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Invited Clinical Article

Step wise approach in diagnosis of dermatological disorders in small animals

B. Nagarajan
President, Association for Veterinary Dermatology, India

Many times Patient data itself will give a clue for clinician to guess the underlying pet skin diseases. A careful dermatologic history is critical to interpret the physical examination findings and choose appropriate diagnostic tests. A detailed history, physical examination, and appropriate diagnostic tests are very much needed for a definitive diagnosis of various skin diseases of pets.

Important Clues about Patient data

Species: Many owners may not aware that unlike dogs, cat would not scratch in front of others unless it is severely affected which lead the owner to say his cat is not scratching. Insect Bite Hypersensitivity is commonly seen in Horses.

Age: Young pups are commonly affected with skin issues like verminous dermatitis juvenile cellulitis, food allergy. Age of first onset in Canine atopy is generally from 6 months to 3 years. Majority of endocrine disorders are seen in adults.

Breed: Clinician should think about the breed predisposition of wide variety of skin diseases like German Shepherd– GSD Pyoderma, West High Land white terrier, Labrador for atopy, Cocker spaniel for Primary seborrhoea, Feline urticaria pigmentosa in Sphynx cats.

Sex: Male feminising paraneoplastic syndrome occurs only in male dogs in approximately 70% of Sertoli cell tumors arising in functional abdominal testes. In female dogs, in Hypoestrogenism, there is a gradual loss of hair due to lack of new hair growth over the under-surface of the belly, around the vulva and the lower chest and neck. The skin becomes soft, smooth, and nearly hairless.

Dogs with hypoestrogenism due to ovarian dysregulation, ovarian cysts, or ovarian neoplasia will have constant oestrus, with numerous comedones on the vulvar and ventral skin, and have flank, perineal, and caudal ventrum hypotrichosis-to-alopecia in the older. Regularly cycling bitch will develop an androgen patterned hair loss i.e. collar region, rump, perineum and ventrum. This hair loss is associated with signs of overt pseudo-pregnancy and when it is over, the hair will regrow spontaneously but a relapse can be expected at next oestrus

Important Clues from History

Clinician should concentrate more on proper history taking since it is possible to get 80 percent of correct clue to identify the type of pet skin disease from the history. The following points should be noted:

General history

- How long the problem has been present? Age of onset
- Where it started & how was its spread?
- Type of lesions noticed first and how was the change?
- Any other pet in pet Family affected?
- Scratching Y/N? (Owner/companion involved Y/N)
- Seasonal/Non seasonal?
- When was the Vaccination and Deworming done?

Feeding history

- Protein source - (Y /N); Dairy Products - (/Y/N); Wheat Products - (Y/N); Nuts - Y/N; Meat Products (Chicken/Mutton/Beef/Any Treats given) - Y/N
- Recent change in the diet and from how long?
- Previous history of allergy to food

Environmental exposure

- Indoor/outdoor
- Plant/pollen

Chemical exposure to and Frequency of exposure

- Eye/ear drops
- Floor cleaning
- Bath -Soap/shampoo

Prior surgery

Previous and present illness

Drugs used past/current and Response to previous therapy

Opinion of owner

General examination: Before the detailed dermatologic examination, perform a complete general physical examination, by which patient's overall body condition should be assessed. Thoracic auscultation and abdominal palpation must be carried out in all patients for quick assessment of the internal problems.

Physical examination: Detailed clinical examination of the skin should be carried out by analyzing distribution and pattern of primary and secondary skin lesions. It is done from head to tail.

- Check for Ticks/Lice/Flea/Mite; Odour, Hypertrichosis/hypotrichosis/hemosiderosis ; Localized/generalized/individual or cluster.
- Distribution of lesions and pattern of lesions (which lesion pattern is predominating) in the body will give a clue in diagnosis. Distribution of different" Reaction pattern "like miliary, head and neck lesions, symmetrical alopecia, eosinophilic granuloma complex signs will give important diagnostic clue in cats

Primary Lesions: Directly associated with diseases, not pathognomonic, valuable clue to assess the type of disease

Macule/Patches: Flat areas of discoloration<1cm/>1cm

Depigmentation: Vitiligo, discoid lupus eryhtematosus (DLE), uveodermatologic syndrome (UDS), Mucocutaneous pyoderma

Hyperpigmentation: Lentigo, Hormonal, Post inflammatory Erythema, Inflammation, Vasculopathy coagulopathy

