Equine Veterinary Education

EQUINE VETERINARY EDUCATION
Equine vet. Educ. (2018) **30** (7) 377-385
doi: 10.1111/eve.12598

Review Article

Clinical pathology in equine dermatology

M. M. Sloet van Oldruitenborgh-Oosterbaan*† and G. C. M. Grinwis‡

†Department of Equine Sciences; and ‡Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.
**Corresponding author email: m.sloet@uu.nl*

Keywords: horse; pathology; dermatology

Summary

Clinical pathology is an essential part of the investigation of skin disease in the horse. It is useful to confirm a diagnosis. After a short introduction, skin grooming and scrape, trichogram, adhesive tape technique, cytology, fungal and bacterial cultures, PCR tests, allergy testing and different ways of taking skin biopsies will be discussed.

Introduction

After a history has been taken and a physical and dermatological examination performed, the clinician should be in a position to compile a 'problem list' and formulate a list of differential diagnoses. The type of diagnostic procedures will depend on the history and presenting signs, e.g. if paraneoplastic syndrome is the tentative diagnosis, a rectal exploration and/or radiographic examination of the thorax will be necessary.

The wishes of the owner (e.g. financial constraints) may dictate the choice of additional examination(s). There is a wide range of techniques available from simple and inexpensive to advanced and sometimes costly. Some techniques are poorly validated in the horse (e.g. intradermal skin test), or are regarded as yet insufficiently reliable in horses. The latter is the case, for instance, with immunofluorescent testing, immunohistochemical methods, enzyme-linked immunosorbent assays (ELISA) and most intradermal tests. The results of the additional examinations have to confirm the tentative diagnosis, or else rule out other causes or establish these are less probable.

The following procedures are discussed: skin grooming and scrape, trichogram, 'adhesive tape' method, bacteriological test, cytological test, test for skin fungus, PCR for bovine papilloma virus, tests for allergies and biopsy.

Skin grooming and scrape

Collection of hairs, crusts and, if present, parasites can be done making a skin grooming or a skin scrape. A skin grooming is a very simple procedure requiring little equipment. Examination of a superficial skin grooming or scrape under a microscope can reveal mites and lice, as well as traces of skin fungi or yeasts. The grooming can be taken using a spoon curette (**Fig 1**) or possibly a clean toothbrush, but preferably not a scalpel blade. The use of a scalpel blade involves a slight risk to the examiner and/or case when the horse makes a sudden movement. A scrape with a scalpel blade is only advisable if there is a tentative diagnosis of scabies or demodicosis.

The collected material can be placed in a paper bag or on a Petri dish and submitted for further testing to a designated diagnostic lab or be examined by the clinician.

Another method for collecting ectoparasites is to use a lice (or a coarser-toothed) comb smeared with a drop of oil for capturing the ectoparasites. A third method involves a vacuum cleaner with a piece of very fine cloth (for instance, a folded piece of gauze) attached between the nozzle and hose. The collected material is then inspected under a microscope. It is said to be easier to find lice on a long-haired horse if the horse has first been worked briefly. Lice will then move to the tips of the hairs.

A small amount of the harvested material is placed on an object glass in 2 drops of indifferent oil (for example, liquid paraffin or bicycle oil). The use of a 10% KOH solution for making slides is an alternative and has the advantage, possibly after the specimen has been heated, of brightening the mites making them easier to detect. However, the solution is an irritant and should not come in contact with the skin.

When a specimen is examined under a microscope, the lowest magnification (20 or 40× = ocular × objective) with sufficient contrast is adequate. If nothing is discernible, it is advisable to allow the slide to stand for a while with the microscope lamp on. The slide will heat up and any mites or lice will try to escape and thus move which makes them much easier to detect.

