Hypersensitivity and Intradermal Allergy Testing in Psittacines

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ABSTRACT: Pathologic feather picking in companion avian species has been attributed to multiple underlying disorders, and many of these birds are thought to be pruritic. Allergies have been discussed as possible contributors to this common and devastating dermatologic disease. This article reviews published information and research currently being conducted on hypersensitivity disorders in avian species. Allergic dermatitis is given special emphasis, with a focus on diagnostic techniques, such as intradermal allergy testing and histopathologic findings in feather-picking birds.

Avian dermatology is an important segment of companion bird practice, with feather picking being by far one of the most common, but also most challenging, dermatologic disorders in psittacine pet birds. Pathologic feather picking occurs when a bird damages its feathers or traumatically removes them from areas on the body that are accessible to its beak (Figure 1). In severe cases, the underlying skin can be considerably damaged, even to the point of fatal hemorrhage. Feather picking is not a specific disease but is rather the result of an underlying disease process or psychologic disorder. The most common causes of self-mutilation include nutritional deficiencies, toxins, infections, internal organ pathology, and psychologic factors. However, identification of an underlying disease often proves difficult, and many cases remain undiagnosed.

Some feather-picking avian patients are believed to be pruritic, with allergies being proposed as the underlying etiologic conditions in these birds. Avian practitioners are trying to establish intradermal allergy testing as a tool to help in diagnosis and in development of specific treatment protocols for avian species. Such intradermal testing is the gold standard for determining offending allergens in type I hypersensitivity conditions in various mammalian species, such as dogs, cats, horses, and humans.
HYPERSENSITIVITY REACTIONS IN BIRDS

Many feather-picking birds are thought to be pruritic, although this has been difficult to document. Pruritus can cause dramatic self-trauma and mutilation in mammals (e.g., dogs, cats, horses, humans) and is often due to an underlying allergic condition, such as food allergy, atopic dermatitis, insect bite hypersensitivity, or flea bite allergy. Many comparisons have been drawn between birds and domestic animals with regard to hypersensitivity reactions. Like mammals, birds have immediate type I hypersensitivity reactions in which epidermal mast cells undergo degranulation and release inflammatory mediators in response to bound allergens. Mast cells have been identified in birds and are known to release cell products, including histamine and serotonin, on degranulation. However, in birds the predominant immunoglobulin was designated IgY; it has antigenically distinct features compared with the mammalian IgG and IgE.

IgY has a molecular mass of 180 kD and is a truncated molecule. It is believed to be the ancestor and functional equivalent of the mammalian IgG. Moreover, molecular cloning techniques provided clear evidence that IgY is the evolutionary ancestor not only of IgG but also of IgE. IgY appears to combine the two major functions of IgG and IgE. Like IgG, it is the major low molecular weight serum antibody and hence is the major defense mechanism against systemic infections. However, similar to IgE, it possesses the ability to mediate anaphylactic reactions. It is now known that IgY is the predominant low molecular weight serum antibody not only in birds but also in reptiles, amphibians, and probably lungfish. Many of these species, little is known about the functional capabilities of IgY. Nevertheless, in birds, IgY is known as a skin-sensitizing antibody that can mediate anaphylactic reactions.

Anaphylactic reactions, which are type I hypersensitivity reactions, have been documented in birds that have collapsed shortly after vaccination. Poultry studies have documented cutaneous delayed hypersensitivity reactions, consisting of local activation or proliferation of T cells and subsequent local recruitment of inflammatory cells (heterophils and mononuclear cells), after mitogen injections. Delayed hypersensitivity is also believed to contribute to the formation of postvaccination granulomas in psittacines and chickens. No consistent clinical pathologic data exist that would help in the diagnosis of allergies, such as the typical tissue eosinophilia seen in mammals. However, anecdotal reports suggest that steroid therapy, antihistamines, and changes in environment and diet may resolve the clinical signs in some feather-picking avian patients. These observations have led to speculation that an allergic condition may be an underlying cause of feather picking in some companion birds.

