**Point-of-care ketone meters may be used to estimate serum β-hydroxybutyrate concentrations in healthy African penguins (*Spheniscus demersus*).** *American Journal of Veterinary Research*. 2022. 83(6):1-8.

Laura Martinelli

**Abstract:**

OBJECTIVE: **To evaluate the agreement between 3 point-of-care (POC) devices and a reference laboratory for measuring β-hydroxybutyrate (β-HB) concentration in African penguin (*Spheniscus demersus*) whole blood (WB) and plasma samples** and the precision of each POC device for measuring β-HB concentration in plasma samples.

ANIMALS: 48 healthy African penguins.

PROCEDURES: Blood was obtained from the right jugular vein of each penguin, and β-HB concentration was measured on each POC device using fresh WB and heparinized plasma and at the reference laboratory using plasma. β-HB concentration was measured in plasma on each POC device.

RESULTS: **All devices overestimated serum β-HB concentrations on average by 0.46 mM relative to the reference laboratory.** **WB samples had less error than plasma** for meters A and C. Meter A had the lowest total error observed (26.4%) and the lowest mean difference (0.19 mmol/L) relative to the reference laboratory. Controlling for other factors, **the magnitude of disagreement was not affected by sex, age, packed cell volume, or serum total solids concentration.**

CLINICAL RELEVANCE: **WB, not plasma, should be used for measurement of β-HB concentration on the POC meters tested.** Meter A showed good correlation with the reference laboratory for WB. The use of POC devices for the measurement of β-HB concentration may be acceptable when laboratory analyzers are not available. Further research is needed for clinical application and the diagnostic value of POC meters compared with reference laboratories.

**Key Points:**

* Ketosis is the physiologic state in which insufficient supply of glucose leads to an increased β-oxidation of fatty acids and the production of ketone bodies (acetone, acetoacetate, and β-HB) as an alternative primary energy source
* β-HB concentrations are important indicators of metabolic condition and have been assessed in birds. Have been shown to be linked to several ecologically relevant factors including migratory decision-making, starvation, and parasitic load.
* In prior studies, penguins with *Aspergillus fumigatus* infection had significantly elevated lipoproteins, fatty acids, and ketone bodies, including β-HB, compared with healthy birds.
* POC β-HB devices used in this study on healthy African penguins and compared to labatory analyzer
  + Meter A = Kiss My Keto Blood Ketone Monitoring System
  + Meter B = Precision Xtra Blood Glucose & Ketone Monitoring System
  + Meter C = STAT-Site WB Dual Analyte Measurement System
* All POC meters overestimated β-HB compared to the reference lab/standard
* The absolute difference between meters and reference standard was unaffected by sex, age, PCV, and serum TS concentration
* There was greater bias between the POC devices and reference laboratory for plasma samples as compared to whole blood samples. POC devices were more accurate with whole blood than plasma in this study.
* Meter A was the most precise compared to the other point-of-care meters (then B, and last C)

**Take Home Point:** β-HB concentrations measured in African penguins with human point-of-care analyzers significantly overestimated actual β-HB concentration relative to the reference laboratory for both whole blood and plasma. Results suggest that whole blood, not plasma, should be used for measurement of β-HB concentration on a point-of-care meter if you are going to use one. The most precise point-of-care meter was the “Kiss My Keto Blood Ketone Monitoring System” and that would be the meter to use if needing one.

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**The effects of critical care nutrition on weight gain in African Penguin (*Spheniscus demersus*) chicks.** *Journal of Avian Medicine and Surgery.* 2023. 37(3): 217-225.

Laura Martinelli

**Abstract:** Nutritional support in malnourished animals is an essential aspect of wildlife rehabilitation; this support is especially relevant when providing lifesaving nutrition to endangered species such as the African penguin (*Spheniscus demersus*). **This study investigated the short-term effects of a commercially available, semi-elemental, critical care diet compared with a hand-made fish formula.** Twenty-one African penguin chicks were selected on admission to the Southern African Foundation for the Conservation of Costal Birds in Cape Town, South Africa, in November 2015. Initial assessment included body weight, a full clinical exam, white blood cell count, packed cell volume, and total plasma protein. **Ten animals received the commercial critical care diet, whereas a control group of 11 animals were fed hand-made formula for the 2-week study period.** All animals were weighed daily and blood sampling was repeated after 14 days. **The median weight of both groups increased significantly over 14 days** (critical care diet χ2 = 10.1, *P* = 0.002; control χ2 = 7.4, *P* = 0.006). **The difference was not significant between the groups for start weight (χ2 = 0.1, *P* = 0.725) or end weight (χ2 = 0, *P* = 1.000) and was not significantly different in the change over time for either absolute numbers (χ2 = 1.7, *P* = 0.193) or percent gain (χ2 = 0.8, *P* = 0.36).** The values for packed cell volume, total plasma protein, and white blood cell count increased in all animals after the 14-day study period was complete. **On the basis of the results of this study, it was determined that the differing diets led to similar weight gain.**