Papule: Small, solid, elevated upto1cm

- Allergic dermatitis
- Parasitic dermatoses
- Bacterial/fungal folliculitis
- Autoimmune diseases
- Early neoplastic lesions

Plaque: Flat, solid, elevated>1cm infiltration, proliferation eg: drug eruption, eosinophilic plaque in cat

Nodule: solid elevation>1cm

Tumor : large growth

Wheal: Irregular, elevated, edematous skin-change in size & shape

- Urticaria
- Insect bites
- Hypersensitivity reactions
- Drug eruptions

Cyst: enclosed cavity with membranous lining with liquid/semisolid material -cystic basal cell tumor

Secondary Lesions

- Result of trauma, time, degree of insult
- Primary lesions evolve secondary lesions
- *Epidermal Collerate:* A circular lesion with a circular rim of scale with a peeling edge. This is seen in superficial pyoderma cases.
- *Comedones:* Sebaceous and epidermal debris blocking a hair follicle. Eg: Hyper adrenocortism
- *Scales:* cast off/dead superficial epidermal cells E.g., Keratinization disorders
- *Crust:* cells and dried exudate - e.g. pemphigus foliaceus cat
- *Erythema:* reddening of the skin due to increased blood flow. e.g., Malassezia, Atopy, Pyoderma, ectoparasites
- *Erosion*: loss of superficial epidermis
- *Excoriation:* self trauma
- *Scar*: abnormal fibrous tissue that replaces the normal tissue after injury-burn
- *Fissure*: A linear break in the skin, sharply defined with abrupt walls
- *Furunculosis*: Deep folliculitis in which the follicular sac breaks and became necrotic, releasing keratin, bacteria and inflammatory cells in dermis. Painful erythematous nodule with sinus / fistula.
- *Lichinification:* thickening of skin seen in chronic skin diseases
- *Ulcer:* loss of epidermis and basement membrane, exposing the deeper dermis
- *Thinning of Skin*: hyper adrenocortism
- *Hyper Pigmentation, Hypo Pigmentation*
- *Induration*: Localized hardening of soft tissue of the body
- *Inter Digital Granuloma*: seen in between the digits due to common primary causes include allergies and foreign bodies. Persistent licking pushes the hairs into the dermis and subcutis resulting in inflammation, hair follicle rupture and free keratin, all of which can result in granuloma formation. Obesity, spinal disease and conformational and gait

abnormalities contribute to formation of false pads and in the long term progress into cystic lesions. Poor conformation, such as widely splayed toes are predisposing factors

- Alopecia –
- *Inflammatory alopecia*, follicle loss is faster than the follicle can replace it. There is nothing fundamentally wrong with the follicular unit either in the signalling for the production of a new shaft or in the production of the shaft. e.g. allergies, infection by bacteria, yeast , fungi and parasitic infestation
- *Noninflammatory alopecia* develops because of either an abnormality in the formation of the hair shaft (dysplasia) or a failure of the follicle to continuously cycle. Follicular Dysplasia of Siberian Husky and Malamute, Black Hair Follicular Dysplasia, Cyclic Flank Alopecia, Colour Dilution (Mutant) Alopecia, *Pattern Baldness,* Testosterone-responsive dermatosis, Congenital adrenal hyperplasia (adrenal sex hormone imbalance) in Pomeranians(black skin disease), Chow Chow, Keeshond, Samoyed, Sex Hormone Dermatoses, Hyperadrenocorticism, Hypothyroidism
- Presence of inflammation distinguishes hypersensitivity conditions from endocrine disorders. Allergic pets will generally have inflamed skin as a result of pruritus, erythema and warm skin. Sites usually affected by atopic dermatitis and food allergy include the face, pinnae, feet, medial brachial region and the inguinal, axillary and perineal areas and Flea allergy with typical lumbosacral ,dorsal caudal trunk in dogs.
- Internal manifestations or systemic signs such as changes in weight, exercise tolerance, hunger or thirst will not be there in allergic pets except in food allergy may suffer from GI signs like periodic vomiting, diarrhoea, flatulence or frequent bowel movements which may influence alteration in weight to some extent. No abnormalities on blood work, except for the occasional hyper eosinophilia or sick euthyroid in allergic dogs.
- Patients with an endocrine disease generally won't be pruritic.
- Since both allergies and endocrine disease often pick up secondary infections with *Staphylococcus* or *Malassezia*, leading to pruritus, diagnosis is challenging one.

