54.
[21] Biel MA, Sievert C, Usacheva M, Teichert M, Balcom J. Antimicrobial photodynamic therapy treatment of chronic recurrent sinusitis biofilms. Int Forum Allergy Rhinol 2011;1:329—34.
[22] Miyabe M, Junqueira JC, Costa AC, Jorge AO, Ribeiro MS, Feist IS. Effect of photodynamic therapy on clinical isolates of *Staphylococcus* spp. Braz Oral Res 2011;25:230—4.
[23] Sellera FP, Sabino CP, Ribeiro MS, Fernandes LT, Pogliani FC, Teixeira CR, et al. Photodynamic therapy for pododermatitis in penguins. Zoo Biol 2014;4, http://dx.doi.org/10.1002/zoo.21135.