SCIENTIFIC SESSION PRESENTATIONS

SUBLINGUAL IMMUNOTHERAPY: A Physician's Perspective

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Allergen-specific immunotherapy (ASIT) has been used in human beings for over 100 years, subcutaneous immunotherapy (IT) is supported by well-controlled studies showing its effectiveness with both environmental and venom allergens.

Sublingual immunotherapy (SLIT) or "allergy drops" is a form of ASIT that historically has been favored in Europe more so than in North America. SLIT has been used in humans for over 50 years. A growing body of evidence and research supports the use of SLIT for human allergy. The World Allergy Organization endorses its use. [1]

In the United States, allergenic extracts for human injection are FDA-registered, and therefore SLIT represents "off-label" use. This is concerning to some physicians, and is a major factor that has limited the use of SLIT for human beings in the USA. In southern Europe 80% of ASIT is given by the sublingual route. In the US the use of SLIT is only about 6%[2]. In Europe many extracts and specific products, even including oral lozenge-type products, are registered for use in SLIT. Studies have been completed in the US for grass and ragweed pollen products. These single antigen products should be FDA approved and available in the near future.

SUBLINGUAL IMMUNOTHERAPY IN HUMANS

Review of Mechanism

Over the last several years, a great deal of understanding has been gained on the mechanisms involved in humans, specifically the sublingual route. Sublingual immunotherapy allows specific antigens placed under the tongue to induce immunologic tolerance. Multiple mechanisms are involved and include the production of anti-inflammatory cytokines such as IL-10 and the induction of regulatory T-cells.

The mucosal area under the tongue is a privileged immunologic site with unique characteristics. It consists of a physical barrier with integrated immunologic elements that allow the uptake of antigens while preventing the invasion by pathogens.[5] Local immune cells must constantly differentiate between harmless antigens and harmful pathogens and must tolerate a broad range of food antigens for normal function. There is a high concentration of dendritic cells and T-cells and a low concentration of mast cells, basophils and eosinophils. Dendritic cells present in the oral cavity appear to have unique functional properties as well as differences in cell surface markers compared to other dendritic cells, which may explain part of the difference in response between injection immunotherapy and SLIT.[3] Both immediate and delayed allergic responses at this site are muted, which contrasts with other mucosal surfaces. The oral cavity is a unique, immunologically active area which tolerates foreign antigens and thus is ideal for immunotherapy.

During sublingual immunotherapy, a small portion of the antigen is taken up by dendritic cells in the mucosa. IgE bound to high affinity receptors on dendritic cells facilitates antigen uptake and has the effect of concentrating the antigen 100–1000 fold in sensitized individuals. Dendritic cells partially mature and migrate to the basal lamina where the antigen is presented to T-cells, directly inducing an effector response. Dendritic cells also migrate to regional lymph nodes where they prime naive T-cells and induce regulatory T-cell formation. [4]

Regulatory T-cells modulate Th1 and Th2 responses directly and indirectly through cell-cell contact and by cytokines including IL-10 and TGF-β. Very early effects of immunotherapy are related to mast cell and basophil desensitization.[5] Non-specific effects of sublingual immunotherapy may be seen within the first 4 weeks of treatment and are mediated by IL-10 produced by dendritic cells and regulatory T-cells. An important observation from Marogna's study on multiple- versus single-antigen sublingual

immunotherapy was that suppression by IL-10 was non-specific; treating a major allergen sensitivity also resulted in some symptomatic benefit even to allergens that were not included in the treatment mixture. [6]

IgG4 induced by immunotherapy is thought to act as blocking antibody to antigens and is associated with immunologic tolerance.[7] Secretory IgA may play a crucial role in the immunologic benefits of sublingual immunotherapy at lower doses where changes in IgG4 and IgE are not seen. [8, 9]

A decrease in end-organ sensitivity, such as to bronchial and nasal provocation, is seen at all ranges of sublingual immunotherapy doses. As treatment progresses, increases in IgA and IgG4 induced by immunotherapy may require an increase in antigen dosing. IgE may be transiently increased during the early phase of sublingual immunotherapy, before the IgE levels eventually decline. With higher doses of immunotherapy, clonal deletion and anergy among reactive T-cells are seen.[10]

Allergen-specific immunotherapy has been shown to sustain disease-modifying effects even after discontinuation of active treatment. IgG4 develops throughout the course of treatment and has shown persistence for an additional year after SLIT (as well as SCIT) has been stopped. IgG antibodies appear protective with ability to block IgE.[11,12]

Review of Evidence for Efficacy

Efficacy of sublingual immunotherapy depends on antigen choice, frequency, and dose, as a variety of studies over the past several decades indicate. Since 1999, more than 80 double-blind, placebo-controlled studies on SLIT were published in peer-reviewed journals, though most were European. The studies showed SLIT to be safe and effective for adults and children, indicated that SLIT reduced asthma symptoms, sometimes prevented asthma from developing, and showed lasting benefit after treatment was stopped. In general studies have shown an efficacy similar to SLIT with less serious adverse reactions. In a 1998 position paper, the World Health Organization endorsed SLIT as a "viable alternative to injection therapy." The Allergy Rhinitis and its Impact on Asthma Guidelines (ARIA) endorsement of SLIT followed in 2001.

In 2003, a Cochrane Report panel of experts reviewed 22 "grade A" clinical trials on SLIT involving 979 patients. The experts reviewed six SLIT studies on dust mites, five on grass pollen, five on *Parietaria* (a common European pollen), two on olive pollen and one each on ragweed, cat dander, tree pollen, and cypress pollen. Each study was double-blind and placebo-controlled. Cochrane's conclusion: For these allergies, SLIT significantly reduced symptoms and need for symptom-relieving medication. Across all trials, SLIT reduced symptoms by 42 percent and reduced medication need by 43 percent. No adverse reactions occurred. SLIT's benefits persisted for at least three years after treatment stopped.

An update to the Cochrane review was completed in 2011. This confirmed the efficacy and safety of SLIT.[13]

Favorable research continues today, with ARIA noting in 2007 that there is more research being done on SLIT than there is on SCIT, and that SLIT studies are higher quality as defined by WHO study design guidelines (see table below). A full bibliography can be found at www.allergychoices.com/bibliography.

Studies on SLIT in human allergic disease have focused mostly on allergic rhinitis and asthma, and less on atopic dermatitis (eczema) – perhaps because historically there has been a feeling among physician allergists that immunotherapy in general is not as useful for atopic skin disease. However, several recent studies have re-examined the possibilities for SLIT in human atopic dermatitis and have concluded that it can be clearly effective.[14,15,16]

Safety

In humans SLIT is considered to have a better safety profile than SCIT. With SLIT most reactions are local and transient and do not require dose modification or cessation of treatment. More than 1 million doses have been administered sublingually in clinical trials. It is estimated that 1billion doses have been administered worldwide since 2000. There have been 11 published case reports of anaphylaxis with no fatalities. This equates to 1 case of anaphylaxis per 100,000 doses sublingually.

In contrast, the American Academy of Allergy Asthma and Immunology in a survey from 1990-2001 fatalities occurred at a rate of 1 in 2-2.5 million injections. This was confirmed in 2008. There were 10.2 systemic reactions per 10,000 injections. 3% of those were classified as "life threatening anaphylaxis". This corresponds to 1 case of anaphylaxis per 33,000 injections[17].

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NOTES

SUBLINGUAL IMMUNOTHERAPY IN VETERINARY DERMATOLOGY

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Allergen-specific immunotherapy (ASIT) is a treatment for atopic dermatitis (AD) in dogs and cats, wherein extracts of allergenic substances to which the patient is sensitive are administered, in gradually increasing amounts, to lessen the hypersensitivity state. It is the only proven treatment for allergies that actually works by reversing the underlying immunopathogenesis of the disease, instead of merely covering up clinical signs with anti-inflammatory therapies. ASIT has a strong advantage of being nearly free of serious adverse effects in the great majority of dogs and cats, even with prolonged use, and can produce substantial, long-lasting relief in many patients. In human beings, ASIT is recommended as early as possible in the course of the disease, as it may prevent development of additional sensitivities and prevent progression of disease severity. These factors have not been studied in animals, but nevertheless, the potential lifelong benefits of ASIT make it a preferred treatment for AD that should be discussed with owners earlier rather than later in the management process.

Sublingual immunotherapy (SLIT) involves administration of allergen extract into the oral cavity, under the tongue, as opposed to by injection. It is commonly used for human allergy in Europe, particularly for atopic rhinitis and asthma. Historically, there are conflicting reports of efficacy, which may be explained in part by the extreme variation in protocols used for dosing, administration, intervals, vehicle, etc. in the different studies reported. Consideration of recent evidence has led authoritative bodies to conclude that, when used correctly, it is clearly efficacious and in fact has a response rate similar to subcutaneous ASIT. Its use in animals is very new, and it has only recently become widely available for veterinary use.

HOW DOES SLIT DIFFER FROM INJECTION ASIT?

There are many similarities between SLIT, as currently used for pets, and allergy shots. As with injections, SLIT formulations are typically supplied in 3 bottles of increasing concentration, and the cost of SLIT vs. shots is approximately the same. Concurrent medications do not appear to interfere with efficacy, and are typically used during the initial few weeks or months of treatment while waiting for SLIT to become effective. The mechanism(s) by which SLIT works are somewhat different than allergy shots, implying that they may be more or less effective than injections for a given patient. Major practical differences include the specific ingredients: while shots typically consist of phenol-saline based extracts, many SLIT formulations use glycerin-based extracts prepared in special vehicles which purport to stabilize the allergens and/or facilitate uptake through the oral mucosa. Stabilizers included in some formulations allow concurrent mixing-in of mold allergens, and room temperature storage. The other major difference is administration frequency: SLIT formulations are typically administered every day, often several times per day, for the duration of therapy with no tapering.

EXPERIENCE IN ANIMALS:

Evidence for efficacy: Studies on SLIT and other non-injection methods of ASIT for use in pets are only just being reported. One study in an experimental model of canine AD failed to show evidence for efficacy of orally-administered allergen in laboratory beagles experimentally sensitized to dust mite; however in this study the allergen was fed to the dog rather than applied to the mucosa.[1] Another small open trial of atopic canine clinical patients with dust mite allergy treated with SLIT reported clinical benefit in 80% of dogs, and that clinical benefit was usually accompanied by measurable immunologic changes, including significant increases in allergen-specific IgG and decreases in allergen-specific IgE.[2-3] Marsella et al.[4] reported some efficacy of SLIT in a laboratory model using sensitized beagle dogs, including significant changes in cytokines such as TGF-beta and IL-10 in treated animals. Finally, a multicenter, uncontrolled open trial of 217 dogs, reported preliminarily by the author, indicated

approximately 60% response to SLIT therapy, including approximately 50% of "injection failure" dogs responding.[5]

There are many reasons why discrepant results have been reported with non-injection ASIT methods, but central to them may be the same principle that has plagued SLIT research in human beings for decades: different studies use widely differing protocols for dosing, frequency, treatment set, vehicle and preparation, etc. These protocol differences are the most obvious explanation for differing results; it makes empirical sense that variations in dosing and formulation of the treatment sets may impact effectiveness.

In the author's clinical experience with atopic dogs, treated by many veterinary dermatologists in varying geographic areas of the USA since 2009, approximately 60% of dogs with AD that have not had prior immunotherapy attempts will have substantial improvement of their clinical signs with this formulation. The response rate for dogs that HAVE had prior immunotherapy failure is also substantial – about 50% of dogs that are "shot failures" due to lack of efficacy, difficulty with administration, or anaphylactic reactions can be successfully treated with SLIT. It's especially encouraging that we've seen dogs that completely failed "allergy shots" often respond very well to SLIT. This is consistent with experimental evidence that shows that the mechanism of SLIT is somewhat different than that of injection immunotherapy. SLIT is not just a different route of administration to produce the same effect, it's actually in some ways a different treatment altogether.

Advantages and Disadvantages of SLIT: One big advantage of SLIT is in ease of administration. We've found that though many owners "don't mind" giving injections to their pets, most owners clearly don't relish it, and are delighted to be presented with an alternative to giving injections. Most dogs accept administration easily, even viewing it as a treat, which increases compliance. On the other hand, successful SLIT requires faithful twice-daily administration, and owners with busy travel schedules may find it much more convenient to give an infrequent injection. "Head-shy" dogs may also resist treatment.

In human beings, anaphylactic reactions to SLIT are rare to nonexistent, and SLIT can be used in humans with a prior history of reaction to allergy shots. In our experience, the same is true for dogs; we've treated numerous patients with SLIT who have had anaphylactic reactions to allergy shots.

Additionally, with many SLIT formulations, you can include mold extracts with pollens in the same vial without fear of losing efficacy of non-mold allergens, and SLIT treatment bottles can be stored at room temperature for a shelf-life of 6 months; refrigeration is not necessary.

PROTOCOLS FOR SUBLINGUAL IMMUNOTHERAPY IN DOGS:

Workup and Testing: In summary, do what you have always done! Initial diagnosis of AD and workup to eliminate secondary infections, parasitism, and a food component of the disease is no different than in any other atopic dog. Dogs should be evaluated for different sensitivities in exactly the same manner that the individual clinician is comfortable and familiar with for treatment using injection ASIT. Following establishment of a firm clinical diagnosis of AD, any combination of serologic or intradermal testing techniques may be used to establish the individual sensitivities of each patient.

Allergen Selection and Formulation: Following careful testing, again, principles for choosing the allergens in the prescription are exactly the same as those employed for choice of allergens for injection ASIT mixtures, and are completely familiar to every veterinary dermatologist, including:

- History of exposure of the patient to the allergen in question
- Cross-reactivity of allergens, including consideration of botanical groups of related weed, tree, or grass pollens
- Empirical observations on the significance of a particular allergen in relation to others, such as may be suggested by the "score" of serologic or intradermal tests

A few considerations that may be unique to formulating a SLIT prescription include the following:

- SLIT prescriptions in human beings tend to follow a "less is more" principle. There is much greater use of "mixes" of related allergens rather than combining many different individual extracts that are antigenically-related, and use of fewer allergens in the mix rather than a greater

number. Consider limiting the number of allergens in your prescription to a maximum of the 10-12 you believe are most important for the patient. Remember, there is substantial documentation in other species that part of the mechanism of SLIT is allergen-specific, *and part is nonspecific*.