Trichogram

In horses, the use of a trichogram (microscopic examination of a few hairs) is not yet commonplace, though it does have

Fig 1: Sharp spoon curettes for collecting skin scrapes.

a number of advantages. For instance, it can show the growth stage (anagen or telogen) of the hair root (**Figs 2** and **3**). Assessment of the growth phase can be useful, for example, in establishing hormonal alopecia, or a more serious problem with which all the hairs may appear in the telogen phase (telogen effluvium). The quality of the hair structure, as well as indications for the presence of dermatophytes, may also be studied. With hair loss, the condition of the hair tip should be assessed if it is uncertain if the animal has been rubbing the hairs off by itself or if the hairs are dropping or breaking off spontaneously. Remove some hairs from the affected spot with tweezers (be careful or cover the serrations with paper) and put the hairs in the KOH 10%, paraffin or oil, covering them with a cover slip. Low magnification (objective 10×) is usually sufficient for a good image.

Adhesive tape technique

The method using adhesive tape is usually employed to test for pinworm (*Oxyuris equi*) eggs (**Fig 4**). A strip of adhesive tape is placed on the anus, then pulled off and examined under a microscope (**Figs 5** and **6**). The adhesive tape method can also be used to reveal any yeasts (Malassezia) in chronic skins lesions or to collect ectoparasites and eggs from other areas, e.g. lice. It may help to add a drop of methylene blue to the slide and place the adhesive tape on it: the yeast cells will appear as bluish-purple peanut-like shapes.

Cytology

The cytological examination requires some experience, otherwise it is best to send the sample to a specialist. Impression smears, smears from biopsies taken with fine needle aspiration (FNA, **Fig 7**) or swabs rolled onto a slide can all be evaluated. Cytology can be used to decide whether lesions are infected, inflammatory or neoplastic. Although not quantitative, cytology gives an impression of what is happening on or in the skin. Notably, cytology is often inconclusive in the horse.

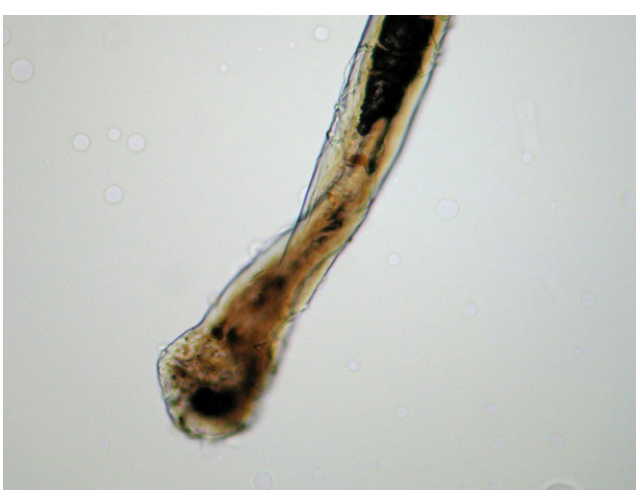

Fig 2: Hair in anagen phase.

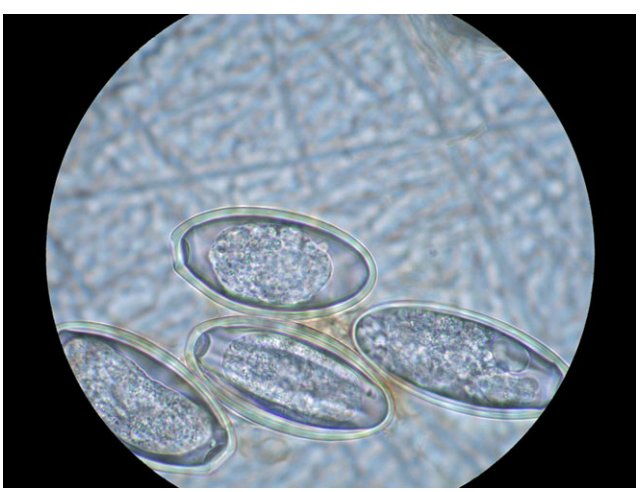

Fig 4: Oxyuris equi eggs found round the anus of a horse (seen here on an object glass in oil).

Fig 3: Hair in telogen phase.