Because of variable responses to empirical therapy used for presumed allergic conditions in feather-picking birds, intradermal allergy testing may be a useful tool for identification of offending allergens. In mammalian species, intradermal allergy testing is widely used to diagnose hypersensitivity reactions. On the basis of positive reactions and the patient’s pertinent history, allergens are chosen for specific immunotherapy, or the information may be used to facilitate avoidance of allergens.

Recently, allergic conditions in normal and feather-picking psittacines have been studied by means of intradermal allergy testing. The data obtained support the hypothesis of underlying allergies as the cause of feather picking in some birds. However, at this time, the feasibility of clinical intradermal allergy testing in avian species is not clear. Current studies are evaluating new protocols and establishing correct dilutions of allergens. Should skin testing ever become a recognized and feasi-
ble technique in avian dermatology, specific treatment plans, including allergen avoidance and hyposensitization to offending allergens, could be developed.

PROTOCOLS FOR AVIAN INTRADERMAL ALLERGY TESTING

Two previous studies investigated intradermal allergy testing in captive psittacines. Results from both studies showed that gross evidence of positive reactions to injected allergens proved difficult to evaluate, and the authors emphasized the need to practice this technique to ensure accurate intradermal placement of the injected allergens. It is unknown whether the amount of injected fluid, the thin skin of birds, or endogenous cortisone production is responsible for the weak skin reactions.

Study by Macwhirter and Mueller

Macwhirter and Mueller used skin testing on 41 healthy birds of various species and 15 Psittaciformes showing evidence of self-mutilation. Histamine was used as the positive control, and saline was used as the negative control. Nine allergens were chosen, and 0.02 ml of each substance were injected into the skin of the sternal apterium (Figure 2). The injection sites were examined visually at various times after injection, and reactions were considered positive if wheals exceeded the diameter of the negative control. Reactions in normal and affected birds were subtle and evanescent. Reactions at 5 and 15 minutes were similar; however, some reactions disappeared during the 15-minute interval.

The response to histamine was highly inconsistent, with only one-third of the healthy birds and two-thirds of the feather-picking birds showing wheals. This inconsistency may be due to the lack of histamine receptors on endothelial cells. When histamine binds to these receptors, endothelial cells are activated, leakage of serum occurs, and exocytosis of inflammatory cells is promoted. Another explanation may be the release of endogenous corticosteroids during the testing procedure, which would suppress histamine-induced wheal formation. In a recent study of healthy psittacines undergoing skin testing, Heatley et al. showed that serum corticosterone concentrations increased dramatically in a linear manner over time. These observations agree with findings in cats, in which handling, skin testing, and anesthesia caused a rise in plasma cortisol concentrations, which resulted in short-lived wheals.

Macwhirter and Mueller observed that positive reactions to one or more of the tested allergens occurred in only one healthy bird but in most of the affected birds, with wheal formations seen most frequently in response to sunflower, house dust mite, grain mill dust allergens. This observation may suggest a hypersensitivity reaction as an underlying disease. However, inflamed skin of clinically affected birds may be more prone than healthy skin to irritant reactions after intradermal injections. Nevertheless, retested birds showed the same positive reactions as had occurred with the initial skin testing, which makes response to specific allergens in individual birds more likely than the wheals’ being a nonspecific irritant reaction.

Protocol of Colombini and Coworkers

Colombini and coworkers established a protocol for intradermal allergy testing in psittacines. The authors tested 40 healthy parrots with various positive and negative controls. The proventer (sternal) region adjacent to the keel was found to be the most suitable skin test

<table>
<thead>
<tr>
<th>Generic Drug</th>
<th>Trade Name</th>
<th>Concentration Used</th>
</tr>
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<tbody>
<tr>
<td>Codeine</td>
<td>Codeine Phosphate Injection USP (Abbott Laboratories, North Chicago, IL)</td>
<td>1:100,000 wt/vol</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>Fluorescite 10% (Alcon Laboratories, Fort Worth, TX)</td>
<td>1%</td>
</tr>
<tr>
<td>Histamine</td>
<td>Histatrol, histamine base 0.1 mg/ml (Center Laboratories, Port Washington, NY)</td>
<td>1:100,000 wt/vol</td>
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site because it allowed the most space for testing. This area is also the largest apterium (featherless skin part), so feather plucking to prepare for the skin test was unnecessary, and hemorrhage, which could obscure skin test reactions, could be avoided (Figure 2). The proventer region was prepared by using alcohol swabs to moisten and part the feathers, but in a following study the alcohol swab was changed to a water swab because the alcohol was found to irritate the birds’ skin.22 Intradermal injections were made above and below black ink marks (Figure 3). Several different test agents, including histamine, codeine phosphate, compound 48/80, anti-avian IgG, and deionized water, were used to establish a reliable positive control to serve in future intradermal allergy testing in presumed allergic psittacines. Phosphate-buffered saline and rabbit serum served as negative controls. Various volumes of each test substance were injected, and skin test sites were evaluated after 5, 10, and 15 minutes.

