

7<sup>TH</sup> WORLD CONGRESS

of Veterinary Dermatology

JULY 24-28 • 2012

# Proceedings of the Continuing Education Programme

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# **HOW TO MAKE A COST-EFFICIENT AND RELIABLE DIAGNOSIS IN EQUINE DERMATOLOGY?**

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## **1. Introduction**

To make a diagnosis in skin disease it is important to establish the signalment of the patient and to obtain a comprehensive anamnesis (history). Many skin conditions are presented initially at an advanced stage and many have had some (or more) attempts at treatment. A thorough clinical examination should then be performed. These steps should provide a “problem list” and from this it should be clear if and what further tests or examinations need to be performed.

## **2. Signalment**

A good signalment (breed, age, gender, colour etc.) as this description of the horse is important because it narrows the diagnostic possibilities considerably.

- Breed matters because there are some important breed related skin diseases such as “hypotrichosis and fading syndromes” in Appaloosa horses, hereditary equine regional dermal asthenia (HERDA) in Quarterhorses and the “pinky Arab syndrome”. Some skin diseases are a “disaster” in specific breeds, not necessarily for the horse but for the owner. For example e.g. leucotrichia in a Friesian show horse.
- Age matters because some conditions such as the congenital skin defects will normally present from birth (while acquired diseases may not); hirsutism in the horse always resulting from equine Cushing’s disease occurs only in older horses; grass warts (viral papilloma) are commonest in young grazing yearlings and 2-year olds.
- Gender matters less in skin diseases primarily because there are few if any conditions that are restricted to one or other sex; however, there are some venereal skin infections that could be related to gender.
- Colour is an important aspect also because there are some diseases such as melanoma that are often colour linked. Furthermore non-pigmented skin has predispositions to inflammatory disorders and to squamous cell carcinoma for example.

### **3.Anamnesis (history)**

In taking a good history it is important that the veterinarian really asks the questions of the person who knows most about the patient, the other horses on the farm or stud and the management. Often this is neither the owner of the horse nor the owner of the farm

Important questions that have to be asked during history taking are

- When did the problem first become apparent

Veterinarians are frequently not presented with the early primary problem; more often they are consulted after several weeks or even months by which time the primary lesions may have disappeared or be complicated by other secondary issues. The duration of the problem and the onset can provide information on the likely primary disorder.

- How and where did it start

This too can help to clarify the likely primary cause of the problem, especially when the disease has spread all over the body. When the original site actually has remaining diagnostic features it is very useful but even simply knowing the location of the primary problem can be a great help in diagnostic process.

- Did the initial lesions appear suddenly or gradually and what did the first lesions look like
- Have the lesions deteriorated or improved (with or without any treatment attempts)

In the majority of cases owners will have ignored the early clinical signs or made some attempt to treat it themselves. Some may be reluctant to admit that they have attempted to treat the disease and so the clinical syndrome may be confused and distorted by secondary changes arising from delays, self trauma or errors in treatment. It is important not to apportion blame or criticism since that is usually counterproductive. The information gained should be used to improve the diagnostic process.

- Is the horse pruritic (biting, itching, kicking) or has it been at any stage

It is important to ask this question several times in different ways; owners or caretakers do not always understand what pruritus is; if they see urticaria they think (comparing to themselves) that it has to be pruritic and may therefore give a misleading answer .

- What is the method and regularity of grooming, and has each horse got its own dedicated grooming equipment

Information about the grooming gives an idea about the possibilities that a disease may spread between different individual animals.

- Are more animals similarly affected, are if so what possible ‘connections’ do they have in terms of management, housing and pasture access

This information will reveal whether aspects of contagion and infection or common management, toxic or environmental contacts are involved.

- Are humans working with the horses affected□

There are very limited zoonotic diseases that are transmitted between horses and humans. The commonest is of course dermatophytosis.

- □as the animal been treated, if so what was the therapy, and was it successful□

This is one of the most important aspects of the history and one that is again subject to some misleading answers. For example an owner might report that the problem is acute when in reality the acute component was caused by over-strength application of medication for a milder, chronic problem. The owner may not be willing to admit to the use of irrational or any treatments.