Diagnostic Tests:

They are performed to differentiate and confirm the specific skin disease which can be done easily without much investment in the clinic itself. They include: A good microscope, glass slides, cellophane tape,number10 BP blade, mineral oil, cotton ear swab, coat brushing comb, artery forceps for trichogram, Diff -quik or Field A&B stain, disposable tooth brush, woods lamp, sterile swab for culture, Dermatophyte Test Medium,2ml syringe needle for Fine Needle Aspiration Cytology(FNAC), biopsy punch 4mm and 6mm, scissors, thumb forceps, formal saline in labelled containers ,gauze pieces, 2-0, 1-0 nylon sutures, a marking pen and a good cell phone with good pixel camera for documentation.

Performing skin scraping to rule out the skin parasites involved and skin cytology to understand and identify the physiological and pathological skin environment are the two important primary steps in diagnostic tests. Cytology can be obtained by different methods like skin scrap, tape impression, direct and indirect impressions.

Skin Scrapings

Skin scraping should be the first step in diagnosis to rule out skin parasites. The skin scraping technique used can vary depending on the specific ectoparasite suspected, such as superficial or deep skin scraping.

Superficial skin scraping

To identify infestation with *Sarcoptes* mites, obtain multiple wide superficial scrapings at crusted, papular, or alopecic lesions on elbows, pinnal margins, and the ventral trunk. *Sarcoptes* mites live in the stratum corneum and are often few in number. Therefore, false negative scrapings are common. So any animal with intense pruritus with classic clinical signs of scabies should be subjected to treatment trial with appropriate acaricidal therapy.

To identify surface-living *Cheyletiella* mites, often found along the dorsum obtain wide superficial scrapings of scaly lesions, and place these scrapings in mineral oil for microscopic examination in 10X magnification. As in cases of suspected scabies, empiric acaricidal therapy is often prescribed in cases of suspected *Cheyletiella* infestation.

In cats Superficial scrapes are used to detect *Demodex gatoi* and *Notoedres cati*,

Deep skin scraping

Deep skin scrapes are used to diagnose dogs with *Demodex canis* and cats with *Demodex cati* mites.

Place a few drops of mineral oil on a glass microscope slide. Hold a #10 scalpel blade firmly in one hand after scooping small quantity of mineral oil. With the other hand, gently collect a fold of skin on the trunk, or evert a pinna or interdigital space and scrap the skin, intermittently squeeze the scraped area to bring out deep seated mites. In a single direction, scrape the skin with constant pressure over a single site until a small amount of capillary oozing occurs. Apply the collected material and exudate to the glass slide containing mineral oil. Evaluate under 10× magnification for mite evaluation.

For cytology, scraped material can be smeared in glass slide directly without adding mineral oil and stained before examining.

Coat Brushing is performed with a comb or disposable tooth brush to collect the hair and scales which is collected in a white paper and aggregated in a glass slide to examine under low power for parasites, eggs and nites

Tape Impression

It is done most commonly to recognise the parasites, yeasts, bacteria, and to identify the type of cells. *Cheyletiella* mites, demodex mites and Malassezia dermatitis are readily recognised by tape impressions. It can be used to sample erythematous, dry, lichenified skin, lesions at skin folds and interdigital areas.

Procedure

Clear, sticky side of acetate tape is pressed over the skin lesion areas. Stick the tape by folding at the distal end of glass slide with sticky area exposed outer side and dip 10 times in each red and blue stain of modified wright's stain(diff-quik). Wash with water, air dry it. While staining, fixation with methanol should not be done to avoid denaturing of acetate tape strips and directly we can use red and blue stain. Spread and place the stained tape strip over the glass slide after placing a drop of mineral oil. Spreading over the oil will adhere the tape in slide. Over the stained acetate tape also place a drop of mineral oil and place a coverslip and see under oil immersion for cytology ,low power for parasites. Malassezia organisms are identified as round to oval budding yeasts under high-power field.

Direct Impression

A superficial skin cytology examination by direct impression is indicated in all conditions that present:

- Papules, pustules and/or epidermal collarettes.
- Erosions, ulcers and/or crusts.
- Dry or oily scales.
- Increased secretions or exudate present in the ear canals

Procedure: This technique involves directly pressing the slide gently on the affected skin surface. Before collecting the sample it is best to clip the hair surrounding the lesion with scissors.