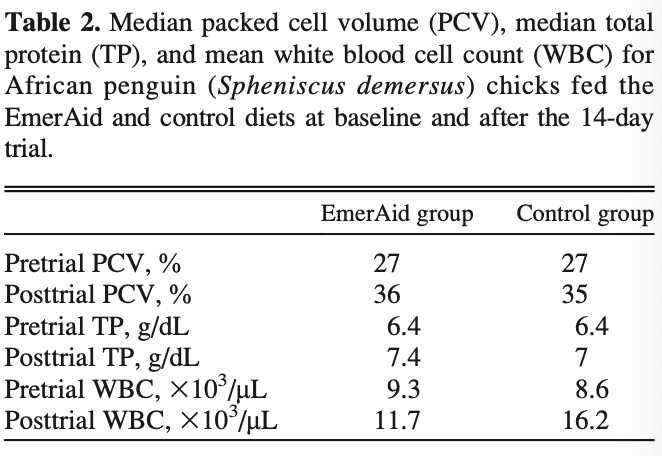
**Key Points:**

* Most rapid periods of growth in African penguin chicks reported to be 25 to 55 days old, with significant increase in food intake during this period
  + Increased amounts of calcium and Vit D are recommended during rapid growth periods
* Neonate penguins more than 3 days old get total volume of liquid food at 10% body weight per feeding up to 6 times per day. Once chick is 100 grams and 7 days old, can gradually add in whole fish.
* Compared EmerAid Piscivore Care to a Fish Formula
  + Fish Formula = minced defrosted sardine (tails and fins removed), water, fish-eating bird tablets, brewer’s yeast tablets, thiamine tablets, cod liver oil, Protexin (probiotic), Cani-Cal powder (calcium supplement), Premolt 5 powder (amino acid supplement)
* While not significant, chicks in the EmerAid group displayed a slightly higher weight gain per day as compared to Fish formula but overall there was no difference in weight gain each day or over the entirety of the study
* While not significant, chicks in the EmerAid group exhibited a greater PCV and TP increase
* In one study, TP is a prognostic indicator in African penguin chicks
  + High (>5.8 g/dL) had a protective effect against natural death in African penguin chicks
  + Low (<4 g/dL) contributed to natural death in chicks and juveniles

**Take Home Point:** Both EmerAid Piscivore Care and a hand-made fish formula led to similar weight gain over seven days in African penguin chicks.

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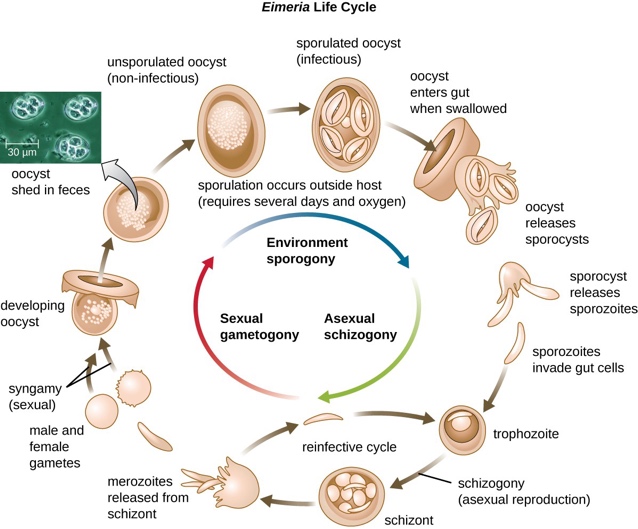
*Journal of Wildlife Diseases, 58(4), 2022, pp. 836–846*

*Summarized by MR*



PREVALENCE AND PATHOGEN LOAD OF EIMERIA IN WILD YELLOW- EYED PENGUINS (MEGADYPTES ANTIPODES) AND THE MORPHOLOGIC CHARACTERIZATION OF A NOVEL EIMERIA SPECIES

Emily Kay,1,2,4 Melanie J. Young,3 Chris Muller,1 Laryssa Howe,1 Wendi Roe,1 and Brett D. Gartrell1

Abstract: Coccidia infections in wild birds rarely cause clinical signs; however, disease and mortality can occur with predisposing environmental and host conditions. The Yellow-eyed Penguin (*Megadyptes antipodes*) is an endangered species endemic to New Zealand that has seen significant ongoing population decline. The aim of this study was to examine the **host–pathogen dynamics of coccidian parasites in two wild populations** of Yellow-eyed Penguin: the mainland (South Island) population and the sub-Antarctic (Enderby Island) population. There was weak evidence for a **difference in the prevalence of the *Eimeria sp.* in birds from Enderby Island** (76.6%; 36/47; 95% confidence interval [CI] 62.78–86.4%) and the South Island of New Zealand (58.54%; 24/41; 95% CI 43.37–72.24%). The mean pathogen load in penguins on Enderby Island was 9,723 oocysts/g of feces (SE1⁄45831 oocysts/g) and from the South Island of New Zealand was 1,050 oocysts/g (SE1⁄4398 oocysts/g). **No evidence of an association was found between pathogen load and body weight** in either study population. The morphology of the sporulated coccidial oocysts was consistent with a **novel species of Eimeria**. There was statistically significant variation between the oocysts collected from the two sites in all measurements apart from the oocyst wall thickness. However, the standard technique of assessing linear regressions of the length and width of oocysts from both sampling sites was 0.80, and therefore above the standard R2>0.5 used to indicate variation within a single population of oocysts, suggesting that **only a single species of Eimeria was present at both sampling locations**. The **prevalence and pathogen load of Eimeria sp. was substantially higher than previous reports of coccidial oocysts in Yellow-eyed Penguins and free-living Sphenisciformes globally**. This host–parasite relationship deserves further investigation, as the **impact of this novel organism on the population remains unclear.**

