SCIENTIFIC SESSION
PRESENTATIONS
DIAGNOSIS AND TREATMENT OF FUNGAL DISEASE

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SPECIMEN COLLECTION, TRANSPORT, AND PROCESSING

The proper collection, transport, and processing of clinical specimens is of utmost importance in determining the etiology of fungal disease. An list of appropriate sites is available in several texts \(^1,2\), and those cited in reference #1 would apply to several animal species as well. As a general rule, the active site of infection is preferred and the larger the specimen, the greater likelihood of recovery of fungal species. Sites removed (such as blood) may also provide a diagnosis. Most specimens should be set up within 2 hours or maintained on a transport medium at room temperature. Exceptions include skin, hair, and nails (clean, dry envelope for extended periods), and CSF specimens (set up within 15 min or 30°C storage no longer than 24 hours). Specimens with heavy bacterial contamination may be refrigerated if processing is delayed. Significant delays in processing increases the potential for non-viability in culture. Sterile body fluids may be concentrated through membranes or centrifuged and the sediment use for culture. Tissue for the recovery of *Histoplasma capsulatum* should be ground, however tissue for other fungi, particularly mucoraceous genera, should only be minced. \(^1\)

CULTURE SET UP AND SAFETY

The battery of fungal culture media used for primary isolation may vary, however non-sterile specimens should be placed on media containing antibacterial agents. Common fungal media includes Sabouraud dextrose agar, potato dextrose agar, brain heart infusion agar (with or without sheep cells), and selective media containing cycloheximide (Mycosel or Mycobiotic). Some isolates fail to grow on selective media so a non-selective medium should always be included. Set ups for dermatophyte species in the genera *Epidermophyton*, *Trichophyton*, and *Microsporum* typically include a medium containing cycloheximide. The use of dermatophyte test medium (DTM) agar alone may be misleading as other genera other than dermatophytes turn the agar red. CHROMagar Candida, selective and differential, may be used for specimens suspected of containing yeasts. \(^3\) Incubation (at 25°C to 30°C) beyond three weeks is seldom necessary for fastidious organisms and moulds, or beyond 7 days for yeasts. Appropriate personal protective equipment (PPE) should be used and direct specimens should be manipulated within a biological safety cabinet if available. All filamentous organism recovered in culture must be examined in a biological safety cabinet. \(^4\)

DIRECT MICROSCOPIC EXAM AND HISTOPATHOLOGY

The value of direct examination of specimens should not be underestimated. Many different techniques including the Gram stain, KOH preparations, fluorescent calcofluor white stains, etc., provide early clues in the diagnosis of fungal disease. Positive histopathology, however, is usually necessary to document an etiologic agent due to the ubiquitous nature of fungal organisms. Histopathological stains facilitate visualization of fungal elements in tissue. Common fungal stains, in addition to the hematoxylin and eosin (H &E), include the Gomori methenamine silver stain (GMS), the Masson Fontana stain for melanin in phaeoid genera, the Periodic acid-Schiff reaction (PAS), and the Mayer’s mucicarmine stain, to name a few. \(^6\) Reference # 6 provides excellent photomicrographs of the more common fungal genera in tissue. Do note, however, that many organisms mimic *Aspergillus* by histopathology, so the statement “suggestive of aspergillosis” should be used judiciously. In addition to submitting biopsy specimens for histopathology, culture, culture, culture!! Fungal identification by means of DNA extraction from formalin-fixed paraffin blocks is fraught with difficulties and/or not routinely available.
RADIOLOGY AND NON-CULTURE-BASED DIAGNOSTICS

Diagnostic radiology, in conjunction with the clinical evaluation, is integral to the diagnosis and management of most fungal infections. The more common imaging techniques include radiographs, magnetic resonance imaging (MRI), and computed tomography (CT). Although culture-based techniques to recover the etiologic agent remain the gold standard, non-culture-based diagnostics have become available in an effort to provide an earlier diagnosis and more timely antifungal therapy. “Tests are classified into four groups according to which component of the invading pathogen or host immune response they target. These include detection of host antibody, fungal antigen, fungal metabolites, or fungal nucleic acid. Overall, despite these multiple potential targets and extensive efforts toward development, only a handful of non-culture based tests have proven clinically useful, and even fewer have reached commercial availability.” They also all vary in sensitivity and specificity. Pan-fungal (1,3-ß-D-glucan testing, a major cell-wall component of many fungi, and galactomannan assays, a polysaccharide component of Aspergillus, are commercially-available, however several fungi cross-react with the latter and both may be cost-prohibitive in some settings.

PHENOTYPIC AND/OR MOLECULAR CHARACTERIZATION OF ISOLATES

The level of fungal identification that can be provided in any given site is often dependent upon the facilities available and the expertise/experience of individuals in the laboratory. While yeast identification for common species is typically performed by determining a battery of physiologic tests (manual or automated), mould identification still relies heavily on the observation of diagnostic phenotypic features, both macroscopic and microscopic, and a limited number of ancillary tests, including temperature tolerance. Identification of an isolate to the species level may depend upon the genus of the organism isolated or the level of identification required for appropriate patient management (i.e., varying susceptibility patterns for different species within the genus). With the recent evaluation of many genera by molecular characterization and the discovery of many cryptic species, the reporting of a “species complex”, rather than the species itself, provides a better assessment of the organism isolated. An example might be reporting an isolate as a member of the “Aspergillus terreus species complex” rather than as “Aspergillus terreus”, a known pathogen in dogs. This type of reporting would include A. alabamensis in this complex, which could subsequently be determined by molecular sequencing, if need be. Many reference books and identification guides are available which provide the salient features of the more common fungal pathogens. A particularly useful guide is the Atlas of Clinical Fungi, 2nd Edition.

Molecular characterization of isolates may provide a more definitive identification and typically relies on DNA target sequencing and a comparison with published databases, particularly those in GenBank. Common targets include ITS1 and ITS2, the D1/D2 domains, ß-tubulin, actin, calmodulin, translation elongation factor 1α, and others. It’s important to note that over 10% of these deposits may be incorrect so results should always be evaluated in light of phenotypic features. Also, isolates in GenBank are deposited under their teleomorph (sexual) names, so one must be able to correlate the anamorphic features in culture with the molecular identification name. Isolates that remain sterile in culture (i.e., fail to produce spores or conidia) are often impossible to identify with certainty, even by molecular methods, as there are no diagnostic features to support the sequence identification.

TREATMENT OPTIONS AND ANTIFUNGAL DRUG LEVELS

Treatment options include antifungal agents in several classes of drugs, the more common being the polyenes (amphotericin B, nystatin, natamycin), the antimetabolite pyrimidine (5-fluorocytosine), the azoles including the imidazoles and triazoles (fluconazole, itraconazole, voriconazole, posaconazole), the echinocandins (caspofungin, micafungin anidulafungin), the allylamines (terbinafine) and a few others such as griseofulvin. Their use in veterinary medicine is often based upon human pharmacokinetic data so the dose, class of agent, and length of administration are all major therapeutic considerations. Antifungal drug level testing is available in some reference laboratories and may be useful in monitoring therapy. Typically, drug levels above the MIC/MEC values for the isolate are desired. On a research basis, antifungal drug levels may help established pharmacokinetic parameters in various animal species.
TAXONOMY AND NOMENCLATURE

While this is the part of mycology that most would prefer to ignore, i.e. “delete”, (Why do they keep changing the names?), it’s also the part that helps separate these organisms into relatively large, identifiable groups. The following is a very simplified schematic of the Kingdom *Fungi* for most human/animal pathogens.

![Kingdom Fungi Diagram]

The Kingdom *Fungi* (Eumycota) (grossly simplified to illustrate placement of common moulds)↓ Sexual form (Teleomorph) ↓ Asexual form (Anamorph) (Synanamorph)

**ASCOMYCOTA**

**MITOSPORIC FUNGI**

**“ZYGOMYCOTA”**

**ASCYMYCOTA**

**MITOSPORIC FUNGI**

**HYPOMYCETES**

**COELOMYCETES**

**A Few Definitions**

Conidia – Asexual reproductive propagules

Spores – Sexual reproductive propagules, include ascospores, basidiospores, zygospores

Homothallic – only one strain required to produce teleomorph

Heterothallic - requires two mating strains to produce teleomorph

Hyphomycetes - bear their conidia free, various colors, methods of conidiogenesis, growth rates, etc.

Coelomycetes – bear their conidia within some type of asexual structure known as a conidioma, otherwise as above

Recognition of the overall placement of clinically significant fungi along with a few mycologic terms simplifies the complex area of taxonomy and nomenclature. A few caveats are in order, however. The term “Zygomycota” was first published without a Latin diagnosis in 1954 and is therefore currently considered invalid, as is the term “Zygomycetes”. The Order *Mucorales*, in the Subphylum *Mucormycotina*, houses several families that encompass the most common genera. Contained in the family *Mucoraceae* are *Rhizopus*, *Mucor*, *Lichtheimia* (formerly *Absidia*) and the lesser known genera *Rhizomucor*, *Apophysomyces*, and *Actinomucor*. Other families such as *Thamnidiaceae* and *Saksenaeaceae* include the genera *Cokeromyces* and *Saksenaea*, respectively, also seen in veterinary medicine. Until a comprehensive phylogenetic reclassification of the Kingdom *Fungi* is resolved, we should probably call the diseases caused by members of the *Mucorales* as mucormycoses rather than zygomycoses. Mucormycosis is often a rapidly progressive mycosis typically requiring surgical intervention and/or appropriate antifungal therapy with amphotericin B and/or possibly posaconazole. The other sexual Phyla, Ascomycota and Basidiomycota, remain.

The asexual or mitosporic fungi include the Hyphomycetes and Coelomycetes. In Hyphomycetes, conidia are borne free (not within some type of enclosed or semi-enclosed structure). Many genera of both hyaline (non-pigmented) and melanized (dark, phaeoid, dematiaceous) filamentous fungi are included under this umbrella. Most are connected to ascomycetous genera, but are heterothallic (require compatible mating strains to form their teleomorph = sexual stage) in culture so only produce their anamorphic state in the laboratory. These organisms make up the bulk of filamentous fungi inciting disease in both humans and animals and many are typically acquired by inhalation. Common examples of such genera include *Aspergillus*, *Fusarium*, *Paecilomyces* (hyaline) and *Phialophora*, *Alternaria*, *Curvularia* (melanized), to name a few. In Coelomycetes, conidia are borne within some type of enclosed or semi-enclosed structure known as a conidioma, and their method of acquisition is more commonly by traumatic implantation.

**ANTIFUNGAL SUSCEPTIBILITY TESTING**

Several methods for antifungal susceptibility testing are now available. Those with the most rigorous standardization include the Clinical Laboratory Standards Institute (CLSI) methods for yeast (M27-A3, M44-A2) and mould (M38-A2, M51A21) testing. While the pharmacokinetics of antifungal agents are unknown for most animal species, and defined breakpoints for “susceptible” or “resistant” are mostly unavailable, we can, however, compare MIC/MEC data for the isolate in question to a large battery of similar isolates (the same species) to help determine potential efficacy of the compound, *in vitro*. Anecdotal case reports are also common sources of determining clinical efficacy, *in vivo*. Antifungal susceptibility data may suggest more appropriate regimens when empiric therapy fails. Combination therapy with more than one antifungal agent may also be evaluated, *in vitro*, by synergy testing.
REFERENCES

AGENTS OF MUCORMYCOSIS

**Cokeromyces recurvatus.** A sixteen-year-old male, castrated, Siamese cat presented with a 4 week history of progressive lethargy and loss of appetite. The cat had been obtained in Minnesota as a kitten and had not traveled outside the state. Abdominal radiographs demonstrated a movable mass in the mid-ventral abdomen, and a large amount of abdominal effusion. There was a moderate leukocytosis with a left shift; tests for feline leukemia p48 and feline immunodeficiency virus antibody were negative. Large (to 100 µm) thick-walled organisms seen by abdominocentesis were most consistent with spherules of *Coccidioides immitis.* An abdominal ultrasound revealed an intestinal mass at the ileocecal junction. A FNA (fine needle aspirate) was inconclusive and a tentative diagnosis of fungal peritonitis and a small intestinal mass was made. Cat was started on itraconazole. He was subsequently diagnosed with a perforated viscus, and underwent an exploratory celiotomy. Turbid, yellow peritoneal fluid was present, and the mass was found to consist of omentum surrounding an area of perforated jejunum; no discrete mass was found. Lymph node and liver biopsies were obtained, and the peritoneal fluid was submitted for bacterial and fungal cultures. Serologic tests for cryptococcus, histoplasmosis, blastomycosis, and coccidioidomycosis were submitted and were negative. Biopsy samples revealed intermediate-grade jejunal lymphosarcoma of T-cell origin based on immunohistochemistry; omentum and mesentery showed severe pyogranulomatous inflammation, focal fat necrosis, and abundant fungal-yeast-like elements up to 100µm in diameter suggestive of *C. immitis* spherules. Cat deteriorated over the next 5 days and humane euthanasia was elected based on declining clinical status and a diagnosis of intestinal lymphosarcoma and fungal peritonitis. Fungal cultures from the peritoneal fluid grew *Cokeromyces recurvatus.* Necropsy samples from the abdominal wall, the omentum, and the liver demonstrated yeast-like organisms.¹ “Widespread fungal peritonitis probably developed after a perforated viscus went undiagnosed for almost 24 hours….” “The initial diagnosis of an abdominal mass and coccidioidal peritonitis resulted in medical management and significantly delayed discovery of and surgery for the perforated viscus”.²⁻³

