Proceedings of the 10th International Congress of World Equine Veterinary Association

Jan. 28 – Feb. 1, 2008 - Moscow, Russia

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THE PRINCIPLES OF DERMATOLOGIC DIAGNOSIS

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The collection of appropriate specimens forms a cornerstone of equine dermatology but failure to select the right specimens and to collect them and submit in the best form often leads to a disappointing outcome. The correct sample selection and collection and the use of experienced dermatopathologists makes dermatology a most satisfying speciality. There is probably more chance of resolution of skin disease in horses than in small animals and so there is a general expectation of success. However, success depends on a diagnosis and a diagnosis depends on a thorough clinical assessment and proper investigative procedures.

a) Skin Scraping:
This is primarily designed to identify burrowing skin mites, which are in any case uncommon in horses. The commonest mite in the UK is *Chorioptes equi*, which is a surface feeding mite and so groomings are usually better. Scrapings can also be useful in dermatophytosis (ringworm) diagnosis but pluckings are probably better and easier.

b) Skin Groomings:
This is used to harvest ectoparasites from a relatively large area of the body into a concentrated form so that even low numbers can easily be seen.

This is a simple and effective aid for location of most of the smaller ectoparasites including *Chorioptes*, *Psoroptes*, *Harvest / Forage* and *Poultry mites* and for lice (both *Damalinia* and *Haematopinus sp.*). They can readily be identified using a black tile or a simple magnifying glass. A dissecting / stereo microscope is very useful – movement of the skin dander is an obvious signal of living creatures! However, identification is important because some are incidental!

c) Hair Plucking:
This is the standard method for identification of dermatophyte infections but can also be used for some of the bacterial infections when a plucking is easily dislodged (e.g. dermatophilosis). Where dermatophytes or Dermatophilosis is suspected there is usually some scaling or crusting and hair loss.

d) Acetate tape preparations: (Sellotape)
Used primarily to identify *Oxyuris equi* eggs on the peri-anal region.

e) Skin Biopsy:
The value of biopsy should not be overstated - many different conditions can induce an almost identical histopathological effect (see notes later). This makes it difficult or impossible for the pathologist to state a definite diagnosis. It is unreasonable to expect him to be able to help in every case! It is very helpful to provide the pathologist with as much helpful information as possible (possibly sending in a copy of your history and clinical findings sheet). Pathologists are very willing to help but do not take kindly to being challenged unnecessarily and then derided because they cannot make a diagnosis for you - you are in the same team not competitors on opposite sides of the field!

Skin biopsies are taken for the following reasons:
- to establish a specific diagnosis
- to eliminate defined clinical conditions
- to monitor the course of disease
- to confirm the completeness of surgical excision of tumours

A single biopsy will seldom answer all four of these questions. It is useful therefore to obtain multiple samples from defined types of lesions (the pathologist should be told the site and the nature of the lesions as far as possible). The exception to this is the vesicle which must be
biopsied as early as possible - many are pruritic and so are subject to early self inflicted damage which seriously alters the pathology and others rapidly become secondarily infected or altered after bursting - again affecting the diagnostic value.

- Biopsies of papules or pustules are prime samples
- Ulcers and crusts are less definitive
- Chronic lesions, superficial inflammatory changes and lichenified crusted dermatoses gain little from biopsy.

**Conditions for which biopsy are useful:**

1. Autoimmune diseases (pemphigus foliaceus and bullous pemphigoid - must be biopsied in unruptured state by SHAVE BIOPSY. Whole lesions should be removed if possible. Biopsy of crusts and ulcers can be useful in Pemphigus foliaceus as often primary vesicles are transient and not often visible. Samples are dispatched for histopathology and immunohistology. Check with lab before obtaining samples to ensure correct fixative.
2. Granulomas caused by bacteria, fungi, and other parasites. Sections can be stained specifically to identify the organism e.g. Onchocerca, deep mycosis, Demodex etc.
3. Neoplasms. These require suitable careful biopsy. If considered safe a wedge biopsy is best in which normal skin and tumour are included with the interface between them.
4. Parasitic Diseases are less suitable except for Habronema and Onchocerca infestations.
5. Infectious Diseases can be helped by biopsy - particularly if the sampled tissue is divided for culture and for histological examination. Staphylococcus and Dermatophilosis and Dermatophytosis may be easily identified from both modalities.
6. Specific Diseases including cutaneous amyloidosis, eosinophilic granuloma, nodular collagen necrosis and several others can provide specific diagnoses.