On the basis of the results of their study, Colombini et al19 confirmed the proventer region as an acceptable region for skin testing. They further proposed using codeine phosphate 1:100,000 wt/vol, at 0.02 ml, as a positive control agent and reading skin test sites at 5 minutes (Table 1). Although this study provided statistically significant results between positive and negative controls because of a consistent injection technique and a small standard deviation, the clinical impact of the proposed protocol is actually doubtful because gross inspection of injection sites did not allow grading of the reactions and made subjective reading of the skin test impossible and unreliable. Furthermore, even reading with digital calipers provided only rather small differences between negative and positive control sites.

Results of the available studies show that intradermal allergy testing still lacks clinical feasibility as a diagnostic tool in avian allergies.

Studies in Cats

In cats, appropriate grading of skin test results can be challenging because of a lack of erythema and the occurrence of short-lived reactions. Therefore, an IV fluorescent dye has been used before injection of allergens for better visualization of skin test sites.23 Using fluorescein before testing led to the accumulation of the dye and visualization of fluorescence with a Wood’s light, which permitted distinctive subjective and objective differentiation between positive and negative reaction sites (Figure 4). Interpretation of intradermal allergy testing in cats became easier and more reliable with the use of fluorescein. This same technique may also help with testing in avian species.

INTRAVENOUS FLUORESCIN AND INTRADERMAL ALLERGY TESTING

Fluorescein sodium is a highly fluorescent chemical compound synthesized from petroleum derivatives.24 It absorbs blue light, with peak absorption and excitation occurring at wavelengths between 465 and 490 nm. To excite the fluorescein, a blue light source is used. The Wood’s light, which is readily available and widely used to reveal the manifestation of ringworm in domestic animals,11 provides light at the appropriate wavelength to excite fluorescein.

Fluorescein in low concentrations may be safely injected IV. Approximately 80% of the dye molecules are bound to serum protein in the circulation. The remaining free dye molecules fluoresce when they are excited with a light source of the appropriate wavelength. Because of these physical properties, fluorescein
has been used extensively in diagnosis and treatment of various vascular conditions in humans and domestic animals and has become an important diagnostic tool in ophthalmology.\textsuperscript{24–26} After IV injection, free fluorescein molecules reach capillaries and invade interstitial tissue through vascular fenestration.\textsuperscript{27} The dye rapidly diffuses out of capillaries and into extravascular tissue. The uptake and distribution pattern of fluorescein in the skin thus reflects both local blood flow and capillary permeability.\textsuperscript{28} The dye is metabolized by the kidneys and is eliminated via the urine 24 to 36 hours after administration. A protocol for fluorescent angiography in raptors, in which fluorescein was used at 40 mg/kg IV, was only recently established. The fluorescein was injected before evaluation of filling patterns in retinal capillaries.\textsuperscript{29} Fluorescein reached the ophthalmic tissue within a few seconds and could be verified by ophthalmoscopic observation for up to 28 hours.\textsuperscript{27}

Fluorescein has been reported to have minimal side effects, in both animals and humans. In humans, the incidence of complications after IV administration of fluorescein sodium is about 5%. Mild gastrointestinal adverse reactions (nausea and vomiting) are the most frequent side effects; severe reactions are extremely rare.\textsuperscript{30,31} In previous studies, IV fluorescein at different dosages was used for ophthalmic investigations in various bird species. Minor side effects, such as salivation, vomiting, head shaking, and somnolence, were encountered at only high fluorescein dosages (50 mg/kg) and did not occur when the dosage was lowered (10 to 40 mg/kg).\textsuperscript{28,29,32}