*Cokeromyces recurvatus* is a homothallic mucoraceous organism producing both anamorphic (fruiting structures with vesicles, recurving stalks, sporangia and sporangiospores) and teleomorphic (zygospores) phases in culture. It is also thermally dimorphic, so may pose considerable problems from an identification standpoint.² In the host and at 37°C, a large yeast with multipolar budding similar to that seen with *Paracoccidioides brasilienensis* is produced.³ This organism has also been recovered from the peritoneal and pleural fluid in a 64-year-old man with a history of alcohol abuse who presented with severe abdominal pain and a ruptured duodenal ulcer⁴ and has been misdiagnosed as *C. immitis/posadasii* in a fatal *C. recurvatus* pneumonia.⁵

**Apophysomyces and Saksenaea species in marine mammals.** Over a ten year period (1999-2001) a killer whale (*Orcinus Orca*), two Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), and two captive bottlenose dolphins (*Tursiops truncatus*), all from the same facility, were infected with agents of mucormycosis. In four of the five cases, the fungi were identified as either *Apophysomyces elegans* or *Saksenaea vasiformis.* The primary site of infection was the subcutaneous tissue or skeleton muscle in dolphins and the placenta and uterus in the periparturient whale. The last of case in the bottlenose dolphin was treated with liposomal nystatin. All animals died or were euthanized between 23 and 39 days after clinical signs.⁶ In 2003, an adult captive female bottlenose dolphin and her 5-week-old female calf were both diagnosed with *A. elegans* at another marine park. Necropsy findings in the mother showed skin lesions and dissemination to the brain due to *A. elegans.* Primary skin lesions in the calf were surgically resected on two occasions, and she was placed on 5mg/kg posaconazole PO BID. Therapy with posaconazole for 17 months halted the infection and the calf survived more than two and a half years after the initial diagnosis with no further recurrence of the disease.⁷ Additional isolates of *A. elegans* from dolphins have subsequently been received at the Fungus Testing Laboratory (FTL) for fungal identification and/or antifungal susceptibility testing.
Apophysomyces elegans and/or Saksenaea vasiformis are aggressive, angioinvasive, and mostly lethal pathogens in bottlenose dolphins and killer whales. The species are difficult to identify in the laboratory due to lack of sporulation on routine media, and are likely underreported. Growth on Czapek Dox agar used for the aspergilla or growth in a 10% yeast extract water culture is usually necessary for sporulation. Apophysomyces and Saksenaea are recognized by their prominent apophysis and vase-shaped sporangium, respectively. Both genera have been characterized by only one species. They are also notorious for dying off with extended storage, so large numbers of isolates have been unavailable for study. Two recent studies involving multilocus sequence analysis and a reevaluation of phenotypic features have resulted in the description of new species in both genera. A recent study of 16 isolates of Apophysomyces elegans including the Type strain as well as human and dolphin strains (three isolates submitted to the FTL between 2003 and 2006) showed that this species was a complex made up of 4 different clades and representing 4 spp.: A. elegans, A. variabilis, A. trapeziformis, and A. ossiformis. Two dolphin isolates were identified as A. variabilis and one as A. trapeziformis. No human or animal isolates matched the Type strain from Indian soil previously considered to be pathogenic. It is currently unknown whether these new species display varying degrees of pathogenicity and/or whether they exist in some particular ecological niche. The susceptibility patterns for strains of A. variabilis (n=7), A. elegans (n=2), A. trapeziformis (n=5) and A. ossiformis (n=2) against amphotericin B and posaconazole do not vary significantly with geometric mean MICs in µg/ml of 1.0 and 1.1, 0.5 and 0.5, 0.8 and 0.8, and 1.4 and 0.7, respectively. A similar study with 11 strains of Saksenaea vasiformis separated this species into 3 clades including the S. vasiformis complex, S. erythrospora, and S. oblongispora. Again, with the 9 isolates tested against antifungal agents, no apparent difference were noted between species, however the sample size was small. High geometric mean MICs in µg/ml were noted for amphotericin B (4.1), voriconazole (4.7) and the echinocandin drugs (MEC = minimum effective concentration 4.0) while itraconazole, posaconazole and terbinafine were 0.2, 0.1, and 0.1, respectively.

BASIDIOMYCETES

Oxyporus corticola. A six-year-old spayed female German shepherd presented with a painful boney mass on the right distal tibia after limping for four weeks. Lab work was unremarkable, however chorioretinal lesions of unknown origin were observed on retinal examination. Radiographs demonstrated a proliferative mass and a FNA of the lesion demonstrated macrophages and branching hyphae with parallel walls suggestive of aspergillosis. Rare fungal hyphae were also present on a prescapular lymph node by FNA indicating a disseminated infection. A biopsy of the tibial lesion was cultured for bacterial and fungal pathogens. No bacterial growth was noted, however a sterile, white, filamentous mould was recovered on the Sabouraud dextrose agar. The isolate was referred to the FTL where it was tentatively identified as a basidiomycetous organism. The dog was administered oral compounded itraconazole again at 200 mg BID. Ten months later the tibial lesion had progressed down the limb into the joint with purulent inflammation observed in a hock joint. Microbiologic studies were negative. Amputation of the limb and a change to amphotericin B was suggested but refused. Terbinafine was then discontinued and the dog was placed on itraconazole again at 200 mg BID. Worsening of the lesions, progressively worsening ataxia, and lesions in the brain detected by CT led to the euthanization one year and eight months after the initial presentation. Necropsy samples demonstrated hyphae in the heart, endocardium, kidneys, endocrine glands (adrenal and thyroid) and multifocal small granulomas in medulla of the bone. In vitro antifungal susceptibility testing of the isolate suggested susceptibility to amphotericin B and itraconazole with MICs of 0.5 and 0.06µg/ml, respectively. The organism was subsequently identified as Oxyporus corticola by D1/D2 sequencing and comparison of the isolate to known strains of O. populinus and O. corticola obtained from the Forest Products Laboratory at the USDA Forest Service in Madison, WI.

This organism is a white-rot decay fungus of various woody angiosperms and gymnosperms characterized by leathery fruiting bodies with a cream to light brown pore surface. In the laboratory the isolate is a white, rapidly growing filamentous mould that remains sterile in culture. The growth of such isolates on benomyl agar is suggestive of a basiomycete. Very few filamentous basidiomycetous fungi are documented agents of human or animal disease. They are likely underreported as most fail to make diagnostic structures in the laboratory.
Schizophyllum commune is an exception in that dikaryons may be recognized by spicule formation, clamp connections, and occasionally, basidiocarps. S. commune is the only basidiomycete to have been previously reported in the veterinary literature in a mongrel dog from Japan.12

**HYPHOMYCETES**

*Geosmithia argillacea*. A four-year-old spayed female German shepherd presented in February 2008 for acute-onset glaucoma of the right eye. Vitreal debris and exudative retinal detachment were also noted and an intraocular pressure of 27 mmHg by rebound tonometry. A diagnosis of panuveitis and secondary glaucoma was made and topical steroids were administered. The dog was suspected of having an underlying systemic disease and was further evaluated. Antibody titers for *Leptospira* and *Brucella canis* were negative as was the urinary antigen for *Blastomyces dermatitidis*. Radiographs of the spine showed osseous proliferation and lysis of the vertebral endplate of the thoracic vertebrae four, five, and six consistent with discospondylitis. Similar changes were also seen in multiple sternebrae. In March 2008 the dog was blind in the right eye with end-stage glaucoma and an intraocular pressure of 50 mmHg. Globe was enucleated and biopsies of multiple sternebrae taken. Bacterial cultures of the vitreal aspirate, sternebral biopsy, and urine were negative. Gomori methenamine silver stains of the lens, retina, and sternebrae showed dichotomously branching hyphae compatible with aspergillosis. A cystocentesis urine sample for fungal culture grew a *Penicillium*-like organism subsequently identified as *Geosmithia argillacea* by morphologic features and D1/D2 sequencing.13,14 The dog became increasingly agitated over the next month, developed a head tilt and nystagmus, had a retinal detachment in the left eye, and humane euthanasia was elected. Necropsy samples from the lungs, pancreas liver, kidney, and cerebrum had multifocal regions of granulomatous inflammation with some granulomas containing fungal hyphae with bulbous ends. Cultures from all tissue except the brain were positive for an organism identical to the one recovered from the urine. Isolates from the urine and the necropsy sternebrae were identical by molecular characterization. Post-mortem antifungal susceptibility testing suggested susceptibility to itraconazole, posaconazole and caspofungin.15

The salient features of *Geosmithia argillacea* include the lack of a green color, growth at 45°C, roughened stipes, metulae, and phialides, and cuniform (wedge-shaped) to ellipsoidal conidia borne in long chains. The organism is superficially similar to other genera such as *Aspergillus* and *Paecilomyces*, also seen in dogs. Following this report, it also appears to be an emerging human pathogen in with cystic fibrosis patients, as well as those with chronic granulomatous disease.16-18

**Waterborne Exophiala species.** From the period January 2002 to March 2007, infections by melanized fungi were identified with greater frequency in aquarium-maintained leafy seadragons (*Phycodurus eques*) and weedy seadragons (*Phyllopteryx taeniolatus*). These species are pivotal to the educational and environmental concerns of the aquarium industry and conservation groups. Many of these isolates were referred to the FTL and were the focus of a large study of waterborne *Exophila* spp. and the doctoral dissertation of Akinyi Nyaoke at the University of Connecticut.19 Clinical signs in both species included “...weakness, loss of appetite, lethargy, increased respiratory rate and effort, abnormal buoyancy, listing, piping at the surface of the water, and death. Fungal dermatitis was diagnosed antemortem in some cases via cytology or biopsy of lesions, and antemortem fungal culture isolates of *Exophila* sp. nov. in 2 such cases.”20 Necropsy samples revealed multiple, well-demarcated, and occasionally extensive black foci in the kidney, swim bladder, and intestinal wall. Systemic necrotizing lesions and invasion of blood vessels were consistent features. Microscopically, hyphae were 2-3 µm in diameter and stained dark in hematoxylin and eosin and Fontana-Masson stains. Isolates were identified as either *E. angulospora* or a previously unidentified sp., i.e., *Exophila* sp. nov. A large study of waterborne species is soon to be published in Studies in Mycology (www.studiesinmycology.org).

*Exophila* spp. (in the Order *Chaetothyriales*)21 are melanized (dark, phaeoid, dematiaceous) fungi and agents of phaeohyphomycosis. Waterborne spp. are documented etiologic agents of cutaneous and disseminated infections in cold-blooded animals and fail to grow at 37°C. Species produce a dark, yeast synanamorph as well as filamentous growth. Sequencing is typically required for identification as morphologic features are similar between species.
**Chrysosporium ophiodiicola.** An adult black rat snake (*Elaphe obsolet obsolete*) was found in an old barn in Sparta, GA by his current owner of 4 years, a wildlife educator. The snake was used in public educational performances, and presented with a history of prolonged anorexia and slow-growing facial masses on the right ventral mandible and the right eye. The submandibular mass was a discrete capsule and was removed in its entirety. The eye mass was friable, locally extensive, and only partially resected. Both masses were submitted for histopathology and culture. Snake was treated with meloxicam and enrofloxacin until the histopathology report demonstrated fungal hyphae. Enrofloxacin was discontinued and replaced with ketoconazole 50mg/kg administered daily. Snake expired two month after surgery.22

Both masses consisted of multi-focal granulomas containing hyphae that were broad, parallel-walled, and occasionally branching. The fungus recovered produced white to pale yellow granular colonies, produced conidia borne on stalks as well as arthroconidia, and gave off a strong, pungent odor. The isolate resembled a *Chrysosporium* sp. but did not match known species. ITS and D1/D2 sequencing identified the isolate as a new species, *C. ophiodiicola* (Entymology: Greek ophio, snake.)22 The *Chrysosporium* anamorph of *nanniziopsis vriesii* (CANV) was closest species which has been associated with infections in a variety of reptiles.

**COELOMYCETES**

**Mycoleptodiscus indicus.** An eight-year-old outdoor, male, castrated pointer dog presented in April 2009 for blood work 2 months after diagnosis of immune-mediated hemolytic anemia. Immunosuppressive therapy consisted of prednisone and cyclosporine. Physical exam revealed potbellied appearance, hepatomegaly, moderate to marked cachexia, a swollen left rear leg with pitting edema and a draining tract on the lateral aspect of the hock, a markedly enlarged left popliteal lymph node, and several area of minor dermal excoriations along the nasal planum. There was a weight loss from 38.5 to 35 kg in two months. Clinical differentials for the draining tract included phaeohyphomycosis, zygomyccosis, pythiosis, lagenediosis, sporotrichosis, nocardiosis, actinomycosis, and mycobacteriosis. FNA of draining lesions showed septate hyphae. Dog started on itraconazole 5 mg/kg and terbinafine 32 mg/kg. Culture of the aspirate produced woolly gray colonies, unrecognizable hyaline conidia at 10 days (25 and 35°, and no acceptable percent identity in the NCBI GenBank database using the BLASTn algorithm. In May 2009 dog had multiple new subcutaneous nodules along rib cage and distal limbs which waxed and waned during treatment, and subsequently presented to the emergency service for lethargy, regurgitation, and aspiration pneumonia secondary to megaesophagus. The dog developed severe clinical signs of iatrogenic hyperadrenocorticism, was discharged to hospice care at the end of May, and expired mid-June, 2009.