**Fine needle aspirate:**

Fine needle aspirates rely upon cytological examination but in some cases such as a large abscess filled with pus aspiration is simply diagnostic in its own right. Bacteriological culture can help considerably in these conditions.

In most practice conditions aspiration of nodules or other tumour or inflammatory masses the procedure has little to commend it. In some cases however, significant information can be gained.

**Impression / scrape smear:**

This technique relies upon the harvest of diagnostic cells from the surface of a lesion – it is sometimes possible to make an incision into the lesion and then obtain the smear. However, again the technique is seldom used except in corneal lesions where surgical biopsies may be problematical.

**Shave Biopsy:**

Shave off epidermis in layers parallel to the surface of the skin. No sutures are usually required. This technique is useful for bullous diseases and in places where excisional biopsies might have long term harm e.g. the coronary band.

**Punch Biopsy:**

Use disposable skin punch (6 / 8/ 9 mm diameter available) - use smallest punch consistent with requirement but remember that very small specimen may not be diagnostic and may distort significantly making both the collection and the handling problematical. Use 25g needle to remove biopsy from underlying fat - do not grasp with rat tooth or plain forceps. Useful to obtain a normal biopsy from adjacent area if interface is not obtained. No need to suture sites if small biopsy.

**Wedge Biopsy:**

Used for larger lesions.

Full thickness cut through abnormal tissues and normal skin including the interface. Careful selection of site, which can be cleaned and sutured after biopsy, is taken.
**Excisional Biopsy:**
Both abnormal and normal skin is required in one sample. Useful for vesicles, pustules. Elliptical incision is made to include all tissues down to panniculus muscle. Wound is cleaned and sutured after biopsy is taken.

It is suggested that biopsies are laid down on a small square of card for about 1 minute to allow them to adhere to it before placing in the fixative. This helps to prevent curling and distortion of the biopsy in fixative. Larger pieces of skin should be pinned to card in natural state. Commercially available specimen meshes are helpful. ALWAYS consult with pathologist if in any doubt as to what the best specimen and fixative are.

(a)       (b)

Fixatives:
1. Formal saline (Buffered Neutral Formalin) - always at least 10x volume of specimen.
2. Michel’s Medium: used for immunofluorescence
3. Boulin’s Medium
4. Glutaraldehyde - for electron microscopy (1 mm cubes maximum)

Laboratory Techniques:
Examination should always be undertaken as soon as possible after sampling. Some fixatives e.g. formal saline requires at least 24 - 36 hours for full fixing.

Direct Smears:
**Dermatophilosis** primarily (also pastern and cannon leucocytoclastic vasculitis / folliculitis). Clip off the hairs from crust sample and place skin-side down onto a drop of saline. Allow to specimen soak for several minutes and gently macerate the specimen. The saline should become patently milky in appearance. Remove excess debris and lumps. Heat fix after allowing to air dry and stain with Gram, Giemsa or Wright-Giemsa (DifQik). Examine under oil immersion. Direct impression smears can be taken from the moist form of the condition and stained with Methylene blue or Wright Giemsa. Isolations from older lesions are more difficult and may only show mixed bacteria and degenerate inflammatory cells. In such cases culture is essential. Small pieces of scab/scale or crusts are placed in a bijou bottle with 1 ml distilled water. Allow standing for 3 - 4 hours at room temperature with top loosely applied. Remove the top of the bottle and place in a bell jar under 20% CO₂ produced by burning a candle in the jar. After 15 minutes a drop of the
saline is seeded onto blood agar and incubated at 37°C in 20% CO₂ incubator for 24 - 48 hours. Abundant small colonies are obtained (usually in pure culture). Examine stained smears from colonies for characteristic branching hyphae. (Haalstra, 1965)

**Dermatophytes:** Remove samples from container and culture on Sabouraud’s dextrose agar medium with phenol red indicator and antibiotics to control bacterial overgrowth (available commercially as Fungassay). Samples should be pressed firmly into the surface of the medium and not buried in it. Incubate for up to 14 days at room temperature and at 37°C (best for *Trichophyton spp.*). Earlier indications are gained by red coloration of the medium (alkali change from fungal growth), which occurs at 24 - 36 hours. Dermatophytes always produce white powdery or fluffy colonies (never dark/black). Mucoid, dark or very light colonies are artifacts, which can suggest false positive results.

⇒ Potassium Hydroxide Extraction: Place sample of hair scab or skin onto slide and add several drops of 10% KOH. Warm gently (do not boil!) for 15 - 30 sec. Allow standing for 15 minutes at room temperature. Apply cover slip and examine. All negative samples should be cultured as above.

*Staining the extracted sample is helpful.*