- □nformation about specific management and routine procedures

The management of a horse may have a significant bearing on skin disease. For example, are there plants such as *Hypericum perforatum* (St John's Wort) in the pasture? It may be necessary to examine the pasture carefully and then of course the veterinarian needs to be able to recognise the potential toxic plants. Similarly horses that are in poor condition with poor nutrition over winter are more liable to develop pediculosis (louse infestation) and dermatophilosis (rain scald). A mare that has recently returned from stud may have contracted coital exanthema. Horses that are repeatedly washed for shows may have dry scurfy coats if specific equine shampoos are not used. Horses mixing with others at shows etc can contract infectious (or other) skin diseases.

The vaccination status and the deworming status should be established. For example, horses that have been dewormed regularly with an avermectin compound are unlikely to have *Onchocerca* dermatitis.

#### **4.Clinical examination**

The clinical examination of the patient is always a vital part of the diagnostic process. It is easy to be drawn in to a restricted and restrictive dermatological examination and ignore the animal as a whole. However, many skin conditions are secondary to other underlying problems. For example a photosensitised horse may have serious liver disease; whilst it may be possible to simply bring the horse inside and so relieve the clinical emergency of the major presenting sign, failure to address the more serious problem can be disastrous.

The clinical examination may include superficial or detailed examination of other in contact horses and possibly even other species. After having completed the normal body systems examination a more detailed dermatological examination should be performed.

The findings of all these examinations should be recorded carefully. A prepared proforma is useful because it reduces errors and omissions and provides an accurate record of the health status of the horse.

A good case record allows accurate and helpful follow-up and in the event that a colleague or a referral centre examines the horse the full clinical history can easily be provided. It also permits

an objective assessment of the progress of treatment. A photographic record is sometimes very useful and can save time.

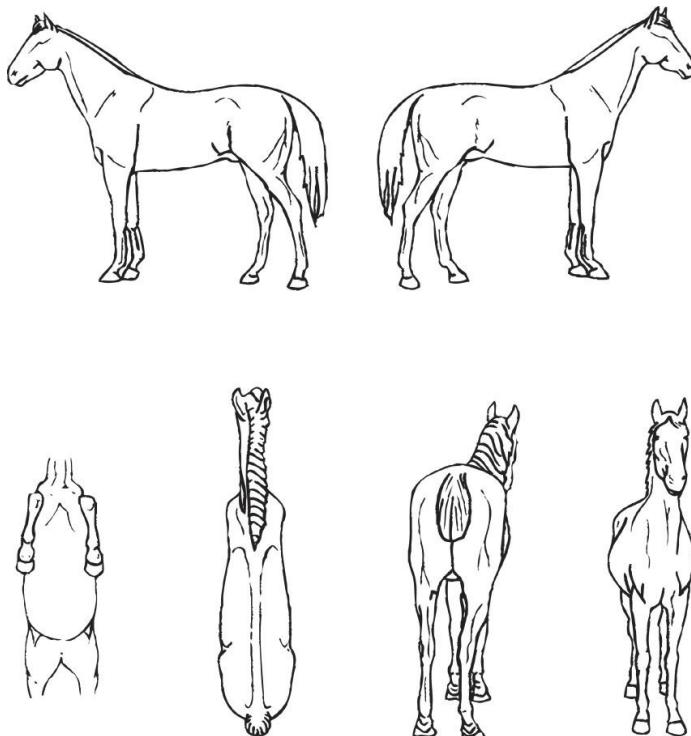


Figure □ Proforma for the clinical examination of a horse with a dermatological problem

The dermatologic examination consists of a general (overall) inspection and a local more detailed inspection.

During the skin inspection it is important to identify□

- □oose or broken hairs
- Dirt or parasites
- Specific lesions

The general inspection consists of□

- Type of coat

The summer and winter coats are distinctly different. A horse with a long non-shedding coat in summer is probably suffering from Equine Cushing's Disease.