A skin cytology sample can be obtained in a variety of ways, depending on the type of lesion present.

a. If pustules are intact they should be gently broken with the 25-gauge needle. The purulent material found is then collected by gently pressing the slide on the surface.
b. Crusts must be lifted with a fine-gauge needle to expose the surface exudate.
c. In the presence of erosive-ulcerative lesions, it is recommended to dry the exudative surface by rubbing it and then collect fresh exudate.

In general, apply samples to a microscope slide and stain with Diff-Quik. Then scan the sample at 10X magnification for a representative area, and examine that area under oil immersion at 100X magnification.

Interpretation

a. Normal -Keratinocytes ,corneocytes and melanin granules . A small number of cocci (<2 per immersion field (1000x))and Malassezia (<1-2 per dry field (400x)) may be present. Absence of bacilli and inflammatory cells.
b. Bacterial infection, Neutrophilic or pyogranulomatous inflammation -degenerated neutrophils with intracellular cocci .
c. The presence of an elevated numbers of cocci or the presence of bacilli in the absence of inflammatory cells is diagnostic of bacterial overgrowth
d. Non-degenerated neutrophils without bacteria -sterile inflammation
e. Non-degenerated neutrophils and numerous acantholytic keratinocytes -pemphigus complex

,chronic bacterial or dermatophyte infection

f. The presence of an elevated number of Malassezia is diagnostic of overgrowth due to Malassezia

g. The presence of eosinophils -initially suggests allergic dermatitis and ectoparasitosis. Eosinophils may occasionally be seen inside pustules, suggesting an immune-mediated origin of the pustule, such as pemphigus foliaceus, pemphigus erythematosus or sterile eosinophilic pustulosis.

h. The presence of macrophages, lymphocytes or plasma cells together with degenerated neutrophils in a deep lesion sample (ulcers) suggests a deep pyoderma (furunculosis). In deep pyoderma there are very few bacteria and they are very difficult to identify.

Ear Swab Examination

Ear swab examination is carried out with a cotton swab tip is placed within the ear canal with a gentle movement, so that discharge or debris is picked up by the swab. This is then smeared on a glass slide, stained and examined under a microscope. Separate swab is used for each ear sample to determine if infections are bilateral or unilateral. Ear swab is inserted into the ear deep enough to reach the junction between the horizontal and vertical ear canals and rotated several times. The swabs are gently rolled on a clean slide, with complete revolutions of the swab tip to avoid rupture, of cells. Samples are allowed to air dry before staining. Using methanol as a fixative is sufficient to fix any microorganisms to the slide before staining. Stained slides are scanned under the 40× objective for cells and larger structures. Microbes are further evaluated using the 100× objective.

If mites are suspected, swab material is mixed into a drop of mineral oil on a separate slide. Mite evaluation slides are not stained. Instead, the slide is cover-slipped and examined under the 10× objective for the presence of mites or mite ova. The most common ear mite observed is *Otodectus cynotis* in dogs, cats.

Bacterial numbers (rods /cocci) be reported as average number per 40× objective; as occasional, mild, moderate, or marked; or by using a scale of 1+, 2+, 3+, etc. to denote increasing numbers. These classifications may depend on patient species. For example, in dogs, an average of >25 bacteria per 40× field may be reported as "marked;" in cats, a "marked" number may be >15 bacteria per 40× field. Ear sampling and cytologic evaluation is a rapid, inexpensive test to identify parasites, cocci and rod bacteria, inflammatory cells, and yeast organisms, which are common in otitis externa

Trichogram

Trichogram is performed to diagnose Hair abnormality, Self mutilation, Colour dilution, Dermatophytes, Demodex and Endocrine disorders. Epilate hair completely with in lesion close to periphery and place on the glass slide with mineral oil.

Hairs in anagen (growing) phase have a rounded, curled, bent and often smooth and pigmented root. Hairs in telogen (resting) phase are spear-shaped and lack pigmentation, although the base of the hair may show a roughened or brush-like edge. Assessment of anagen/ telogen ratios- helpful in ascertaining the cause of alopecia although care must be taken in interpreting these ratios.

Nordic breeds have 'telogen dominated hair cycles'; Poodle and Bichon frise have 'anagen dominated hair cycles'. Absence of anagen seen in hair growth cycle disorder (eg endocrinopathy). Normal hair tips taper to a fine point. Fractured hair shafts indicate self-trauma, which is often due to pruritus. Dermatophytosis-affected hairs may be fractured and covered with spores and penetrated by hypha.