**Background:**

* Two genetically distinct and geographically isolated subpopulations: mainland NZ and sub-Antarctic
  + Declining mainland population despite more recent colonization than island, fewer than 250 breeding pairs
  + Occupy different habitat ranges: different ecological stressors
* Eimeria sp. – obligate intracellular protozoan. Most infect intestinal or renal epithelial cells
  + Significance is unknown: variable findings in existing literature

**Summary:**

* Objectives:
  + examine host-pathogen dynamics of coccidian parasites in the two populations
  + provide morphometric description of *Eimeria* sporulated oocysts in this species
* Methods
  + Opportunistic collection of individuals from sub-Antarctic/Enderby Island (n = 47) and mainland population (n = 41) between 2015-2017
  + A microscope view of a cell

    Description automatically generatedFecal samples collected from each bird when voided during handling or digital expression, then PE + weight (BCS by one operator)
  + Fecal floatation and light microscopy of concentrated samples
* Island population 76.6%, mainland 58.5% - no statistically significant difference in coccidial load between populations
* Morphology consistent with novel species of Eimeria – distinct from *Eimeria pygosceli* identified in chinstrap penguins
  + Molecular analysis is required to complete the description of the novel Eimeria sp. reported here and confirm that these observed oocysts are distinct from *E. pygosceli.*
* Some variation in pathogen size, but regression analysis and morphologic examination supports a single species
* The Yellow-eyed Penguin colonized the mainland relatively recently, after the extinction of another penguin species: supports that the *Eimeria* identified is capable of replication and transmission between penguin hosts in a variety of environmental conditions (co-evolution with changes in species range)
* Pathogen load is higher than previously described and higher than reported in other wild penguin species
  + Unknown clinical significance at this time (co-evolution = tolerance?)
  + Unknown tissue tropism at this time
  + Mean BCS of infected penguins significantly lower in sub-Antarctic island pop. than mainland – did not definitively conclude that there is a clinical impact of infection (BCS impacts can be multifactorial)
* High chronic Eimeria loads within avian populations have been shown to reduce reproductive success and survival

**Take Home Points:**

* Despite higher *Eimeria sp.* load than previously described in this population and other penguin populations, clinical significance of the pathogen and tissue tropism is unknown
* Morphology consistent with novel Eimeria sp. – molecular analysis required to complete description

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*Journal of Zoo and Wildlife Medicine 55(3): 585–594, 2024*

*Summarized by MR*



EFFECT OF SUBSTRATE AND WALKING SURFACES ON CENTRAL METATARSAL FOOT PAD WEIGHT LOADING IN MAGELLANIC PENGUINS (*SPHENISCUS MAGELLANICUS*) WITH AND WITHOUT PODODERMATITIS: AN EX VIVO STUDY

Su Hyun Faith Yang, Jessica Aymen, DVM, DVSc, DACZM, and Hugues Beaufrère, DVM, PhD, DACZM, DABVP(Avian), DECZM(Avian)

Abstract: **Pododermatitis is common** in penguins kept under human care. Substrate optimization plays an important role in prevention and treatment; however, there is **limited information on biomechanical properties of commonly used substrates on penguin feet**. The objectives were to test the ability of different substrates to decrease weight loading on the central metatarsal pad of penguin feet in an ex vivo model using **feet with and without bumblefoot harvested from two Magellanic penguin (*Spheniscus magellanicus*) cadavers**. **Penguin feet were attached to a digital force gauge mounted onto a stand for compression testing at 2.5 and 5 kg. Forces at the central metatarsal pad were measured** in triplicate using small force sensors. Tested substrates included five granular surfaces (**sand, wet sand, pea gravel, wet pea gravel, and crushed ice**), three compliant surfaces (**short-leaf Astroturf, long-leaf Astroturf, and neoprene**), and three firm surfaces (**tile, rubber drainage mat, and 3M Safety- Walk Wet Area Matting**). Data were analyzed using linear mixed models. There were multifaceted effects of applied pressures, substrate surfaces, and pododermatitis on central metatarsal measured pressures. In general, doubling compression forces resulted in higher measured pressures in all firm and compliant surfaces but not in granular surfaces. Firm surfaces were associated with higher recorded plantar pressures at 2.5 kg, but different significance groupings emerged at 5 kg with a high-, medium-, and low-pressure cluster of surfaces. **Pododermatitis lesions resulted in significant alterations in statistical significance clustering among substrate surfaces and unique substrate behaviors**. The results of this study could help in making recommendations pertaining to foot health for penguin exhibits.