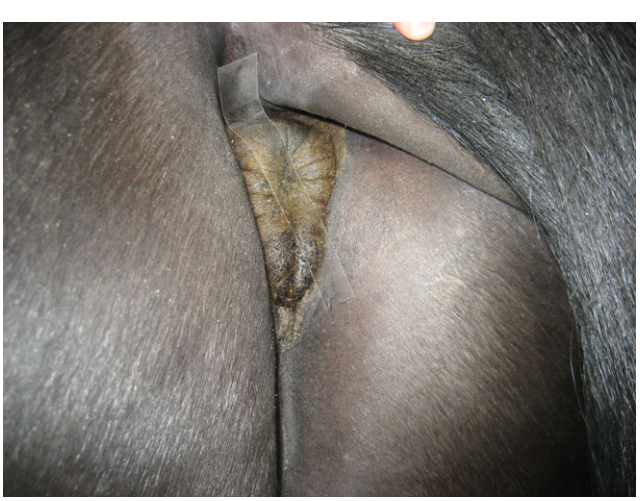

Fig 5: A strip of adhesive tape placed over the anus; it is removed after a few seconds.

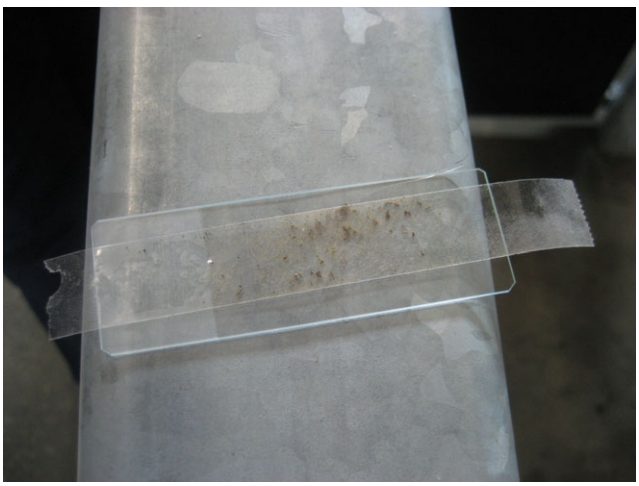

Fig 6: The strip of adhesive tape is then placed on an object glass for examination under a microscope.

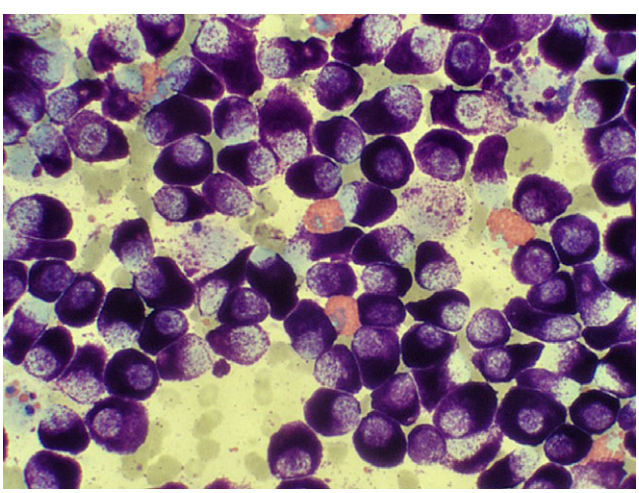

Fig 7: A fine needle aspiration biopsy (FNAB) smear of a small nodule of an 11-year-old Andalusian mare with mastocytoma (Courtesy of Drs J. Ensink and E. Teske).

Testing for dermatophytosis

In horses it is often possible to diagnose dermatophytosis on the basis of the clinical picture, especially if typical ring-like lesions are present. However, nontypical presentations do sometimes occur and, to exclude an underlying dermatophyte infection, further testing with the time-consuming (>10 days) fungal culture is required. Lately, some diagnostic laboratories are offering PCR-based tests for veterinary dermatophytes which reduce the sample-to-result delay considerably (1 day). However, one has to make sure that a thoroughly evaluated (comparatively tested) procedure is applied, since issues with its sensitivity may occur.