- Attachment and disposition of the hair

Apart from the natural shedding of the coat it should not be possible to pull out the hairs easily. Attempts to pluck out a few hairs may reveal that some hairs are easily lost and others are firmly attached. Close examination of these under the microscope will reveal whether the shafts have been broken or if the hairs have become dislodged from their follicular base. A trichogram is a method of establishing the proportion of anagen and telogen hairs and the presence of broken shafts as well as the quality of the hair shafts themselves. The proportions of “exclamation mark / bulbed” hairs is noted in the construction of a trichogram. Regrettably not many equine veterinarians use trichogram information, so we know little about the way in which variations occur in different disease conditions. However, it can be useful; for example in Insect Bite hypersensitivity (Sweet itch) there are many broken hairs and these include the larger hairs as well as the fine ones, whereas in alopecia areata the few hairs that are present have poor bulbs and very thin shafts.

In some cases the hairs may be of a different colour or consistency and this may be a local change or may involve wider areas of the body.

- Coat density

Alopecia and hirsutism are important clinical signs and the disposition of the abnormal areas often provides clues as to the possible underlying cause. Areas of alopecia may show concurrent skin abnormalities such as thickening or scaling or exudation.

- Sheen and colour of the hairs

Although localised alterations in colour and sheen are regularly seen in dermatological disease, generalised alterations such as a harsh starring coat with rough hair quality, may more often be a reflection of underlying systemic disease. The old maxim “the skin reflects the inner state of health” is worth remembering.

- Presence or absence of pain at the site of lesions

Some conditions are characterised by pain while others can elicit a “scratch response” when they are touched (manifest as an impression of “pleasure” by mouth twitching or posturing).

When examining focal lesions it is important to consider:

- Number uniformity and configuration

Some conditions will show more or less identical lesions (e.g. viral papilloma) while others may have lesions that are either older or younger and will reflect the range of appearance characteristic of the progression of disease. An example of the latter is dermatophytosis (ringworm) in which the oldest lesions may be larger and may have a central area that is more normal (although alopecic). The equine sarcoid for example has 6 morphologic variations and it is easy to mistake the differing forms of the condition for other disorders.

- Distribution over the body

The distribution of the lesions may help considerably especially if the original site can be established. For example dermatophytosis may occur first around the girth where it is commonly associated with contaminated tack and rubbing trauma, but it may be spread from here to other sites on the horse during grooming or to other horses sharing the same tack or grooming equipment. The pattern of the lesions is sometimes almost pathognomonic; for example staphylococcal saddle dermatitis (saddle rash) is usually restricted to the saddle area while dermatophilosis (rain Scald) may follow the wettable areas of the dorsum and sides.

- Size, shape, and depth of the lesions

While many skin diseases have no characteristic shape of lesion others do. Dermatophytosis (ringworm) is often (but by no means always) roughly circular (at least in ten early stages) and the occult sarcoid may present with similar shaped lesion. Sometimes the characteristic shape is only present in the early lesions and so it is important to glean this information from the history stage of the examination. Some conditions are epidermal while others are dermal or subcutaneous. The equine sarcoid is a good example of the variations in depth and cutaneous location that can be encountered.

- Nature of the lesions (moistness, exudate, necrosis, pus etc.)

The nature of lesions will often reflect the type of inflammation or ulceration present. Exudation may be associated with infection but may also arise from non-infectious inflammation. Vert pus is a cardinal sign of infection but many serious and important equine skin infections do not induce exudates that are readily recognisable as pus. A dry thickened skin may arise from chronic self-trauma such as is seen in chronic *Culicoides* hypersensitivity (Sweet itch).

When examining specific lesions it is important to use a magnifying glass and if necessary the hair may be clipped off. This may for example reveal a single purple focus in the centre of an oedematous wheal which can then quite reasonably be assumed to be the result of an insect bite or other focal insult.

Usually careful clinical assessment will identify the primary condition. It is important to establish this because failure to recognise and eliminate the primary problem can result in a poor therapeutic outcome. The face may be severely ulcerated as a result of tear overflow and this may be due to a physical or neurological defect of eye function or to excessive lacrimation. A mare may drip milk onto her legs and cause scalding but the real problem is that the foal is not feeding effectively.