**Background:**

* High prevalence of pododermatitis in penguins under managed care
* Risk factors may include obesity, decreased swimming time, substrate choice, trauma, nutrition, and diseases of the contralateral limb
* Chronic systemic inflammation → systemic amyloidosis
* Bandaging options for penguins are extremely limited: debridement, neoprene shoes, significant husbandry alterations (dry docking)
* Current AZA recommendations include providing a wide variety of materials and textures, and exhibit design should encourage natural aquatic behaviors

**Summary:**

* Objectives:
  + evaluate effects of common substrates on weight loading at the central metatarsal pad of cadaver feet when placed under compressive forces – mimic standing, walking, jumping.
  + Compare feet with and without pododermatitis
* Methods:
  + Four total cadaver legs radiographed for bony lesions (none noted) and stored frozen. Two legs were normal, one had a mild lesion, another severe
    - No pododermatitis grading in this species, adapted raptor scale used = 2/5, 4/5 respectively
  + Limb attached to digital force gauge and manual test stand – weight loading measured at the central MT pad using a small force sensor
    - 2.5 and 5 kg applied based on average weights in the zoo collection (mean 5kg, (range up to 7.6kg) half the weight distributed to each foot in the average standing penguin = 2.5)
  + 11 substrates tested (see abstract, wet and dry)
    - Adjustments were made if repeated measurements altered the compression of the substrate surface (repeat zero’ed, pressure points changed on substrate piece)
* The amount of force applied to the feet and changes in conformation from pododermatitis significantly affected force patterns
* Granular surfaces tended to better decrease central MT pad measured pressure especially for nonpathological feet/at higher forces
  + Low granularity (sand, crushed ice) performed better than pea gravel – better at cushioning the foot
* Neoprene was the most effective compliant surface tested, and provided sufficient cushioning on pathological feet – previous literature supports the use of neoprene shoes and pads
  + Further evidence that neoprene shoes effectively redistribute weight loading away from central MT pad in penguins with pododermatitis
* It can be reasonably inferred based on results for firm surfaces in this study that it likely increases the risk of developing pododermatitis lesions
* Given anatomic conformation changes with bumblefoot – weight is redistributed abnormally and exposes the central MT pad to increased pressure (self-perpetuating cycle if not for accommodating substrate changes)
* Force patterns. Describe in this study suggest that forces redistribute over the larger foot surfaces and pedal tissues “flatten”
  + doubling the compression pressure did not lead to a twofold increase in measured pressure when using granular surfaces.
  + This may also be related to foot webbing starting to take some of the load as the center of the feet sinks into the granular surface types
* Husbandry recommendations
  + Granular surfaces (small as possible, granular sand > pea gravel) are likely the best outdoor substrate option
  + Neoprene padding performed best and is recommended for orthotic shoes and high traffic or standing areas
  + Firm surfaces should be avoided in most instances, at least in high traffic or standing areas
  + Considerations for ramps and indoor habitats should be made both from high traffic and congregation area considerations

**Take Home Points:**

* Granular surfaces (small as possible, granular sand > pea gravel) are likely the best outdoor substrate option
* Neoprene padding performed best and is recommended for orthotic shoes and high traffic or standing areas
* Firm surfaces should be avoided in most instances, at least in high traffic or standing areas
* Considerations for ramps and indoor habitats should be made both from high traffic and congregation area considerations

Journal of Zoo and Wildlife Medicine, 55(3): 595-601, 2024.

**ADAPTATION OF A COMMERCIALLY AVAILABLE WESTERN BLOT KIT FOR THE DETECTION OF ANTIBODY TO *ASPERGILLUS* IN PENGUINS IN FRANCE AND THE UNITED STATES** - reviewed by HSS

Antoine Leclerc, Raphaël Piarroux, Adriana Callico, Ellen Bronson, Carolyn Cray

**Abstract:**

**Antemortem serodiagnosis of aspergillosis remains challenging in Sphenisciformes. Protein electrophoresis, serology (antibody, antigen) by ELISA, and gliotoxin detection provide variable diagnostic value. In the present study, a commercially available Western blot (WB) validated for use in humans and dolphins was adapted for use with penguin samples.** Using the same method and reagents, samples were analyzed from multiple institutions in the United States and one facility in France. This was inclusive of normal juvenile African penguins (*Spheniscus demersus*, n = 10) and various species of penguins in the United States with confirmed infection (n = 9) as well as 52 samples from Humboldt penguins (*Spheniscus humboldti*) in France. **Cumulative WB scores (based on reactivity to different antigens) were found to be significantly higher in the group of penguins with confirmed infection (p < 0.0001).** Significant differences were also observed between the clinically normal penguins in the two populations, with higher scores in the United States (median score 1.0, 95%CI [0–5], min 0, max 11) compared to France (median score 0,95%CI [0–0], min 0, max 5). **The utilization of the WB as a diagnostic tool is inconclusive due to the use of samples from varying institutions, environmental background, age, and stages of infection. However, this tool may provide an overview of antigen reactivity in penguins infected with *Aspergillus* to help design a more robust serology assay and further understand the humoral immune response during infection.**

**Key Points:**

* Culture and identification of *Aspergillus* spp. from associated lesions is considered the gold standard diagnostic method. Alternative diagnostic procedures include hematology, blood chemistry, diagnostic imaging, and endoscopy. However, these procedures have a limited and variable diagnostic value. Antemortem serodiagnosis of aspergillosis in penguins remains challenging.
  + The combination of adjunct testing including 3-hydroxybutyrate levels and plasma protein electrophoresis provides high specificity and negative predictive value.
* Western blotting (WB) is a diagnostic technique used to measure antibody reactivity to specific antigens through the use of a membrane for immunodetection. A commercially available WB kit for the detection of IgG antibody to *Aspergillus* has been validated for use in humans and dolphins
* The avian IgG, better noted as IgY, is believed to be the precursor to mammalian IgG and IgE.
* Unaffected penguins in France had significantly lower scores compared to unaffected penguins in the US
* Regardless of the country and institution of origin, unaffected penguins had lower scores than penguins with confirmed aspergillosis
* Preliminary data indicates that repeated measures of positive penguins may be reflective of clinical condition; additional studies may indicate that this new serological method could provide prognostic information.
* Study serves as proof of concept that the immunoblot methodology may have application in the diagnosis of aspergillosis in penguins. This type of assay may help to better understand the humoral immune response of penguins to this fungal infection, which may be beneficial for development of future ELISAs or other immunoassays.