Additional phenotypic and molecular characterization at the FTL identified the isolate as *Mycoleptodiscus indicus*.23 Isolate is a coelomycete, typically considered a plant pathogen, characterized by the formation of appressoria, and the production of curved, two-celled conidia formed in small, sporodochial aggregates. This isolate extends the morphologic features seen in this species (septate conidia with lateral appendages) and is the first report of infection in a dog. Antifungal susceptibility data for this isolates suggested susceptibility to amphotericin B, itraconazole, voriconazole, posaconazole, and terbinafine.23
References

Cyclosporine (CsA) has potent immunosuppressive properties that result from blocking the transcription of cytokine genes in activated T cells. It has been used for years in people to prevent organ transplant rejection and more recently for the treatment of immune-mediated diseases such as rheumatoid arthritis, psoriasis, and atopic dermatitis. Cyclosporine is used infrequently for the prevention of renal transplant rejection in cats, but is now frequently being prescribed for the treatment of feline allergic dermatitis, eosinophilic plaques and granulomas, allergic cervico-facial pruritus, other immune-mediated dermatosis, stomatitis, inflammatory bowel disease, allergic bronchitis, and some immune-mediated cytopenias.

Because of the potent immune suppressive effect of CsA, activation of infectious agents is a significant clinical consideration, particularly in cats currently treated with glucocorticoids. The majority of reported cases of CsA activation of infections have been in cats undergoing renal transplantation. In a retrospective study of 169 cats, approximately 25% had infectious disease complications; bacterial, viral, fungal, and protozoal infections were noted in some cats (Kadar et al, 2005). For renal transplantation, high doses of CsA combined with prednisolone are usually prescribed. Whether activation of infectious agents is common in cats treated with lower, anti-inflammatory doses of CsA is currently unknown (5.0 mg/kg, PO, q24-48 hr). Compared to dogs, CsA in cats has a higher bioavailability and longer clearance and elimination half-life. Plasma concentrations can vary among littermate cats given the same dose of CsA which may in part explain the apparent variation in risk of infectious side-effects. Monitoring trough plasma CsA levels to identify cats with high CsA concentrations in an attempt to lessen the potential for activating infectious diseases should be considered by clients that can afford repeated testing. The following is a summary of infectious agents that have been or potentially could be activated by the immunosuppressive effects of CsA.

**Cutaneous infections.** In cats undergoing renal transplantation, bacterial infections at the site of feeding tube placement was common. Additionally, use of high doses of CsA appears to predispose these cats to local or disseminated *Mycobacterium* spp. infections. Administration of CsA has lead to the activation of dermatophytosis in cats that were either not previously infected or were subclinically infected. Bacterial pyoderma should be treated in cats to be administered CsA. Housing CsA treated cats indoors in an attempt to avoid exposure to soil-associated bacteria and fungi as well as cat fights may be indicated.

**Gastrointestinal infections.** In previous studies, the most common gastrointestinal agents (prevalence rates of approximately 5%) found in cats include *Giardia* spp. and *Cryptosporidium* spp.; infected cats are frequently clinically normal (Hill et al, 2000; Spain et al, 2001). In addition, adult cats were frequently shedding *Toxocara cati* eggs (approximately 4%) and kittens were rarely shedding *T. gondii* oocysts (usually < 1%). In the same studies, *Salmonella* spp. and *Campylobacter* spp. were uncommon (approximately 1%). Each of these infectious agents is zoonotic and so if shedding was exacerbated by CsA, human health could also be affected. Other infectious agents commonly inhabiting the gastrointestinal tract include *Cystoisospora* spp. and *Clostridium* spp. In cats with diarrhea, a complete gastrointestinal workup including a fecal flotation, fecal wet mount examination, and *Cryptosporidium* spp. screening test like IFA should be considered prior to administration of CsA (Brown et al, 2003). A complete diagnostic evaluation is also indicated in cats that develop diarrhea while being administered CsA. Whether there is clinical benefit to performing fecal diagnostic tests or administering drugs with activity against enteric pathogens prior to the administration of CsA to cats with normal stools is unknown. However, prescribing *Dirofilaria immitis* drugs that also aid in the prevention or control of select internal and external parasites may be of benefit and is considered good preventative medicine by many veterinarians (Brown
et al, 2002; www.capcvet.org). Housing CsA treated cats to restrict hunting behavior and feeding processed foods should be considered to attempt to lessen exposure to enteric pathogens.

**Polysystemic infections.**

*Bartonella spp.* Cats have been proven to be infected by *Bartonella henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. quintana* and *B. bovis* by culture or DNA amplification (Brunt and colleagues, 2007). *Bartonella henselae* is the most common cause of cat scratch disease, as well as bacillary angiomatosis and peliosis hepatitis, common disorders in humans with AIDS. Based on results of seroprevalence studies, culture, or polymerase chain reaction (PCR) assay, cats are commonly exposed to or infected by *Bartonella* species. The organism is transmitted between cats by *Ctenocephalides felis* and so prevalence is greatest in cats from regions where fleas are common. The seroprevalence rate in cats likely to have been exposed to fleas can be as high as 93%. In a recent study in the United States, we collected fleas from cats and attempted to amplify *Bartonella* species DNA from flea digests as well as the blood of the cat (Lappin et al, 2006). The prevalence rates for *B. henselae* in cats and their fleas were 34.8% and 22.8%, respectively. The prevalence rates for *B. clarridgeiae* in cats and their fleas were 20.7% and 19.6%, respectively. Results are similar in other studies performed around the world. *Bartonella henselae* survives in flea feces for days after being passed by infected *C. felis*. Infected flea feces are likely to contaminate cat claws during grooming and then *Bartonella* are inoculated into the human when scratched. It is also possible that open wounds are contaminated with infected flea feces and so working with cats with fleas can be an occupational health risk for veterinarians. In addition, *Bartonella* species DNA can also be amplified from the mouths of healthy cats and those with gingivostomatitis, and so bites and scratches should be avoided (Quimby et al, 2008). *Bartonella* spp. infection of cats has been linked to fever, lymphadenopathy, hematuria, and uveitis; other manifestations are proposed but not proven. While *Bartonella* spp. infections are extremely common in cats, it is currently unknown whether performing diagnostic tests or administering antibiotics with anti-*Bartonella* effects to cats to be administered CsA has clinical benefit. It appears unlikely that *Bartonella* spp. infections of cats can be cleared with routine antimicrobial drugs and there is no permanent immunity. Thus, testing or treating healthy cats for *Bartonella* spp. infections it is currently not recommended (Brunt et al, 2007). However, if cats with a history of fleas develop clinical signs consistent with *Bartonella* spp. infection while treated with CsA, diagnostic tests or treatment may be indicated. In cats, doxycycline (10 mg/kg, PO, q24hr) or fluoroquinolones are generally effective for the treatment of bartonellosis. Use of flea control products and housing CsA treated cats indoors to avoid fighting may lessen potential for exposure to *Bartonella* spp.

*Haemoplasmas.* The new names for *Haemobartonella felis* are *Mycoplasma haemofelis* (Mhf), ‘*Candidatus Mycoplasma haemominutum*’ (Mhm), and ‘*Candidatus M. turicensis*’ (Mtc). It is likely all 3 organisms infect cats worldwide. Mhf is apparently the most pathogenic of the organisms but disease has been detected in infected with any of the agents. In a recent study, we collected fleas from cats and attempted to amplify hemoplasma DNA from flea digests as well as the blood of the cat. The prevalence rates for Mhf in cats and their fleas were 7.6% and 2.2%, respectively. The prevalence rates for Mhm in cats and their fleas were 20.7% and 23.9%, respectively. Transmission by biting has been hypothesized and we have recently documented hemoplasmas in the mouths of cats with and without fleas. Clinical signs of disease depend on the degree of anemia, the stage of infection, and the immune status of infected cats. Coinfection with FeLV can potentiate disease associated with Mhm. Clinical signs and physical examination abnormalities associated with anemia are most common and include pale mucous membranes, depression, inappetence, weakness, and occasionally, icterus and splenomegaly. Fever occurs in some acutely infected cats and may be intermittent in chronically infected cats. Evidence of coexisting disease may be present. Weight loss is common in chronically infected cats. Cats in the chronic phase can be subclinically infected only to have recurrence of clinical disease following periods of stress. While haemoplasmas are common, only one cat undergoing renal transplantation has been reported to have activated hemoplasmosis. Haemoplasma infections should be considered in cats administered CsA that develop classical clinical signs of disease. Currently, the diagnostic test of choice is PCR which is more sensitive and specific than cytological examination of a blood smear. Doxycycline (10 mg/kg, PO, q24hr) and fluoroquinolones have anti-haemoplasma effects. It appears unlikely that haemoplasma infections can be cleared and there is no permanent immunity. It is currently unknown whether performing haemoplasma PCR or administering antibiotics to cats to
be administered CsA has clinical benefit. As for *Bartonella* spp., use of flea control products and housing CsA treated cats indoors to avoid fighting may lessen exposure.

*Retroviruses.* Cats are still commonly infected with FeLV and FIV (approximately 2% in the United States). Cats have become FeLV positive after administration of CsA for renal transplantation, suggesting activation of latent infection. While the effects of anti-inflammatory doses of CsA on cats with subclinical FeLV or FIV infection are unknown, it seems prudent to assay all treated cats prior to initiation of CsA. If positive, the potential for activation of FeLV or FIV associated clinical syndromes or exacerbation of retroviral associated immunosuppression should be discussed with the owners. It is currently unknown whether administration of interferon or other compounds with anti-viral activity is indicated.

To *Toxoplasma gondii.* In a recent study of 12,628 clinically ill cats tested by our laboratory, we showed that 31.6% of cats of the United States are seropositive for *T. gondii* IgM or IgG (Vollaire et al, 2005). Oocysts shed by *T. gondii* infected cats can sporulate and be infectious to humans. While we previously showed that cats with acute or chronic *T. gondii* infection did not repeat oocyst shedding when administered clinical doses glucocorticoids (Lappin et al, 1992), there is no similar published information in cats treated with CsA. In one unpublished study in our laboratory, we showed that *T. gondii* oocyst shedding was not reactivated by administration of anti-inflammatory doses of CsA. In addition, cats administered CsA before *T. gondii* shed similar numbers of oocysts for a similar duration as cats infected *T. gondii* and not administered CsA. *Toxoplasma gondii* infection of cats results in tissue infection of a variety of organs including liver, brain, lungs, and muscle (Dubey et al, 1998). Most cats are subclinically infected but bradyzoites remain in the tissues for life. If activated by extreme immune suppression, the organism replicates as tachyzoites which destroy infected cells, often resulting in death. Recently, activated toxoplasmosis has been recognized cats undergoing renal transplantation and cats with dermatological disease treated with CsA. In an unpublished study in our laboratory, we showed that cats infected with *T. gondii* prior to CsA administration failed to develop clinical illness after administration of CsA. However, cats that have high CsA concentrations when first exposed to *T. gondii* can develop fatal infection. Thus, it is imperative that *T. gondii* seronegative cats treated with CsA avoid exposure. Further data is needed to determine whether *T. gondii* seropositive cats will have frequently have exacerbation of subclinical infection. It is likely that potential for *T. gondii* activation may relate to CsA concentrations in individual cats and so plasma concentrations should be monitored in seropositive cats or cats allowed to hunt. *Toxoplasma gondii* is not cleared from the tissues of cats treated with clindamycin, potentiated sulfas, or azithromycin. Thus, the benefit of treating *T. gondii* seropositive cats prior to administration of CsA is unknown. However, some renal transplantation programs recommend chronic clindamycin administration in *T. gondii* seropositive cats while on CsA and prednisolone. Attempts should be made to avoid *T. gondii* exposure in all cats to be administered CsA. This can be accomplished in most cats by restricting hunting behavior (including potential transport hosts that may enter the house) and feeding processed or cooked foods.

**Respiratory tract infections.** Information concerning activation or potentiation of respiratory tract infections in cats treated with CsA is minimal. However, cats undergoing renal transplantation have developed suspected viral rhinitis or cryptococcosis. Cats can be subclinical carriers of feline herpesvirus 1, feline calicivirus, *Bordetella bronchiseptica*, *Mycoplasma* spp., and *Chlamydia felis*. Thus, potential for activation of respiratory tract disease after administration of CsA is possible. However, it is currently unknown if there is clinical benefit to performing diagnostic tests for these agents as the positive and negative predictive value of PCR panels and cultures are low. It is also unknown whether treating subclinical carriers (eg. lysine for feline herpesvirus 1 or doxycycline for *C. felis*, *Mycoplasma* spp. or *B. bronchiseptica*) prior to the administration of CsA is indicated. Cats treated with CsA should not be housed with cats with active signs of respiratory tract disease if possible. If vaccinations for feline herpesvirus 1, feline calicivirus, and panleukopenia are deemed necessary in CsA treated cats, an inactivated product should be used.

**Urinary tract infections.** While bacterial urinary tract infections in cats are rare, cats treated with CsA after renal transplantation have developed urinary tract infections or had subclinical infections exacerbated. An
urinalysis to evaluate for bacteriuria or pyuria followed by aerobic bacterial culture and antimicrobial susceptibility testing in appropriate cases could be considered prior to administering CsA.

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FOOD ALLERGY IN ANIMALS

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INTRODUCTION
Practising veterinarians all recognize animals in which pruritus, otitis or gastrointestinal symptoms are ameliorated by a change in diet and clinical signs will return after oral provocation with a previously fed dietary item. What is unknown however, in the majority of cases, are the mechanisms underlying this apparent adverse reaction to food and whether these cases truly represent a food allergy. The majority of allergic reactions to food in people are mediated by IgE and although this is assumed in the dog for example there is scant evidence to support this theory.