## 5. Problem list

Following the history and the clinical examination the clinician should be able to define the specific (skin and other) problems that the horse has. At least some of these may not be either specifically or indirectly related to the dermatological condition. A major inclusion in the problem list should be the specific complaint from the owner. It is quite easy to lose sight of this as more problems are identified. The problem list focuses attention on the salient aspects of the health of the horse. Having all the data it may be possible to refine the diagnosis with a list of differential diagnoses of the skin problem and it may even be possible at this stage to make a probable diagnosis. This differential diagnosis list is usually compiled from the problem list and

should be listed in order of likelihood. Common things occur commonly and so the most likely conditions will be those that are relatively common. Thus in Europe certain conditions, such as Glanders and Epizootic Lymphangitis are extremely unlikely and so even when the clinical signs may resemble the disease it can be allocated a less prominent place in the list. However, the difference between a good clinician and an ordinary one is the way in which the unusual case is identified and confirmed.

The advantage of a good differential diagnosis list is that it allows a logical progression to diagnosis and the careful selection of further tests that can help significantly with the management.

## 6. Laboratory tests and further examinations

### A. Introduction

A specific diagnosis is a satisfying outcome of the clinical investigation but many dermatological disorders require further investigation. Some conditions are very distinctive and further examinations are not really required. For example, melanomas under the tail of grey horses are very distinctive. However, other conditions are less characteristic; a nodule in the skin at the base of an ear in a grey horse could reasonably be assumed to be a melanoma but it could easily also be a sarcoid. Further examinations and tests are necessary to rule-in or rule-out the various diagnostic options from the differential diagnosis list.

The process is also complicated by the fact that the skin has only a limited number of responses that it can make to a wide range of insults. Thus a pruritic patch around the tail base could manifest clinically simply as an inflamed eroded area of skin with broken hairs and some alopecia. This could be due to *Culicoides* dermatitis but could also be due to *Oxyuris equi* infestation or even louse infestation. The time of the year, the management of the horse, the deworming programme of the horse will help in refining the diagnosis. Then, specific tests may rule-in or rule-out this diagnosis. There are not only obvious therapeutic advantages to a defined diagnosis but a confirmed diagnosis is also a very satisfying outcome for the owner and the clinician. However, a good probable diagnosis or a short list of differential diagnosis will inevitably save time and money. It is important to select specific tests that will add to the diagnostic information and so establish a definitive diagnosis.

The collection of the correct specimens from the most appropriate sites is essential if they are to be of diagnostic value. Therefore, the decision to collect specimens must be made after all the lesions have been examined and the best, most representative lesions have been identified. For example where a condition causes severe pruritus the clinician may be drawn to the most inflamed ulcerated area but this may not reveal anything useful diagnostically. Rather an adjacent area may provide the best opportunity to identify parasites such as lice or mites. Similarly hair pluckings from the central part of an aging dermatophytosis (ringworm) lesion may not yield any active fungi while pluckings from the outer margin may be markedly positive. In many cases biopsies are helpful but it is important to realise that even such a definitive sample may be grossly misleading. A biopsy may be useless when there are long-standing chronic changes or where there is superimposed self-trauma or infection. By contrast skin tumours and other nodules are often very rewarding subjects for biopsy. However, the question then arises whether this

should be a true sample biopsy or total extirpation (excisional biopsy) with histopathology afterwards.

The available tests and examinations include

- Microscopic examinations for parasites, fungi and bacteria
- Bacterial and fungal cultures
- Skin biopsy
- Haematology, blood biochemistry and other blood tests
- Skin (intradermal and patch) testing

#### *B.Specimens for parasite-examination*

A deep skin scraping is primarily designed to identify burrowing skin mites such as *Sarcoptes equi*, which are in any case extremely uncommon in horses. The technique is therefore probably much less useful in horses than in many other species. The commonest mite is *Chorioptes equi*, which is a surface feeding mite and so 'groomings' (pluckings) are usually better.

For deep skin scrapings the selected areas are shaved using a 22 scalpel blade directly into sterile containers. Another method is to moisten the areas slightly with mineral oil and apply the material directly to a slide. This is only useful if the microscopic examination is performed immediately.

In the laboratory, either type of sample should be covered with enough mineral oil to allow the placement of a cover slip and the sample is examined under x10 and x20 objectives. *Demodex* spp will only be found if the scrapings are deep and taken with gentle squeezing of the skin during the procedure. *Demodex* is a commensal in the eyelids and muzzle regions of some horses, and is rarely found in East Europe.

Hairs, crusts and scales can be collected using a curette on the edge of a recent lesion. In this way a little bit more material will be collected than with "grooming", but it is less damaging and less painful than a deep scraping with a scalpel blade. This type of scraping should not continue down to the appearance of blood.