**Take-Home Message:**

* Regardless of the country and institution of origin, unaffected penguins had lower scores than penguins with confirmed aspergillosis
* Study serves as proof of concept that the immunoblot methodology may have application in the diagnosis of aspergillosis in penguins.

Journal of Zoo and Wildlife Medicine, 55(2): 479-489, 2024.

**FATAL ACUTE HEMOLYSIS FOLLOWING TRIAZOLE THERAPY IN AFRICAN PENGUINS (*SPHENISCUS DEMERSUS*)**- reviewed by HSS

Courtney N. Patson, Elizabeth J. Elsmo, Lauren Trepanier, Michael M. Garner, Michael J. Murray, Ellen Bronson, Lorelei L. Clarke, Sherry K. Cox, Robert J. Ossiboff, Marley E. Iredale, Bryce M. Miller, Lindsey Waxman, Eric Littman, Mary I. Thurber



**Abstract:**

**Aspergillosis is a major cause of morbidity and mortality in penguins, with triazole antifungal drugs being commonly used for prophylaxis and treatment.** This report describes 15 cases of **fatal hemolysis associated with liquid itraconazole and voriconazole formulations administered to African penguins (*Spheniscus demersus*)** from four institutions. All penguins underwent stressful events (e.g. relocation, induced molt) and were administered commercial liquid itraconazole formulations or compounded voriconazole liquid suspension. Observed clinical signs in affected penguins prior to death included **hyporexia, weight loss, lethargy, dyspnea, red-tinged droppings, and obtunded mentation.** **Intra- and extravascular hemolysis and hemoglobinuric nephrosis** were the primary pathologic manifestations on postmortem examination. The concentration-dependent hemolytic potentials of itraconazole, voriconazole, and commercial and compounded vehicle suspensions were evaluated in vitro by **exposing chicken whole blood as a surrogate for penguin blood**. Hemoglobin content in blood plasma was then measured by spectrophotometry. Neither itraconazole nor voriconazole alone induced hemolysis in vitro. The vehicle ingredients sorbitol and hydromellose induced hemolysis, but not at predicted plasma levels in chicken erythrocytes, suggesting **neither the azole antifungals nor their major vehicles alone were likely to contribute to hemolysis in vivo in these penguins. Potential mechanisms of toxicosis include generation of an unmeasured reactive metabolite causing hemolysis, preexisting erythrocyte fragility, or species-specific differences in hemolytic thresholds that were not assessed in the chicken erythrocyte model.** More research is needed on the potential for toxicosis of azole antifungal drugs and carrier molecules in this and other avian species.