- Generally, on the prescription just indicate a list of the relevant allergens; the correct dose of these allergens will be included in the final prescription. Though this may vary my manufacturer, typically the prescriptions do not "double-up" on a particular allergen that is felt to be more "important" than others; all treatment sets are usually prepared with a uniform and standard dose of relevant allergen.
- If you believe molds or fungi (including *Malassezia*) are important allergens for a patient, ask the supplier if they may be included in the mixture – with some formulations, they can be and there is no need to give them by separate administration.

Dosing: As with many injection ASIT protocols, dosing is done with a set of three bottles of gradually increasing concentration. *If the patient has a history of prior anaphylactic reaction* to allergy shots, to be cautious we advise starting at an even weaker treatment dilution.

Ideally, the allergen solution should remain in contact with the oral mucosa for as long as possible. Humans are instructed to hold the solution under the tongue for 1 minute before swallowing. Obviously, we cannot request the same of our canine patients, but it is important that the solution is dispensed into the oral cavity, *not in food*, and that the pet refrain from eating or drinking for a short period after the dose is given.

A key difference with SLIT is this basic principle of treatment: *the allergen must be dosed regularly and frequently*. Multiple daily administrations are required for efficacy in human beings, and we strongly recommend that owners be counseled to administer the "allergy drops" TWICE DAILY, EVERY DAY. If they forget to give a dose in the morning, give one in the afternoon and one before bed.

This twice-daily dosing schedule is indefinite for the duration of therapy. The schedule does not "taper" to once daily, every other day, etc. Administration continues twice daily for the duration of treatment.

When switching from "shots to drops" there are three possible situations. If the patient has had no response to shots, we recommend starting SLIT with the lowest concentration vial and escalating from there, just like a dog that had never had ASIT at all. On the other hand, if the patient is stable and has been doing well on shots (owner is just tired of giving them!) one can typically start directly with the maintenance vial of SLIT, with no need for the escalation phase. Finally, if the patient is being switched to drops because of prior shot reactions, we recommend very cautious administration of the lowest concentration vial at first; if there seems to be any reaction or worsening, reduce the concentration even further.

At this time, the ideal total duration of treatment is not known in dogs. In human beings, multiple-times daily administration is continued for a period of 2-5 years. After this time, if the patient is stable, the treatment can be discontinued *and the effect appears to be permanent in nearly all cases*. Whether this is true for a canine patient is yet to be determined.

Follow-up Evaluations: As with injection immunotherapy, it is important to re-evaluate patients on SLIT on a regular basis, for example after 3, 6, and 12 months on treatment. Our subjective clinical impression is that response to SLIT often occurs quite rapidly - some dogs are improved at 3 months, and most who will respond show at least some improvement, if not substantial improvement, by 6 months.

Adverse Reactions: A few dogs may rub or scratch at their mouth after administration, perhaps analogous to the oral itch that some human SLIT patients experience. Almost always, this will disappear after the first few treatments. Likewise, occasional vomiting has been observed in a few dogs for the first few doses. In a few cases with very sensitive animals, we've seen worsening of clinical signs with SLIT administration – actually causing a flare of the disease. If any of these reactions occur or persist, it may require lowering the allergen dose. Contact the SLIT supplier for specific instructions as to how to accomplish this for their specific formulation.

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Shots or Drops? Considerations in Selection of Injection vs. Sublingual ASIT in Dogs

Client schedule and convenience factors. Some clients may find it easier to give an injection every 14 days or so than to consistently administer SLIT every day; others may find regular daily administration easier. Some may find it desirable that some SLIT formulations do not require refrigeration (injection formulations do).

Client aversion to needles. Some clients find injections very easy to give to their pets; others are fearful of needles and will be delighted to have the availability of oral SLIT administration.

Patient cooperation factors. Most pets tolerate injections at home quite well, though some may be extremely resistant. Most pets find SLIT formulations palatable and view administration as a 'treat,' though some head-shy animals may be difficult to medicate.

Importance of mold/fungal allergens. If molds are an important allergen, most suppliers recommend that fungal extracts for injection should not be mixed in with others, but rather given by separate injection. Fungal extracts can be included within the same vial for some SLIT formulations (i.e., those that include stabilizers in the vehicle), so separate administration is not necessary.

Anaphylactic reactions to injections. In humans, anaphylactic reactions to SLIT formulations are much less common than to injections. For pets, SLIT formulations can be safely used even in pets that have had reactions to injection ASIT; advise supplier of this history as they may recommend a modified administration schedule for such patients.

History of failure of injection ASIT. For pets that have experienced no clinical benefit from injection immunotherapy, SLIT is still a consideration. A substantial number of 'injection failure' dogs improve with SLIT.

NOTES

ALLERGEN IMMUNOTHERAPY FORMULATION: THE HUMAN PERSPECTIVES

Raweewan Hoontrakoon, M.D.

SPECIFIC ALLERGENS:

Immunotherapy is effective for pollen, animal danders, dust mite, and mold/fungi. The most recent NIH guidelines for asthma treatment in 2007 recommends immunotherapy for specific allergens as a treatment in persistent asthmatic patients.

1. Pollen: Pollen extracts have been shown to be safe and effective.[1] Although the vast majority of clinical immunotherapy trials have been with single allergens, mixed pollen extracts have shown clinical effectiveness in clinical studies.[2]
2. Mold/fungi: *Alternaria* and *Cladosporium* extracts have been shown to be effective in several studies.[3,4] The clinical challenge lies in the variability of the allergen content in most commercially available extracts.[5] More over, the options for commercially available mold extracts are limited.even for those that are dominant airborne allergens with significant clinical impact on allergic and asthmatic patients. Mold extracts contain proteolytic enzymes that could render other allergens ineffective. Thus, it is recommended that mold extracts be separated from other extracts that doe not have high proteolytic enzymes.[6]
3. Animal dander: Major allergen content of cat extracts is relatively low. Therefore, larger amounts are required to achieve an effective dosing.[7] Dog extracts contain even lower amount of major dog allergen. Using an extract containing AP extract from Hollister-Stier appeared to result in a significant dose response.[8]
4. Dust mites: *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* contain 2 major allergen groups that are immunologically cross-reactive: Der p1 and Der f1 and Der p2 and Der f 2. Therefore, only 50% of the projectd amounts of each of the 2 house dust mites needs to be included when preparing an allergen immunotherapy extracts.
5. Cockroach: Efficacy of cockroach extracts is suggested in one clinical study.[9] Only glycerinated extracts should be used.

MULTIPLE ALLERGEN IMMUNOTHERAPY:

Although immunotherapy using multiple allergens is a common practice, data on its efficacy has not been consistent.[10] Further research is needed. It is important to treat the patients with only relevant allergens.

BASIS OF ALLERGEN EXTRACT SELECTION:

The section of allergen extracts is based on a history that supports a clinical correlation between the allergen exposure and clinical symptoms, evidence of specific Ig-E antibodies against the allergens, and an understanding of potential aeroallergen exposures in the patient's environment based on local and regional aerobiology.

History:
1. Timing of symptoms
2. Patient's life style
3. Environment history

Evidence of specific Ig-E antibodies:
1. Skin testing utilizing standardized extracts remains the primary diagnostic tool. Based on nasal/bronchi challenge test results, skin tests have greater sensitivity than serum specific Ig-E

measurement.[11,12] A percutaneous test consistently produces reproducible results and is the recommended test for diagnosis of allergy to aeroallergens.
2. Serum specific Ig-E antibodies can be considered in patients with dermatographism.

Arobiology: The clinical relevance o fan aeroallergen depends on certain key properties:
1. Intrinsic allergenicity
2. Aerodynamic property
3. Quantities produced
4. Buoyancy
5. Prevalence of such plants

Information on regional and local aerobiology is available on various Web sites or through the National Allergy Bureau at http://www.aaaai.org/nab.

ALLERGEN EXTRACT SELECTION:

1. Nonstandardized extracts: Variable allergen contents and should not be considered equipotent.
2. Standardized extracts: include cat hair, cat pelt, *D pteronyssinus*, *D farinae*, short ragweed, Bermuda grass, Kentucky bluegrass, perennial rye grass, orchard grass, timothy grass, meadow fescue, red top, and sweet vernal grass.

PRINCIPLES OF MIXING ALLERGEN IMMUNOTHERAPY:

1. Cross-reactivity: Select a single allergen to represent the cross-reactive genus or subfamily.
   - 1.1. Tree:
     - 1.1.1. Cypress family (juniper, cedar, cypress) strong cross reactivity among these members.
     - 1.1.2. Betulaceae family (birch, alder, hazel, hornbeam, and hop hornbeam) strongly cross react with Fagaceae family (oak, beech, and chestnut)
     - 1.1.3. Ash strongly cross react with European olive tress
     - 1.1.4. Maple and Box elder should be considered separately.
   - 1.2. Grass: Temperate pasture grasses (subfamily Pooideae; fescue, rye, timothy, blue and orchard) strongly cross react. Other subfamilies such as Bermuda, Bahia, and Johnson should be considered separately.
   - 1.3. Weed:
     - 1.3.1. Sages are strongly cross react
     - 1.3.2. Chenopod and Amaranth strongly cross react. Russian thistle appears to have the most cross-allergenicity.
2. Proteolytic enzymes and mixing: Extracts with high proteolytic enzymes such as cockroach and mold/fungi could cause a significant loss of potency in extracts with lower proteolytic enzymes such as pollen, animal dander, and dust mites. Therefore, separation of extracts with higher proteolytic enzymes from other extracts is recommended. However, short ragweed appeared resistant to the effects of proteolytic enzymes.
3. Allergen extract expiration dates: Determining factors include
   - 3.1. Storage temperature: Extracts should be kept at 4 degree centigrade.
   - 3.2. Presence of stabilizers and bactericidal agents: Phenol can denature proteins. Addition of human albumin might protect extracts against this effect. At 50% concentration, glycerin may prevent loss of allergen potency although this must be weighed against discomfort concentrated glycerin may cause.
   - 3.3. Concentration: Highly concentrated extracts are more stable.
   - 3.4. Presence of proteolytic enzymes

4. Dose selection:

Probable effective dose range for standardized and nonstandardized US-licensed allergen extracts

Allergenic extract	Labeled potency of concentration	Probable effective dose range
Dust mites	3,000, 5,000, 10,000, and 30,000 AU/ml	500-2,000 AU
Cat hair	5,000 and 10,000 BAU/ml	1,000-4,000 BAU
Cat pelt	5,000-10,000 BAU/ml	1,000-4,000 BAU
Grass, standardized	1000,000 BAU/ml	1,000-4,000 BAU
Bermuda	10,000 BAU/ml	300-1,500 BAU
Short ragweed	1:10, 1:20 wt/vol, 100,000 AU/ml	6-12 □g of Amb a 1 or 1,000-4,000 AU
Nonstanderdized extract: AP dog	1:100 wt/vol	15 □g of Can f 1
Nonstandardized extract: dog	1:100 wt/vol	15 □g of Can f 1
Nonstandardized extract: pollen	1:10 to 1:40 wt/vol or 10,000-40,000 PNU/ml	0.5 ml of 1:100 or 1:200 wt/vol
Nonstandardized extracts: mold/fungi, cockroach	1:10 to 1:40 wt/vol or 10,000-40,000 PNU/ml	Highest tolerated dose

NONINJECTION ROUTES OF IMMUNOTHERAPY: Currently subcutaneous immunotherapy

1. Sublingual immunotherapy (SLIT): Several studies have demonstrated its efficacy in treating allergic rhinitis and allergic asthma.[13,14] However, the appropriate dose for SLIT and the relative efficacy of SLIT versus SCIT have not yet been established. [15] Of interest is the result from studies that demonstrated SLIT's efficacy in increasing tolerance to hazelnuts in allergic subjects, some of whom have had anaphylactic reactions. [16] Clinical trials with peanut, egg, and milk showed that SLIT could result in a clinical tolerance to these food allergens. [17-20]
2. Intranasal immunotherapy: Efficacy has been shown with dust mite and pollen extracts. Local adverse reactions limit its use in clinical practice.
3. Intralymphatic:
4. Epicutaneous: No significant difference in nasal provocation scores when compared to placebo.

NOVEL FORMULATIONS:

1. Adjuvants: Adjuvants may enhance the effectiveness of allergen immunotherapy by shifting the immune response toward Th1 production through their action on toll-like-receptors (TLRs). Linking TLR agonist to an allergen may improve the treatment's efficacy and reduce its side effects. Clinical efficacy from treatments utilizing adjuvants may depend on the type of TLR agonists. [21-23]

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NOTES

ALLERGEN IMMUNOTHERAPY MANAGEMENT: THE HUMAN PERSPECTIVES

Raweewan Hoontrakoon, M.D.

INTRODUCTION:

Immunotherapy has long been an integral part of allergy treatments in human. The history of immunotherapy dates back to 1911 when Dr. Noon and Dr. Freeman proposed grass pollen inoculation as therapy for hay fever. [1-2] Few treatments have stood such a test of time. The celebration for the 100th year anniversary of immunotherapy in 2011 reminded us not only of great scientific advances we have achieved in the past century but also of the need to further elucidate our understanding of the basis on which our immune system functions in order to continue our stride forward into the future.

IMMUNOLOGIC RESPONSES TO IMMUNOTHERAPY:

The immunologic response to immunotherapy is characterized by a decrease in the sensitivity of end organs and changes in the humoral and cellular responses to the administered allergens. [3-5] The initial immunologic changes include an increase in CD4+ CD25+ regulatory T lymphocytes secreting IL-10 and TGF-β.[6-7] These cells are associated with immunologic tolerance which is defined as a long-lived decrease in allergen-specific T-cell responsiveness. As the treatment progresses, deviation from TH2 to TH1 cytokine response to the allergens used in immunotherapy occurs. [8] Humoral response to SCIT begins with an initial rise in specific Ig-E levels which then gradually decreases. A rise in the levels of specific IgG1, IgG4, and IgA follows. However, these changes in antibody levels do not consistently correlate with clinical improvement or duration of efficacy of treatment. [9-10] However, functional alterations in allergen-specific IgG levels such as changes in avidity, affinity, or both for allergen might play a role in determining clinical efficacy.