Skin groomings are also a very useful method of finding small numbers of ectoparasites. The technique is limited to parasitological diagnosis only. For "skin groomings" a small dustpan or petri dish is used and a stiff brush is used to harvest the hair and scales which are groomed into the dish or onto a black tile and carefully observed for moving mites and lice. This is a simple and effective aid for *Chorioptes*, harvest forage and poultry mites and for lice (both *Damalinia* and *Caematinus*).

When sending hair specimens to a laboratory for parasitological examination, the material should not be packed airtight as saprophytes will grow easily in an airtight bag or container.

A dissecting stereo microscope is very useful for examining hair and grooming samples – at low power the movement of the scales and hair (dander) can easily be seen and then the power can be simply increased to reveal the cause of the "movement".

Alternatively a small vacuum cleaner can be used to collect hairs, scales, and crusts and is especially useful for *Sarcoptes* mites.

To identify *Oxyuris equi* eggs on the peri-anal region “acetate adhesive tape preparations” (Sellotape) can be made. This technique sometimes also picks up lice and other ectoparasites. This involves the simple use of 4 cm lengths of clear adhesive tape applied to the (usually around the anus and perineum or on the haired skin). The tape is then applied to a glass slide onto which a drop of mineral oil has been applied (this helps to disperse the bubbles and artefacts which can be confusing). This specimen can easily be examined under low power microscope for the characteristic oval-triangular operculate eggs ( $\square \square \text{m} \times \square \square \text{m}$ ) of *Oxyuris equi*.

### *C.Specimens for the diagnosis of fungal infections*

The use of an ultraviolet (Wood's) lamp is not usually useful in horses as only *Microsporum* species fluoresce yellow-green as a result of the tryptophan metabolites produced by this fungus. Skin scrapings can also be useful in dermatophytosis (ringworm) diagnosis but plucking is probably better and easier. The best way to collect material for a fungal culture (“plucking”) is by cleaning the lesions and its surrounding carefully with alcohol and waiting for the area to dry thoroughly. Hairs are then collected from the fresh margins of young lesions using a sterile pair of haemostats and putting the hairs into a sterile non -airtight container or bottle. Pathologic fungi live along the shafts of the hairs, while saprophytes such as mucor live on the surface. The latter cause overgrowths which makes a culture valueless. Sterile pluckings give much better results than scrapings.

The microscopic examination of the sample can be done directly and it may be possible to see the fungal hyphae. However, it is usually better to digest the keratin first with  $\square \square$  or  $2 \square$  potassium hydroxide ( $\square \square \square$ ) solution; either heat the slide gently for  $\square + 2 \square$  seconds or wait for  $\square \square$  minutes  $\square \square \square$  before examination. All equine dermatophytes are ectothrix and conidia may be found along the hairs.

When the fungi are not found in a direct microscopic examination a dermatophyte culture is necessary. Here again careful sample collection is essential as the pathologic fungi live along the shafts of the hairs, while saprophytes live on the surface.

Commercially available dermatophyte tests (e.g. Fungassay®) contain Sabouraud's dextrose agar medium with a phenol red indicator and antibiotics to control bacterial overgrowth. When using this medium samples should be pressed firmly into the surface of the medium and not buried in it. Then it should be incubated for up to 14 days at room temperature and at  $37 \square \text{C}$  (best for *Trichophyton* spp.). Earlier indications are gained by red coloration of the medium which results from alkali change due to fungal growth, which usually occurs at  $24 - \square 6$  hours. Dermatophytes always produce white powdery or fluffy colonies and mucoid, dark/black or very light colonies are artefacts and not positive results.

In a specialised laboratory fungal culture is done on a selective medium at  $2 \square \text{C}$  and on a Sabouraud B agar at  $\square \square \text{C}$ . A fungal culture usually takes  $\square - \square$  weeks to become distinctive. If there is no growth of specific colonies after  $\square$  weeks the fungal culture is considered negative. The definitive species of dermatophyte can be identified by its cultural characteristics and its macroconidia and this information can be used to support the origin of the infection. Thus if a *T.*

*verrucosum* is cultured, it is likely to have derived from cattle and if *T. mentagrophytes* is found it is probably derived from cats and rodents. Control measures may be helped significantly.