**Key Points:**

* Itraconazole is the most commonly used medication for prophylaxis and treatment of aspergillosis in penguins. However, because of reports of drug resistance to itraconazole among *Aspergillus* species, the use of voriconazole in birds has become more common for the treatment of aspergillosis.
* All cases occurred in penguins that were undergoing stressful events, with most having been relocated to temporary indoor facilities. These birds were administered itraconazole for antifungal prophylaxis (n=13) or voriconazole for treatment of suspected aspergillosis based on clinical signs and abnormalities detected via protein electrophoresis (n=2)
* 28 additional penguins were also administered itraconazole and voriconazole simultaneously across all four institutions without mortality
* **Clinical signs:** Hyporexia occurred in all penguins with an onset of 1–7 d after initiation of antifungal therapy; in 5/15 penguins, hyporexia was the only clinical sign prior to death. In the remaining penguins, the most common clinical signs were lethargy (8/15), weight loss (6/15), increased respiratory effort (7/15), and red-tinged droppings (2/15). These clinical signs were observed from 10 to 20 d after initiation of azole antifungal therapy. Clinical signs progressed rapidly over 12 h to 2 d until death.
* **CBC/Chem:** increase in AST/CK, most samples moderately to markedly hemolyzed, 2 had evidence of increased RBC production on blood smears, one had decrease in HCT over time
* **Itraconazole concentrations:**
  + Plasma itraconazole + hydroxyitraconazole concentrations determined in 4/15 cases 🡪 all greater than the conc in which there is increased risk of toxicosis in humans (>1 ug/mL)
  + Tissue itraconazole + hydroxyitraconazole and voriconazole determined in 4/15 🡪 values in 3 penguins were 5x higher than published PK and tissue distribution studies in other species (duck, hawk, chicken)
* **Necropsy findings:** 
  + The liver was enlarged and mottled or pale in 9/15 penguins. Histologic examination revealed mild to moderate erythrophagocytosis in Kupffer cells (sinusoidal macrophages) in 14/15 penguins. Extramedullary hematopoiesis was present within the liver in 10/15 birds.
  + Kidneys were bilaterally enlarged and mottled or pale in 10/15 penguins, and gross pigmenturia within ureters was identified in one penguin. Histologic examination revealed varying degrees of acute tubular necrosis in all penguins. In 14/15 penguins, proximal renal tubular epithelial cells contained hemoglobin consistent with hemoglobinuric nephrosis
  + Cardiac muscle necrosis in 6/15 penguins and skeletal muscle necrosis in 4/9 penguins
  + **Intravascular and extravascular hemolysis, hemoglobinuric nephrosis, and muscle necrosis (skeletal and cardiac) were the primary lesions in these mortality events.**
* Despite intensive investigation into nondrug causes of hemolysis, bacterial and viral cultures and PCR revealed no primary pathogenic organisms, and no toxic agent was found through heavy metal and micronutrient analysis
* In vitro hemolytic activities were evaluated for itraconazole, voriconazole, and drug vehicles used in commercial and compounded formulations of these drugs (hydromellose and sorbitol; cyclodextrin was not available for evaluation). Whole blood was collected in heparinized tubes from healthy domestic chickens as a surrogate for penguin blood
  + Neither itraconazole nor voriconazole alone resulted in hemolysis in vitro. Although sorbitol and hydromellose resulted in dose-dependent hemolysis in vitro, the threshold for hemolysis was about 1,000-fold higher than predicted in vivo plasma exposures, making both vehicles unlikely to reach hemolytic concentrations in vivo in these penguins.
* Ddx for hemolysis in birds:  heavy metal or micronutrient toxicosis, hemoparasites (e.g. *Plasmodium*, *Babesia*, and bacteria *Anaplasma*, *Ehrlichia*, and *Borrelia*), bacterial septicemia (e.g. *Salmonella*, *Clostridium*, *Colibacillus*, *Chlamydia*, and *Leptospira*), respiratory viral disease, rhabdomyolysis, and drug-induced toxicosis.
* Voriconazole toxicosis has been reported in multiple penguin species, including African penguins. Clinical signs included anorexia, lethargy, weakness, and ataxia
* A definitive mechanism of presumptive toxicosis remains unclear, but potential mechanisms include generation of an unmeasured reactive metabolite causing hemolysis, preexisting erythrocyte fragility, or species-specific differences in hemolytic thresholds that were not assessed in the chicken erythrocyte model.
* The differences in azole absorption between liquid formulations and capsules may account for the increased tissue levels observed in the penguins in this series.

**Take-Home Message:**

* The administration of liquid azole antifungal therapy, potentially in combination with physiological stressors, may have played a role in multiple African penguin mortalities. Intravascular and extravascular hemolysis, hemoglobinuric nephrosis, and muscle necrosis (skeletal and cardiac) were the primary lesions in these mortality events. Monitor penguins undergoing itraconazole and voriconazole treatment for changes in appetite and behavior and to discontinue therapy immediately if signs of toxicosis are observed.

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**Effects of Alfaxalone on the Induction and Maintenance of Total Intravenous Anesthesia in Gentoo Penguins (Pygoscelis papua).** JAMS 2023. Ono K, Yamasaki M, Ichijo T, Satoh H. - review by LMumm

Systemic anesthesia in penguins is often achieved using inhalation anesthetic agents alone, and information on injectable drugs for systemic anesthesia is limited. General anesthesia with a minimal effect on circulatory dynamics is necessary to perform noninvasive examinations and treatments in animals, including penguins. In this study, alfaxalone (ALFX), an injectable anesthetic agent, was examined to establish the optimal anesthetic method for gentoo penguins (*Pygoscelis papua*). Alfaxalone was administered intravenously through the metatarsal vein, and anesthesia was maintained by a constant rate infusion (CRI). A biological monitor was used to record numerous clinical indices, and the anesthetic depth was evaluated every 5 minutes during anesthesia; the CRI was adjusted until the optimal anesthetic depth was obtained. Anesthesia depth was assessed, and the CRI rate was adjusted. The CRI was stopped, and the time until recovery was recorded. Blood samples were collected to analyze plasma concentrations of ALFX. The mean total dose of ALFX required for anesthetic induction was 9 ± 1.9 mg/kg, the intubation time was 126 ± 21 seconds, and the maintenance infusion rate of ALFX was 0.3 ± 0.08 mg/kg/min. The time from discontinuation of anesthesia to extubation was 42 ± 23 minutes, and the time to recovery was 90 ± 33 minutes. **Significant changes in the heart rate and blood pressure were not observed during the anesthetic events.** The plasma concentration of ALFX under stable anesthesia was 6734 ± 4386 ng/mL (range, 3315-14 326 ng/mL). **Although anesthesia using ALFX tended to result in a prolonged time to recovery in gentoo penguins, rapid induction of anesthesia and stable hemodynamics during anesthetic maintenance were achieved**. Therefore, ALFX may be considered a suitable anesthetic method for noninvasive examinations and treatments in penguins.

