



Letter

Occupational sensitization to African penguin serum and mucus proteins

The African penguin (*Spheniscus demersus*) is an endangered species. Populations have decreased from 1.5 million to 60,000 penguins on the southern African coast during the past 50 years. However, it is popular in aquariums around the world because of its ability to thrive in warmer climates, unlike other antarctic penguins.

Animal behaviorists working in aquariums have been reported to become sensitized to fish feeds, fish, and crustaceans by direct contact or by the airborne route in poorly ventilated areas. Sensitization resulting in clinical symptoms when exposed to penguins has not been previously reported.

We report sensitization resulting in allergic symptoms in a 42-year-old woman, an animal behaviorist, who cared for a small African penguin colony at an aquarium. The patient was known to have atopy, having had eczema, asthma, and allergic rhinitis since early childhood, and was previously found to be sensitized to fish, shellfish, cat, dog, peanut, and soy by skin prick testing.

Before taking up employment at the aquarium, she had worked at a dolphinarium for approximately 10 years, where she developed severe eczema of the hands, which was aggravated by handling fish, requiring topical treatment with steroids and azathioprine. She also was unable to ingest fish. Her hand eczema was alleviated when she wore gloves in the work environment.

When she became the curator of the African penguin colony at the aquarium in 2009, in addition to conducting talks and demonstrations of the penguins to the public, her duties included cleaning the penguin enclosure with a powered water hose and feeding the penguins. Her arms had numerous scars from the pecking of penguins when she did the feeding demonstrations. Within 2 years she developed severe asthma attacks when entering or cleaning the penguin enclosure and particularly when examining the penguin nests.

She had no asthma symptoms when working in other areas of the aquarium, indoors or outdoors, or during the weekends at home or when away from the penguins. Peak expiratory flow rate readings at home ranged from 440 to 480 L/min. Baseline lung functions were completely normal (3.2 L/min of forced expiratory volume in 1 second [111% predicted], 82% for the ratio of forced expiratory flow to forced vital capacity, 1.32 for forced expiratory flow at 25% [72% predicted]) when away from the penguins, but peak expiratory flow rate monitoring showed a 24% decrease in peak expiratory flow rate in the mornings (from 470 to 360 L/min) when entering the penguin enclosure. She required rescue fenoterol inhalation to treat these episodes of bronchospasms. The patient found that her episodes of bronchospasm were particularly severe when working with the penguin nesting material. Her asthma in the work environment also decreased on twice-daily combination inhalation therapy with 250 mg of fluticasone and 50 mg of Serevent

(salmeterol; GlaxoSmithKline, Brentford, United Kingdom), which offered bronchial protection when entering the penguin area.

She also reported transient erythematous patches on her arms and legs and allergic conjunctivitis when exposed to the penguins, which was worse in the mornings when the enclosure was cleaned, the penguin excretion became airborne, and the enclosure became smellier because of poor ventilation.

She had positive skin prick test reactions (wheal size) to white fish (5 mm), blue fish mix (8 mm), sardine (10 mm), tuna (11 mm), *Alternaria* species (12 mm), *Aspergillus* species (4 mm), and wheat (5 mm).

Her total IgE was 25,800 kU/L. ImmunoCAP (ThermoFisher Scientific, Waltham, Massachusetts) reactions were positive to pigeon droppings (20.5 kU/L), parakeet (46.5 kU/L), duck feathers (67.2 kU/L), grass pollen (210 kU/L), hake (649 kU/L), squid (362 kU/L), pilchard and sardine (100 kU/L), langoustine (2.38 kU/L), egg white (13.7 kU/L), *Anisakis* species (1.36 kU/L), *Aspergillus* species (45.6 kU/L), *Alternaria* species (27 kU/L), latex (5.5 kU/L), and *Lepidoglyphus destructor* (68 kU/L).

Immuno Solid Phase Allergen Chip (ISAC; ThermoFisher Scientific) assays to 52 recombinant allergen sources confirmed IgE sensitization to tree and grass profilin, egg serum albumin, mouse lipocalin, *Alternaria* enolase, mite cysteine proteases, latex rubber, elongation factor and profilin, Bermuda grass group I, lipocalin in cat and dog allergens, *Aspergillus* paroxysmal protein, *Anisakis* tropomyosin, kiwi traumatoin (Act d 2), cod parvalbumin, and timothy grass (group I).

These investigations confirmed her known allergies to grass pollen inhalants, fish, and mice but also suggested IgE sensitization to cross-reacting recombinant protein epitopes. Initially it was not clear whether the development of severe bronchospasm, which she experienced when entering the penguin enclosure or cleaning the enclosure, was due to fish or seafood or possible fungal allergen exposure or the protein products of the penguins.

To investigate further, samples were collected from the penguin enclosure, penguin feces, and nesting material. The aquarium veterinarian obtained blood and mucus (from the penguin esophagus) from the penguins for western blot analysis.

Western blots of penguin serum, mucus, feces, and nesting material are shown in Figure 1. Incubation of the blots with the patient's serum (1:30 dilution) overnight and detection of IgE binding used a 1:1,000 dilution of antihuman IgE (labeled with alkaline phosphatase). Clear (patient-specific) specific IgE bands were detected to 25- and 66.70-kDa proteins on the blot and to 12-kDa proteins in the penguin feces and nesting material. No binding to penguin proteins was detected using control sera from other allergic subjects. Western blots also confirmed patient-specific IgE binding to several proteins in hake, pilchard, and squid using the same techniques (data not shown) and was confirmed by ImmunoCAP testing.