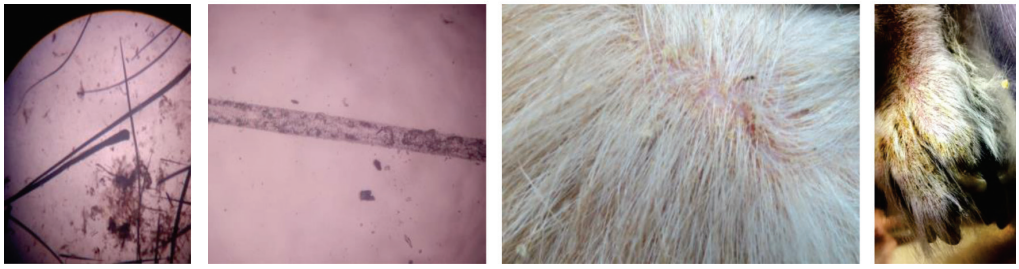
The presence of follicular casts (accumulations of keratosebaceous material around the hair shaft) indicates a follicular cornification disorder of the hair follicle. Follicular casts are usually seen in dogs with sebaceous adenitis. Structural defects: Colour dilution alopecia or black hair follicular dysplacia seen with melanin clumps. Care should be taken while interpreting Weimaraner which may normally have melanin clumps along the hair shaft. If dermatophytosis fungal spores efface the clear shape of the hairshaft.

Fine Needle Aspiration Cytology

Indication

The procedure is a minimally invasive, simple, accurate, fast, and economical technique which does not typically require general anesthesia or sedation. FNAC technique is useful in collecting samples from cutaneous and subcutaneous nodular lesions.

Aspiration is advantageous as the masses are extremely firm and the added force of the negative pressure generated in the syringe may pull firmly attached



Examination of hair bulbs

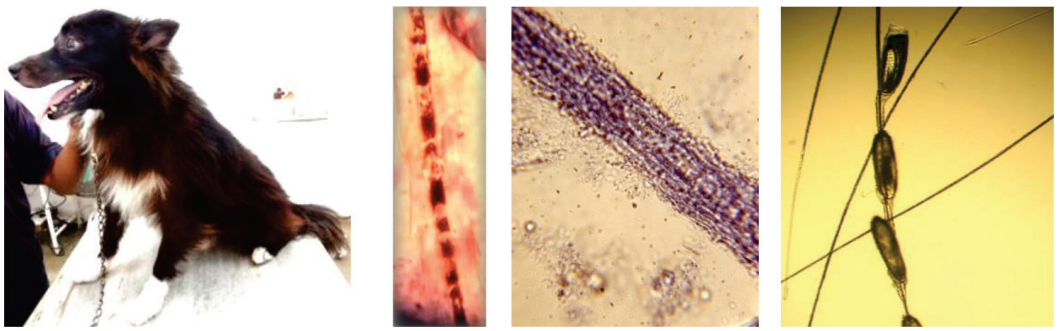
cells into the needle. Aspiration is also helpful if the areas to be sampled are extremely sensitive or painful or are near a critical structure that should not be punctured, such as the eyelid margin or an inflamed digit.

Procedure

- Attach the 5-mL syringe to the needle and insert the needle into the tissue. Hold the syringe in one hand and the mass in another.
- Once the needle is inserted, the plunger is pulled back to obtain 2 to 3 mL of negative pressure.
- Gently remove the needle from tissue.
- Remove the needle from the syringe and aspirate 4 to 5 mL of air into the syringe. It is necessary to remove the needle before drawing air into the syringe, otherwise the sample will be pulled into the syringe and the amount expelled will be poor.
- Expel the sample onto a slide.
- For cytologic assessment, make smears that are thin and evenly distributed without rupturing the cells.
- Stain with an in-house stain (Diff Quik) for assessment

Intrepretation

- Neutrophilic (suppurative or purulent) inflammation contains primarily neutrophils. This type of inflammatory response is nonspecific but warrants careful examination for organisms such as bacteria or fungi. Culture may be required if the neutrophils are degenerated. Noninfectious causes of neutrophilic inflammation include trauma, chemical injuries, immune-mediated diseases (e.g. pemphigus complex), neoplasia, or foreign body reactions.
- Eosinophilic inflammation occurs with arthropod bites, parasitic infections, allergic or hypersensitivity reactions, eosinophilic granuloma complex, fungal infections, collagen necrosis, or some types of neoplasia (e.g., mast cell neoplasia).
- Macrophagic (granulomatous) inflammation is characterized by an influx of macrophages and may contain numerous multinucleated giant cells. Macrophages are often intermixed with neutrophils (pyogranulomatous inflammation), eosinophils, and/or lymphocytes/plasma cells and considerations are similar. This is associated with infections (often more complex bacteria, protozoa, dermatophytes, or localized or systemic fungi), foreign body reactions, nodular panniculitis/steatitis, lick granuloma, sebaceous adenitis, calcinosis circumscripta, injection site reactions or cutaneous xanthoma.