EFFICACY OF IMMUNOTHERAPY:

Immunotherapy is effective for the treatment of allergic rhinitis, allergic asthma, and stinging insect hypersensitivity. [11-13] With proper patient selection and treatment formulation and management, immunotherapy yields a significant efficacy. Outcome measures used to assess the efficacy of immunotherapy include quality of life (symptom and medication scores, organ challenge) and immunologic changes. In one systemic review of 88 trials involving 3,459 asthmatic patients, subcutaneous immunotherapy (SCIT) resulted in significant reductions in asthma symptoms, medication use, and improvement in bronchial hyper-responsiveness. This meta-analysis determined that it would have been necessary to treat 3 patients with SCIT to avoid 1 deterioration in asthma symptoms and 4 patients with SCIT to avoid 1 patient requiring increased medications. These meta-analyses strongly support the efficacy of allergen immunotherapy. In addition to treating clinical symptoms of allergy and/or asthma, it has been suggested that immunotherapy may also prevent the development of new allergen sensitivities in mono-sensitized patients.

PATIENT SELECTION: patients who demonstrate evidence of specific IgE antibodies to clinically relevant allergens

<u>Indication</u>:

1. Patient's preference/acceptability
2. Adherence
3. Medication requirements
4. Response to avoidance measures

5. Adverse effects of medications
6. Co-existing allergic rhinitis and asthma
7. Possible prevention of asthma in patients with allergic rhinitis

Potential indication:

1. Atopic dermatitis if associated with aeroallergen sensitivity

Conditions for which immunotherapy is investigational:

1. Food hypersensitivity

Conditions for which immunotherapy is NOT indicated:

1. Urticaria and angioedema

SPECIAL CONSIDERATIONS IN IMMUNOTHERAPY:

1. Pediatric patients: Immunotherapy is an effective treatment for children. Indications for immunotherapy in pediatric populations are similar to those for adults. More over, immunotherapy used to treat allergic rhinitis in children may offer protection against development of asthma. [14] Generally, immunotherapy is considered for children who are 5 years old or older. Although there is no universal consensus on the lower age limit for initiation of immunotherapy, considerations need to be given to (1) the child's ability to effectively communicate his/her symptoms that may suggest systemic reactions and (2) emotional trauma that may result from injections.
2. Geriatric patients: Significant co-morbid medical conditions such as hypertension, cardiovascular disease, and/or cerebrovascular disease may increase the risk for adverse outcomes from immunotherapy in this population. However, this treatment can provide significant benefits and each case should be considered individually.
3. Pregnancy: Immunotherapy can be safely continued in pregnant women, however, the treatment should not be initiated in during pregnancy.

SAFETY OF IMMUNOTHERAPY:

1. Local reactions: Local reactions are common side effects from SCIT. Individual local reactions do not predict subsequent reactions. However, patients with recurrent large local reactions (defined as wheal size of 25 mm or greater) may be at an increased risk for future systemic reactions. [15] Glycerin concentrations of up to 50% are not associated with significantly higher local reaction rates although higher glycerin concentrations are associated with injection pain. Antihistamines are effective in reducing local reactions during cluster and rush protocols and leukotriene antagonists are effective in a rush protocol. [16-17] Their efficacy for conventional dosing has not yet been extensively studied.
2. Systemic reactions: The risk of systemic reactions per injection with conventional schedule is approximately 0.2%. [18] The estimated fatality rate was 1 per 2.5 million injections. [19]
   2.1. Risk factors: for systemic reactions include poorly controlled asthma, injections during periods of exacerbation of symptoms, high degree of hypersensitivity, use of β-blockers, injections from new vials, and dosing errors. Pre-injection health screening can minimize some of these risk factors.
   2.2. Timing: Almost all severe systemic reactions began within 30 minutes after an injection. Patients should remain in the physician's office for at least 30 minutes after receiving an injection. Delayed systemic reactions, defined as reactions occurring after 30 minutes of receiving injections, can occur but usually are not severe. Biphasic anaphylactic reactions which are defined as complete resolution of the initial reaction with recurrence at 2-14 hours, were reported in up to 23% of patients who experienced a systemic reactions. Women and patients who require more than 1 dose of epinephrine during the initial reaction appear to be at risk for biphasic reactions. Counseling on the possibility of immediate and delayed systemic reactions and an action plan for such an event is recommended prior to initiation of immunotherapy.
   2.3. β-Blockers and ACE inhibitors: β- Blockers do not appear to increase the frequency of systemic reactions from inhalant immunotherapy and venom immunotherapy (VIT), however, their effect

on increasing the severity of systemic reactions and/or rendering the reactions refractory to treatments cannot yet be determined. ACE inhibitors have been associated with greater risk for more severe reactions from VIT. ACE inhibitors discontinuation should be considered for patients receiving VIT. No evidence exists that angiotensin receptor blockers are associated with greater risk for anaphylaxis from immunotherapy.

2.4. Treatment: Adequate equipment and medications should be immediately available to treat systemic reactions. See "The diagnosis and management of anaphylaxis practice parameter: 2010 update." [20] Intramuscular Epinephrine is the first-line treatment. There is no contraindication to epinephrine in patients with anaphylaxis.

SCHEDULES, DOSING, FOLLOW UP CARE, AND DURATION OF TREATMENT:

1. Schedules: There are different protocols for building up doses of immunotherapy. Once a patient reaches a maintenance dose, the interval between injetions often can be progressively increased, as tolerated, up to 4 weeks for inhalant allergens and up to 8 weeks for venom.
   1.1. Conventional: 1-3 injections per week
   1.2. Cluster: 2 or more injections per visit on non-consecutive days
   1.3. Rush: More rapid than cluster.
2. Dosing: It is recommended that the dose be reduced after a systemic reaction. Once the patient tolerates a reduced dose, a cautious increase can be attempted. Lowering the maintenance doses may also be warranted. Dosing modifications when injections are given during the increased exposures to inhalant allergens may also be considered for patients with high degree of sensitivity. Although there is no evidence-based guidelines on dose adjustments for missed immunotherapy doses, it is customary to repeat or even reduce the dose when a substantial time interval between injections occurs.
3. Follow Up Care and Duration of treatment: Clinical improvement can be demonstrated very shortly after the patient reaches a maintenance dose. Patients should be evaluated at least every 6-12 months while receiving immunotherapy. If clinical improvement is not seen after 1 year of maintenance therapy, discontinuation of immunotherapy should be considered. At present, there are no specific tests or clinical markers that will distinguish between patients who will relapse and those who will remain in long-term clinical remission after discontinuing effective inhalant immunotherapy. Studies suggest that a 3-to-5-year treatment duration may be sufficient.

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NOTES

COMPARATIVE OTOLOGY

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HISTORY OF OTOLARYNGOLOGY-HEAD & NECK SURGERY

In the early 20th century Otolaryngology and Ophthalmology were a combined specialty and society. In 1908 the first specialty board was proposed by the President of the American Academy of Ophthalmology and Otolaryngology, but it wasn't until 1917 the Board of Ophthalmology actually happened. This was followed in 1924 by the Board of Otolaryngology. Separate training programs became more common in the 20's and 30's and after World War II the EENT programs disappeared. In the early 40's as antibiotics became available, Otolaryngology was considered a dying specialty as most of the conditions they treated were for infectious problems and their complications. This included things like acute otitis media, mastoiditis, acute sinusitis with its complication, recurrent tonsillitis, laryngitis, epiglottitis, diphtheria etc. Interest in the specialty and resident trainee applications dropped off in the late 40's and 50's, but out of the ashes a new vigorous specialty developed. It started with the introduction of the microscope for ear surgery in the early 40's. Head and neck cancers were usually diagnosed by Otolaryngologists, but if major surgery was required they were referred to a general surgeon in most communities. In the 50's several Otolaryngology centers started to do their own major Head & Neck surgery and by the 70's and 80's the majority of Head & Neck surgery in the US was done by Otolaryngologists. In the 90's and early 21st century this expanded to include thyroid and parathyroid surgery. Rhinoplasties and otoplasties were always part of Otolaryngology, but in the 60's and 70's a new subspecialty was born as several Otolaryngologists moved into doing other cosmetic procedures like face lifts, blepharoplasties etc. and then began to teach others in the field. The specialty developed further subspecialties in allergy/ immunology, rhinology, laryngology and pediatric otolaryngology, so that today it is one of the most sought after residencies.

As I look at Veterinary Medicine and see how it has changed in the 50+ years since I graduated, I see the development of specialties, but otolaryngology is not one of them. The question I asked myself is why and is it a viable specialty for Veterinary Medicine? Certainly I cannot answer this, but I did do a little thinking about it. In human otolaryngology it is estimated that 40% of the patients seen by primary care specialties are for disease of the head and neck. For pediatricians, ear infections, colds, sore throats etc. are their bread and butter especially in the winter, but I don't see the same spectrum of disease in animals (1). Companion animals don't smoke and drink, so there goes one large segment that human Otolaryngology treats. They aren't vain, except maybe their owners are, so except for otoplasties another large segment disappears. I think if the specialty was to be viable it would have to start with those of you in this room who are interested in ear disease, but it would require that you include surgical procedures and develop surgical treatments for some of the things you now treat medically which could be improved with surgery. You would also need to evaluate what are the diseases of the nose, throat, upper respiratory tract, esophagus and some neurological problems in all fields of Veterinary Medicine which might be better treated by specialists in the area. I hope you will think about this and I will look forward to hearing of the American Academy of Veterinary Otolaryngology-Head & Neck Surgery in the future. Maybe you will let me be an associate member.

ANATOMY OF THE HUMAN EAR (2,3)

The auricle is made of elastic cartilage and is relatively stiff and immobile. The shape of the auricle and concha help collect and focus sound waves to the tympanic membrane(TM). It tapers medial to form the cartilaginous external ear canal and becomes continuous with the bony canal. The human cartilaginous canal is lined with epithelium and subcutaneous tissue containing hair, cerumen glands and sebaceous glands. In the bony canal the subcutaneous tissue is thin and closely attached to the periosteum with a thin epithelium which is easily traumatized. Because of the tightly attached external canal lining and its abundant neural supply, touching the canal with instruments is quite painful.

The ear is contained in the temporal bone. We divide the middle ear into the epi-, meso- and hypotympanum. The division is made by the TM. Below the most inferior part of the TM is the

hypotympanum, which does not have any vital structures, but in the posterior floor is the jugularbulb and anterior-medial the carotid artery. Medial to the TM is the mesotympanum which contains the malleus handle, long process of the incus, stapes and in the medial wall the tympanic plexus, round window niche, promontory, oval window and the horizontal portion of the facial nerve. Anterior superior the Eustachian tube (auditory tube) joins the middle ear with the nasopharynx. It is about 1/3 bone and 2/3 cartilage. In the epitympanum are the malleus head and body of the incus. Posterior the aditus is a connection between the mastoid antrum and epitympanum. The middle ear and Eustachian tube are lined with a thin respiratory type of epithelium, some ciliated and some cuboidal.

The TM laterally is divided into 4 quadrants based on the malleus handle. A vertical line down the handle creates anterior and posterior segments. A horizontal line though the umbo at right angles to the vertical line creates the superior and inferior segments. This creates an anterior- superior and anterior-inferior quadrant and a posterior-superior and posterior-inferior quadrant. The TM itself is divided into the pars tensa and the pars flaccida. The former has circular and radial connective tissue fibers which give it its tensile strength. These fibers condense around the circumference of the TM to form the annular ligament, which fits into the tympanic (annular) sulcus like an O-ring. Superior is the pars flaccida which lacks the connective tissue layer of the pars tensa. This will allow negative middle ear pressure to produce retraction pockets and can lead to cholesteatomas. The outer surface of the TM is covered by squamous epithelium and the medial surface is covered by mucosa.

The inner ear contains the cochlea, vestibule with the semicircular canals and the internal auditory canal. These provide the hearing and balance mechanism of the ear which we will not discuss in detail today.

The mastoid connects with the middle ear via the aditus. It is made up mainly of a large air cell called the antrum. The rest of the mastoid is made of several collections of smaller air cells. The facial nerve passes from its horizontal portion in the middle ear to a vertical portion that passes through the anterior mastoid to exit the temporal bone by the stylomastoid foramen.

MAJOR ANATOMICAL DIFFERENCES

The human external ear canal is made up of about 1/3 cartilage and 2/3 bone. In looking at the skull of a dog it looks like the bonycanalisrelatively short. The cartilage has a vertical component and a horizontal component which makes visualizationof the TM in dogs difficult. The longer bony canal in humans is important for surgical approaches to the middle ear.

The major difference in the middle ear is a large bulla in dogs, cats and some laboratory animals, which is equivalent to the hypotympanum in humans. In humans there is a relatively large mastoid, which is diminutive in the animals mentioned. This changes the spectrum of disease and potential surgery. As an example the facial nerve exits superior and superficial in animals where in humans it exits the temporal bone inferior and medial where it is protected by the mastoid tip. It would appear that the ossiclular shapes vary from animal to animal.

In otology a detailed knowledge of the anatomy is the key to successful otological surgery. The facial nerve is the key to all of the surgical procedures we can do. The second most important are the ossicles which we can remove and reconstruct with autologous or synthetic materials. The TM is also important, but is the easiest for us to successfully reconstruct using temporalis fascia or perichondrium.

The sensory nerve supply to the auricle and external canal comes from branches of the greater auricular nerve, lessor occipital nerve and auriculo-temporal branches of the V nerve. There is one small branch from the VII nerve to the external canal. Why is this important? Because of the nerves we can block the sensation to the auricle by doing a ring block around the auricle. We can also block sensation to the external canal by injecting around the cartilaginous canal and down to the bony canal. With this, surgery can be done on the auricle or we can lift a flap in the bony canal, lift the annulus out of the tympanic sulcus and do surgery in the middle ear or on the ossicles totally under local anesthesia.