#### D. Specimens for bacterial cultures.

Direct smears are usually used for dermatophilosis diagnosis but may also useful for other bacterial infections and pastern and cannon leucocytoclastic vasculitis or folliculitis. To make a direct smear the hairs over the lesion should be clipped away and the crust sample placed skin-side down onto a drop of saline on a slide. The specimen is left to soak for several minutes and then gently macerated into the saline. The saline should become patently milky in appearance and the excess debris and lumps should be removed. The slide is then heat fixed at 60°C over a flame after allowing it to air dry. The slide can be stained with Gram, Giemsa or Wright - Giemsa (Diffék) and examined 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 much more difficult and may only show mixed bacteria and degenerate inflammatory cells.

All samples for culture should be taken into transport media. Plain swabs are useless unless they reach the laboratory at very short notice. A bacterial culture may take 2 to 5 days and a sensitivity assay may add an extra 2 days to the delay. A bacterial culture is not always useful except for *Dermatophilus* spp. since the skin abounds with commensal organisms. However, *Staphylococcus pseudintermedius* is an important pathogen and it may also be useful to have a sensitivity profile of all the bacteria involved. A special technique for *Dermatophilus* is:

- Small pieces of scab or scale or crusts are placed in a bio bottle with 2ml distilled water and allow standing for 2-4 hours at room temperature with top loosely applied
- The top of the bottle is removed and the bottle is placed in a bell jar under 2% CO<sub>2</sub> produced by burning a candle in the jar
- After 24 minutes a drop of the saline is seeded onto blood agar and incubated at 37°C in 20% CO<sub>2</sub> incubator for 24 - 48 hours.
- Abundant small colonies are obtained (usually in pure culture) and determined in stained smears from colonies for characteristic branching hyphae.

#### E. Specimens for histopathology

For histopathological examination a skin biopsy is required. Skin biopsies are usually taken to establish a specific diagnosis, to eliminate defined clinical conditions, to monitor the course of disease and/or to confirm the completeness of surgical excision of tumours. However, the diagnostic value of a biopsy should not be overstated. Many different conditions can induce an almost identical histopathological effect. This makes it difficult or impossible for the pathologist to provide a definite diagnosis. It is unreasonable to expect a pathologist to be able to help in every case but the best opportunities arise when a detailed history and clinical description are provided with carefully selected samples of the affected skin. The most representative and most typical samples should be provided; in general early lesions give the best results. Pathologists are very willing to help but do not take kindly to being challenged unnecessarily and then derided because they cannot make a diagnosis.

A single biopsy will seldom answer all 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 (e.g. pemphigus), which must be biopsied as early as possible since it will usually disappear quickly.

Further many skin diseases are pruritic or become secondarily infected at an early stage and so the histological features may be misleading. Thus, biopsies of nodules, papules or pustules are prime samples for biopsy, while ulcers and crusts are less definitive. There is little to be gained by biopsying chronic lesions, superficial inflammatory changes and lichenified crusted dermatoses.

A biopsy can be taken using local anaesthesia and suitable physical restraint. The disadvantage of using local anaesthesia is that it may damage the biopsy, especially when lignocaine hydrochloride with adrenaline is used. In some cases a biopsy can be taken with a nose twitch alone or with sedation using alpha-2 agonists and sometimes opioids (e.g. Dolorex).

Importantly, the skin should not be shaven and the site should be gently washed rather than scrubbed for aseptic surgery. Scrubbing of the skin may induce misleading changes and may remove the diagnostically significant material. A biopsy punch or a scalpel blade should be used as scissors can cause severe crushing artefacts of the biopsy. Shave biopsies, done by shaving off the epidermis in layers parallel to the surface of the skin, is not performed very often. No sutures are required.