In chickens, the intradermal injection of histamine and several other known inflammatory agents produced a marked increase in vascular permeability.\textsuperscript{33} This finding may provide evidence that fluorescein could be applied to aid visualization of hypersensitivity reactions in avian skin testing. In an unpublished study, we used IV fluorescein before intradermal allergy testing in healthy psittacines to develop a clinically feasible protocol that would permit subjective reading of positive skin test sites. Healthy psittacines were given fluorescein at 10 mg/kg IV immediately before skin testing, together with one negative control and two positive controls (phosphate-buffered saline, histamine, codeine phosphate; Table 1). At 5 and 10 minutes after the injection, sites were evaluated with the aid of the Wood’s light. Fluorescence was observed at all injection sites, and statistical analysis showed increased wheal diameters at histamine injection sites compared with saline or codeine phosphate injection sites. However, subjective differences between positive and negative controls were not consistent, and at this point IV fluorescein cannot be recommended for skin testing of psittacines.

Figure 5—A nontranslucent tape is placed on the selected biopsy site before the punch biopsy is performed through the tape and the adherent skin. The sample with the adherent tape is removed for routine processing and dermatopathologic evaluation.

HISTOPATHOLOGY IN AVIAN HYPERSENSITIVITY DISORDERS

One of the diagnostic tools available to avian practitioners for determining a diagnosis in feather-picking birds is a full-thickness biopsy. In small animal dermatology, histopathologic study is an important and widely used tool for establishing a definitive diagnosis in many dermatologic conditions, and it can also assist in diagnosing allergic skin disease when the pattern of inflammation in the superficial dermis is characteristic.\textsuperscript{11}

However, the avian skin is very thin, and thus obtaining skin biopsy samples can prove exceedingly difficult because the skin tends to ball up and roll after release of punch or wedge biopsy tools. This is especially true for skin specimens with no feather follicles. Correct orientation of the skin surface with its appendages is essential for adequate trimming and subsequent reading of skin biopsy specimens to ensure a correct histopathologic diagnosis. Correct diagnosis is very difficult with rolled specimens, and skin specimens should be obtained that remain flat during fixation, to improve the histopathologist’s ability to establish a proper diagnosis.

A new punch biopsy technique to help provide such flat skin specimens was developed at our laboratory.\textsuperscript{34,35} A 2- to 3-cm-long, nontranslucent self-adhesive tape (Scotch tape, 3M) was placed on the chosen biopsy site. The biopsy was then performed through the tape by applying gentle force to the biopsy punch as it was twisted clockwise and counterclockwise at the same time (Figure 5). Once the tape was punched, only min-
resolved after the bird’s diet was changed, and therefore a diagnosis of food allergy was postulated. In chickens sensitized to Mycobacterium tuberculosis, repeated exposure resulted in edema at the injection site and subsequent infiltration of heterophils and mononuclear cells, which indicated acute inflammation, compatible with a hypersensitivity reaction.

In a retrospective study, more than 200 skin specimens from different avian species were analyzed and categorized into 13 groups on the basis of morphologic diagnoses after histopathologic examination. Hypersensitivity was diagnosed in almost one-third of all examined biopsy specimens, with cockatoos, macaws, Eclectus parrots, African grey parrots, Amazon parrots, and conures being overrepresented. Unfortunately, the histologic patterns on which the morphologic diagnoses were based were not provided in this study.

Pathologists have occasionally seen skin lesions with granulocytic infiltrates but noted the difficulty in differentiating heterophils from eosinophils. Other reported histologic patterns included multifocal perifollicular dermatitis and peripheral feather necrosis. In one recent study, multiple skin biopsy specimens from healthy birds, which had undergone intradermal allergy testing, were examined for histopathologic patterns suggestive of allergic conditions. At positive control sites (codeine phosphate injection), small nodules composed of lymphoid cells and heterophils were described. The adjacent vessel walls contained lymphoid cells and granulocytes (Figure 6). These findings indicate hypersensitivity reactions and are similar to those for superficial perivascular dermatitis, which are observed in specimens from mammals with allergic skin conditions.