Collection of objective data from client owned animals is challenging, especially when large numbers of animals would need to be examined to account for breed variations. Additionally selection of animals with an allergy to a specific foods can be time consuming and may not result in a homogenous population as some individuals are additionally sensitized to environmental allergens.

Adverse food reaction: any clinically abnormal response attributable to the ingestion of food or food additive
Food intolerance: abnormal physiological response to food with no immunological basis
Food allergy: Immunologically mediated adverse food reaction.

What evidence would we require to be confident in a diagnosis of food allergy?
1. Repeatable improvement with a change in diet and relapse on challenge with previously fed food.
2. Allergen specific activation of the immune system associated with clinical deterioration.

Probably most research in this area has been performed in the field using animal models. In general animal models of food sensitization have required non-physiological routes of allergen exposure (subcutaneous, intra-peritoneal etc) along with alum adjuvants. Dogs have been selected for high IgE antibody production following viral infection or immunization. Subsequent serum allergen specific IgE titres and positive intradermal and gastroscopic food sensitivity testing are described after sensitization.

The pivotal question in consideration of these models is to what degree they mimic the naturally occurring disease?

DOGS
Spontaneous
Dogs with reported in the literature with AFR have been identified by feeding limited antigen diet containing novel or hydrolysed proteins which is selected after detailed review of the individual’s dietary history. A challenge with previously fed foods is performed and clinical deterioration demonstrated. On the basis of these clinical observations we can only describe these animals as having dietary response disease. These papers are reviewed in my second lecture.

Canine adverse food reactions (AFR) often looks clinically similar to canine atopic dermatitis (CAD). Although a sub-population of dogs with different clinical presentations and age of onset also appears to exist. In a Swiss study in which the allergic population was compared with all registered dogs, West Highland White Terriers, Rhodesian Ridgebacks and Pugs were predisposed. Gastrointestinal signs were more common in the population and clinical signs tended to develop earlier 48% <1 year as compared with 16% of dogs with CAD (Picco, Zini et al 2008)¹. These findings are similar to a study carried out by the author in North Carolina (38% < 1year).

Intradermal skin test reactivity to food antigens can be performed and circulating food allergen specific IgE can be measured in dogs with suspected food allergies although at this time these tests are unreliable in the diagnosis or prediction of canine food allergy. Whether this relates to the test methodology, allergens employed or the lack of IgE involvement in canine AFR is unclear.
There is some evidence that AFR may be mediated by IgE in some dogs. Increased IgE specific to bovine serum albumin was identified in dogs with clinical hypersensitivity to beef but not in normal dogs (Ohmori et al 2005). Additionally, increased histamine release after food antigen specific stimulation of peripheral blood leucocytes harvested from affected dogs supports a role for for IgE (Ishida et al 2004).

Canine models

The Maltese x beagle; dogs with naturally occurring food allergy at North Carolina State University. This colony was originally established to express an autosomal recessive glycogen storage disease. Dogs fed on a regular canine diet from weaning developed allergies to components of that diet, notably corn, soy, milk and pork. Food allergies manifest as pruritus of the feet, limbs, face, ears and ventrum as early as 4 months of age and within hours of ingesting specific proteins. An allergen specific IgE response has also been measured in these dogs after oral challenge leading us to conclude that, at least in this group of dogs food allergy is IgE mediated (Jackson et al 2003). Furthermore, treatment with oral cyclosporine failed to ameliorate that acute response to oral challenge with food allergen supporting a role for acute histamine release (Jackson).

Non-physiological sensitisation of colonies of high IgE responder dogs to food antigens has facilitated by-pass of normal immune tolerance. The timing of sensitisation has been shown to be critical to the subsequent development of a robust IgE response. Predictable outcome measures (clinical and immunological) allow for the testing of novel therapeutic strategies such as testing the immunogenicity of genetically modified foods, or treatment strategies for nut allergies in man (Day 2005).

CATS

Although AFR is recognized as a clinical entity in the cat the clinical dermatological manifestation can be variable. It has been suggested that facial pruritus may be more indicative of AFR but a recent large multicentre study did not support this theory. There does not appear to be a specific age of onset in this species. In one study 55 cats with GI and/or dermatological signs improved with dietary restriction and clinical signs recurred with provocation. Serum allergen specific IgE measurements had limited value as a screening test and gastroscopic food sensitivity testing was not helpful. (Guilford et al).

HORSES

Adverse food reactions in the horse have not been demonstrated definitely although there is clinical testimony to the existence of the condition. Although classically AFR is considered a non-seasonal problem, seasonality may be recognized in this species dependent on grazing and feeding practices. The clinical presentation can be variable. The horse may present with focal or generalized pruritus and chronic urticaria. As for other species the diagnosis rests on demonstrating an improvement with dietary restriction and relapse on challenge.

PIGS

Pigs develop transient post-weaning allergy to soy allergens which can be prevented by pre-weaning feeding of soy protein in sufficient quantity. Pigs have also been used as an experimental model of food allergy as they develop cutaneous and enteric clinical signs similar to those in humans (Rupa et al 2009).

RODENTS

There are a number of rodent models which have been developed to study hypersensitivities to food allergens (Takeda & Gelfand 2009). Sensitisation is often performed parenterally combined with an adjuvant although oral sensitization has been described. Although much has been learned from these models there are limitations in translation to similar diseases in other species.

Clinical implications
Adverse food reactions are recognized as a clinical entity in client owned animals but good data supporting an immunological basis for this disease is lacking. Most robust information is derived from canine and rodent models which may not necessarily reflect the spontaneous disease in the companion animal population.

References
FOOD ALLERGY IN HUMANS

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Mechanism of Human Food Allergy

Adverse reactions to foods in humans are characterized as either immune mediated or non-immune mediated. A food allergy is defined as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food. Therefore, food allergies are immune mediated responses to food. 1

Most food allergies in humans are induced by two major immunologic mechanisms, IgE mediated and/or non-IgE mediated. Conceptually, it is most practical to group diseases into three groups, IgE-mediated, non-IgE mediated and mixed disorders. Classically, IgE mediated disorders occur when food specific IgE antibodies on the surface of mast cells and basophils bind circulating ingested food allergens and activate the cells to release cytokine and other potent mediators, like histamine. The typical classic symptoms in these reactions occur immediately after food ingestion resulting in urticaria, angioedema, wheezing, cough, nausea, vomiting and, in some cases, hypotension. This is the mechanism which mediates anaphylaxis after food ingestion.

Non-IgE mediated food allergies are considered the result of the production of mediators like cytokines IL-4, IL-5, and IL-13 which further the allergic response (called T\textsubscript{H}2 cytokines). Eosinophilic inflammation can result from this cascade of events. These reactions are generally slower in onset (greater than 4 hours after ingestion) and are primarily gastrointestinal reactions. Examples of non-IgE mediated conditions include food protein enterocolitis syndrome, eosinophilic proctitis, dermatitis herpetiformis, celiac disease and contact dermatitis. 2 Both IgE and non-IgE mechanisms can work together to exacerbate diseases like atopic dermatitis and eosinophilic gastrointestinal disease.1,2

Food Allergens in Humans

Food allergens are defined as those specific components of food or ingredients within food (typically proteins, but sometimes also chemical haptens) recognized by allergen specific immune cells that cause specific immunologic reactions resulting in characteristic symptoms. 1 Although many food proteins are theoretically capable of producing allergic responses, the true clinically relevant number of food allergens is quite small. The vast majority (greater than 85%) of significant food allergic reactions occur to milk, egg, peanut, wheat, soybean, tree nuts, shellfish and fish.3,4

Prevalence of Food Allergy

Food allergies in humans are important in public health because they affect adults and children and may be increasing in prevalence. Self reported food allergy occurs in 12-13% of humans and, when food challenges are used to confirm food allergy, the prevalence decreases to 3%.1 Prevalence of food allergy is higher in certain high risk groups. These include individuals with atopic dermatitis, certain pollen sensitivities and latex.5 Up to one third of patients with atopic dermatitis have skin exacerbations after the ingestion of food.5 In individuals with urticaria and/or angioedema of less than 6 weeks duration, the prevalence of food allergy is 15-20%.6 Four to eight percent of individuals with asthma have food allergies.7
Risk Factors for Human Food Allergy

Risk factors for the development of food allergy include a younger age, as the prevalence in children, especially young children under 3 years old, is higher than that in adults. A family history of atopic disease increases the rate of food allergy for individual four-fold. Familial atopic diseases which place individuals at risk include asthma, allergic rhinitis, atopic dermatitis and food allergy. Atopic dermatitis is the highest risk factor of all of these diseases.

Diagnosis of Human Food Allergy

The diagnosis of food allergy begins with an accurate history with attention to pertinent details. Some general guidelines apply for the evaluation of food allergy. It is important to note that, despite the most skilled medical history taking, the parent’s history is notoriously inaccurate in identifying food allergies. This is demonstrated by the fact that 13% of people believe they have an allergy to a food but only 3% of these suspected allergies are confirmed with positive oral challenges. Also, reacting to three or more foods is very rare. Most food induced IgE mediated allergic reactions occur within minutes to a few hours after ingestion. IgE mediated food allergy is essentially excluded if symptoms occur > 4 hrs after ingestion. The historical details which may help delineate the causative food include the quantity ingested, time course of reaction, activities or other medications surrounding the ingestion (i.e. exercise, aspirin, alcohol), reaction consistency, treatment and the nature and time course of the response to the treatment.

True IgE mediated food allergies involve the classical signs and symptoms affecting the skin, gastrointestinal tract and respiratory systems. Anaphylaxis, urticaria, angioedema, wheezing, cough, difficulty breathing from bronchoconstriction, cutaneous pruritus, recurrent vomiting, diarrhea and hypotension can all be clinical symptoms of food allergy. If a person eats a food, is suspected to have an IgE mediated reaction afterwards, and subsequently tolerates the food, this food should be removed from the list of potential offending foods. Also, it is reasonable that a food which is ingested infrequently is more likely to be responsible for reactions than a food which is regularly ingested. The ingredients on the label of a processed ingested food may be important in identifying the suspect allergen. It is rare, but occasionally added spices may be the culprit for a reaction. Only a very small number of additives have been implicated in food adverse reactions.

All physicians must be aware of the possibility of ingestion of one of the major food allergens (cow’s milk, egg, soy, wheat, peanut, tree nuts, shellfish or fish) through cross contamination or through “hidden ingredients.” An example of cross contamination may occur when sufficient milk contamination may occur provoking an allergic reaction when a “boxed” fruit drink is packaged on a “non dedicated” line used to package milk drinks. Another example would be a shellfish contaminated hamburger cooked on a grill which was previously used to cook shellfish without cleaning of the grill between preparations. “Hidden ingredients” may also be peanut or nut products added to flavor or to thicken sauces (i.e. spaghetti sauce, gravies and barbecue sauces) in baked goods.

The medical history for chronic disorders triggered by food allergies (atopic dermatitis, asthma, and allergic eosinophilic gastroenteritis) has a poor predictive value for the identification of food allergic patients. Acute reactions after the isolated ingestion of a single food, like peanuts, have a much higher predictive value. Acute urticaria is more likely than chronic urticaria (urticaria lasting greater than 6 weeks) or asthma to be associated with food allergies. For individuals with atopic dermatitis and allergic eosinophilic esophagitis, diet diaries may be helpful in identifying a trigger food. Behavioral changes are not manifestations of food allergy. Headaches are also not typical manifestations of food allergies.

The physical examination is used to evaluate the cutaneous, gastrointestinal and respiratory systems. The presence of atopic dermatitis would increase the chances that the patient has food allergies since up to 34% of patients with atopic dermatitis have a food allergy. Physical findings, like allergic shiners, conjunctival injection, clear rhinorrhea, nasal congestion with a pale, edematous nasal mucosa, a transverse nasal crease, wheezing, and xerosis or patches of eczema suggest the presence of other atopic disease and increase the
likelihood of coexistent IgE-mediated sensitivity to foods. Evidence of weight loss or failure to thrive is more common in non-IgE mediated allergy or gastrointestinal enteropathies than in IgE mediated food allergy. After the history and physical examination delineates the likely clinical syndrome, and whether the reaction was acute (<4 hours), late 6-48 hours or chronic in nature, and the severity of disease, the next step is the determination of the general approach for testing and management. If an immunologically mediated process is suspected, the reaction can be categorized as IgE mediated, non-IgE mediated or a mixture of both. The determination of the presence of IgE to the suspected foods is helpful to diagnose an immunologically mediated condition.

**Testing Methods for Human Food Allergy**

The two methods of measuring specific IgE to food are the **immediate hypersensitivity skin prick test and the in vitro serum specific IgE test**. These tests are highly sensitive (>90%) but only modestly specific. (50%) Therefore, panels or broad screening should NOT be performed without supporting history because of the high rate of false positives. These tests should only be performed when the clinical suspicion is very high for allergy to a food. Both the modalities detect the presence of IgE to specific foods which is not synonymous with clinical reactivity. When a person has the presence of food-specific IgE, this is called “sensitization.” The amount of specific IgE which correlates with clinical reactivity differs depending upon the specific food.