Microscopic examination may be helpful to detect dermatophytosis (**Fig 8**), but this technique is often insufficiently sensitive to exclude dermatophytosis. Examination with a Wood's lamp cannot be applied to horses, because their common dermatophytes do not fluoresce.

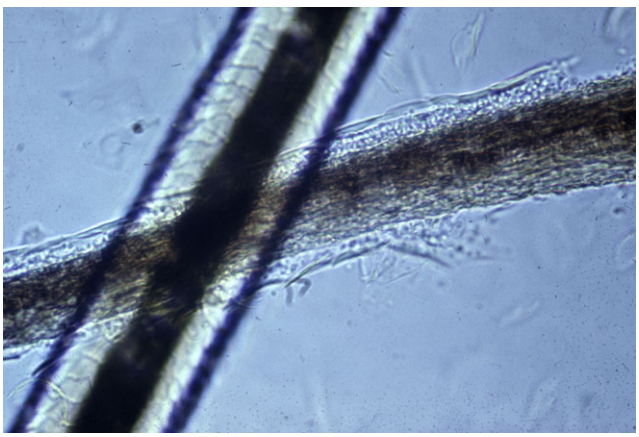

Fig 8: Ectothrix conidia of Trychophyton verrucosum along hairs.

When collecting the samples (hairs, flakes of skin, horn material) for fungal culture, it is important to cleanse the sampling site with alcohol (70%) and let it dry before actually taking the sample. This cleansing procedure is to reduce the contamination with saprophytic fungi (**Fig 9**) which may interfere with the culturing of dermatophytes. Polymerase chain reaction testing does not require this cleaning procedure. Samples should consist of hairs and flakes taken from the rim of the lesion, collected using tweezers or by scraping off with a scalpel. A convenient way of sampling is using a new travel toothbrush to rub the rim of the lesion and using its case as the transport casing.

In house culture can be done using commercially available ready made culture dishes containing dermatophyte test medium (DTM) which are sometimes combined with other specific media. It is paramount to place as much of the sample as possible in contact with the media and follow exactly the directions for use. Only if the medium

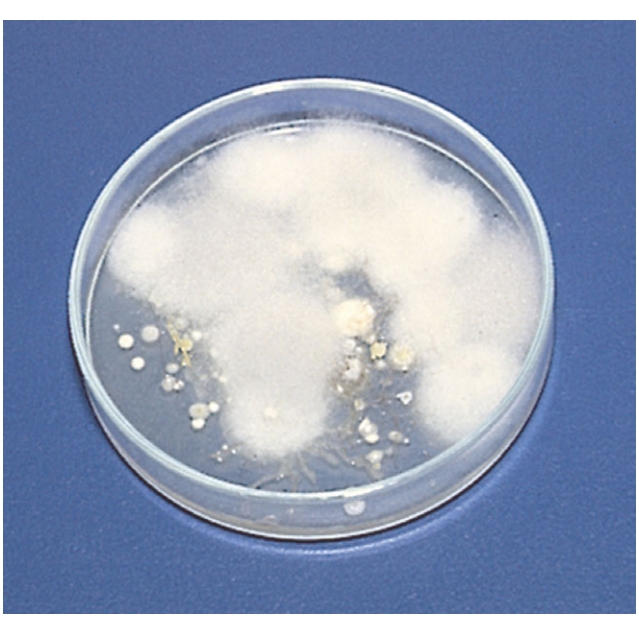

Fig 9: If the skin is insufficiently cleaned before material is collected for a fungal culture, the Sabouraud B agar culture plate with cycloheximide can become overgrown with mucor.

or media show a colour change within 10–14 days at room temperature and the growing colonies are flat with a whitish surface, one may conclude to have cultured dermatophytes. The advantage of submitting the sample to a specialised laboratory is that microscopic species identification is carried out. In equine enterprises it is sometimes important to know which dermatophyte is present to understand the implications.

Although the use of PCR-based tests for dermatophyte diagnostics has several advantages, their diagnostic reliability still needs thorough evaluation. In general, positive results can be considered more reliable than negative results, unless the sensitivity of the procedure has been established.