Disclosure: Authors have nothing to disclose.

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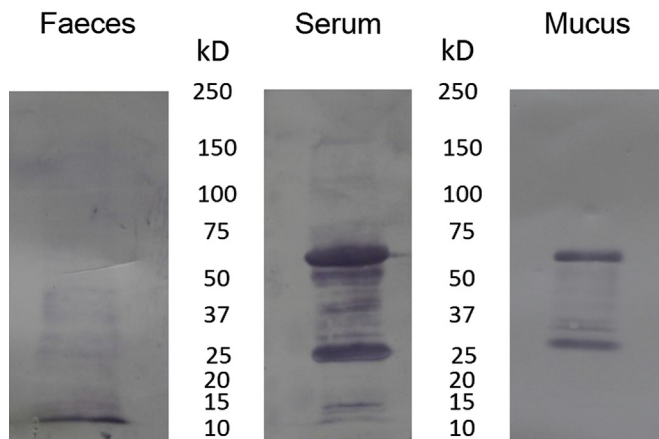


Figure 1. Specific IgE binding to 66.7- and 25-kDa proteins in African penguin serum and mucus and to 12-kDa protein in African penguin feces by western blotting.

The compensation commissioner for occupational diseases was notified of this novel allergic sensitization as an occupational allergy to penguin after peak expiratory flow rate monitoring confirmed a decrease in lung functions when the patient was exposed to the penguin enclosure in the mornings and started wheezing but was free of symptoms when working in other areas of the aquarium and at home. The patient was advised to wear a 3M mask (3M, St Paul, Minnesota) and to avoid direct exposure to aerosolized penguin proteins at cleaning times. Her asthma medication was adjusted and steps were taken to avoid exposure to fish proteins by direct contact or inhalation in the work environment.

An assistant was employed to clean the enclosures, allowing the patient to continue her overall curatorship of the penguins, rather than being offered redeployment. After avoiding direct contact with the penguins, the patient made excellent progress and has been relatively asymptomatic after an 18-month follow-up and still able to work at the aquarium. The patient has been able to handle fish and fish feeds in the aquarium using gloves, with no adverse, local, or respiratory events.

This is the first report of the acquisition of sensitization and the development of asthma and allergic conjunctivitis in an animal behaviorist after direct exposure to and working with penguins and cleaning of the enclosures.

The nature of the dominant 66.70- and 25-kDa IgE binding proteins encountered in this patient to penguin serum and mucus is intriguing and requires more detailed characterization. Very little has been published on the characterization of penguin serum proteins. Allergenicity has not previously been reported. Western blotting also showed strong specific binding to 12-kDa protein in the penguin feces (Fig 1). Further characterization of this protein is necessary in the future.

Osuga et al¹ in a comparative study of penguin vs chicken and bovine serum albumin (70 kDa) found that penguin serum albumin has properties similar to bovine serum albumin. It is of interest that this patient's serum recognized recombinant chicken egg serum albumin but did not bind to horse serum albumin or bovine serum albumin in the ISAC test, confirming that IgE binding in the ISAC and ImmunoCap assays was not nonspecific in view of the patient's elevated total IgE level.

It is of interest that sensitivity to the Gal d 5 α -livetin serum albumin (molecular weight 69 kDa) is responsible for the bird-egg syndrome in egg yolk and bird feather extracts.²

Major allergenic bands of similar size to those identified in our patient to penguin serum (molecular weights 20–30 kDa and 67

kDa) have been found in IgE immunoblots of budgerigar, parrot, pigeon, canary, and hen sera, which cross-react with other bird species.³ Previous sensitization and pigeon serum proteins could have increased the risk of our patient's reaction to cross-reacting or novel African penguin proteins, because the molecular weights identified in the penguin serum (25 + 66 kDa), mucus, and feces are similar to those identified in bird-egg syndrome and bird fanciers asthma, but this would require enzyme-linked immunosorbent assay inhibition studies for further exploration.

One of the reasons for the decline in penguin populations has been the illegal consumption of penguin eggs by local populations. To date, allergy to penguin eggs has not yet been reported. Penguin serum albumin has a molecular weight of 66,000 kDa (identified to bovine serum albumin), and a unique pen albumin (61,000 kDa) in antarctic (eg, Adelie) penguin eggs has a high carbohydrate content (15%) and antifreeze properties.⁴ These proteins have not been characterized in the African penguin.

Occupational contact dermatitis resulting from handling fish food (eg, herring in a dolphinarium)⁵ and other foods⁶ has been reported, and we believe our patient's chronic eczema was acquired previously from fish handling without glove protection.

The acquisition of work-related asthma to exposure to the penguins and their aerosolized feces in our patient indicates that atopy might be a risk factor for asthma development in penguin curators in aquariums around the world. It is recommended that penguin enclosures be well ventilated to decrease the risk of inhalation and sensitization and clinical allergy to penguin, but also to fish proteins, in atopic workers. Further characterization of the IgE binding to penguin serum and mucus and fecal proteins should be conducted.

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