Background:

* Alfaxalone = neurosteroid anesthetic agent
  + Y-aminobutyric acid type A receptors
  + Known for rapid induction of anesthesia, smooth recovery, minimal CV effects

Key Points:

* Induction was rapid with ~ 9 mg/kg IV (similar dose to other species)
  + Intubated in ~2 minutes
* Maintenance infusion ~0.3 mg/kg/min + IPPV
  + One animal did not reach adequate depth at 0.5 mg/kg/min and removed from study
* Time from discontinuation of CRI to extubation 42 + 23 mins
* Time to recovery was 90 + 30 minutes
* Adverse effects
  + **Apnea** occurred in 8/10 animals at time of induction
  + Excitement/escape behavior in 1/10 during inducton
  + **Cough** in 10/10at time of intubation (induction graded “poor”), and 4/10 during maintenance and recovery
  + **Large amount of saliva** noted in ~50% of animals
  + 50% required **reintubation during recovery** due to hypoventilation/apnea
  + Metabolic alkalosis (higher pH, BE, HCO3) post vs. pre-anesthesia
* No significant changes in HR (decreased in time dependent manner)
* No significant changes in NIBP (unknown efficacy of monitor)
* Normal behavior and feeding were confirmed in all the day after anesthesia

**TLDR: Alfaxalone TIVA (IV induction and maintenance CRI) in gentoo penguins provides rapid induction of anesthesia and stable hemodynamics, though can expect apnea, coughing, drooling, and prolonged recoveries.**

**Capillary Zone Electrophoresis in Humboldt Penguins (Spheniscus humboldti).** J Avian Med Surg. 2024. Leineweber C, Lücht M, Gohl C, Steinmetz HW, Marschang RE. - review by LMumm

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Electrophoresis is a useful diagnostic tool for detecting inflammation, including inflammation associated with infectious diseases (eg, aspergillosis in penguins). To our knowledge, reference intervals are not available for plasma proteins via electrophoresis in Humboldt penguins (Spheniscus humboldti). Therefore, preliminary reference intervals for blood plasma proteins measured by capillary zone electrophoresis were calculated for Humboldt penguins from a single zoological collection, and possible differences between the sexes and the ages of the birds were evaluated. Lithium heparinized plasma samples from 39 Humboldt penguins were analyzed. The following sex- and age-independent reference intervals were calculated: total protein 33.8-70.4 g/L, prealbumin 1.9-4.9 g/L, albumin 12.9-31.1 g/L, albumin: globulin ratio 0.7-1.7, α-globulins 4.5-11.6 g/L, β-globulins 5.6-20.6 g/L, and γ-globulins 2.6-8.4 g/L. Male penguins had a significantly (P = 0.047) higher albumin: globulin ratio and lower percentage of β-globulins (P = 0.015) in comparison with female penguins. Prealbumin (g/L) significantly (P = 0.021) decreased with increased age of the penguins. These results showed some differences between the sexes and ages of the penguins, which should be considered when interpreting the results. Further studies are needed to determine whether differences in other age groups or seasons exist, and also to evaluate which infectious diseases affect plasma proteins and how the reference values calculated here may deviate in ill penguins.

Background

* Protein electrophoresis = helpful tool for diagnosing inflammatory changes (generally) and only method to accurately measure albumin in birds
* Prev study (JZWM 2018): African penguins with asper had decreased prealb, A:G ratio, and α1 globs + increased α2, β, and γ-globulins due to increased APP and Ab in plasma (used AGE)

M&M: hep plasma from n=29 apparently healthy captive Humboldts → capillary zone electrophoresis

Key Points

* CZE resulted in 5 different major plasma protein fractions
  + Prealbumin, albumin, α-globulin, β-globulin, γ-globulin
* **Minor differences between sex - males had higher A:G ratio, and lower beta-globs**
  + May expect more differences during breeding season (sampled outside of breeding)
* **Prealbumin had significant NEGATIVE correlation with age (decreased prealbumin with increased age)**
  + **Age trends also reported in flamingos (JAMS 2021 Leineweber et al.**)
    - Greater flamingos have increased γ-globs with increasing age
    - American flamingos have decreasing albumin and increasing α-globs and β-globs with increasing age
  + Increased pathogen exposure with increasing age, and metabolic/growth hormones can influence protein fractions
* Humboldts (CZE) had higher albumin, A:G ratios, α-globs and β-globs compared to African penguins (AGE) but used different EPH methods (JZWM 2018)

Related articles: Leineweber C et al. Comparison of capillary zone electrophoresis in greater flamingos (Phoenicopterus roseus) and American flamingos (Phoenicopterus ruber). JAMS 2021; 35:180–186.