Figure 6—Histopathologic sample of bird skin from the proventer region, which shows superficial dermal blood vessels with a mixed perivascular infiltrate. (Hematoxylin–eosin)

CONCLUSION

Pathologic feather picking in birds is multifactorial, and, even though there are consistent overt physical manifestations, underlying diseases are many and varied. Hypersensitivity reactions to allergens, such as pollens or dietary ingredients, are thought to contribute to this common syndrome. Possible tools for diagnosing such allergic conditions include histopathologic study and, potentially, the use of intradermal allergy testing.

Intradermal allergy testing protocols still lack clinical feasibility because of subtle and short-lived reactions and the difficulty of administering the intradermal injections.
On the basis of available statistical evidence, codeine phosphate seems to be the most consistent positive control agent for psittacine skin testing. In the future, improving the gross subjective grading of positive skin test reactions may be possible with the use of an IV fluorescent dye immediately before the intradermal injection.

Much is still unknown, however, and further research in the field of avian allergy is needed to achieve scientific diagnosis of the disease condition known as pathologic feather picking.

ACKNOWLEDGMENTS

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REFERENCES


1. Pathologic feather picking refers to a condition in which
   a. a bird loses its feathers on the head and neck.
   b. a bird damages its feathers or traumatically removes
      them from areas on the body that are accessible to
      its beak.
   c. a bird plucks its feathers for nesting.
   d. a bird plucks its feathers for a breeding pouch.
   e. feathers exhibit dystrophic growth.

2. Which statement does not apply to the avian IgY?
   a. IgY is the major defense mechanism against sys-
      temic infections.
   b. IgY possesses the ability to mediate anaphylactic
      reactions.
   c. IgY is the predominant low molecular weight
      serum antibody not only in birds but also in repti-
      les, amphibians, and lungfish.
   d. IgY is the evolutionary ancestor of IgG and IgA.
   e. IgY is a skin-sensitizing antibody.

3. Which statement may contribute to the theory that
   birds might suffer from allergies?
   a. In birds, mast cells release cell products, including
      serotonin and histamine.
   b. After vaccination, birds collapse, which suggests an
      anaphylactic reaction.
   c. After mitogen induction, cutaneous delayed hyper-
      sensitivity reactions have been documented in
      poultry.
   d. Steroids, antihistamines, and dietary and environ-
      mental changes have helped some feather-picking
      pet birds.
   e. all of the above

4. Which substance seems to be the most reliable positive
   control for intradermal allergy testing in birds?
   a. histamine
   b. rabbit serum
   c. compound 48/80
   d. codeine phosphate
   e. anti–avian IgG

5. Which region was suggested to be used for intradermal
   allergy testing in psittacines?
   a. dorsal neck
   b. under the wings
   c. sternal region (proventer)
   d. head and tail
   e. dorsum

6. Fluorescein
   a. is a highly fluorescent compound synthesized from
      petroleum derivatives.
   b. absorbs blue light at wavelengths between 465 and
      490 nm.
   c. can be excited via the Wood’s light.
   d. is 80% bound to serum protein.
   e. all of the above

7. Fluorescein has been used for
   a. diagnosis of various vascular conditions in humans.
   b. diagnosis of various vascular conditions in domestic
      animals.
   c. diagnosis of ophthalmologic diseases in humans
      and domestic animals.
   d. angiography in raptors.
   e. all of the above

8. Recognized side effects of fluorescein in birds do not
   include
   a. vomiting.
   b. diarrhea.
   c. salivation.
   d. head shaking.
   e. somnolence.

9. Observed histopathologic patterns supporting hyper-
   sensitivity reactions in birds do not include
   a. angiocentric accumulation of lymphoplasmacytic
      cells.
   b. edema at injection sites of allergens.
   c. infiltration with heterophils and mononuclear cells.
   d. tissue eosinophilia and epidermal hyperplasia.
   e. multifocal perifollicular dermatitis.

10. _________ may aid in the diagnosis of allergies in
    birds.
    a. Therapeutic trials and elimination diets
    b. Intradermal allergy testing
    c. Alterations in the environment
    d. Histopathology
    e. all of the above