Physiology of the Human Ear (2,3)

The skin of the external canal is unique compared to the rest of the body as it migrates rather than flaking off. If it were the latter the canal would fill with squamous debris. The epithelium starts growing from an area on the anterior-inferior surface of the TM. It migrates in all directions as I like to explain, similar to a snake shedding its skin. This migration take place at about 0.05 mm/day and as it migrates

carries cerumen and debris to the opening where it will come out on its own if a person does not push it back with a Q-tip. Cerumen has a high lipid content and is hydrophobic which is considered to be part of the protective mechanism of the external canal. There are also lysozymes and immunoglobulin's produced, along with a slightly acidic pH which helps suppresses the growth of bacteria, especially Pseudomonas. When the cerumen production is reduced or the canal is constantly traumatized with things like Q-tips or fingers, this protective mechanism is lost and the patient is more susceptible to infections. The character of cerumen is controlled genetically. Caucasians tend to have 85% soft cerumen and 15% dry cerumen. In my experience Asians tend to be the opposite with mostly dry flaky cerumen.

Middle ear health is dependent on a functioning Eustachian tube (ET). When this is not functioning, oxygen is absorbed from the middle ear, creating a negative pressure. The TM is retracted and if the situation is not corrected middle ear fluid will develop. The chronic stretching of the retracted TM will result in loss of some of the middle connective tissue layer making it lose tensile strength and can lead to retraction pockets and cholesteatoma. This is seen especially in children with cleft palates. Ligating the ET in animals has been shown to produce the same result.

The mastoidantrum and air cellsare felt to act as a pressure regulating mechanism for the middle ear. In children with chronic recurrent otitis media, especially with a persistent effusion the mastoid does not develop normally and the child will end up with what we call a sclerotic mastoid with a small antrum. These children will many times end up with lifelong ET dysfunction and chronic ear disease. Other things like allergies can also cause ET obstruction. The question I have is does the bulla serve a similar function in animals?

We do not have time to go into the physiology of hearing and vestibular function which would take up another whole session.

EXTERNAL OTITIS

In the next session we will discuss in more detail comparative external canal diseases.

Comparison of Middle Ear Disease (2, 3)

As previously mentioned the ET is the key to middle ear disease. In children from birth to about 5 years of age, acute otitis media is a common problem. About 2/3 are related to bacterial infections and the rest are assumed to be viral. The most common organisms are Strep. pneumoniae, Hemophilus sp., Moraxella sp. and a variety of viruses. The symptoms usually have a sudden onset with fever, severe pain and sometimes otorrhea. On exam the TM will be erythematous and bulging. Treatment is usually with antibiotics, although now there is a movement to wait 24-48 hours assuming a viral infection will start to improve by that time. I am not sure you see this in animals although in lab animals we can certainly induce suppurative otitis media (1).

Over the past 50+ years we are seeing more chronic otitis media with effusion. The effusion may vary from a watery transudate to a thick tenacious fluid we sometimes refer to as a glue ear. It is felt that the treatment of acute otitis media with antibiotics may be responsible for this since it usually prevents a rupture of the TM. The main problem with persistent fluid is hearing loss, which in young children will delay speech development and permanent damage to the TM as mentioned earlier. If the fluid does not clear in 3-6 months we will usually recommend a myringotomy to remove the fluid and place a ventilation tube in the TM.

One complication of chronic otitis media is the development of cholesteatomas. There are a few which are congenital where there is a nidus of epithelium in the middle ear which gradually forms a cyst of keratin which will need to be removed surgically. Most cholesteatomas are acquired and due to chronic otitis media with the development of retraction pockets especially in the pars flaccida or posterior quadrants. The pockets enlarge with desquamationof the epithelium and trapping of keratin debris with repeated infections. They can erode bone, extend into the mastoid, destroy ossicles and create a chronic draining ear. Treatment will require surgery which is usually some type of mastoidectomy with reconstruction of the TM and/or missing ossicles. One question I have is do you see a similar pathology occur in the bulla of dogs and cats?

If cholesteatomas are not treated and are frequently infected, the can cause complications. These are usually infections of surrounding areas including a sigmoid sinus thrombosis, meningitis and brain,

subdural or epidural abscesses. A fulminant untreated acute otitis media can result in a mastoiditis or sigmoid sinus thrombosis. All of these conditions will usually require high dose intravenous antibiotics and surgical treatment.

Inner Ear Disease

Diseases of the inner ear are associated with hearing loss and vertigo. There are a large number of different pathologies which are beyond the scope of this talk.

Otological Surgical Procedures (4)

Surgical procedures on the external ear canal are usually associated with middle ear surgical procedures. When we do a mastoidectomy, we frequently need to do a meatoplasty to enlarge the cartilaginous part of the ear canal for good visualization of the TM and cleaning of the canal and/or mastoid cavity. It reminds me a little of a procedure I remember being done on dogs to open the vertical portion of a dog's ear canal when they had chronic infections. All I really remember was that it wasconsidered a difficult procedure and was something I never did. We usually remove some of the conchal cartilage, but preserve the skin so we cover the exposed cartilage by creating flaps.

Another procedure which is sometimes done for a chronic stenosing external canal is to remove all of the canal skin, enlarge the bony canal with a drill and replace the skin with a split thickness skin graft (STSG). This does not make sense to me as you replace the migrating skin of an ear canal with skin that flakes off, so the ear needs to be cleaned frequently. I prefer to treat these patients medically.

Finally we will occasionally see a totally atretic ear canal with or without auricular deformities, most commonly unilateral, but occasionally bilateral. It is a congenital malformation of the first and second branchial arch. Surgical treatment is for cosmesis if there is a deformed auricle and the creation of a new ear canal for hearing. The latter is a technically difficult procedure with limited success as often there are also ossiclular and middle ear abnormalities. This surgery was recommended mainly in bilateral cases since a person can do reasonably well with one normal hearing ear. Today most people prefer what is called a BAHA procedure. This is a bone anchored hearing aid which gives much better hearing with less risk to the facial nerve and no ear canal to keep clean or get infected.

Operations on the tympanic membrane are mainly a myringotomy with ventilation tube and tympanoplasties (reconstruction of a new TM). The former is the most common surgical procedure performed in children which we discussed earlier. Basically a small myringotomy incision is made in the anterior inferior quadrant of the TM and the middle ear effusion removed with suction. A ventilation tube is placed through the TM which has some type of flange on the inner side to hold it in the middle ear. The tube is not to allow the fluid to drain, but to bypass the ET and allow air directly into the middle ear giving the mucosa time to normalize. Depending on the style of ventilation tube and the individual, the tubes will gradually migrate out of the TM which then heals spontaneously. About 5 % have to be removed if they stay in longer than 2-3 years and maybe 2-3% will leave a persistent perforation, which can later be repaired with a tympanoplasty.

Tympanoplasties are to repair a TM perforation. There are a variety of techniques, but probably the most frequent one done today is what is called the underlay technique. Basically the rim of the perforation is debrided to break the mucosal-epithelial junction, which occurred when the perforation healed. Next a fascial graft is taken from the lateral connective tissue covering the temporalis muscle. A 180 degree flap is made on the posterior canal wall, one cm from the annulus. The flap is elevated to the tympanic sulcus and then the annular ligament is carefully elevated out of the sulcus which then allows the whole posterior half of the TM to be rotated forward as far as the malleus handle. In some cases we may even elevate the TM off the handle of the malleus for more exposure anterior. The middle ear is then filled with gelfoam, an absorbable sponge like gelatin, to the level of the TM. The fascia graft is then trimmed to cover the perforation by 2-4 mm with a tail which comes up the posterior canal for 5-6 mm. This is laid under the TM and over the gelfoam, bringing the tail up onto the posterior canal. The TM is then laid back into position with the canal skin covering the fascia tail. The canal is then packed with gelfoam. The annular ligament will migrate back into the tympanic sulcus as healing takes place. The graft is incorporated into the fibrous layer of the TM and acts as a scaffold for the epithelium to migrate across the perforation. The overall success rate is about 95%. Several variations are used in different situations, depending on the size of the perforation, whether a mastoidectomy has been done etc.

Mastoidectomies are done for several reasons, but most are done for cholesteatoma. Again there are a variety of techniques with the main difference being whether the posteriorcanalwall is left intact or removed. If the canal wall is left intact the mastoid cells are removed with a drill and the cholesteatoma is removed. One can also reconstruct the ossiclular chain with this technique. Post operatively the canal and TM will look normal. The problem is that if there is a recurrence of the cholesteatoma in the mesotympanum or mastoid, it cannot be visualized until it is quite large and potentially has caused more problems. If it is removed then the patient is left with a skin lined cavity that will require regular cleaning the rest of their life. There are two different types when the posteriorcanal is removed. The most common is a modified radical mastoidectomy where the TM is reconstructed, but the incus and head of the malleus are removed. Ideally the new TM will attach to the stapes head which will give good hearing. If a radial mastoidectomy is done all of the ossicles except the stapes are removed, the TM is removed , an attempt is made to remove all of the middle ear and mastoid mucosa and the ET is plugged, so the mastoid andmiddle ear become lined with squamous epithelium from the ear canal skin. The hearing will be poor.

The main goal for a mastoidectomy is to create a safe dry ear. This means all of the cholesteatoma must be removedor exteriorized so that there will not be recurrent infections or progression of the cholesteatoma. This requires that all the matrix of the cholesteatoma be removed and none be buried where a new cholesteatoma can develop. This should result in a dry ear.

A cortical or simple mastoidectomy is done for complications of acute otitis media, such as acute mastoiditis, sigmoid sinus thrombosis etc. Mastoidectomies are also used for removing a variety of external canal, middle ear and inner ear tumors, both benign and malignant.

Finally ossiculoplasties are used to reconstruct the ossiclular chain if ossicles have been damaged or dislocated by cholesteatomas, infections or trauma. A variety of materials may be used ranging from the patients remaining ossicles or cartilage to synthetic materials of metals, plastic or hydroxyapatite. The results are variable. but are best for replacing the incus using some type of connection between the malleus handle and the head of the stapes. The poorest results are a total ossiclular replacement when there is no stapes superstructure, only the footplate and the tympanic membrane.

Personally ear surgery was one of my favorite surgeries. Patients recovered quickly with good results most of the time. It requires an excellent knowledge of the three dimensional anatomy of the temporal bone and infinite patience. The general surgeons could never understand how surgery limited to a space not much larger than a quarter could take 3-4 hours, but that was what I thought made it so interesting and challenging. Plus I liked to sit down for surgery using the microscope.

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NOTES

COMPARATIVE EXTERNAL OTITIS (OTITIS EXTERNA)

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DEFINITION

External Otitis (EO) is an inflammatory process of the auricle and/or external ear canal caused by a variety of infections, allergies or trauma.[1, 2]

ACUTE BACTERIAL EXTERNAL OTITIS (ABEO)

ABEO in humans is most often associated with warm humid weather. It is sometimes referred to as "swimmers ear", as it is frequently seen in swimmers. It is usually manifested by itching which rapidly progresses to severe pain, especially on any motion of the auricle or things like chewing. There may be associated otorrhea. On examination the cartilaginous ear canal will be swollen, erythematous and have purulent discharge present. Any manipulation of the auricle or tragus will be painful. One variation is an isolated furuncle in the cartilaginous canal which will be painful and look like a small pimple or abscess. The most common organism found is *Pseudomonas aeruginosa*, followed by *Proteus* sp. In recent years, MethicillinResistant*Staphylococcus aureus* (MRSA) is also a common finding. Other organisms found less frequently may be a variety of Gram negative organisms.

The key to treatment is careful cleansing of all purulent debris and foreign material. This is usually done with suction, but I prefer to use a cotton swab soaked in Cresylate otic solution (Recsei Laboratories LLC, Goleta, CA 93117). Cresylate is a keratolytic agent and also has antifungal and antibacterialproperties. This must be done gently as it will be quite painful. Once the canal is clean, I use a rolled up wick of cotton or a preformed Merocel wick (Pope Wick) and place in the ear canal as far medial as possible. I then prefer to use an antibiotic-steroid otic drop bid or tid. The wick keeps the drops in contact with the canal wall and will wick them down the canal, which might not happen if the canal is badly swollen. Within 48-72 hours the swelling will go down and the wick will fall out or it will be removed in the office in 5-7 days. The drops should be used for a minimum of 10-14 days or until the patient is symptom free for at least 3 days. If the tympanic membrane (TM) is intact one does not need to worry about ototoxicity, so aminoglycosides like neomycin, streptomycin, gentamycin or tobramycin may be used safely. The choice of antibiotics is usually based on the assumption that the most common organism will be*Pseudomonas* or other Gram negative. Cultures are done only when there is no response to therapy. Unless there are systemic symptoms such as fever, lymphadenopathy or surrounding cellulitis, I do not use systemic antibiotics.

For patients who have chronic recurrent EO, I will use an alcohol-vinegar solution as a preventative, especially for swimmers. I have them fill the ear canal with the solution after swimming. I have them make up the solution by taking a pint bottle of rubbing alcohol, pour out a small amount and replace this with two tablespoons of white vinegar. The alcohol will help dry the canal and the vinegar makes the canal acidic, preventing the growth of *Pseudomonas* sp. It burns if the external otitis is acute, so it works better as a preventative.

Veterinary perspective: There is no standard acceptable definition in veterinary dermatology to classify otitis externa as acute vs chronic. One review of otitis externa in 100 dogs in Greece defined acute otitis as otitis present for less than 2 months.[3]Other studies have defined chronic otitis, usually when the otitis is greater than 6-7 months in duration, while not addressing the definition of acute otitis.[4]It is clear that acute bacterial otitis externa, defined as bacterial infection in the ear for less than 2 months duration, does in exist in canine patients; however, the incidence is unknown. The condition is seen infrequently in veterinary referral practice, but since referral centers are logically more likely to see (referred) chronic and recurring cases of otitis externa, the incidence is likely higher than observed by veterinary dermatologists. Swimming is generally considered a predisposing factor of otitis, and it is not clear what the incidence of bacterial otitis is in dogs that swim regularly. Certainly, exposure to water contaminated with known pathogens seems more likely to initiate infection in an ear prone to inflammation. The more commonly referred cases in veterinary dermatology practice are chronic,

recurring bacterial otitis or bacterial otitis cases where the organism is multi-drug resistant. The organism isolated most frequently from dogs with bacterial otitis is *Staphylococcus pseudintermedius,* with *Pseudomonas* spp., *Proteus* spp., *Streptococcus* spp. *Enterococcus* spp., *Corynebactrium* spp., and other species isolated less frequently.[4-6]*Pseudomonas* spp. are usually the second most frequently isolated bacteria from dogs with otitis.

