Common Pitfalls in Veterinary Dermatology
Sheila Torres, DVM, MS, PhD, DACVD
University of Minnesota
Madison, WI

Skin and ear disorders account for the large majority of cases clinicians examine routinely in private practice. In contrary to most specialties, it is possible for any veterinarian to diagnose the common dermatologic conditions of dogs and cats with very little operating costs. All that is needed is a good microscope, a good handheld otoscope, glass slides, coverslips, scalpel blades, mineral oil, immersion oil, acetate tape, Diff-Quik stain, new methylene blue stain, cotton-tip applicators, 6 or 8 mm punch biopsy instrument, scissors, lidocaine, formalin jars, needles, syringes, cotton balls, gauze, a few bags of novel or hydrolyzed protein diet, among few other things. The most valuable asset, that unfortunately is not always available, is the knowledge regarding when to use these materials, how to use them and, how to interpret the results.

Common shortcomings of clinicians in general practice when working up a dermatological case include the failure to perform, perform correctly, or interpret properly the following diagnostic tests: cytology; skin scraping, bacterial culture and susceptibility, skin biopsy, and food trial. In addition, more frequently than not, antibiotic, glucocorticoid, and shampoo therapies are not instituted properly. Nonetheless, the good news is that these pitfalls can be easily corrected. We will review here not the pitfalls per se but how to properly perform some dermatological tests and to use a few commonly prescribed drugs.

Cytology
Cytology is the microscopic examination of skin and ear samples collected from various lesions and, in many different ways according to the lesion. I can certainly say that dermatologists perform cytology in at least 90% of the cases with skin or ear problem; therefore, if you are not routinely performing this very simple test, you are missing a quite valuable diagnostic tool.

What do I need to perform cytology?

Very little is required including: glass slides, coverslips, Diff-Quik or any Romanovsky-type stain, and a microscope.

When to perform cytology?

Samples for cytology should be collected in every dog or cat with otitis externa at the first visit and during follow up visits. In addition, samples should be collected in the presence of pustules, vesicles, bullae, crusts, epidermal collarettes, exudative lesions (i.e. fistulous tracts, eroded, and ulcerated lesions), oily or scaly skin surfaces, nodules, and plaques.

How to perform cytology?

The method of collection will vary depending on the type of lesion present. These methods are well described in many textbooks; however, the clinician can be creative and modify them as needed, by asking the question: “What is the easiest way to collect a sample from this lesion for cytology?”

Let’s review some of the various techniques that are used based on the different types of lesions. Pustules – Remember, pustules are small lesions and you want to collect their content without causing bleeding. Use a 25 gauge needle or the edge of a glass slide to gently rub the top of the pustule with the intent of rupturing it (do not angle or “stab” the pustule with the needle to avoid bleeding). Transfer any exudate present in the needle or slide edge directly to a glass slide (this method is called “direct smear”). Thereafter, perform direct imprints by pressing the slide against the lesion multiple times. This technique can be also used to collect samples from other fluid-containing lesions such as vesicles or bullae. In addition, direct imprints are commonly used to collect exudate from underneath crusts, fistulous tracts (press the lesion to bring the exudate to the surface; cotton applicators can also be used to collect samples from draining tracts), surfaces of eroded or ulcerated lesions, and edges of epidermal collarettes. It is also an effective technique to transfer greasy samples to a glass slide.

Scaly or greasy skin surface

The acetate tape test is often used in these circumstances and mainly when lesions are present in areas where a direct imprint (or other technique) cannot be performed such as, interdigital spaces, skin folds, etc. The method is easy to perform: (i) take a piece of acetate tape (shorter than the length of the glass slide), place the sticky part on the lesion surface and using your indicator finger, press and scratch the tape against the skin very firmly; (ii) use Diff-Quik stain but, only the dark blue stain; (iii) after gently rinsing the tape with water, allow it to air dry (or blotch dry); (iv) place a drop of immersion oil on the surface of a microscope slide and then place the tape sticky side over the oil; (v) apply another drop of oil on the non-sticky surface of the tape and examine under oil immersion. As mentioned before, direct imprint can also be used to collect samples from greasy lesions.