A punch biopsy is taken using a disposable skin punch (available in 4 or 6 mm diameter). Very small specimens may not be diagnostic and may distort significantly during collection making both the collection and handling problematical. Although in theory the smallest punch biopsy possible should be used it is far better to get a truly representative sample first time and so wherever possible the largest biopsy that is consistent with the region and the condition should be taken. I prefer 4 mm punch biopsies for the lower limbs and the head, and 6 or 8 mm punch biopsies for the body. Often it can be wise to take several biopsies. This will enhance the possibility of detecting subtle pathological changes. It is best to use a scalpel blade to remove biopsy from underlying fat. Grasping the biopsy specimen with rat tooth or plain forceps is poor technique and can cause crush or other artefacts. It may also be useful to obtain also a normal biopsy from adjacent area; this sample must of course be identified separately. With punch biopsies is it not wise to try to obtain the interface as the pathologist may miss the lesion this way. It is not necessary to suture punch biopsy sites. They will usually heal rapidly without any significant scar.

A wedge biopsy can be used for larger lesions if necessary. A full thickness cut is taken through abnormal tissues and normal skin including the interface. Careful selection of site is important as it should be possible to clean and suture the site after biopsy. An excisional biopsy is particularly useful for vesicles and pustules. Here an elliptical incision is made to include all tissues down to the panniculus muscle. The wound is cleaned and sutured after the 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. The pathologist should be consulted if there is any doubt about which site to biopsy and which type of biopsy to take. It can be useful to

photograph the biopsy site before and after so the pathologist can orientate the sample correctly when planning the sections.

Excisional biopsies may require several sutures or staples or both depending on the size and location of the biopsy site. Although formalin (4% formaldehyde) is the standard fixative, some others may be required for special purposes. Additional points for taking a biopsy include

- The sample must be handled gently to avoid stretch or compression artefacts.
- A scalpel rather than scissors should be used to excise the skin; the latter cause marked artefactual damage, especially to small pieces of skin.
- A fine needle should be used to lift the biopsy rather than forceps, which also induce crushing injuries.
- Samples should be reduced to 1cm in size to allow good fixative penetration.
- For electron microscopy (e.g. poxvirus etc.) 1mm cubes of tissue are placed directly into glutaraldehyde; suitable arrangements for examination should be made in advance of collection.
- Bacteriological or virological cultures should be taken from the biopsy specimen as soon as possible; the swabs or samples should be placed into suitable transport media and delivered to the laboratory as quickly as possible
- For an immunological examination the biopsy specimen should be placed on a saline soaked tissue and transported immediately to the laboratory.

#### *F. Specimens for immunological examination*

Immunohistochemical methods are not widely used yet in equine dermatology but there is increasing interest in this area as more is known about the way the skin responds to insult. Usually these methods are used to diagnose specific tumour types and particular cell types in inflammatory or allergic disease.

#### *G. Intradermal skin test*

The intradermal skin test is not yet reliable in horses. It is used by some specialists but may not add greatly to the diagnostic process. It is not currently possible to determine reliable thresholds for the different allergens in the horse and so the tests are inherently unreliable.

#### *H. Radio-allergo-sorbent-test (RAST)*

A blood test requiring a single sample of plain (clotted) blood or serum is used to detect and quantify specific monoclonal IgE's. This is presumed to reflect the presence of allergic responses and the natural corollary to this is that hyposensitisation can then be targeted directly at those allergens that are causing problems (either to the skin or other organs). The problem here is that the test is very crude in so far as it does not confirm the exact allergen that is actually responsible and in many cases does not test all the available options. Therefore a pool of possible allergies is reported. This results in multiple desensitisations and frequently a very disappointing outcome. Many of the more significant allergens show only mild alterations in the IgE concentrations and

so may be ‘hidden’ in the large numbers of partial or moderate sensitivities. Unless a more definitive test can be established or unless a more satisfactory, safer, desensitisation protocol can be established, the test is probably not worth the financial cost.

### I. *Testing the effect of removing instigating factors*

In some circumstances test treatment or avoidance of possible contacts with allergens or instigating factors can be used to confirm a diagnosis. Thus where urticaria is a result of allergic sensitivity to oats or barley, simply removing the offending material from the diet may resolve the issue. In order to confirm the diagnosis the offending material should be reintroduced. Recurrence of the typical signs will then be confirmatory. The same applies for pruritus as a result of food allergy. If the instigating factor has been removed the clinical signs should disappear. Animals at pasture suspected as showing insect hypersensitivity should be stabled for 4 weeks and if the clinical symptoms disappear the diagnosis is quite likely.

### **Additional reading**

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