FUNGAL EXTERNAL OTITIS

Fungal external otitis is less common and tends to be more chronic or occur after prolonged treatment of ABEO with antibiotic drops. Also it is more common in tropical climates. Symptoms are usually less severe and tend to be more itching with only mild pain and minimal discharge. On examination the findings may range from a few whitish cotton like strands on the surface of the canal skin to moist paper looking debris partially filling the canal. If it is *Aspergillus* sp., one will be able to see black spores on the surface. Sometimes it may be just a small amount of purulent or milky discharge containing yeast organisms.

The key to treating fungal external otitis is carefully cleaning the canal and this is where cresylateswabbing is especially useful. Usually swelling is not a problem, so a wick is not always used. Antifungal drops like Chlortrimazole or Nystatin will be used. There are also creams with antifungal drugs which can be used. In chronic recalcitrant cases I will sometimes fill the canal with an antifungal powder like Tolnaftate and then see the patient back in a week, swab the ear and refill the canal. Sometimes gentian violet will be used to paint the canal in chronic recurrent cases. One problem with fungal cases is that they tend to be recurrent when exposed to moisture and heat like in tropical settings.

Veterinary perspective: The most common fungal infection found in dogs and cats are due to *Malassezia* spp. yeast. Other fungal organisms are found infrequently in the ears of normal dogs and dogs with otitis externa.[7]Otitis associated with an *Aspergillus* spp. infectionhas been reported infrequently in the dog.[8,9]

ALLERGIC EXTERNAL OTITIS

In humans this can usually take one of two forms. The most common is more of an eczematoid reaction that is seen in older patients who have seborrhea. It usually presents with the complaint of itchy ears. On examination in the lateral part of the canal one can see some flakywhite debris and in severe cases some minor fissuring. This is easily treated with just a steroid crème bid for 2-3 weeks and then prn when the itching recurs. Occasionally patients with psoriasis will have similar findings.

The second form is what I think of as a contact dermatitis. Most commonly in human medicine it is iatrogenic and caused by the drops we prescribe. Dermatologists are most concerned about aminoglycosides which are potent skinsensitizers. They are aghast at how we as otolaryngologists use them so freely. Although I have seen this, the thing I have seen missed the most is a propylene glycol sensitivity. Propylene glycol is ubiquitous in almost all ear canal medications. Patients will be treated for months with antibiotic ear drops and not clear up. The symptoms are usually a mildly swollen, erythematous ear canal with some moist debris. Cultures may show no growth or scant yeast infection. Sometimes the drops will cause a burning sensation.

The treatment starts with stopping all drops. I then clean the canal carefully, but if I am suspicious of a propylene glycol sensitivity I do not use cresylate as the base is propylene glycol. I clean with hydrogen peroxide or just saline and then use a steroid eye drop preparation. If I suspect propylene glycol and not aminoglycosides, I will use antibiotic eye drops for bacterial infections as they do not contain propylene glycol. If the symptoms are very severe with pain and swelling, I have occasionally resorted to a 10- 14 day tapering course of oral systemic steroids such as prednisone to break the cycle and then switch to topical ophthalmic steroid preparations.

Other things that can cause similar symptoms are hair sprays and occasionally animal dander. In these situations it is usually the whole auricle that is involved with erythema and sometimes even blisters and weeping skin, not just the canal.

Veterinary Perspective: When the term allergic otitis is used in veterinary dermatology, we immediately relate that to the high prevalence of otitis in dogs with atopic dermatitis and food hypersensitivity. In those conditions, various reports indicate that up to 83% and 80% of atopic or food hypersensitivity patients will have pinnal erythema and/or otitis.[10,11]Adverse contact reactions in the ears

are often discussed, but poorly documented in veterinary literature. The concave aspect of the pinna as the most commonly mentioned site of involvement. Unfortunately, there are few studies to 1) document the precise substance responsible and 2) to confirm the nature (allergic *vs* irritant) of those reactions. Substances most often mentioned is association with adverse reactions in ears of dogs and cats include medications, such as neomycin and tetracaine, and vehicles used in commercial formulations, such as propylene glycol.[12,13]Anecdotally, contact dermatitis is a common finding in dogs treated with ear preparations containing propylene glycol. Those patients may present with inflamed external ear canals with a mildly ceruminous exudate. Cytology shows no inflammation but large numbers of keratinocytes.

KERATOSIS OBTURANS (KO)

This is an unusual condition where the ear canal becomes filled with keratin debris. The etiology is unclear. The self-cleansingmechanism of the external ear canal is the result of a coordinated process of keratin maturation and lateral cell migration. In KO there seems to be an increased rate of desquamation of the epithelial cells and an interruption of the lateral migration of the epithelial cells from the lateral surface of the TM. This results in the external canal becoming packed with keratin debris with some cerumen. It may interfere with hearing when there is total occlusion. This keratin debris can be infected and result in a chronic external otitis. With continued impaction there can be resorbtion and remodeling with enlargement of the bony canal. It is more common in younger age groups and may be associated with chronic bronchiectasis and/or sinusitis.[14]

On examination the bony canal is filled with a keratinous plug, which when removed leaves an erythematous ear canal and sometimes granulation tissue. It can also be painful secondary to an underlying external otitis. Treatment is conservative with frequent removal of the keratin debris and tropical treatment for the underlying external otitis.

External canal cholesteatoma may present in a similar way, but with a history of otorrhea and chronic dull pain. It is usually secondary to an invasion of squamous tissue into a localized area of periosteitis. Again treatment starts with local debridement and topical drops, but in advanced cases may require surgical treatment.

Veterinary perspective: Impaction of the external ear canal with keratin debris is a common finding in veterinary medicine.The anatomical differences between the dog and human ear canal may account for the tendency of the dog to develop impaction in the horizontal ear canal. However, in most cases, the accumulation of keratin debris is presumed to be a secondary effect of chronic otitis. Infection is often, but not always, present in these situations. The material is usually easily removed with vigorous flushing or mechanical extraction with a loop or curette. Histopathological changes in the skin underlying the impacted keratin have not been described in dogs. Many of these cases do appear to be analogous to keratosis obturans. There have been several reports of middle ear cholesteatomas, also referred to as aural keratinizing cysts, in dogs; however external ear canal cholesteatoma has not been described in the veterinary literature, to the author's knowledge. [15-17]

MALIGNANT EXTERNAL OTITIS

This is an unfortunate name for the condition as it has nothing to do with cancer. When it was first recognized by Dr. Chandler in Miami, Florida in the early 60's the mortality rate was over 60%. Thus the name. This condition occurs in primarily diabetic patients, but also in other immunologically compromised patients such as bone marrow transplant patients or others receiving chemotherapy. A frequent inciting cause is trauma to the external ear canal such as someone removing cerumen and traumatizing the canal skin. In contrast to other externalotitis it usually starts in the bony canal. The hallmark symptom is severe deep boring pain in the ear of a diabetic patient, which is out of proportion to the findings of mild erythema, scant or no discharge and maybe a little granulation tissue in the bony canal. The pathophysiology is almost always a *Pseudomonas* osteomyelitis. If not treated it is a relentlessly progressive osteomyelitis of the temporal bone, which may progressively spread across the skull base and pick off cranial nerves. The first one to be involved is usually the facial nerve.

The diagnosis is usually presumptive on the basis of a diabetic with symptoms of severe pain and some granulation tissue in the bony canal. Adjunctive studies include a CT scan, but positive findings are later in the disease process. A MRI may be helpful. Also bone scans and an elevated sedimentation rate can be useful to follow progression. These should always be cultured, preferably before initiating

therapy. Treatment is high dose intravenous anti-pseudomonas antibiotics for 6-8 weeks. In general it is not considered a surgical disease, although in the 60-80's we did do surgery for refractory cases. The incidence has decreased with the availability of oral fluroquinolones. Even today it will carry a 5-10% mortality rate.

Veterinary perspective: This condition, per se, is not recognized in veterinary practice. *Pseudomonas*spp. infections of the ear do result in painful, ulcerated ears. Osteomyelitis can occur in conjunction with *Pseudomonas*spp. infection of the ear, but the prevalence of underlying osteomyelitis is unknown.

CHONDRITIS AND PERICHONDRITIS

Chondritis of the auricle can be a devastating problem, because of the resultant cosmetic problems and the difficulty with treatment. Because of the low metabolic rate and lack of blood supply to cartilage, antibiotics do not get to the cartilage when it is infected. Most of the time chondritis is secondary to some type of trauma, such as ear piercing, lacerations, post-surgery or occasionally from a chronic external otitis. Sometime it is hard to distinguish perichondritis, which is an infectionof the perichondriumversus true chondritis. The former will respond to antibiotics, wherechondritis usually has to be treated with a combination of surgery and antibiotics. Perichondritis is usually manifested by pain, erythema and swelling over the auricle with preservation ofthe landmarks. Chondritis will also have pain, erythema and swelling, but there is loss of the landmarks which are replaced with a soft doughy feeling to the auricle. Usually there is no fever with either condition. Again the most common and feared organism is *Pseudomonas* sp. Perichondritis may also be from *Streptococcus* or *Staphylococcus* sp.

Treatment for perichondritis may be either oral or intravenous anti-pseudomonas antibiotics. Sometime one may want to also include gram positive coverage for Staph and Strep. Treatment for chondritis will also start with intravenous antibiotics, but if there is no improvement within 24-48 hours or there is considerable involvement of the auricle surgical debridement will need to be carried out. This will consist of bivalving the auricle, removing the soft mushy cartilage back to normal appearing cartilage and then a soft conforming compression dressing to coapt the dead spaces. Some people also recommend placing a small catheter between the two layers of skin so you can irrigate with an antibiotic solution. Intravenous antibiotics will need to be continued for at least two weeks. With the availability of oral anti-pseudomonas antibiotics one can now switch to oral forms after a few days. The dressing needs to be changed every 1-2 days and watch for improvement. If there is progression of erythema and swelling despite antibiotics, repeat debridement may be necessary.

Veterinary perspective: Aural chondritis is a rare condition affecting the pinnae of dogs and cats.[18]This condition affects young to middle-aged cats, resulting in swollen, curled and painful pinna. The condition is even less frequently described in dogs, but may result in or swelling of the pinna or the development of firm papules on the concave aspect of the pinna.[19]It has only rarely been reported as a component of polychondritis.[20]The pathogenesis of the condition is unclear but has been presumed to be immune-mediated.[15]Infectious causes of aural chondritis have not been described for dogs and cats. Treatment may include the use of anti-inflammatory drugs or pinnectomy. Aural chondritis has also been reported in the alpaca. [21]Perichondritis may be a component of severe, chronic, proliferative otitis externa, especially in ears where the cartilage becomes ossified. The mechanism for those changes is not clear.

FOREIGN BODIES (FB)

Foreign bodies are most commonly self-induced especially with children. Other less common FB's are bugs such as moths or beetles. Bugs especially moths are extremely bothersome as their wings flutter against the TM and create a horrible racket. For live bugs we usually drown them with something like olive oil drops or some people use ether. FB's may then be removed with an alligator or cup forceps with magnification or sometimes irrigation. When removing any foreign body one must be careful to not traumatize or perforate the TM.

Veterinary perspective: Foreign bodies are common causes of otitis in dogs and cats. Plant materials are most common and are somewhat geographically dependent, but other foreign bodies found in the ear canal include: hair, parasites, assorted household items, medical dispensing materials. Clinical signs are generally, but not always, limited to one side. Manual extraction, using an appropriate level of sedation, is recommended.

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NOTES

COMPARATIVE OTITIS: A PANEL DISCUSSION

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INTRODUCTION

The purpose of this discussion is to compare and contrast conditions known to occur in veterinary medicine with conditions that may have a counterpart in human medicine.

PRIMARY SECRETORY OTITIS MEDIA- (Cole LK)

Primary secretory otitis media (PSOM) is a disease that has been described in the Cavalier King Charles spaniel (CKCS). Signs suggestive of PSOM include hearing loss, neck scratching, otic pruritus, head shaking, abnormal yawning, head tilt, facial paralysis, or vestibular disturbances; however, none of these signs may be considered pathognomonic for PSOM. A large, bulging pars flaccida identified on otoscopic examination confirms the diagnosis. In many CKCS with PSOM, the pars flaccida is flat, and radiographic imaging (e. g. computed tomography, magnetic resonance imaging) is needed to confirm the diagnosis. Currently, the cause of PSOM is unknown but has been speculated to be due to a dysfunction of the middle ear or auditory tube – increased production of mucus in the middle ear or decreased drainage of the middle ear through the auditory tube or both.Auditory tube dysfunction is implicated in the pathogenesis of otitis media with effusion in humans and may occur secondary to craniofacial abnormalities such as cleft palate. Changes in the nasopharyngeal soft tissues (greater thickness of the soft palate and reduced cross-sectional area of the nasopharynx)have been identified in CKCS with PSOM. The association of these changes in relation to the development of PSOM is not known but these anatomic changes in the nasopharynx may impair auditory tube drainage.

Although there are two reports in the veterinary literature in regards to the use of tympanostomy tubes for treatment of PSOM,no prospective studies have been published on the outcome after extrusion of the tympanostomy tubes as far as the length of time the bulla remains effusion-free or the efficacy of long-term tympanostomy tubes. Current treatment includes performing a myringotomy into the caudal-ventral quadrant of the pars tensa with subsequent flushing of the mucus out of the bulla.

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PSEUDOMONAS OTITIS IN DOGS –(Rosychuk RAW)

Pseudomonas spp. (most commonly *Pseudomonas aeruginosa*) are cultured from up to 35% of cases of otitis externa and/or media in the dog[1]. *Pseudomonas* otitis is only uncommonly encountered in the cat. *Pseudomonas spp*. are not isolated or seen cytologically in normal canine and feline ears. *Pseudomonas spp*. are noted alone in about ½ of cases and in combination with other bacteria (most commonly *Staphylococcus spp*.) in the rest; concurrent *Malassezia spp*. are uncommon.