Nodules, masses, plaques or cysts

In these cases it is important to sample the core of the lesion; therefore, you need to perform fine needle aspirations. Fluid-filled lesions can be aspirated with 20- or 22-gauge needles and a 3 ml syringe. Firm lesions should be aspirated with 20-gauge needles and 6 or 10 ml syringes to obtain better suction. Small solid lesions can be aspirated using 22-25-gauge needles. Fibrotic or dense masses may necessitate the use of an 18-gauge needle to get an adequate sample. The needle is introduced into the lesion, and then suction is gently applied by withdrawing the plunger of the syringe. Little withdrawing is necessary for fluid-filled lesions. In solid lesions, the
plunger is withdrawn one half to three fourths of the syringe volume. Suction is then interrupted while the needle is redirected into another area of the mass. Suction is again applied, and this procedure is repeated three to four times. Suction is then released, and the needle is withdrawn from the lesion. The syringe and the needle are separated, air is introduced into the syringe, the needle is reattached, and the contents of the needle and hub are expelled onto the surface of a glass slide. The material is then streaked across the microscope slide surface with another glass slide or the needle; allowed to dry and then stained. An alternative to aspirating the lesion content is to just use a fine needle to collect the sample (i.e. “fine needle sampling”). This method is identical to fine needle aspiration without using a syringe. The needle is introduced into a mass and redirected multiple times to sample a representative portion of the tissue. Short and fast movements with the needle can be made to optimize sample collection of solid masses. After adequate sample is collected, introduce air into a syringe, attach the needle onto the syringe and push the syringe plunger to expel the needle content onto a glass slide. The material is then streaked across the microscope slide surface with another glass slide or the needle, allowed to dry and then stained. This method is easier to perform and the sample quality is arguably better.

How to interpret the test results?
If intra and extracellular cocci-shaped bacteria are present, it is likely a case of staphylococcal pyoderma. If the pyoderma is superficial, degenerated neutrophils will be present; if it is deep, other inflammatory cells such as, lymphocytes, plasma cells, macrophages, and eosinophils will be seen, in addition to red blood cells. In deep pyoderma the number of bacteria is much smaller compared to superficial pyoderma. Be careful when interpreting the presence of rod-shaped bacteria on cytology. These bacteria rarely cause skin infections but, if they are seen inside inflammatory cells and the samples were collected from areas where the animal cannot lick (they are normally present in the mouth), the findings suggest an infection. Large number of bacteria without inflammatory cells indicates an overgrowth. Cytology of lesions caused by malassezia will show variable numbers of yeast organisms without inflammatory cells, also indicating a malassezia overgrowth. The presence of anaplastic cells (i.e. immature keratinocytes) with non-degenerated neutrophils suggests pemphigus complex (these cells can also be found less often in severe pyoderma or dermatophytosis cases typically caused by Trichophyton spp). If neutrophils and macrophages are the predominant cells look for fungal or atypical bacterial organisms, but the process may be sterile. The presence of groups of anaplastic cells indicates neoplasia (these can be mixed with inflammatory cells).

Skin scrapings
Skin scrapings are cheap, easy and quick to perform and are remarkably informative; therefore, perform skin scrapings every time you are faced with alopecic lesions in a dog or cat. It should be also performed when the differential diagnosis includes microscopic ectoparasitic diseases (e.g. demodicosis, sarcoptic mange, and cheyletiellosis). Do not embarrass yourself by missing a case of canine follicular demodicosis where mites can be easily found on properly done skin scrapings.

What do I need to perform skin scrapings?
A dull clean # 10 scalpel blade (smaller blades may be used for periorbital scrapings) or a spatula, mineral oil, glass slides, and a microscope.