Prick skin tests using commercial extracts are typically used in the evaluation of food allergy, but fresh extracts must be used for fruits and vegetables since the proteins in these foods are easily degradable and labile. The prick/puncture skin test is performed by placing a drop of the allergen extract on the skin. One of several available devices is used to puncture the skin through the drop, and results are read in 15-20 minutes. The wheal and flare around the puncture is measured to determine positivity. There is a strong correlation between the wheal size and the likelihood of a clinical reaction and positive tests are considered those with a mean wheal diameter of greater than 3 mm above the saline control prick test. Intradermal testing (insertion of 0.1 mL food extract subcutaneously) for food allergies are not recommended secondary to the high degree of false positivity and poor positive predictive accuracy. Positive predictive values have been determined for prick skin testing for milk, egg and peanut.

There are **limitations to the skin prick/puncture methodology** of detecting specific IgE to foods. A clear surface for testing is required and this is not always possible in a child with severe eczema. In order for the skin test to be performed accurately, the individual’s histamine responses must be intact so they must discontinue antihistamine therapy prior to the visit. Highly allergic patients cannot tolerate the increased symptoms while off of the antihistamines in preparation for the testing. Test results may vary depending upon the prick device, pressure and location of the test placement, as the back is approximately 20% more reactive than the arm. There is also some variability in the protein content of commercial extracts for easily degradable proteins, as seen in raw fruits, nuts and vegetables.

**In vitro serum food specific IgE testing can be performed if the limitations of skin prick/puncture immediate hypersensitivity skin testing prevent its use.** In the most frequently used assay, a serum or plasma sample is incubated with a solid immobilized preparation containing one allergen. In all commercial assay systems based on immobilized allergens, a standard curve is established and used to convert the results to International Units (IU) per mL of serum or plasma. Many commercial assays are available including the Phadia ImmunoCAP, Agilent Turbo-MP, and Siemens Immulite 2000. For each different food assayed, there may or may not be correlation between the assays. Clinicians must be careful not to make the mistake of comparing absolute values from differing assays. The correlation or lack of correlation between the assays must be considered. The ImmunoCAP FEIA is the method that has been most extensively investigated in the context of food allergy in humans.
If the history and physical exam suggest a non-IgE mediated immunologic reaction to a food, a clinician may consider other tests to confirm their suspicions. Other tests which are appropriate include endoscopy and biopsy of the GI tract to diagnose eosinophilic gastrointestinal disease or celiac disease. Patients with severe allergic eosinophilic gastroenteritis may have anemia, blood in stool and decreased serum protein, albumin, and IgG levels.

**Food Challenges**

After taking a detailed history, examining the patient and obtaining testing for specific IgE or evidence of non-IgE mediated immunologic reactions to food, **food challenges are helpful to determine if food allergy is causing clinical symptoms.** Three types of challenges may be performed: open, single blind, or double blind, placebo-controlled. The open challenge is an unblinded feeding with a food in its natural form if the concern for patient bias is low and objective symptoms like urticaria and wheezing are expected to occur with a reaction. Both the patient and the physician are aware of the challenge content and, therefore, the challenge is subject to bias. It is indicated to eliminate potential food culprits when the history or laboratory testing indicates the food is unlikely to be causative.

In the single blind placebo controlled challenge, only the patient is unaware of the challenge content and the physician is aware. The double blind placebo controlled food challenge (DBPCFC) remains the gold standard for diagnosis of food allergy for both clinical and research purposes. Neither the patient, parents nor physician are aware of the challenge food content. All challenges are best performed with children having discontinued any medications which could mask symptoms of an allergic reaction to the food such as antihistamines and beta adrenergic bronchodilators.4

For patients with a history of delayed responses to foods, such as in chronic diseases like atopic dermatitis and gastrointestinal syndromes, **elimination diets** can be very useful. Elimination of the food for up to 8-12 weeks with improvement in symptoms followed by recurrence of symptoms with reintroduction can delineate causative foods. There are three types of elimination diet which are useful in these situations. In the first, the suspected food is eliminated from the diet. In the other types the patient is instructed to eat either a limited “eat only” diet or an elemental diet.17 If elimination diets are prescribed or children are allergic to a large number of foods, a nutritionist is important to involve in patient care to monitor growth at a minimum of every 3 months.

**Management of Food Allergies**

Once the diagnosis of food allergies is established, the **strict avoidance** of the specific food allergen is the best preventative therapy. Patients and caregivers must be educated about food allergen avoidance through label reading, avoiding high risk situations like buffets and the early management of allergic reactions.4 Antihistamines are helpful to alleviate pruritus for IgE mediated skin symptoms. These agents, however, do not block systemic reactions. **Epinephrine is still the most effective therapy for systemic reactions.**1 Topical corticosteroids or, in severe cases, systemic corticosteroids are helpful in chronic syndromes like atopic dermatitis and eosinophilic esophagitis.

Several new therapies are under investigation for the treatment of food allergic disorders. Sublingual and oral immunotherapy with standard food allergens like milk, egg, peanut, fish, and hazelnut are currently in clinical trials.18 A Chinese herbal remedy, Food Allergy Herbal Formula (FAHF-2) which is a mixture of 9 herbs which completely blocked anaphylaxis in a mouse model of peanut allergy, is also currently in trials in humans.19 These strategies may be helpful in the future for food allergic individuals to alleviate the risk of anaphylaxis with exposure to trace amounts of food allergens.

**Summary**
Food allergies are an important disease in humans, causing many clinical manifestations, including anaphylaxis, urticaria, angioedema, wheezing, cough, difficulty breathing, cutaneous pruritus, recurrent vomiting, diarrhea and hypotension. Both IgE and non-IgE mechanisms are important in the pathogenesis of food allergies in humans. Diagnosis is performed by taking an excellent medical history, performing a physical exam looking for classic atopic diseases, and using testing for specific IgE, food challenges and, in chronic diseases, elimination diets to confirm food allergies. Avoidance of the food is the best preventative therapy and epinephrine is the most effective therapy for systemic reactions.

Resources
Guidelines for the Diagnosis and Management of Food Allergy in the United States
References
Information on Food Allergy from the National Institute of Allergy and Infectious Disease
http://www.niaid.nih.gov/topics/foodallergy/Pages/default.aspx
Food Allergy and Anaphylaxis Network (FAAN) www.foodallergy.org
Food Allergy Initiative (FAI) www.faiusa.org

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Introduction
The *Staphylococcus* genus contains an impressively diverse group of species that are common commensals of the skin and mucous membranes of humans and a wide range of animal species. While commonly found in or on healthy individuals, they are also important causes of opportunistic infections. The clinical relevance of different *Staphylococcus* species is quite variable, with some being important causes of infection and others minimally pathogenic. Coagulase positive staphylococci are the most important clinically. In dogs and cats, *S. pseudintermedius*, *S. aureus* and *S. schleiferi* subsp *coagulans* are the main coagulase positive species and main staphylococcal pathogens. Coagulase negative staphylococci (CoNS) are common commensals that can cause disease but are more often found as skin contaminants. In general, they are considered minimally pathogenic, however certain CoNS might be more pathogenic, particularly *S. schleiferi* subsp *schleiferi* and *S. epidermidis* in pyoderma and otitis externa and *S. felis* in urinary tract infections.

While the title of this presentation is “MRSA and MRSP”, they are not the only two methicillin-resistant staphylococci that are of relevance, particularly in regions where *S. schleiferi coagulans* is common. Further, while MRSA tends to receive the most public attention, MRSP is actually of much greater animal health relevance, particularly in dermatologic infections.

Methicillin-resistance
From the first introduction of antimicrobials, staphylococci have demonstrated an impressive ability to develop antimicrobial resistance. Early in the ‘antibiotic era’, the obvious approach to overcoming clinical problems with penicillin-resistant staphylococci was development of new antimicrobials. New drug development outpaced resistance initially, but the ability of staphylococci to become resistant was repeatedly demonstrated as the introduction of new drugs was typically followed shortly by identification of resistant strains. Included in this pattern was resistance to methicillin. Unlike penicillin-resistance, which was caused by secretion of beta-lactamase, methicillin-resistance was caused by production of an altered penicillin binding protein (PBP) with a poor affinity for beta-lactam antimicrobials that conferred resistance not just to methicillin, but to virtually all beta-lactams; penicillins, cephalosporins and carbapenems. Production of PBP2a is mediated by the *mecA* gene, a gene that is located on a staphylococcal chromosomal cassette (SCCmec). This site also has the ability to acquire other resistance genes, and methicillin-resistant staphylococci are often resistant to a wide range of other antimicrobials. While the evolution of methicillin-resistance has been best studied in MRSA, the same mechanism is present in all methicillin-resistant staphylococci. Methicillin-resistant staphylococci, particularly MRSA and methicillin-resistant *S. pseudintermedius* (MRSP) are emerging as serious problems in veterinary medicine.

*Methicillin-resistant Staphylococcus pseudintermedius* (MRSP)
MRSP has rapidly emerged as a critically important problem in companion animal practice. This organism appears to have emerged and disseminated internationally at a truly amazing rate, with rapid development of a very high level of drug resistance. MRSP infections are being identified virtually everywhere that people are looking, and the increase in incidence of disease, while not objectively studied, seems to be great. It has been called a serious emerging problem in small animal veterinary medicine and one that requires urgent action to control its spread.

The predominance of MRSP over other MR-staphylococci is not surprising given the major role of *S. pseudintermedius* in canine and feline skin disease. There is no evidence that methicillin-resistant staphylococci are more likely to cause disease than their susceptible counterparts, so the dominance of *S. pseudintermedius* with lower numbers of infections caused by *S. aureus* and other staphylococci should be similar with MRSP compared to other MR-staphylococci. Indeed, that is the case as MRSP infections have now become an important cause of skin and ear infections in dogs and cats internationally.

As with methicillin-susceptible strains, MRSP can be found in or on healthy dogs and cats (albeit at lower rates). Carriage rates of 0-17% in dogs and 0-1.2% in healthy cats have been reported, and it appears that the rate
of colonization is increasing in many regions. Risk factors for MRSP colonization have not been adequately investigated.

As with susceptible staphylococci, MRSP is an opportunistic pathogen and colonization does not necessarily lead to disease. Indeed, it is likely that the vast majority of colonized animals never develop a clinical infection. The risk of infection in MRSP carriers has not been reported, but it is reasonable to assume that MRSP carriers are at some increased risk of MRSP infection, at least in certain situations (e.g. after undergoing surgery, if they have underlying skin disease). Limited study of risk factors for infection has been performed but antimicrobial administration, hospitalization or surgery within 30 days prior to the onset of infection were associated with MRSP versus methicillin-susceptible S. pseudintermedius infection in one study.1

**Methicillin-resistant *Staphylococcus aureus* (MRSA)**

Emergence of MRSA in companion animals appears to be a direct reflection of changes in the prevalence of MRSA in people in the general population. As MRSA became more common in people, it started to spread into pet populations. MRSA can be found in healthy animals, particularly in the nasal passages, intestinal tract and perineum. Reported colonization rates are variable but tend to be 0-3.3% in healthy dogs and 0-6% in healthy cats. Being owned by a human healthcare worker and participation in hospital visitation programs have been identified as risk factors for MRSA colonization in dogs, and are logical based on the increased likelihood of exposure to colonized people. Contact with children has also been identified as a risk factor. While these, and potentially other, risk factors should be considered, MRSA can be identified in any animal and absence of known risk factors should not lead to excluding MRSA from consideration.

Most animals that are colonized with MRSA have no signs of infection and may never develop a clinical infection. In humans and horses, MRSA colonization is known to be a risk factor for clinical MRSA infection in certain circumstances (e.g. after admission to hospital). It is reasonable to assume that this also applies to dogs and cats yet this is not proven.

The hypothesis that MRSA in companion animals is intimately linked to MRSA in humans is supported by the recurring observation that MRSA strains found in companion animals are the most common human strains in any given region. Virtually all MRSA isolates from pets are recognized human epidemic clones and identification of other strains in dogs and cats is rare.

**Staphylococcus schleiferi**

*Staphylococcus schleiferi* consists of two subspecies, the coagulase positive *S. schleiferi* subsp *coagulans* and coagulase negative *S. schleiferi* subsp *schleiferi*. These are less common causes of infection compared with *S. pseudintermedius*, however failure of a many diagnostic laboratories to differentiate these organisms from *S. pseudintermedius* (*S. schleiferi coagulans*) and other coagulase negative staphylococci (*S. schleiferi schleiferi*) hampers proper assessment of their role in disease. The role of *S. schleiferi coagulans* appears to vary greatly between regions, based on limited comparisons between laboratories where proper identification is performed.

*Staphylococcus schleiferi coagulans* can be isolated in 0.8-4% of healthy dogs and 0-2% of healthy cats. Colonization with methicillin-resistant *S. schleiferi* (MRSS) has been identified in 0-2% of dogs and MRSS infections are being increasingly reported.2-4

**Coagulase negative staphylococci**

Coagulase negative staphylococci (CoNS) are very common and generally of limited virulence. Methicillin-resistance is not uncommon in commensal CoNS. Studies have reported MR-CoNS colonization prevalence ranging from 5-13% in healthy dogs and 5% in cats. As with other staphylococci, methicillin-resistant strains are inherently no more pathogenic than methicillin-susceptible strains, and the implications of colonization with MR-CoNS are typically minimal. While colonization with CoNS is common, infection is not. CoNS infections may be overdiagnosed because CoNS can be isolated as contaminants from various superficial body sites. In human medicine, CoNS are primarily a concern in hospitalized individuals. Community-associated CoNS infections in humans are usually UTIs caused by *S. saprophyticus*. The situation may be similar in dogs and cats, with most CoNS being of minimal pathogenicity but some species (i.e. *S. schleiferi schleiferi*, *S. epidermidis* and *S. felis*) being potentially important causes of community-onset disease (including skin infections).
**Clinical presentation**

Staphylococcal infections caused by different species are not distinguishable. The main difference between *S. pseudintermedius* and *S. aureus* is the incidence of disease, not disease location or severity, although it has been suggested that *S. schleiferi coagulans* may tend to produce more superficial skin disease compared to *S. pseudintermedius* and *S. aureus*. An interesting difference between MRSA in humans and companion animals is the rarity (or absence) of the ‘contagious carbuncle, a classical form of community-associated MRSA infection in humans, in animals. There is no indication that methicillin-resistant infections are more serious than infections caused by methicillin-susceptible strains, however they may be more difficult to treat.