In the dog, *Pseudomonas* is most frequently seen with chronic otitis, especially when proliferative changes are noted within ears (*Pseudomonas* well adapted to warm, moist environment of proliferative ears). The most common primary factors producing these changes are allergies (atopy and/or food sensitivity). Breed predispositions include pendulous ears or dense hair within or around the entrance to the ear canals, both of which may reduce aeration and promote moisture retention. Other primary factors potentially resulting in a higher incidence of secondary *Pseudomonas* infections include foreign bodies, immunocompromising diseases such as hypothyroidism or hyperadrenocorticism, erosive autoimmune diseases, such as pemphigus foliaceus and neoplasia. The presence of a *Pseudomonas* infection is usually heralded by the presence of purulent exudate. Affected ears often become very inflamed, swollen and painful and may become eroded or ulcerated. Tympanum perforation is common. The diagnosis of a *Pseudomonas* component to the problem is suggested by physical examination, otoscopic and cytologic findings ("rods") and confirmed by culture.

Pseudomonas is intrinsically resistant to many antibiotics and rapidly develops multi-drug resistance, especially with periodic topical otic antibiotic use (as is commonly part of the history in our canine otitis patients). There is only one evidence based review of *Pseudomonas* otitis therapies (10 studies) which showed insufficient evidence for or against recommending the use of any of the 13 treatments included [1]. However, this was largely because there was only one trial supporting the use of each treatment option and none were randomized controlled studies. Potentially effective therapies, based on a compilation of these studies, personal and anecdotal experiences include:

1. Thorough deep ear cleaning to remove debris, bacteria and their mediators of inflammation and tissue damage; significant benefit may be associated with repeat deep ear cleanings.
2. "At home" ear flushes to facilitate debris removal and/or to take advantage of ingredients with variable antimicrobial effects: e.g. Tris-EDTA noted to increase the susceptibility of *Pseudomonas* to several antibiotics, including fluoroquinolones and aminoglycosides [1] ; Tris-EDTA potentiated chlorhexidine; acetic acid noted to have unique anti-pseudomonal effects [1]; salicylic acid, lactic acid and PCMX as EpiOtic® [1]; monosaccharides noted to reduce *Pseudomonas* adherence to epithelial cell surfaces .
3. Topical antimicrobial therapies for empiric "first line" use: Polymixin B, gentamicin, enrofloxacin, enrofloxacin and silver sulfadiazine, marbofloxacin, orbifloxacin. These antibiotics are commonly used with Tris-EDTA containing products as "pre" treatments. Some may be mixed with Tris-EDTA for concurrent administration (gentamicin, enrofloxacin, marbofloxacin).
4. Topical antimicrobial therapies for treatment failures/ more resistant infections. Choices are often dictated by culture and sensitivity testing : Silver sulfadiazine, ticarcillin and clavulonic acid; ciprofloxacin, amikacin, tobramycin or ceftazidime .
5. Systemic antibiotic therapy may be of benefit; potentially indicated when ears are proliferative, erosive or ulcerated and /or there is otitis media or it is difficult for the owners to treat the ears. Those antibiotics found to be more effective include marbofloxacin, ciprofloxacin, ticarcillin andceftazidime.
6. Topical and systemic glucocorticoids are usually of significant benefit. They more rapidly reduce inflammation, exudation, pain, discomfort and proliferative changes.
7. Because of contemporary widespread increases in antibiotic resistance, there appears to be an increasing emphasis on the avoidance of antibiotic therapy and the use of antiseptics (as noted in the ear flushes noted above) along with frequent deep ear cleanings to achieve infection resolution.

The successful management of *Pseudomona*s infections puts great emphasis on case follow-up (otoscopic and cytologic examinations +/- culture) to assure that the problem is put in to complete remission. Prevention of recurrence emphasizes the need to resolve / control primary factors (e.g. allergy) and perpetuating factors (e.g. proliferative changes).

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OTOTOXICITY – (Noxon JO, Griffin CE)

There are many possible types of adverse drug reactions that affect the ear. They include any adverse drug effects on the skin of the ear canal, adverse effects within the middle ear, systemic effects of medications applied to and absorbed from the ear canal, and toxicity to the inner ear (both vestibular and cochlear functions). Most commercial otic medications solvents and emulsifiers and have multiple active ingredients, including antibiotics, antifungal agents, topical anesthetics, glucocorticoids, and other agents. This "polypharmacy" increases the chance of adverse effects, not only from the individual effects of the ingredient, but from possible summative or synergistic effects.

Many ingredients of commercially-available otic cleansers and medications are presumed to have adverse effects. These include, but are not limited to, products containing propylene glycol (increase epithelial turnover, irritation); aminoglycoside antibiotics (vestibular or cochlear toxicity); chloramphenicol (hypersensitivity reactions); chlorhexidine (cochlear toxicity); ethanol, and povidone-iodine (vestibular and cochlear toxicity).

Ototoxicity is often defined strictly as damage to the inner ear structures, with loss of cochlear or vestibular function, following administration of topical or systemic medication. The potential for ototoxicity varies with the following factors: the toxicity of the active ingredient, the carrier or solvent used in the product, the concentration of the active ingredient, the dosing variables (route, frequency, duration), the placement of the agent (external vs middle ear), and concurrent medications (e.g., aminoglycosides and loop diurectics; gentamicin and salicylates; detergents and chlorhexidine).[1-4]Ototoxicois may result from administration of drugs commonly used in veterinary dermatology (e.g., aminoglycosides in otic medications) or drugs used in veterinary medicine, but not typically used to manage dermatology problems (e.g., cisplatin, furosemide).

Disruption of the tympanic membrane would likely make ototoxicosis more likely following instillation of medication into the ear canal; however, there have been numerous reports of hearing loss in dogs following application of medications containing gentamicin into ears with intact tympanic membranes. The data concerning ototoxicity in dogs and cats is largely anecdotal or extrapolated from human studies and/or reports of laboratory animal studies, which are commonly used in medicine for this purpose. More recent work in dogs includes documenting ototoxicity utilizing BAER testing to monitor decreased cochlear function (i.e., hearing).[5,6]It is likely that we will learn a lot about ototoxicity in companion animals as more data becomes available. More work is needed to evaluate other adverse effects of topically applied veterinary preparations.

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NOTES

DEEP MYCOSES

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Mycoses of the dermis and subcutaneous tissues arise from a phylogenetically and morphologically diverse collection of fungal and pseudo-fungal organisms. For most of the well-recognized endemic mycoses in which dermatologic lesions are manifestations of systemic infection (such as blastomycosis, histoplasmosis, cryptococcosis, coccidioidomycosis, and sometimes sporotrichosis), the causative agents have innate virulence factors that allow them to infect healthy hosts. In contrast, opportunistic fungi have low inherent virulence and typically cause infection only when normal host barriers or resistance mechanisms are compromised. These fungi comprise a myriad of genera and species with unfamiliar names that have traditionally caused disease only sporadically. Over the past 3 decades, these opportunists have become increasingly important in human patients as immunocompromise associated with chemotherapy, organ transplantation, and human immunodeficiency virus has become more prevalent. Similarly, over the past 5-10 years, the frequency with which opportunistic fungal infections are encountered in small animal patients has increased significantly in association with the use of multi-agent immunosuppressive therapy (especially with cyclosporine) to treat immune-mediated disease in dogs. Thus veterinarians are increasingly likely to encounter patients with deep cutaneous or subcutaneous infections caused by opportunistic fungal pathogens with which they are unfamiliar.

Unlike the more easily recognized endemic fungal pathogens (for which a definitive diagnosis can usually be made simply by visualizing unique morphologic features in cytologic or histologic samples), opportunistic fungi can only be identified to genus and species level by culture or molecular methods. However, they can be assigned to categories based on their morphologic features in tissue, such as pigmentation, hyphal diameter, organism distribution, and frequency of septation:

- **phaeohyphomycosis** - pigmented hyphal or yeast forms
- **hyalohyphomycosis** - non-pigmented hyphal forms
- **eumycotic mycetoma** - fibrosing granuloma with tissue grains containing pigmented (black grain) or non-pigmented (white grain) fungal elements

Although identification of a specific pathogen based on culture is ideal, categorization of opportunistic mycoses is often sufficient for reasonably predicting clinical course and prognosis, and for choosing initial therapies. It should be noted that because many opportunistic fungi are common contaminants and can normally be found on skin, nasal mucosa, and other non-sterile sites, culture or PCR-based identification of a potential opportunistic fungal pathogen from a skin sample, nasal swab, or exudate should not be considered evidence of fungal infection unless there is supportive histologic or cytologic evidence of tissue invasion by a morphologically compatible organism.

The oomycetes and zygomycetes make up a fourth category of fungi or pseudo-fungi that cause deep skin infections. Like the endemic systemic fungal pathogens, these organisms cause disease in previously healthy hosts. However, like the opportunists, these organisms cannot be definitely identified by their morphologic characteristics in tissue, but instead can be placed into a category:

- **pythiosis, lagenidiosis, and zygomycosis** - pyogranulomatous and eosinophilic inflammation associated with wide, infrequently septate, non-pigmented hyphae with non-parallel walls

Because of important differences amongst these three diseases in expected clinical course, prognosis, and appropriate therapy, it is important to follow categorization with specific tests (such as serology for pythiosis or culture for lagenidiosis and zygomycosis) that allow a definitive diagnosis to be made.

Table 1. Morphologic features of fungal pathogens of dogs and cats.

Disease	Causative Agents	Histologic & Cytologic Characteristics
Phaeohyphomycosis	*Alternaria*, *Bipolaris*, *Phialophora*, *Cladophialophora* (*Cladosporium*), *Curvularia*, *Exophiala*, *Fonsecaea*, *Moniliella*, *Ramichloridium*, others	pyogranulomatous inflammation associated with pigmented, irregularly septate hyphae or yeast-like cells that may be solitary or cluster in small groups or chains
Hyalohyphomycosis	*Acremonium*, *Fusarium*, *Geotrichum*, *Paecilomyces*, *Phialosimplex*, *Pseudallescheria*, *Scedosporium*, *Schizophyllum*	pyogranulomatous inflammation associated with hyphal elements that have hyaline (transparent, non-pigmented) walls; *Phialosimplex* may cause yeast-like forms in tissue
Mycetoma (black-grain)	Curvularia, Madurella	pyogranulomatous inflammation associated with pigmented tissue grains (which represent aggregates of fungal organisms)
Mycetoma (white-grain)	*Pseudallescheria boydii*, *Acremonium*	pyogranulomatous inflammation associated with non-pigmented tissue grains (which represent aggregates of fungal organsims)
Pythiosis	*Pythium insidiosum*	pyogranulomatous and eosinophilic inflammation associated with broad (2-7 µ), infrequently septate hyphae
Lagenidiosis	*Lagenidium* species	pyogranulomatous and eosinophilic inflammation associated with broad (4-25 µ), infrequently septate hyphae
Zygomycosis	*Basidiobolus ranarum* *Conidiobolus* species	pyogranulomatous and eosinophilic inflammation associated with broad (5-20 µ), infrequently septate hyphae with thick prominent eosinophilic sleeve
Sporotrichosis	Sporothrix schenckii	pyogranulomatous inflammation associated with round, oval, or cigar-shaped yeast forms, 5-9 µ long, within macrophages or extracellular
Rhinosporidiosis	Rhinosoporidium seeberi	mixed inflammatory response associated with very large (300 µ) sporangia that contain many endospores; released endospores often visible in cytologic samples
Candidiasis	*Candida albicans* other *Candida* spp	suppurative inflammation associated with numerous 2-6 µ oval yeasts, pseudohyphae (chains of oval yeast cells), and true hyphae
Blastomycosis	Blastomyces dermatiditis	suppurative to pyogranulomatous inflammation associated with large (8-15 µ), spherical, thick-walled, broad-based budding yeasts
Cryptococcosis	*Cryptococcus neoformans*	granulomatous inflammation (may be minimal) with 3-7 µ pleomorphic, narrow-based budding yeasts surrounded by a variably thick (1-30 µ) polysaccharide capsule
Histoplasmosis	Histoplasma capsulatum	granulomatous inflammation associated with intracellular, 2-4 µ, round to oval yeast cells characterized by a basophilic center and clear halo
Coccidioidomycosis	*Coccidioides immitis*	pyogranulomatous inflammation associated with very large (20-200 µ), round, thick-walled spherules that at maturity contain many small (2-5 µ) endospores
Aspergillosis	*Aspergillus terreus*, *A. deflectus*, *A. flavipes*, *A. fumigatus*	suppurative to granulomatous inflammation associated with multiple, non-pigmented, 3-6 µ, septate hyphae with parallel walls and 45 degree angle branching

PHAEOHYPHOMYCOSIS

The term "phaeohyphomycosis" refers to cutaneous, cerebral, or disseminated infections caused by pigmented (dematiaceous) fungi. Infection usually results from traumatic implantation. Fungal genera that have been identified as agents of phaeohyphomycosis in include *Alternaria*, *Bipolaris*, *Cladophialophora*, *Curvularia*, *Exophiala*, *Fonsecaea*, *Moniliella*, *Phialophora*, *Xylohypha*, *Ramichloridium*, and *Ulocladium*, among others (Table 1). The most common clinical presentations in immunocompetent small animals are distal extremity, nasal, and pinnal lesions in cats.[1-3] In immunocompromised patients, the most common presentation appears to be multifocal cutaneous lesions in dogs treated with multi-agent immunosuppressive therapy, especially including cyclosporine.[4-6] Patients with phaeohyphomycosis are typically presented with cutaneous nodules or a visible nasal mass. Infected tissues may appear grossly pigmented, and thus may be confused with melanoma. Lesions caused by phaeohyphomycosis tend to be locally invasive and may extend to regional lymph nodes. Dissemination is not common in immunocompetent patients, but has been observed in patients receiving immunosuppressive therapy.