How to perform skin scrapings?
Place a few drops of mineral oil on the scalpel blade/spatula or the skin site to be scraped to facilitate the adherence of the sample to the blade. Place the sample on the microscope slide and add mineral oil enough to float a cover slip. The oil is mixed with the scraped material to prevent clumping and improve visualization of the sample under the microscope. Place a cover slip on the material to be examined to ensure a uniform layer that is more readily examined. The coverslip will also protect your microscope lens. Lowering the microscope condenser causes more light diffraction and contrast, resulting in easier recognition of the mites, especially demodex mites which are transparent. Examine the samples under 20x or 40x objectives. The depth of the scraping will vary according to your diagnostic suspicion. Superficial scrapings covering a broad area are sufficient for surface-dwelling mites such as, Cheyletiella sp, Choriopotes sp, Demodex gatoi, and Demodex cornei, and for mites that burrow in the epidermis such as, Sarcoptes sp and Notoedric sp mites. To assure that adequate sample is obtained, scrape until a small amount of blood from laceration (capillary bleeding) is obtained. Deep scrapings are necessary for deeper-dwelling parasites such as, Demodex canis, Demodex injay, and Demodex cati. Squeeze the skin between the thumb and the forefinger to extrude mites from the hair follicles and scrape until capillary bleeding is visible. Mites are easily found in follicular demodicosis caused by D. canis or D. cati (when scrapings are properly done) but, they can be more difficult to find in demodicosis caused by D. injay. “Dry” superficial skin scrapings can be used to collect surface samples (i.e. stratum corneum) for cytology when suspecting of Malassezia or Staphylococcus overgrowth. This method of scraping can be used for areas of skin that are naturally oily since you are not applying mineral oil to the scalpel blade or spatula. Transfer the sample to a glass slide, heat fix it and stain. The sample will need to be examined with immersion oil under 100x objective.

How to interpret skin scrapings?
The only parasitic skin diseases where skin scrapings have a very good sensitivity are follicular demodicosis caused by Demodex canis and Demodex cati. Therefore, do not rule out any of the other parasitic diseases (i.e. cheyletiellosis, sarcoptic scraping, and demodicosis caused by Demodex injay, Demodex gatoi, and Demodex cornei) if skin scrapings are negative.

General rules when performing skin scrapings include: (i) always perform multiple skin scrapings to increase your chances to find mites; (ii) a positive skin scraping confirms a parasitic skin disease; however, a negative skin scraping does not rule it out; (iii)
squeezing the skin before scraping if suspecting of follicular demodicosis; this will help bring the mites to the skin surface; (iv) scrape until capillary bleeding is obtained; (v) erythematous papules are great lesions to scrape when suspecting of dermatoses caused by parasites, and (vi) lower the microscope condenser to increase contrast and more easily visualize the mites.

**Skin biopsies**

Biopsies are indicated in the presence of nodules, tumors, vesicles, bullae, or any lesions that are unresponsive to symptomatic therapy, or when specific causative factors have not been identified. It is important to tell owners that not always a skin biopsy will be diagnostic!

**What do I need to perform skin biopsies?**

2% lidocaine, 4, 6, and 8 mm punch biopsy instruments, iris scissors, forceps, formalin jars, needles, syringes, suture material, needle holders, and gauze pads.

**How to biopsy**

Skin biopsies are only valuable when performed properly. A few rules need to be followed: (i) select the samples properly! Primary lesions when present (i.e. papules, pustules, vesicles, bullae, etc.) should always be biopsied. Perform multiple biopsies to increase your chances of making a diagnosis; (ii) do not scrub! Surgical preparation of the biopsy site will remove crusts, rupture pustules, vesicles, and bullae and, may introduce new lesions. The non-surgical preparation of the area seldom leads to complications in dogs and cats; (iii) never sample normal skin when using a punch. The lesions are often small, thus, you may end up sampling only normal skin if you try to get samples of both abnormal and normal skin at same time with a punch; (iv) large lesions should be excised completely or have a wedge-shaped sample removed. The longitudinal axis of the wedge should begin at the center of the lesion and end in the normal skin 3-4 mm outside the lesion; (v) when taking biopsies of face, ears, nose, feet, and mucous membranes, use general anesthesia or heavy sedation; (vi) biopsies should be put into 10% buffered formalin immediately after collection; and (vii) submit the samples to a veterinary dermatopathologist or a veterinary pathologist with an interest in dermatopathology, for the best results.