Penguin Summaries

APPLICATION OF A NOVEL ASPERGILLUS LATERAL-FLOW DEVICE IN THE DIAGNOSIS OF ASPERGILLOSIS IN CAPTIVE GENTOO PENGUINS (PYGOSCELIS PAPUA PAPUA)

Abstract: Aspergillosis is the primary fungal disease affecting captive penguins globally. Its diagnosis remains challenging, and currently no tests are both sensitive and specific for the detection of early infection. The present study evaluated a recently developed Aspergillus lateral-flow device (AspLFD) for the detection of Aspergillus spp. antigen in plasma and glottis mucus from captive penguins. In a pilot retrospective study, banked frozen plasma samples from captive penguins were reviewed: samples from 11 gentoo penguins (Pygoscelis papua papua) and 4 king penguins (Aptenodytes patagonicus) fulfilled the inclusion criteria and were used in the analysis. Positive plasma AspLFD test results were found in 80% (four of five) of the aspergillosis-positive cases tested. All of the aspergillosis-negative cases tested negative (10 of 10) on the AspLFD test. In a cohort prospective study, paired plasma and glottis swab samples were opportunistically and nonrandomly collected from captive gentoo penguins. In total, 26 penguins were tested. In the negative control group, AspLFD test was negative on plasma and swab in 100% of birds (14 of 14). In the aspergillosis-positive group, AspLFD test was positive on plasma samples from 33% (4 of 12) of birds, on swab samples from 50% (6 of 12) of birds, and on either plasma or swab samples from 75% (9 of 12) of birds. The AspLFD is currently used for the diagnosis of aspergillosis in humans and also shows promise for use in penguins. Larger prospective studies are recommended.

* Clinical signs of aspergillosis in penguins are nonspecific: anorexia, weight loss, self isolation, death; respiratory signs can include open-beak breathing, coughing, and aphonia
* Galactomannan (GM) has a 67% sensitivity and a 73% specificity
* AspLFD (lateral flow device): is a rapid immunochromatographic test for qualitative detection of an extracellular glycoprotein antigen secreted during the active growth of the fungus
  + Target: antigenic mannoprotein released exclusively in the active growth phase
* In the pilot study (banked plasma samples): the test confirmed ⅘ positives (80%- however the one that was not positive was the chronic case) and 10/10 negatives (100%)
* In prospective study (plasma and swabs): all negatives confirmed 14/14 plasma and 14/14 swabs (100%) In the asper group 4/12 plasma samples were positive (33%) and 6/12 swab samples (50%) and then 9/12 birds (75%- were positive by plasma, swab, or both)
* AspLFD in the pilot study was biased toward the most severely affected cases (those that failed treatment and died)
* Optimal performance was seen by combining the results of AspLFD in plasma and swab (making 75% of cases detectable in the asper group - sensitivity- which is better than GM testing)
* Majority of clinically abnormal penguins present with a depressed albumin-globulin ratio and increased alpha-2, beta, and gamma globulin on PPE
  + This does have high sensitivity however normal PPE have been obtained in clinically or subclinical birds

Take Home: AspLFD may be used to detect Asper (especially if both plasma and swabs are submitted together) but further larger studies are needed to compare AspLFD and GM to understand the performance of AspLFD

DETECTION AND PREVALENCE OF SPHENISCID ALPHAHERPESVIRUS-1 (SpAHV-1) IN A SAMPLE OF HUMBOLDT PENGUINS (SPHENISCUS HUMBOLDTI) AT PUNTA SAN JUAN, PERU

Abstract: Infections with herpesvirus have contributed to respiratory, enteric, and neurological disease reports in avian species worldwide. Herpesviruses have been detected in penguin species before but have not been studied extensively. To better understand the impact of these viruses in free-living populations, an initial retrospective survey was performed on a wild population of Humboldt penguins (Spheniscus humboldti) in the Punta San Juan Marine Protected Area, Peru (158229S, 758129W) using tracheal swabs collected from 28 penguins in 2016 and 34 penguins in 2018. DNA extracted from these swabs was analyzed using a consensus herpesviral PCR assay targeting the DNA polymerase gene, and positive samples were sequenced. A single sample from 2016 was positive for spheniscid alpha-herpesvirus-1 (SpAHV-1), establishing an overall sample prevalence of 1.6% (95% CI: 0–8.6%). The positive animal was an adult male that did not show any clinical signs of herpesviral infection and was otherwise healthy based on physical exam and laboratory findings. This is the first detection of a herpesvirus in penguins at Punta San Juan, Peru, and the first step toward characterizing the implications of SpAHV-1 for Humboldt penguins. This investigation highlights the importance of continual disease surveillance in wild populations over time to monitor for changes that may impact long-term population viability.

* Penguins are among avian family to have show to be affected by infectious pathogens
  + Stressors include predation, habitat loss, weather, human interference or malnutrition which can increase susceptibility to pathogens
* Studies have Identified antibody titers for infectious bronchitis virus, avian reovirus, paramyxoviruses, and Salmonella pullorum
* Penguin populations have been shown to be highly susceptible to West Nile virus, avian malaria, avian cholera and avian influenza
* Herpesvirus is large, enveloped viruses with double stranded DNA genome and are generally host specific
  + Latent infections are in the trigeminal nerve ganglia
* Most avian herpesviruses of non-commercial birds fall into gamma subfamily
* This study surveyed a group of wild Humboldt penguins- took tracheal swab samples as part of their health surveillance
* PCR found one penguin that was positive for spheniscid alpha herpesvirus-1 (an adult male with no clinical signs)
* Sample prevalence was determined to be 1.6% (1/62)
* This is the first detection of herpesvirus in Peru- BUT not in South America
* SpAHV-1: previously isolated from juvenile Humboldt and African penguins observed with penguin-diphtheria-like disease- implying that herpesvirus could play a role in this disease process

Take Home: This study found SpAHV-1(1.6%) in a wild population of humboldt penguins in Peru (sample size only reflected 4.8% of population)