Histologically and cytologically, fungi that cause phaeohyphomycosis appear as dark-walled, irregularly septate hyphae, or as yeast-like cells, solitary or in small groups. The presence of melanin in the walls of lightly-pigmented hyphae can be confirmed by examining unstained sections or by utilizing a Fontana-Masson stain for melanin. Agents of phaeohyphomycosis are readily isolated on routine fungal media. Because pigmented fungi are common laboratory contaminants and can be isolated in healthy animals from non-sterile sites such as skin, positive cultures should only be considered significant when cytologic or histologic evidence of infection with a morphologically compatible organism is present.

Pigmented fungi are often poorly responsive to medical therapy, in part because melanin is a potent virulence factor. Therefore, aggressive surgical resection is the treatment of choice for solitary lesions caused by phaeohyphomycosis, and attempts should be made to obtain wide margins at the time of the initial excision. Digit amputation is usually indicated for lesions involving the distal phalanx, and limb amputation may be necessary when lesions have extended more proximally. Medical therapy with itraconazole or posaconazole is recommended for 3-6 months after surgery because recurrence of disease at the surgery site is common. For lesions that cannot be surgically cured, itraconazole administered orally at 10 mg/kg/day is often initially successful in resolving or improving cutaneous lesions, but recurrence is very common, so prolonged courses (6-12 months) should be recommended. The newer triazoles voriconazole and posaconazole may be more effective than itraconazole for the treatment of phaeohyphomycosis, but are significantly more expensive. In addition, voriconazole should not be used in cats because of significant side effects. Recently, long-term voriconazole therapy (7-10 mg/kg/day for 10-12 months) was successful in resolving intracranial phaeohyphomycosis in one dog[7] and mycotic peritonitis caused by *Exophiala* in another.[8] Amphotericin B lipid complex may be indicated in patients that fail triazole therapy or have rapidly progressive disease.

In the author's experience, cutaneous phaeohyphomycosis that occurs in dogs on immunosuppressive therapy can often be resolved with itraconazole administered for at least 6 months if immunosuppressive therapy can be tapered quickly. However, dissemination of disease may also occur, sometimes despite appropriate antifungal therapy. Discontinuation of cyclosporine or other non-glucocorticoid immunosuppressive medications in these patients appears to be important for achieving a good outcome.

HYALOHYPHOMYCOSIS

The term "hyalohyphomycosis" refers to infections caused by fungi that are non-pigmented (hyaline or transparent) in tissue. Genera that have been described as agents of hyalohyphomycosis include *Fusarium, Acremonium, Paecilomyces, Pseudallescheria, Sagenomella, Phialosimplex*, and *Scedosporium*, among others (Table 1). By convention, infection caused by *Aspergillus* and *Penicillium* species are not included in the term hyalohyphomycosis because aspergillosis and penicilliosis can usually be identified as such based on their clinicopathologic features. In general, hyalohyphomycosis

occurs more often in dogs than in cats.

Animals with hyalohyphomycosis develop lesions ranging from local disease confined to the skin, nasal mucosa, or cornea, to osteomyelitis, pneumonia, and disseminated disease involving kidneys, bone marrow, lymph nodes, liver, spleen, bones, and CNS.[9-12] Traditionally, the disseminated and corneal forms have been most common. Therefore, animals with no overt signs of systemic disease that present with cutaneous or bone lesions that contain non-pigmented hyphae should still be evaluated for occult lesions in the chest and abdomen. Like phaeohyphomycosis, hyalohyphomycosis has become an increasingly common cause of multifocal skin lesions in dogs receiving multi-agent immunosuppressive therapy. These dogs may or may not have accompanying non-cutaneous lesions.

Cytologically and histologically, fungi that cause hyalohyphomycosis appear as non-pigmented, frequently septate, branching hyphae that are often pleomorphic. These fungi are readily isolated on routine fungal media from infected tissues as well as from fine needle aspirate samples from infected lymph nodes, bones, or abdominal organs. It is important to note that these fungi are common laboratory contaminants and can sometimes be isolated from the skin or hair of healthy animals. Therefore, positive cultures from non-sterile sites should only be considered significant when cytologic or histologic evidence of infection with a morphologically compatible organism is present.

Treatment of hyalohyphomycosis has traditionally been unrewarding because most patients have disseminated disease. Drugs used most often to treat hyalohyphomycosis in small animals include itraconazole and amphotericin B lipid complex. The newer triazoles, voriconazole and posaconazole, may have better efficacy than itraconazole based on their broader spectrum, but are significantly more expensive. Although treatment of disseminated hyalohyphomycosis with antifungal drugs can prolong survival, clinical signs usually recur even if signs initially resolve. Therefore, disseminated hyalohyphomycosis generally carries a poor prognosis. In the author's experience, cutaneous hyalohyphomycosis that develops in an animal receiving immunosuppressive therapy may respond well to oral azole therapy, or may rapidly disseminate. Therefore, fungal skin lesions that develop in immunocompromised patients should be treated aggressively, and a guarded prognosis should be offered. This author most often uses itraconazole (10 mg/kg/day) administered orally for at least 6 months. Other options include amphotericin B lipid complex, voriconazole (dogs only), or posaconazole. Discontinuation of cyclosporine and rapid tapering of other immunosuppressive medications in these patients appears to be important for achieving a good outcome.

MYCETOMA

The term “mycetoma” refers to localized, mycotic or actinomycotic infections that are characterized by the presence of colonies or aggregates of organisms that form “grains” in tissue. Actinomycotic mycetomas are caused by bacteria such as *Actinomyces* sp and *Nocardia* sp, whereas eumycotic mycetomas are caused by fungi. Lesions result from traumatic implantation of soil organisms into tissue. The grains or granules associated with eumycotic mycetomas are characteristically pigmented (for black grain mycetoma, caused by dematiaceous fungi) or hyaline (for white grain mycetoma, caused by non-dematiaceous fungi), depending on the type of fungal pathogen involved.

Black grain mycetomas are most often caused by *Curvularia* or *Madurella* species, and typically manifest as chronic non-healing wounds and cutaneous nodules on the extremities.[13] Lesions often develop weeks to months after a traumatic incident in the same area. Draining tracts are often present, and black grains may be observed in the exudate. White grain mycetomas, usually caused by *Pseudallescheria boydii* or *Acremonium* species, most often occur as body wall and/or intra-abdominal granulomas that develop months or even a year or more after surgical wound contamination or dehiscense.[14] Affected dogs may be presented with a draining mass on the body wall, or may develop clinical signs of peritonitis. The treatment of choice for eumycotic mycetoma is aggressive surgical excision of infected tissues, including amputation if clinically indicated. Response to medical therapy is

routinely poor. Dissemination of eumycotic mycetoma beyond local tissues is rare, but local extension of disease within the abdomen may be extensive.

PYTHIOSIS, LAGENIDIOSIS, AND ZYGOMYCOSIS

Pythiosis, lagenidiosis, and zygomycosis are often grouped together because of similarities in their clinical presentations and histologic characteristics (all three cause pyogranulomatous and eosinophilic inflammation associated with large, infrequently septate hyphae with non-parallel walls). Despite their clinicopathologic similarities, however, the pathogens that cause these infections are taxonomically diverse. *Pythium insidiosum* and *Lagenidium* species are water molds in the class Oomycetes, and as such are more closely related to red algae and *Prototheca* spp than to true fungi such as the zygomycetes. Important traits that distinguish oomycetes from fungi include the production of motile, flagellate zoospores that act as infective elements in wet environments, and the fact that the oomycete cell membrane generally lacks ergosterol.[15] In addition, there are clinically relevant differences in prognosis, recommended treatment, and epidemiology that make it important to distinguish between pythiosis, lagenidiosis, and zygomycosis. Although *P. insidiosum* has been recognized as a pathogen in dogs and horses for more than 25 years, *Lagenidium* species have only been recognized as mammalian pathogens since 1999. In comparison to pythiosis and lagenidiosis, infections caused by the zygomycetes are rare.

PYTHIOSIS

Pythium insidiosum is a devastating and often fatal cause of cutaneous or subcutaneous lesions in dogs and cats. In the US, the disease occurs most often in the Gulf Coast states, but has also been recognized throughout the south; along the east coast as far north as Maryland, New Jersey, and Connecticut; in the midwest including Missouri, Kansas, southern Illinois and Indiana, and recently Wisconsin; and in the west in Arizona and California. Globally, pythiosis occurs in tropical and subtropical climates, including Southeast Asia, eastern coastal Australia, and South America. The infective form of *P. insidiosum* is thought to be the motile biflagellate zoospore, which is released into aquatic environments and likely causes infection by encysting in damaged skin. Many dogs with pythiosis have a history of recurrent exposure to warm freshwater habitats. However, some cases are observed in suburban house dogs with no history of access to lakes or ponds. Affected animals are typically immunocompetent and otherwise healthy.

Pythiosis occurs most often in young, large breed dogs and is especially common in outdoor working breeds such as Labrador Retrievers. Affected dogs are presented to the veterinarian more often in the fall, winter, and early spring than in the summer months. In cats, specific breed and sex predilections have not been observed in the few cases reported to date. However, infection of very young animals appears to occur more often in cats than in dogs. Of more than 30 cats diagnosed with cutaneous pythiosis through the author's laboratory in the past 13 years, more than a third were less than a year old.

Cutaneous pythiosis in dogs typically causes nonhealing wounds and invasive masses that contain ulcerated nodules and draining tracts, most often involving the extremities, tailhead, ventral neck, or perineum.[16] In contrast to GI pythiosis, regional lymphadenopathy associated with cutaneous pythiosis often reflects extension of infection rather than just reactive hyperplasia. Extension of cutaneous disease to tissues other than regional lymph nodes is rare, but the author has observed single, focal pulmonary lesions caused by *P. insidiosum* in two dogs with cutaneous pythiosis on a distal extremity. Cats with pythiosis may present with nasopharyngeal lesions, invasive subcutaneous masses in the inguinal, tailhead, or periorbital regions, or draining nodular lesions or ulcerated plaque-like lesions on the extremities, sometimes centered on the digits or footpad.[17,18]

In animals with cutaneous pythiosis, lagenidiosis, or zygomycosis, cytologic evaluation of exudate from draining tracts, impression smears made from ulcerated skin lesions, and fine needle aspirates of enlarged lymph nodes often reveal pyogranulomatous and eosinophilic inflammation. Hyphae are observed occasionally, and their morphologic appearance (broad, rarely septate with tapered, rounded

ends) in conjunction with a typical inflammatory response can provide a tentative diagnosis of oomycosis or zygomycosis. Microscopic examination of macerated tissue that has been digested in 10% potassium hydroxide may be more likely to reveal hyphal elements than other cytologic specimens.

Histologically, pythiosis is characterized by eosinophilic pyogranulomatous inflammation, with multiple foci of necrosis surrounded and infiltrated by neutrophils, eosinophils, and macrophages. In addition, discrete granulomas composed of epithelioid macrophages, plasma cells, multinucleate giant cells, and fewer neutrophils and eosinophils are often be observed. Organisms are typically found within areas of necrosis or at the center of granulomas, and vasculitis is occasionally present. Lesions typically involve the deep dermis and subcutis, necessitating deep wedge biopsies rather than punch biopsies for optimal evaluation. *Pythium insidiosum* hyphae are not routinely visualized on H&E-stained sections, but may be identified as clear spaces surrounded by a narrow band of eosinophilic material. Hyphae are readily visualized in sections stained with Gomori's methenamine sliver (GMS) but usually do not stain well with periodic acid-Schiff (PAS). They are wide (mean, 4 µ; range, 2-7 µ), have non-parallel walls, are infrequently septate, and occasionally branch at right angles.[16]

Isolation of *P. insidiosum* from infected tissues is not difficult but does require specific sample handling and culture techniques. For best results, unrefrigerated tissue samples should be wrapped in a saline-moistened gauze sponge and shipped at ambient temperature to arrive within 24 hours at a laboratory that has experience with pathogenic oomycetes. Small pieces of fresh, non-macerated tissue should be placed directly on the surface of vegetable extract agar supplemented with streptomycin and ampicillin (or an alternative selective medium such as Campy blood agar) and incubated at 37C.[19] Mycelial growth is typically observed within 12 to 24 hours. Isolation of *P. insidiosum* from swabs of exudate collected from draining skin lesions is generally unsuccessful. Because generation of the sexual reproductive structures that are necessary for definitive morphologic classification of pathogenic oomycetes rarely occurs in the laboratory, identification of *P. insidiosum* isolates should be based on species-specific PCR amplification[20] or rRNA gene sequencing. Although production of zoospores is an important supporting feature for the identification of pathogenic oomycetes, it is not specific for *P. insidiosum.* Species-specific PCR amplification can be used to identify *P. insidiosum* DNA in fresh, frozen, or paraffin-embedded tissues as well as DNA extracted directly from cultured isolates.[21]

A highly sensitive and specific ELISA for the detection of anti-*P. insidiosum* antibodies in dogs is currently available through the author's laboratory at LSU.[22] In addition to providing a means for early, noninvasive diagnosis, this assay is also very useful for monitoring response to therapy. Following complete surgical resection of infected tissues, a dramatic decrease in antibody levels is typically detected within 2-3 months. In contrast, antibody levels remain high in animals that go on to develop clinical recurrence following surgical treatment. This same ELISA has been adapted for detection of anti-*P. insidiosum* antibodies in domestic and exotic cats, and although case numbers are too small to generate strong estimates of sensitivity and specificity, in the author's experience they appear to be high.

Aggressive surgical resection of all infected tissues with wide margins is the treatment of choice for pythiosis. In animals with cutaneous lesions confined to a single distal extremity, amputation should be recommended unless there is evidence of regional lymph node infection. Surgeons should attempt to obtain skin margins of 5 cm and deep margins of two fascial planes.[23] Enlarged regional lymph nodes in dogs with cutaneous pythiosis are often infected; therefore, they should be evaluated cytologically before amputation or other aggressive resection is attempted. Unfortunately, many dogs with pythiosis are not presented until late in the course of disease, when complete excision is not possible. In addition, the anatomic location of the lesion (such as tailhead, ventral chest, or trunk) may prevent complete surgical excision. Still, very aggressive surgery (massive resection combined with reconstructive techniques)[23] can sometimes result in a successful outcome.