**Therapy**

Detailed discussion of treatment options for staphylococcal infections is beyond the scope of these proceedings, given the various issues that are present and different treatment approaches for different types of skin and soft tissue infections.

**Underlying Issues**

A critical component of treatment is identification and management of any underlying causes, whenever possible. From a broader standpoint, it is difficult to consider clinical resolution of an individual infection a ‘successful outcome’ if it will likely be followed in short order by another infection, possibly by a more resistant bacterium. While this is not always possible, it needs to be an important part of case management and is critical with recurrent disease.

**Systemic Antimicrobial Therapy**

Systemic administration may be required for many, but not all, infections. Depth of infection, chronicity and underlying disease likely dictate the need for systemic therapy. Broad recommendations for treatment of staphylococcal infections are difficult to make because of the variability in infection types and susceptibility patterns. Beta-lactam antimicrobials, including penicillin/beta-lactamase inhibitor combinations (e.g. amoxicillin-clavulanic acid), should not be used for methicillin-resistant staphylococcal infections. The only exception is a small group of anti-MRSA cephalosporins, however use of any of those in companion animals has not been described. In humans, fluoroquinolones are considered to be contraindicated for the treatment of MRSA infections because of poor clinical response and rapid development of resistance. This has not been objectively investigated in dogs and cats, but there is no reason to suspect that it would be different in these species, so fluoroquinolones probably be avoided whenever possible as treatments of MR-staphylococcal infections. Inducible clindamycin resistance is a potential problem, particularly with MRSA. With this phenomenon, isolates appear to be susceptible to clindamycin *in vitro*, however resistance is induced upon exposure *in vivo* and treatment failure is expected. Inducible resistance is common in erythromycin-resistant, clindamycin-susceptible MRSA from dogs, but appears to be relatively uncommon in MRSP. In the absence of specific testing for inducible resistance, erythromycin-resistant MRSA isolates (or those where erythromycin susceptibility was not reported) should be considered potentially resistant.

Despite multidrug resistance, there is typically one or more ‘reasonable’ option, however as MRSP, in particular, rapidly becomes more resistant, these options are getting limited. Drugs such as trimethoprim-sulfonamide, doxycycline, aminoglycosides and chloramphenicol are often still effective and practical, although not without concerns.

**Topical Therapy**

The proliferation of resistant staphylococcal infections has led to the need to consider approaches beyond systemic antimicrobial therapy. Topical antimicrobial therapy may be useful, depending on the depth of infection. The ability to deliver high concentrations of antimicrobial directly to the site of infection, with minimal systemic exposure, can be very useful for treatment of superficial infections. Resistance of staphylococci from dogs and cats to topical antimicrobials such as mupirocin and fusidic acid is currently rare and the high local antimicrobial levels that can be achieved may reduce the risk of acquired resistance. The main limitation to topical therapy is the ability of topically-applied antimicrobials to reach the infection site. Thus, topical therapy is best reserved as a sole method for treatment of focal superficial infections.
Topical administration of biocides (antiseptics) is another potentially useful alternative for superficial infections. The potential efficacy of biocides involves a balance between the bactericidal activity of the compound and the tissue damage from biocide application, something that may be difficult to assess because of limited information regarding both efficacy and safety. Some compounds have profound antibacterial properties but are not useful because of the degree of tissue damage that can ensue. For some biocides, the cost-benefit of antibacterial properties and tissue damage are not well understood. As with topical antimicrobials, the ability to reach the infection site is the main limitation. Bathing with shampoos containing chlorhexidine, povidone iodine, ethyl lactate or benzoyl peroxide gel can be an effective approach. Other compounds such as accelerated hydrogen peroxide have also been proposed as topical therapies but there is currently little information about their use. Essential oils are gaining popularity as topical therapies. While various essential oils may have antibacterial properties, including activity against MRSA and MRSP, tissue damage is a potential concern as some essential oils can be rather cytotoxic.

Other Options

The use of honey has undergone resurgence for treatment of superficial infections. This has typically involved wound infections but the use of honey in focal skin infections could be considered. There are differences in bactericidal activity between different types of honey, and the best-investigated honey has been Manuka honey, produced by bees feeding from the blossoms of *Leptospermum scoparium* (Manuka). Commercial honey-impregnated bandages can facilitate focal therapy.

The role of autogenous bacterins or commercial bacterial antigens (e.g. Staphage Lysate) is unclear, but there is probably no contraindication.

Infection control

In some practices, particularly referral practices, methicillin-resistant staphylococci now account for a large percentage of pyoderma cases. In general, animals with MRSP and MRSA are isolated and handled with barrier precautions in veterinary hospitals because of the chance for hospital-associated and (predominantly for MRSA) zoonotic transmission. However, the epidemic of MRSP in dermatology creates a conundrum...what do you do when a significant percentage of your population is infected with MRSP, additional animals come in colonized, and when animals that you have successfully treated may become colonized during treatment? It is much easier to be more restrictive and aggressive when controlling an uncommon pathogen than when there is a high endemic rate.

Currently, there is no clear consensus regarding management of these dermatologic cases. Considering the potential (although unquantified) risk to other patients and humans, consideration must be given to both careful application of routine infection control practices as well as the use of enhanced precautions around animals infected or colonized with MRSA or MRSP. The degree of enhanced practices and the aggressiveness in applying them (all infected animals/infected or colonized animals/infected animals or those considered at high risk for infection/previously infected or colonized animals) will vary depending on the type of practice, prevalence of MRSP/MRSA in the population and risk aversion. Detailed discussion of this area is available elsewhere.
References


**INFECTION CONTROL**

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**General Concepts of Infection Control**  
Infection control has been an overlooked and underappreciated field in veterinary medicine, particularly in small animal practice. The concept of ‘biosecurity’ is one that is well developed in some sectors of food animal veterinary medicine, however this differs from ‘infection control’. Biosecurity involves implementation of measures to prevent entrance and dissemination of infectious agents into defined groups of animals. This is not applicable to small animal practice because, inherently, small animal clinics ‘invite’ animals with infectious diseases into their facility. Therefore, the focus is on limiting the impact of infectious diseases that will enter the clinic. This is the practice of infection control, which attempts to control the impact of the inevitable exposure to infectious agents on animals and humans.

While infection control has often been ignored, there is increasing interest in this field in small animal practice. There are many possible reasons for this, but factors such as increased awareness of infectious diseases by the general public, increased information about hospital-associated (nosocomial) infection in human and veterinary hospitals, occupational health and liability concerns about zoonotic infections, and emergence of multidrug resistant pathogens highlight the need for a more organized approach to infection prevention and control.

Every veterinary clinic, regardless of size and type, should have at least a basic infection control program. This may range from a written collection of basic infection control practices to a formal infection control manual with specific training, monitoring, surveillance and compliance programs. Unfortunately, this is rarely the case, which may lead to unnecessary patient morbidity and mortality, and exposure of veterinarians, staff and owners to zoonotic pathogens. The increasingly litigious nature of society could be one of the driving forces towards improved infection control in veterinary clinics. While the potential liability consequence of morbidity and mortality in individual pets is currently limited, the potential consequences of zoonotic diseases in owners and staff are high and require careful consideration. Improved infection control is also a necessity as veterinary medicine evolves. Advances in veterinary medicine mean that animals are living longer and there are more animals at higher risk for infection because of immunosuppression and more invasive treatments.

Despite increasing awareness of infection control in veterinary medicine, the field is still in its infancy with few personnel focusing on clinical infection control or infection control research. Limitations in objective data mean that current guidelines are based on general principles of infection control and infectious diseases, information from human medicine, limited scientific study and anecdotes. While they are reasonable, it is hoped that they will be refined over time as more objective information becomes available. Essentially no objective data are available pertaining to infection control and veterinary dermatology.

**Principles of Infection Control**

In general terms, 3 basic areas must be considered when infection control is approached. These include **decreasing exposure** to pathogens, **decreasing susceptibility** of the host and **increasing resistance** of the host.

**Decreasing Exposure:** Decreasing exposure is the most important aspect of disease control in most situations. If a pathogen is unable to encounter an individual, disease will not occur. Depending on the pathogen, preventing exposure may be easy, difficult or impossible. Organisms that always quickly produce readily apparent infection can be easy to control, but these are rarely the case. Clinically normal animals and people may harbour a variety of primary and opportunistic pathogens, and even clinical evaluation cannot rule out the possibility that an animal is carrying a relevant infectious agent.

**Decreasing susceptibility:** The pathophysiology of disease is multifactorial and in most cases, simple exposure to an infectious agent does not necessarily mean that disease will result. The susceptibility of the individual to an infectious agent plays an important role. While difficult to quantify, certain situations may result in increased susceptibility to disease. Many factors causing increased susceptibility are not preventable, but some are and efforts should be undertaken to address these issues. From a dermatological standpoint, this is a critical infection
control measure, as addressing any underlying skin disease is critical for the control and prevention of opportunistic skin infections. 

*Increasing resistance:* Vaccination is the main technique employed to increase resistance of animals or humans to infection. Vaccination is currently of limited relevance for infection control in dermatology practice, beyond the concept that improving overall health status can likely reduce the risk of opportunistic infections.

**Infection Control and Veterinary Dermatology**

If infection control in veterinary medicine is described as being in its infancy, it may be reasonable to state that the approach in veterinary dermatology is in a ‘fetal’ state. That is not to denigrate the knowledge or interest of individuals involved in practice or research. Rather, it highlights the fact that the dermatology patient (along with most other predominantly outpatient and non-surgical patients) has typically received little consideration as either a source or recipient of hospital-associated infection diseases, and little corresponding objective infection control research has been performed. However, infection control is increasingly important in veterinary dermatology because of the dissemination of antimicrobial resistant pathogens. While multidrug resistant staphylococci may drive the current increase in interest, other transmissible pathogens are also of concern, such as fungi (e.g. *Microsporum canis*) and certain insects.

Some aspects of the general dermatology patient facilitate infection control, such as:
- typically short duration of hospitalization (predominantly outpatient)
- limited use of invasive devices
- concentration of activities in a single room or area
- limitation of contact to a small number of clinic personnel

At the same time, the dermatology patient population has some factors that may increase infection control risks and challenges:
- compromise to the protective skin barrier
- frequent antimicrobial exposure
- comorbidities that may increase the likelihood of disease
- immunosuppressive therapy
- repeated contact with the veterinary healthcare system

There are 4 main areas of concern regarding infectious disease transmission and dermatology practice. The relative risk of each is unclear.

1) **Transmission to other dermatology patients**

We currently have limited information about transmission of pathogens between dermatology patients. Organisms of concern include various bacteria (especially multidrug resistant organisms), fungi and insects. Transmission between patients is of concern because of high rates of infection or carriage and the potential for various types of indirect (e.g. examination room areas, equipment, clothing of veterinary personnel, human hands) or, less likely, direct (e.g. comingling in a waiting room) transmission. One limiting factor in our understanding of this problem may be difficulties in identifying transmission. For example, a dog with superficial pyoderma could acquire a resistant *Staphylococcus* spp during examination, with subsequent secondary infection. The potential for nosocomial superinfection would probably not be considered if there was a poor response to treatment or recurrence of infection. This is particularly true when initial cultures are not taken.

2) **Transmission to patients on other services**

The risk to other patients is similarly unknown, but it is logical to assume that dermatology patients in areas with a high prevalence of antimicrobial resistant infection and colonization could be a focus of infection for susceptible hospitalized individuals, particularly surgical patients. This is an area that needs careful consideration and investigation, particularly because strife has been created in some practices, with conflict between dermatology and surgical services. This is largely based on concerns about the potential risk of transmission of methicillin-resistant staphylococci from dermatology patients (where the prevalence can be high) to surgical patients (where the implications of infection can be high).

3) **Transmission to clinic personnel**

The risk to clinic personnel has not been adequately investigated. Most recent attention has often given to the potential for methicillin-resistant staphylococcal transmission, and high rates of MRSA colonization have
been reported in veterinary personnel.\textsuperscript{1,2} The role of dermatology practice in this is unclear, but it is hard to argue that there is at least some increased risk posed to personnel that routinely work with animals carrying multidrug resistant pathogens. While the focus has been on staphylococci, a variety of other organisms are also of concern, in terms of infection or infestation. It is likely that there is an unreported burden of zoonotic disease in veterinary dermatology practice.

4) Transmission to owners

While pets can be sources of various zoonotic skin pathogens, the role of the veterinary clinic in prevention of this is variable. Animals presenting with a potentially zoonotic infection may have already likely exposed household members, but this is not assured so measures are indicated to reduce exposure. Concurrent colonization of pet owners and pets with the same \textit{Staphylococcus} has been reported,\textsuperscript{2,3} but the role of pets in human disease is unclear and conflicting data have been obtained.\textsuperscript{4} Pets have been identified as sources of various dermatologic infections in households, such as ringworm and parasitic infestations.\textsuperscript{5-7} Additionally, identification of infestation in a patient is sometimes the most important factor for diagnosis of concurrent human disease.