**Bacterial culture and susceptibility (C&S)**

It is never wrong to perform bacterial C&S; however, the following are the situations where they must be performed: (i) recurrent infections; (ii) history of multiple antibiotic therapies; (iii) no response to the current treatment; (iv) history of methicillin resistant or multidrug resistant infections; (v) presence of rod-shaped bacteria on cytology; and (vi) deep infections.

**Proper use of systemic antibiotic therapy**

(refer to the notes on “Antibiotic guidelines for superficial pyoderma” for additional information) – When selecting an antibiotic to treat bacterial skin infections, make sure to use the right antibiotic at the right dose for the right duration of time. An empirical antibiotic selection can be considered for mild and non-recurrent infections when the clinician is not practicing in a geographic area where antibiotic resistance is a concern (review the reasons for performing C&S, mentioned above). In these circumstances, most dermatologists will first select cephalaxin or cefadroxil, with the second choice being typically amoxicillin clavulanate. My recommendation is to not use any other antibiotic to treat skin infections without performing C&S. When treating a bacterial skin infection, use the high end of the antibiotic dose range and never under dose. Make sure to weigh the patient before determining the correct dose and to refer to a drug handbook for the recommended dosage ranges of the various antibiotics. Also follow the recommended frequency of administration taking into consideration if the antibiotic is time or concentration dependent. Most uncomplicated superficial bacterial folliculitis cases will resolve after 2-3 weeks of appropriate antibiotic therapy. However, recurrent or resistant infections may take longer (4-6 weeks). Deep bacterial infections may take at least 6 to 8 weeks to resolve. The widespread rule of thumb currently adopted by dermatologists, is to treat a superficial infection for 7 days past complete clinical resolution and, a deep infection for 14 days. The assessment has to be made by the clinician and not the pet owner. Reevaluations should be scheduled, ideally, every 2 to 3 weeks.

**Proper use of glucocorticoid therapy**

Glucocorticoids (GCs) are wonderful drugs if used properly, otherwise, they can induce a worse disease than the one being treated because of the potential severe side effects associated with GC therapy. Because in veterinary dermatology most inflammatory diseases treated with GCs are chronic, non-curable diseases, we have to carefully consider the following: (i) do not use injectable, long-acting GCs to treat any inflammatory skin or ear disease of dogs because these drugs can have a much stronger effect in the hypothalamic-pituitary-adrenal axis and, typically, their anti-pruritic/anti-inflammatory effect will not last longer than 2 weeks;(ii) use long-acting GCs carefully in cats with skin diseases, because they will induce diabetes mellitus much more easily compared to the short-acting oral CGs such as, prednisolone and methylprednisolone (the only justification for using injectable GCs in cats is because they can be hard to pill); (iii) try to always use oral short-acting GCs such as, prednisone, prednisolone (use prednisolone in cats because they do not efficiently metabolize the pro-drug, prednisone), and methylprednisolone; (iv) use the proper dose and as soon as possible reduce the dose to the lowest possible amount that maintains the disease under control and, administer the drug every-other-day when using short-acting drugs or every-third-day when using intermediate-acting drugs (i.e. oral triamcinolone and dexamethasone); (v) avoid using it on dogs with parasitic skin diseases (e.g. demodicosis, etc.), infectious disease (e.g. fungal diseases, etc.), or wounds.