Local post-operative recurrence of pythiosis is common (especially when wide surgical margins cannot be achieved), and can occur either at the site of resection or in regional lymph nodes. For this reason, post-operative medical therapy is often recommended. In the author's practice, dogs without regional lymphadenopathy that undergo amputation for distal extremity lesions are not routinely treated with post-operative antifungal medication; all other patients with cutaneous pythiosis typically receive

itraconazole (10 mg/kg once daily, PO) and terbinafine (5-10 mg/kg once daily, PO) for at least 2-3 months after surgical resection. Unfortunately, in animals that undergo incomplete resections, lesions typically progress despite medical therapy, and clinical signs recur within weeks to months. To monitor for recurrence, ELISA serology should be performed at the time of surgery and 2-3 months later. In animals that have had a complete surgical resection and go on to have no recurrence of disease, serum antibody levels usually drop 50% or more within 3 months. If this occurs, medical therapy can be discontinued. In the author's experience, surgery is curative in a majority of animals with a distal limb lesion treated with amputation, but is rarely curative in animals with lesions in other locations.

Medical therapy for nonresectable pythiosis is typically unrewarding, likely due to the fact that ergosterol (the target for most currently-available antifungal drugs) is generally lacking in the oomycete cell membrane. Despite this fact, the author has observed clinical and serologic cures in a number of patients treated with a combination of itraconazole (10 mg/kg once daily, PO) and terbinafine (5-10 mg/kg once daily, PO).[24] Although the percentage of animals responding is still very low, the combination protocol appears to be superior to itraconazole or amphotericin B alone.

Dogs with nonresectable GI pythiosis are often treated with anti-inflammatory doses of corticosteroids in an effort to palliate clinical signs and to decrease vomiting so that oral antifungal medication can be administered. Prednisone administered at a dose of 1 mg/kg/day routinely causes clinical improvement in the short term. Surprisingly, the author has observed complete long-term resolution of GI lesions in a small number of dogs treated with prednisone alone that have gone on to have no recurrence of their clinical signs. Although this is certainly not recommended as a primary treatment for animals with resectable lesions, it is a reasonable option in animals with nonresectable GI lesions, especially when financial concerns preclude the use of antifungal medication. Unfortunately, the author has not observed this effect of prednisone in dogs with cutaneous pythiosis.

Mefenoxam, an agricultural fungicide used to control plant-pathogenic oomycetes on crops, acts by preventing RNA synthesis. Initial *in vitro* investigations demonstrated that mefenoxam had a profound effect on the growth of clinical *P. insidiosum* isolates, causing greater than 90% inhibition at a concentration of only 1 μg/ml.[25] Canine toxicity studies performed as part of the manufacturer's pesticide petition to the EPA showed that mefenoxam added to the diet of dogs for 6 months had a no-observable-effect level of 8 mg/kg/day.[26] The author has administered mefenoxam in the form of an aqueous agricultural product (Subdue MAXX®; Syngenta Crop Protection Inc, Greensboro, NC) at a dose of 4 mg/kg orally twice daily to a small number of dogs with pythiosis. Although the compound was well tolerated, it failed to cause significant clinical improvement. In one dog that was treated with mefenoxam in addition to itraconazole and terbinafine,[24] the patient was improving on the antifungal drugs before the mefenoxam was added, so its role in the eventual successful treatment outcome in that dog is uncertain.

An immunotherapy product derived from antigens of *P. insidiosum* has been used successfully to treat pythiosis in horses and people.[27,28] Unfortunately, although controlled trials have not been completed, the efficacy of this product in dogs appears to be poor, and clinical improvement has not been observed in any of the authors' patients. The limited published information available regarding the efficacy of *Pythium* vaccines in dogs is anecdotal.[29] In one canine case report that suggested a vaccine-related therapeutic effect,[30] tissues obtained after the initial diagnostic wedge biopsies but before vaccine administration were submitted to the author's laboratory. Culture of the tissue was negative for oomycetes and multiple GMS-stained sections failed to show any hyphae, suggesting that the disease may have been resolving before vaccine administration. Interestingly, the author is aware of one additional dog in which lesions associated with cutaneous pythiosis resolved completely without additional therapy after incomplete surgical resection.

LAGENIDIOSIS

Most species in the Oomycete genus *Lagenidium* are pathogens of insects, crustaceans, and nematodes. The most well-studied species, *L. giganteum*, is a mosquito larval pathogen that has previously been used as a biological control agent for mosquito populations. Within the past 15 years, two novel oomycetes that appear to belong to the genus *Lagenidium* have been isolated from dogs. The

first of these species causes fatal dermatologic and disseminated disease in dogs in the southeastern US.[31] The second species is less common than the first, and causes chronic ulcerative nodular dermatopathy that has a prolonged course and does not appear to extend past local tissues. More recently, a third *Lagenidium* species has been isolated from two cats with cutaneous lesions. Sporulation and infectivity for the *Lagenidium* pathogens are thought to be similar to *P. insidiosum* and *L. giganteum*, and the epidemiologic features of lagenidiosis are similar in many respects to those associated with cutaneous pythiosis. Affected patients are typically young to middle-aged animals living in the southeastern US. Although most affected dogs and cats have lived in Florida or Louisiana, cases in Texas, Tennessee, Alabama, Georgia, South Carolina, Maryland, Virginia, Indiana, and Illinois have been identified as well. A number of infected dogs have had frequent exposure to lakes or ponds. None have had evidence of immunocompromise or had been treated with immunosuppressive therapy before developing lesions.

Dogs with *Lagenidium* infection are presented for evaluation of progressive, multifocal or focal, cutaneous or subcutaneous lesions involving the extremities, mammary region, perineum, or trunk.[31] Lesions appear as firm dermal or subcutaneous nodules, or as ulcerated, thickened, edematous areas of deep cellulitis with regions of necrosis and draining tracts. These lesions tend to be progressive, locally invasive, and poorly responsive to medical therapy. In dogs infected with the more aggressive species of *Lagenidium*, regional lymphadenopathy is often noted, and may occur without obvious cutaneous lesions. Unlike dogs with cutaneous pythiosis, these dogs typically have occult lesions in the thorax or abdomen, including involvement of the great vessels, sublumbar and/or inguinal lymph nodes, lung, pulmonary hilus, and cranial mediastinum. Animals with great vessel or sublumbar lymph node involvement typically have cutaneous or subcutaneous lesions on the hindlimbs, and often develop hindlimb edema. Sudden death caused by great vessel rupture may occur. In dogs infected with the less aggressive species of *Lagenidium*, lesions progress locally but rarely extend beyond cutaneous and subcutaneous tissues. The clinical course in these dogs is chronic and slowly progressive, with some patients having lesions that are somewhat stable for more than 3 years. The two cats from which a third species of *Lagenidium* was isolated had lesions characterized by miliary dermatitis of the tail or tailhead region. In one of these cats, papules eventually coalesced to form plaques over the caudal dorsum.

Because of its clinicopathologic similarities to pythiosis and zygomycosis, lagenidiosis is often misdiagnosed during cytologic or histologic evaluation. Although there are subtle differences in hyphal size, morphology, and distribution among these three infections, histologic lesions alone do not allow definitive differentiation. Therefore, suspected histologic diagnoses should be followed with serology, culture, or molecular confirmation. In contrast to *P. insidiosum*, *Lagenidium* spp hyphae are often visible on H&E-stained sections. On GMS-stained sections, numerous broad, thick-walled, irregularly septate hyphae are easily recognized. *Lagenidium* hyphae typically demonstrate significant variability in size, but are generally larger than *P. insidiosum* hyphae, with an average of 12 µm for the more aggressive canine pathogen, 7.5 µm for the less aggressive canine pathogen, and 9 µm for the feline pathogen.

Diagnostic imaging is an essential part of evaluating dogs suspected of having lagenidiosis because of the potential for occult lesions in the chest or abdomen. These may include solitary pulmonary nodules, sublumbar, inguinal, and medial iliac lymphadenopathy, thickening and invasion of the wall of the aorta or caudal vena cava (sometimes with associated aneurysm), and retroperitoneal or epaxial masses. Thoracic and abdominal lesions have not been observed in dogs infected with the less aggressive pathogen, and were not observed in the two cats infected with the third species.

In dogs with supportive clinical signs and histologic findings, immunoblot or ELISA serology for the detection of anti-*Lagenidium* antibodies in canine serum can be suggestive of lagenidiosis, but must be interpreted in conjunction with results of serologic testing for *P. insidiosum* infection because of the potential for cross reactivity in serum from dogs with pythiosis. In addition, the author has observed nonspecific anti-*Lagenidium* seroreactivity in dogs with other fungal or non-fungal infections. Therefore, based on currently available data, serology alone should not be used as a basis for the diagnosis of lagenidiosis. The definitive diagnosis of lagenidiosis and differentiation amongst the pathogenic species requires culture followed by rRNA gene sequencing. Isolation techniques for *Lagenidium* spp are similar to those described for *P. insidiosum*, but with peptone-yeast-glucose (PYG) agar. For best results, small

pieces of fresh, non-macerated tissue should be placed directly on the surface of the agar and incubated at 37C. Growth is typically observed within 24 to 48 hours.

As with pythiosis, aggressive surgical resection of infected tissues is the treatment of choice for lagenidiosis when disease is confined to a resectable cutaneous lesion. Unfortunately, the vast majority of dogs infected with the more common (and more aggressive) *Lagenidium* pathogen have nonresectable disease in the thorax, abdomen, or regional lymph nodes by the time the initial diagnosis is made, and in the author's experience, the disease is routinely fatal. In dogs infected with the less aggressive species, surgery that achieves 5 cm margins is often curative. Because the species of *Lagenidium* causing infection is often not known early in the course of diagnostic evaluation, any dog suspected of having lagenidiosis should be evaluated with thoracic radiography and abdominal ultrasonography prior to attempting surgical resection of cutaneous lesions. As with pythiosis, medical therapy for lagenidiosis is usually ineffective. However, a combination of itraconazole (10 mg/kg, once daily, PO) and terbinafine (5-10 mg/kg, once daily, PO) along with repeated aggressive surgical resection was effective in resolving infection caused by the less aggressive pathogen in one dog with recurrent multifocal cutaneous lesions. In one of the cats infected with the third *Lagenidium* species, amputation of the tail was curative.

ZYGOMYCOSIS

The term "zygomycosis" refers to infections caused by fungi in the class Zygomycetes, including the genera *Basidiobolus* and *Conidiobolus* in the order *Entomophthorales*, and the genera *Rhizopus*, *Mucor*, and others in the order *Mucorales*. In human and veterinary patients, the *Mucorales* tend to cause acute, rapidly progressive disease in debilitated or immunocompromised individuals, whereas the *Entomophthorales* typically cause chronic localized infections in subcutaneous tissue or nasal submucosa of immunocompetent patients. Culture-confirmed infections caused by pathogens in the order *Mucorales* have not been well documented in small animal patients. However, *Conidiobolus* spp and *Basidiobolus* spp have been reported in dogs to cause cutaneous pyogranulomatous lesions that are grossly and histologically similar to those caused by *P. insidiosum* and *Lagenidium* spp.

In dogs, humans, horses, sheep, and other mammalian species, conidiobolomycosis occurs most often as a nasopharyngeal infection with or without local dissemination into tissues of the face, retropharyngeal region, and retrobulbar space. Manifestations of infection in dogs may include nasal or facial swelling or deformity, nasal cavity discharge, ulceration of the nasal planum or hard palate, exophthalmus, chemosis, ocular discharge, and sometimes skin lesions near the eye. In animals with retrobulbar disease that extends into the brain, neurologic signs may occur. *Conidiobolus* infection has also been described in a single dog as a cause of multifocal nodular draining subcutaneous lesions and regional lymphadenopathy[32] and as a cause of pneumonia in a dog that was receiving chemotherapy.[33] Basidiobolomycosis is a rare cause of ulcerative skin lesions in dogs, and has also been reported in a single case as a cause of respiratory disease.[34] In cats, culture-confirmed cases of zygomycosis are sparse; a subcutaneous mass caused by a *Mucor* species on the dorsum of the nose of a 14 year old cat was treated successfully with posaconazole.[35]

Because of their histologic similarities, zygomycosis is often confused with pythiosis. Unfortunately, there are no serologic, immunohistochemical, or molecular techniques routinely available for the diagnosis of conidiobolomycosis and basidiobolomycosis, making identification of infected animals reliant on culture of fresh tissues. As a result, a definitive diagnosis of zygomycosis is often elusive. On GMS-stained sections, hyphae appear broad, thin-walled, and occasionally septate. The histologic hallmark of zygomycosis is the presence of a wide (2.5 to 25 µ) eosinophilic sleeve surrounding the hyphae and making them easily located on H&E-stained sections. This finding helps to differentiate zygomycosis from pythiosis and lagenidiosis, in which eosinophilic sleeves tend to be thin or absent. In addition, the hyphal diameter tends to be significantly larger for *Basidiobolus* spp (mean 9 µ; range, 5-20 µ) and *Conidiobolus* spp (mean 8 µ; range, 5-13 µ) than for *P. insidiosum* (mean, 4 µ; range, 2-7 µ).

Recommendations for the treatment of zygomycosis are not straightforward because attempted therapy has only been described in a few patients with culture-confirmed diagnoses. Although anecdotal

information as well as a small number of cases in the literature suggest that cutaneous zygomycosis may be less aggressive than cutaneous pythiosis or lagenidiosis, progression of lesions and sometimes even dissemination despite treatment have also been observed in zygomycete-infected dogs. The author's current recommendation for dogs with nasopharyngeal conidiobolomycosis is treatment with itraconazole (10 mg/kg/day orally) or posaconazole (5 mg/kg q24hr) for at least 6 months. Recurrence is common after medication is discontinued, so a prolonged course should be prescribed. Cutaneous zygomycosis in small animal patients should be treated with aggressive surgical resection of infected tissues whenever possible, followed by itraconazole therapy for 2-3 months. If resection is not possible, therapy with either itraconazole, posaconazole, or amphotericin B lipid complex should be recommended.

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