\textbf{Risk Reduction}

Various options need to be considered when designing an infection control program for dermatology services. An understanding of the most relevant pathogens and their routes of transmission (e.g. direct contact, fomite) is critical to ensure that relevant areas are being addressed. Among the areas that should be considered are:

\textit{Cohorting cases:} Ideally, animals of different risk groups are kept apart, and high-risk individuals (both for being infectious and for becoming infected) are managed with a greater degree of care. Separating dermatology cases from other hospital cases is ideal. This includes dedicated dermatology examination and treatment rooms, separate housing areas and ideally no (or minimal) commingling with other animals in the waiting area. Complete separation is not always practical or possible, but measures to reduce cross-contact as much as possible should be developed. There should be protocols to identify and manage particularly high-risk cases. For example, flagging of medical records can allow for animals carrying specific pathogens to be directly admitted to an examination room or isolation area so that they can be managed properly from the start. Personal hygiene and barrier precautions would typically be indicated. This type of screening can be based on previous diagnoses (e.g. MRSA) or increased likelihood of a high-risk pathogen (e.g. cat recently adopted from a shelter the has developed skin lesions).

\textit{Dedicated examination and procedure areas:} As mentioned above, dedicated rooms for dermatology cases are ideal in referral practices (though not practical in non-specialty practices). Having a dedicated room assists in containing any pathogens that might be brought in. While the potential for transmission of pathogens to other patients that visit the room remains, it allows for more overall containment so that different services are not involved, and decreases the chance that an inpatient (typically with inherently increased risk) is exposed. Further, it allows for better knowledge of what has happened in the room, so that clinicians and technicians can have a more informed and active role in ensuring proper cleaning and disinfection.

\textit{Personal protective equipment:} Personal protective equipment (PPE) is a critical routine infection control tool. It involves the use of routine protective outerwear (e.g. scrubs or lab coat), with enhanced precautions (e.g. gloves, gown) in specific situations. PPE is designed to reduce the risk of infection of the person, transmission of pathogens throughout the clinic and transmission of pathogens home. However, routine PPE can easily act as a fomite if used improperly (e.g. not changed regularly and when soiled, worn home, not laundered properly...). Similarly, enhanced PPE can be useless if not used properly (e.g. wearing gloves but touching common contact surfaces, re-using gowns, hanging used gowns adjacent to labcoats, not washing hands after PPE removal). PPE is relatively simple to use, but similarly simple to mess up. Proper training is required.

\textit{Cleaning and disinfection:} Cleaning and disinfection is a critical component of the infection control program, but it is often performed poorly. Common errors include failing to adequately clean before disinfection, use of inadequate disinfectants and improper use of disinfectants (e.g. inadequate contact time, improper dilution). There is often little scrutiny of cleaning and disinfection practices, and the author has seen many situations where lay or technical staff have changed cleaners or disinfectants (sometimes for the worse) based solely on information from a sales representative or because a product has a better smell.
**Surveillance:** Surveillance is perhaps a less critical component for dermatology cases than surgical cases, where identification of post-discharge surgical site infections is critical. However, surveillance takes many forms and there are some important surveillance aspects to consider. Perhaps the most important is developing an understanding of the pathogens in the practice area. This is important for identifying high-risk situations and for guiding empirical antimicrobial therapy. In particular, knowing general rates for antimicrobial resistant pathogens can be useful for deciding initial treatments and when to empirically apply enhanced precautions.

**Enhanced precautions:** In certain situations, enhanced infection control practices (increased barriers, different animal handling or housing, enhanced cleaning and disinfection…) may be indicated in response to identification of potentially concerning pathogens (e.g. ringworm, MRSA, MRSP). This involves recognition of situations that require enhanced practices and a mechanism to ensure that these practices are followed. Clear guidelines regarding optimal practices for these are lacking, however the general concept that ‘being proactive can’t hurt’ should be considered.

**Development of an infection control program**

Every clinic should have a formal infection control program. This may involve a complex program with detailed policies and dedicated full-time personnel in large specialty hospitals, but typically only requires a modest effort with little to no additional resources, training and time. The size and scope of the infection control program needs to be tailored to the needs and resources of the individual veterinary hospital. However, some common components should be present in all hospitals:

1) A written infection control manual: Written resources are critical. If something is not written down, there may be a loss of consistency as people modify practices, knowingly or otherwise. A central written resource allows people to quickly and easily determine the required practices for both routine (i.e. cleaning and disinfection) and uncommon (i.e. rabies exposure) events. Written documentation is also critical to demonstrate that an infection control program is in place, should there be issues regarding professional or legal liability. The adage “if it’s not written down, it doesn’t exist” is important to remember.

2) Documented training of all personnel: All personnel working in a clinic, from owners to temporary kennel staff, must be trained on infection control practices. This is not only required for optimal patient care. It is also critical for protection of the clinic because failure to properly train and document training of individuals about how to protect themselves, particularly lay personnel who would not be expected to know anything about zoonotic diseases or infection control, could expose the clinic to significant liability risks.

3) A designated central contact person/resource: This ‘infection control practitioner’ (ICP) can be a veterinarian or technician, and should be in charge of developing protocols, ensure protocols are being followed, act as a resource for infection control questions, ensure proper training of new staff and direct any surveillance activities. This is not necessarily a cumbersome or time-consuming job, as the day-to-day responsibilities are typically minimal. The main effort involves establishing the program, and available resources can facilitate this.

4) “Buy-in” from clinic management: An infection control program is bound to fail if people in charge of the clinic do not support it. Failure of senior personnel to follow protocols, to support the general concept and to facilitate with compliance of all personnel will ultimately result in failure of the program. In some clinics, a proper infection control program requires a substantial ‘culture shift’ in attitudes, and this can only be achieved if there is proper support.

5) An ability to adapt: An infection control program cannot remain static. Changes in disease risks, emergence of new diseases, changes in clinic design and operation and improvement in the general knowledge of infection control will result in the need for an evolving program. This should not require extensive and frequent program modification, but the program needs to be designed so that it can respond to any changes.
Resources
- Compendium of Veterinary Standard Precautions by the National Association of State Public Health Veterinarians: http://www.nasphv.org/documentsCompendia.html
- http://www.wormsandgermsblog.com

References
UPDATE ON CANINE AUTOIMMUNE SKIN DISEASES: SELECTED TOPICS

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This lecture will be divided into different sections that cover several issues relevant to disease classification, clinical signs, diagnosis, treatment and pathogenesis of autoimmune skin diseases (AISDs) in animals. As almost all publications and research have been done on canine diseases, the material reviewed will be almost entirely limited to this species.

A REVISED CLASSIFICATION OF CANINE AUTOIMMUNE SKIN DISEASES

At the onset of the recognition of AISDs in dogs and cats, these diseases were separated in “bullous” (i.e. pemphigus and pemphigoids) and “non-bullous” diseases (i.e. discoid and systemic lupus erythematosus) 1. With the recognition of clinically vesicular forms of lupus and often clinically non-bullous variants of pemphigoids in dogs, this original classification is no longer valid. We propose herein a separate nosology based on dominant mechanism of lesion formation. This classification additionally provides a better rationale for treatment approach.

We propose to separate AISDs into those with lesions due – or presumed to be due – to the action of autoantibodies (table 1; antibody-mediated AISDs) and those whose lesions are caused by an attack from (usually cytotoxic) T-lymphocytes (table 2; lymphocyte-mediated AISDs). For each main category of AISDs, entities can then be logically separated among their principal cellular or molecular targets (tables 1-2).

A PROPOSAL FOR CLASSIFICATION OF CANINE CUTANEOUS LUPUS

In the late 1990’s, we proposed to use a classification of canine cutaneous lupus erythematosus (CLE) that was adapted from that designed by Gilliam and later modified by Sontheimer 2.

The Gilliam-Sontheimer nosology proposes to divide all skin lesions associated with lupus erythematosus (LE) into those that have microscopic skin lesions specific for lupus (i.e. a lymphocyte-rich interface dermatitis with prominent basal keratinocyte death by oncotic necrosis or apoptosis) and are thereby named “LE-specific skin diseases” (or CLE sensu stricto) and those that do not share such histopathologic pattern and are named “LE-nonspecific skin diseases”.

In humans, LE-specific skin diseases (CLE) are subdivided into three subcategories based on disease evolution: acute cutaneous LE (ACLE), subacute cutaneous LE (SCLE) and chronic cutaneous LE (CCLE). Lupus erythematosus-nonspecific skin lesions are those that are related to the underlying autoimmune disease, but that are not specific for LE since the same lesions can be encountered also in other diseases. Examples of LE-nonspecific skin lesions are those associated with vasculitis, cryoglobulinemias, or vesicobullous lesions associated with basement-membrane autoantibodies (i.e. bullous SLE). Finally, human patients with systemic LE (SLE) can exhibit cutaneous lesions that are either specific or nonspecific (SLE with or without CLE). Conversely, LE-specific skin lesions can be present with or without systemic involvement (CLE with or without SLE) (Figure 1).

Our proposal is to use the same logic to classify manifestations of LE in dogs (Figure 2). At this time, LE-specific skin diseases (CLE sensu stricto) would include: vesicular cutaneous LE (VCLE), exfoliative cutaneous LE (ECLE) and localized or generalized discoid LE (DLE) and the rarely seen and not well-characterized mucocutaneous LE (MCLE) and oral LE (OLE). In contrast, LE-nonspecific skin diseases would presently encompass vasculitis (including perhaps, lupus panniculitis) and type I-bullous SLE.
DOES THIS DOG HAVE PEMPHIGUS FOLIACEUS OR ANOTHER INFECTIOUS PUSTULAR ACANTHOLYTIC SKIN DISEASE?

As described in a recent review, superficial epidermal acantholysis can occur, not only in the context of canine PF, but also as part of staphylococcal and dermatophyte infections. The recent identification, from *Staphylococcus pseudintermedius* isolated from two dogs with bullous impetigo (BI), of two novel exfoliative toxins from (now renamed ExpA [EXI] and ExpB) that digest canine DSG1 and induce superficial epidermal acantholysis lends further credence to the importance of exfoliatin-induced acantholysis in dogs. Importantly, supernatants from cultures of staphylococci isolated from dogs with exfoliative superficial pyoderma (ESP, also known as “superficial spreading pyoderma”) do not appear to cleave canine DSG1, thereby suggesting that other toxins or mechanisms might be the cause of exfoliation in this disease (Nishifuji K: personal communication).

As the mere demonstration of acantholytic keratinocytes and neutrophils in cytological examination of pustule content, or in superficial pustules on histopathology, can no longer be deemed specific for canine PF, there are diagnostic clues that can help clinicians establish the probability of diagnosis of PF, BI, ESP or pustular cornophilic dermatophytosis (PCD) when looking at pustules.

One can use, for example:

- **the shape of the pustules**: those from BI are typically round, and they slowly expand centrifugally. Pustules from PF normally will arise and not typically expand peripherally; however, they will often coalesce leading to irregular “polycyclic” pustules.

- **the size of the pustules**: those of BI and PF might span multiple follicles, those of bacterial folliculitis are monofollicular.

- **the presence of epidermal collarettes and scaling**: rapidly expanding epidermal collarettes and exfoliation are expected to be the consequence of bacterial exfoliative toxin action; collarettes are not typically seen in PF.

- **the arrangement of the pustules**: those of PF might have an annular or polycyclic arrangement with a normal center, while the center of ESP lesions usually reflect the inflammatory expansion of the epidermal collarettes often leaving hyperpigmented macules and patches.

- **the rapidity of expansion of the lesions**: lesions of PCD slowly expand centrifugally, those of ESP do so more rapidly; lesions of PF rarely expand but they do tend to coalesce.

- **the location of lesions**: it would be rare for lesions of PF and PCD to begin on the trunk and not on the face, while it would be very uncommon for lesions of BI and ESP to begin on the face and/or ears but not on the trunk.

In summary, results of cytology and histopathology must always examined in the context of the clinical presentation before a diagnosis of PF be made!

CAN CLINICAL SIGNS HELP DIFFERENTIATING AMONG RESEMBLING CANINE AUTOIMMUNE SKIN DISEASES?

If skin and mucocutaneous blisters and erosions arise in an adult dog, it is customary to establish an exhaustive list of differential diagnoses that typically includes pemphigus vulgaris (PV) and its paraneoplastic variant (PNP), bullous pemphigoid (BP) and other autoimmune subepidermal blistering dermatoses (AISBDs), erythema multiforme major (EMM) and lupus variants such as VCLE. Pending biopsy results, and as immunological diagnostic tests are not readily available, are there any clinical signs that can be used to narrow the list of differential diagnoses?

A list of such signs can be found in Table 3. The readers are referred to the historical paper recently published by S. Grando for a unique perspective on the use of Nikolskiy’s and related signs.
AN HISTORICAL REVIEW OF THE DISCOVERY OF CANINE PEMPHIGUS FOLIACEUS ANTIGENS

In 1991, Amagai et al. identified the desmosomal cadherin adhesion molecule desmoglein 1 (DSG1) as the main autoantigen in humans with pemphigus foliaceus (PF). As a result, the search for the canine PF autoantigen(s) focused first on this protein.

At the 2nd World Congress of Veterinary Dermatology in 1992, M. Suter presented immunoblotting results from two dogs with PF, both of them having IgG serum autoantibodies that bound to a 148 kDa antigen present in canine lip epithelium. Serum IgG from a human patient with PF also targeted a protein of similar molecular weight, which was later shown to be the canine homologue of desmoglein (1).