Hair Shaft with Nits

- Lymphocytic or lymphoplasmacytic inflammation is relatively uncommon by itself and is usually found as part of a mixed inflammatory response. In inflammatory lesions, lymphocytes are heterogeneous but contain primarily small cells that have a round to oval, densely stained nucleus with a thin rim of cytoplasm.
- Cutaneous lymphoma should be considered if there is a monotypic population of atypical lymphocytes and plasma cells and other inflammatory cells are absent.

Wood's Lamp Examination

Wood's lamp evaluation for dermatophytosis is an easy and useful tool. It is a good screening test, although false-positive and false-negative results often occur. Only 30% to 80% of *Microsporum canis* isolates fluoresce, and *Microsporum gypseum* and *Trichophyton mentagrophytes* do not fluoresce under a Wood's lamp. Occasionally, lint, dander, and organic debris can fluoresce and give a false-positive result. Dermatophytes produce an apple-green color upon fluorescence Other, less common, species of dermatophytes that fluoresce are *Microsporum audouinii*, *Microsporum distortum*, and *Trichophyton schoenleinii*.

Procedure

- UV light filtered through Nickel oxide
- Detects fluorescent pteridine metabolites from *M.canis* (50%)hairs not on scales and claws
- Electric is superior to battery
- The Wood's lamp should be warmed up for 5 minutes before use because the stability of the light's wavelength and intensity depends on temperature.
- The animal is examined under the lamp in a dark room.
- Hairs invaded by *M. canis* may show a yellow-green fluorescence. This fluorescence runs along the hair shafts rather than fluorescing on discrete, individual, occasional scales, as may be seen in normal animals and humans.
- Some drugs, soaps, and bacteria such as Pseudomonas aeruginosa may also cause fluorescence but are usually not associated with hair shafts.
- A lack of fluorescence does not rule out dermatophytosis. Fungal culture and/or biopsy are the next steps.

Bacterial culture

Bacterial culture is required in canine pyoderma cases during the following criteria

1. Presence of intracellular rod-shaped bacteria on cytology
2. Previous history of drug-resistant infection in the dog or in a pet from the same household
3. Less than 50% reduction in clinical improvement within 2 weeks after appropriate systemic antimicrobial therapy has begun
4. Emergence of new lesions (papules, pustules, collarettes) 2 weeks or more after the initiation of appropriate antimicrobial therapy

This technique is also used to collect the secretions inside the ear canals and exudate present in the interdigital spaces or between skin folds,

- Dermatophyte culture
- Scrape , comb , pluck
- Inoculate on to agar gently
- Color change of medium – white colony growth
- Not all white colonies are dermatophytes
- Not all red color change are dermatophytes
- Culture at 25C in the dark is preferred

Skin Punch Biopsy

Indications

1. Acute and severe lesions.
2. Neoplasia (nodule, chronic non-healing ulcerative lesion).
3. Unusual skin lesions.
4. No response to an appropriate therapy.

Procedure

- 6-8mm punch biopsy is used
- No scrub, no forceps
- Select different lesion stages
- No excision for deep lesions
- With firm continuous pressure the punch is rotated in one direction until the dermis is free from its underlying attachment.

- Don’t squeeze the biopsy tissue with artery forceps.
- Samples should be collected in 10% neutral phosphate buffered formalin
- Provide adequate information in the label.
- Dermatopathologists should analyse the sample

Histo Pathological Pattern Analysis

- The pattern of cell distribution in the skin and the cell types present are extremely important diagnostic clues to many dermatoses.
- to generate a list of differential diagnoses.
- more than one pattern of inflammation in a biopsy
- it becomes very important to recognize which patterns are more specific than others in order to identify the primary condition.

Clinician should follow these step wise clinical procedures meticulously and appropriately to have a confirmatory diagnosis of dermatological disorders in small animals. Confirmatory diagnosis only will help the clinician to select the proper treatment schedule for early cure of the patients and make them to lead a good quality life.