In 1997, Iwasaki and colleagues performed immunoblotting with sera from 16 dogs with PF tested on an extract made from a culture of differentiated canine keratinocytes. Sera from 8/16 dogs with PF (50%) recognized a 160 kDa antigen that had the same mobility as the protein identified by a human PF serum. As a result, the 160 kDa DSG1 was suspected to be a major (i.e. recognized by > 50% of affected patients) autoantigen for canine PF. Interestingly, five of these 16 canine PF (31%) sera also had serum IgG that bound to an unidentified 120 kDa antigen while serum IgG from six dogs with PF (38%) did not recognize any antigen using this substrate and technique.

After the cloning and sequencing of canine DSG1 by E. Muller et al., a recombinant baculoprotein encompassing the extracellular segment of canine DSG1 was produced. This recombinant protein was found to be identified by human PF but not canine PF serum IgG.

In 2006, Olivry and colleagues transfected human kidney epithelial cells to ectopically produce extracellular and transmembrane segments of dog DSG1. Using this substrate, only 5/83 canine PF sera (6%) were found to have IgG autoantibodies that recognized DSG1-producing cells. When present, anti-DSG1 IgG autoantibodies appeared to target calcium and glycosylation-dependent epitopes. These studies established DSG1 as a minor autoantigen in dogs with PF.

Using indirect immunofluorescence (IF) performed on canine footpad and buccal mucosa, Bizikova et al. extended previous observations of the heterogeneity of IF patterns in dogs with PF. The most common indirect IF pattern, present in ~80% of canine PF sera, was a suprabasal intercellular fluorescence of dog footpad epidermis without staining of canine buccal mucosal epithelium. Remarkably, this pattern matched that of immunostaining of desmocollin-1 (DSC1), another desmosomal cadherin adhesion molecule.

Finally, at this 2011 NAVDF meeting, Bizikova reported the successful cloning of canine DSC1 and the transfection of human 293T kidney epithelial cells to produce the full-length protein. Using indirect IF, she found that ~80% of canine PF sera with the dominant superficial epidermal staining pattern recognized canine DSC1-producing cells. These studies established DSC1 as a major autoantigen for canine PF.

Because rare PF sera have IgG that recognize unique sections of either footpad and/or buccal mucosal epithelium, it is suspected that other minor canine PF autoantigens might also exist. Our recent observation that many of these sera also recognize canine DSC1 challenges this suspicion, however.

In summary, while human PF is an autoimmune acantholytic blistering skin disease characterized by autoantibodies (IgG, IgM and IgE) that target DSG1 (major antigen) and also some other minor proteins (including DSC1), canine PF is associated with autoantibodies (IgG so far) that recognize DSC1 (major antigen) and DSG1 (minor antigen, < 10%).
THE FUTURE OF AUTOIMMUNE DISEASE DIAGNOSIS: ANTIGEN-SPECIFIC SEROLOGICAL TESTS
In the last 15 years, ELISA using recombinant human DSG3, DSG1, collagen XVII and collagen VII have been set up for the diagnosis and treatment follow-up of human patients with PF, pemphigus vulgaris, bullous pemphigoid and epidermolysis bullosa acquisita (EBA), respectively.

Recent data from our laboratory has shown the value of using recombinant antigens for the diagnosis of canine EBA. We transfected human cells to produce the NC1 aminoterminus of canine collagen VII, and used these cells as substrate for indirect IF testing of sera of 13 dogs with EBA, 26 dogs with other AISBDs and 21 normal dogs. Using this technique, 11/13 dogs with EBA, but none of the other 47 dogs, had detectable IgG serum autoantibodies targeting NC1-producing cells, thereby giving such assay a sensitivity of 85% and a specificity of 100%.

Attempts are being made to develop other immunoassays for this and other canine autoantigen. It is expected that such assays would provide valuable tools to aid in diagnostic and follow-up of immunosuppression of dogs with AISBDs.

IS AZATHIOPRINE SAFE TO USE IN DOGS?
When autoimmune skin lesions fail to respond with high dose glucocorticoid therapy, veterinary dermatologists often add azathioprine (1.5-2.5 mg/kg every 24 to 48 hours) to enhance the potency of immunosuppression. Azathioprine is believed to be relatively safe in dogs as, in spite of its widespread usage, there are only scattered publications of toxic events. It is likely, however, that most adverse effects are not reported.

Myelosuppression has been described in five dogs; this adverse effect was diagnosed after four to 16 weeks of administration at standard dosages. Of interest is that azathioprine-induced myelosuppression in dogs does not appear to be caused by low levels of activity in its metabolizing enzyme thiopurine methyltransferase, as is seen in humans. Pancreatitis has been reported in three dogs treated with combinations of oral glucocorticoids and azathioprine.

An important, fairly common yet rarely reported side effect of azathioprine in dogs is drug-induced hepatitis. A small open trial reported the use of azathioprine monotherapy (2.0 to 2.5 mg/kg once daily) in 12 dogs with atopic dermatitis. Serum levels of alanine aminotransferase and alkaline phosphatases enzymatic activity rose above normal in 10 of 12 dogs (83%), as early as the second week after the trial begun. Clinical signs of hepatitis developed and led to study withdrawal in three dogs (25%), all with high liver enzyme activity. All dogs recovered uneventfully once azathioprine was withdrawn. In other dogs, high liver enzyme activity was not associated with any clinical signs of liver disease.

In summary, the administration of azathioprine to dogs at 2.0 to 2.5 mg/kg once daily appears to cause frequent elevations of liver enzyme activity, occasional clinical hepatitis and rare myelosuppression and pancreatitis. The latter is seen in dogs also receiving oral glucocorticoids.

Based on previous reports of toxic effects of azathioprine in dogs, guidelines for monitoring toxicity after administering this drug can be proposed. The evaluation of liver parameters should be performed at least every two weeks, complete blood counts at least every two to four weeks and pancreatic enzyme levels at least every four weeks. If signs of toxicity are not seen after three months, monitoring of these parameters probably can be reduced to once per trimester. At this time, there is no evidence supporting the routine measurement of red blood cell thiopurine methyltransferase activity in dogs treated with azathioprine, as toxic events do not appear to be associated with low enzyme activity levels (SOR E).
REFERENCES:

### Table 1: Revised Classification of Antibody-Mediated Autoimmune Skin Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abbrev.</th>
<th>Affected Species</th>
<th>Autoantigens Targeted by Autoantibodies Humans</th>
<th>Autoantigens Targeted by Autoantibodies Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antikeratinocyte AIsDs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pemphigus Folliculosis</td>
<td>PF</td>
<td>humans, dogs, cats, horses, geats</td>
<td>desmoglein-1</td>
<td>desmocollin-1, desmoglein-1</td>
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<tr>
<td>Pemphigus Erythematosus</td>
<td>PE</td>
<td>humans, dogs, cats</td>
<td>desmoglein-1</td>
<td>desmoglein-1, desmocollin-1</td>
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<tr>
<td>Pemphigus Vulgaris</td>
<td>PV</td>
<td>humans, dogs, cats</td>
<td>desmoglein-1, desmoglein-3</td>
<td>desmoglein-1, desmocollin-1</td>
</tr>
<tr>
<td>Paraneoplastic Pemphigus</td>
<td>PNP</td>
<td>humans, 3, 4 days</td>
<td>plakins, desmoglein-3, desmoglein-1,</td>
<td>plakins, desmoglein-3</td>
</tr>
<tr>
<td>Pemphigus Vegetans</td>
<td>Png</td>
<td>humans, 1 dog</td>
<td>desmoglein-1, others</td>
<td>desmoglein-1</td>
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<tr>
<td>IgA Pemphigus</td>
<td>IgAP</td>
<td>humans, 1 dog</td>
<td>desmoglein-1, others</td>
<td>unknown</td>
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<tr>
<td>Basal Membrane AIsDs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bullous Pemphigoid</td>
<td>BP</td>
<td>humans, dogs, 1 cat</td>
<td>collagen XVII</td>
<td>collagen XVII, BPAG1-e</td>
</tr>
<tr>
<td>Necropsy Membrane Pemphigoid</td>
<td>NMP</td>
<td>humans, dogs, 3 cats</td>
<td>laminin-332, collagen XVII</td>
<td>collagen XVII, laminin-332, BPAG1-e</td>
</tr>
<tr>
<td>Linear IgA Disease</td>
<td>LAD</td>
<td>humans, 2 dogs</td>
<td>processed collagen XVII</td>
<td>processed collagen XVII</td>
</tr>
<tr>
<td>Pemphigoid of Guttatepe</td>
<td>PG</td>
<td>humans, 1 dog</td>
<td>collagen XVII</td>
<td>unknown</td>
</tr>
<tr>
<td>Epidermolysis Bullosa Acquisita</td>
<td>EBA</td>
<td>humans, dogs</td>
<td>collagen VII</td>
<td>collagen VII</td>
</tr>
<tr>
<td>Type-I Bullous Systemic Lupus Erythematosus</td>
<td>BSL-E1</td>
<td>humans, 1 dog</td>
<td>collagen VII, nuclear antigens, others</td>
<td>collagen VII, nuclear antigens</td>
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<tr>
<td>Acquired Sensational Epidermolysis Bullosa</td>
<td>AEB</td>
<td>humans, 3 dogs</td>
<td>laminin-332</td>
<td>laminin-332</td>
</tr>
</tbody>
</table>

**Mixed Autoimmune Subepidermal Blistering Dermatosis (MABSD)**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abbrev.</th>
<th>Affected Species</th>
<th>Autoantigens Targeted by Autoantibodies Humans</th>
<th>Autoantigens Targeted by Autoantibodies Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed Autoimmune Subepidermal Blistering Dermatosis (MABSD)</td>
<td>MABSD</td>
<td>humans, 3 dogs</td>
<td>collagen VII, laminin-332, others</td>
<td>collagen VII, laminin-332</td>
</tr>
</tbody>
</table>

Note: underline are major autoantigens, i.e. those recognized by serum autoantibodies of more than 50% of patients with the disease; underlying use only done if autoantigens were characterized in more than 10 patients.

### Table 2: Revised Classification of Lymphocyte-Mediated Autoimmune Skin Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abbrev.</th>
<th>Affected Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antikeratinocyte AIsDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesicular Cutaneous Lupus Erythematosus</td>
<td>VCLE</td>
<td>dogs, humans</td>
</tr>
<tr>
<td>Exfoliative Cutaneous Lupus Erythematosus</td>
<td>ECLE</td>
<td>dogs</td>
</tr>
<tr>
<td>Discoid Lupus Erythematosus</td>
<td>DLE</td>
<td>humans, dogs, cats?</td>
</tr>
<tr>
<td>Antimeelanocyte AIsDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uveodermatological (Vogt-Koyanagi-Harada) Syndrome</td>
<td>VKH</td>
<td>humans, dogs</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>none</td>
<td>humans, dogs, cats, horses</td>
</tr>
<tr>
<td>Antifollicular AIsDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alopecia Areata</td>
<td>AA</td>
<td>humans, dogs, horses, cows</td>
</tr>
<tr>
<td>Pseudopelade</td>
<td>PP</td>
<td>humans, dogs, 1 cat</td>
</tr>
<tr>
<td>Antifollicular AIsDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sebaceous Adenitis</td>
<td>SA</td>
<td>dogs, cats, rabbit</td>
</tr>
</tbody>
</table>

### Table 3: Useful Signs to Differentiate Resembling AIsDs

<table>
<thead>
<tr>
<th>Sign \ Disease</th>
<th>PV/PNP</th>
<th>AISBDs</th>
<th>EMM/SJS</th>
<th>VCLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>flaccid blister</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>turgid (tense) blister</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>blood in blister</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>erythematous blister margin</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>marginal Nikolsky’s sign</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>direct Nikolsky’s sign</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pseudo-Nikolsky’s sign</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

+: presence; #: variable; -: absence
AISBDs: autoimmune subepidermal blistering dermatoses
EM/SJS: erythema multiforme major/Stevens-Johnson syndrome
PV/PNP: pemphigus vulgaris/paraneoplastic pemphigus
VCLE: vesicular cutaneous lupus erythematosus
Marginal Nikolsky sign: Ability to split the epidermis far beyond preexisting erosions by pulling the remnant of a ruptured blister or rubbing normal-looking perilesional skin
Direct Nikolsky sign: Ability to split the epidermis by rubbing normal-looking skin areas distant from the lesions
Pseudo-Nikolsky sign: Ability to peel off the epidermis by rubbing erythematous perilesional skin areas
Nikolsky sign definitions modified from Grando et al, JAAD 2003
**Figure 1:**

Gilliam-Sontheimer Classification of Human Cutaneous Lupus Erythematosus

- Lupus Erythematosus (LE)
  - Lupus-Related Skin Disease
    - Lupus-Specific Skin Disease
      - Acute CLE (ACLE)
      - Subacute CLE (SCLE)
      - Chronic CLE
        - Includes DLE
  - Lupus-Nonspecific Skin Disease

- Systemic Lupus (SLE)
  - SLE with Skin Lesions
    - SLE with Skin Lesions
  - SLE without Skin Lesions

**Figure 2:**

Revised Classification of Canine Cutaneous Lupus Erythematosus

- Lupus Erythematosus (LE)
  - Lupus-Related Skin Disease
    - Lupus-Specific Skin Disease
      - Cutaneous LE (CLE)
        - Vasculitis (incl. Lupus Periarteritis?)
        - Autoimmune DM Disease (BSLE-I)
  - Systemic Lupus (SLE)
    - SLE with Skin Lesions
    - SLE without Skin Lesions

*Modified from Sontheimer, 1987*