PCR for bovine papilloma virus

Sarcoids are troublesome tumours in horses and an unequivocal diagnosis is not always possible with a biopsy. If an owner does not wish to embark on treatment immediately, a tissue biopsy of a sarcoid is even undesirable. Recently, PCR has amply proven its value in establishing the presence of a bovine papilloma virus in lesions clinically suspected of an equine sarcoid. Only a small (3 mm) biopsy stored in a dry container (for instance, an Eppendorf tube) is required, but it is even possible to perform the test on material collected with a swab moistened with a physiological saline which has been rubbed firmly over the surface of the tumour for 30 s. With a fibroblast type of sarcoid a swab is very reliable, with other sarcoids false-negative results can occur. False-positive test results are rare, although bovine papilloma virus may occur on normal equine skin.

Testing for allergies

Elimination diet

If a domestic animal is suspected of having a food intolerance, testing with a 'homemade' hypoallergen or elimination diet is the most reliable. Clearly it is much more difficult with horses, although one option is to feed the horse only good quality grass hay or keep it at a decent pasture for 6 weeks, omitting any other feedstuffs and supplements (if longer a good supplement is necessary). If this diet cures the problem, new feed compounds can be added one by one and each for a period of 6 weeks to see the results.

Intradermal and serum tests

Such testing entails the intracutaneous injection of allergens, including a positive and a negative control. After 30, 60, 240 min and 24 h, checks are carried out to ascertain which allergens produce a positive skin reaction (swelling) (**Fig 10**). Probably the 1 and 4 h readings are the most important. This test may determine to which allergens the animal is sensitised. The test is often combined with detection of allergen-specific IgE in serum of the tested animal using an ELISA in a laboratory.

However, both the intradermal allergy test and the specific IgE test are still in their infancy in horses and in the authors' view cannot be reliably performed at present. However, their applicability for tail and mane eczema (or rather, insect hypersensitivity) is currently developing apace. When an equine case is thought to have an allergy, the best solution is often to move the horse to entirely different surroundings for a few weeks and refrain from taking accoutrements from home with the animal, not even a halter. If the problems disappear, various items and means of transportation from the old surroundings can be reintroduced one by one. However, the causal factor is invariably hard to pinpoint.

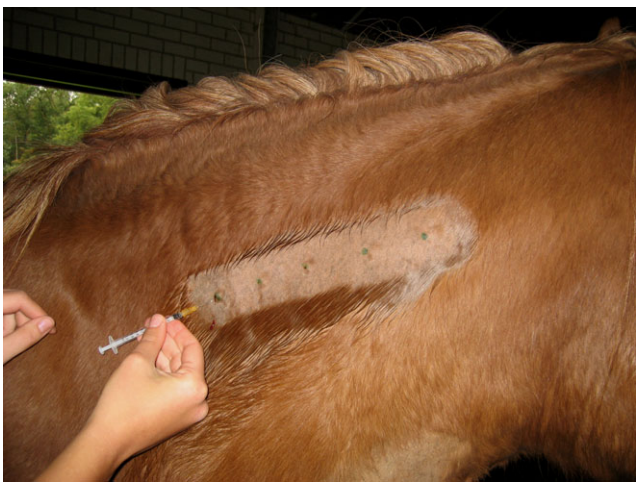

Fig 10: The intradermal skin test with various* Culicoides *antigens is already being used in research settings in horses with sweet itch.

Bacteriological examination

This test comprises a bacteriological culture and, if a relevant agent is found, an antibiotic susceptibility test. Bacteriological testing is useful for cases with deep pyoderma and for cases with (superficial) pyoderma which do not react to initial therapy.

The skin around a pustule is first cleansed with 70% alcohol, after which the skin is allowed to dry. The pustule is opened with a sterile needle. The contents of the pustule is absorbed with a swab. Using special bacteriological swabs with a transport gel is strongly recommended. If deep pyoderma is involved, the entire surface of the sampling site is cleansed with alcohol and left to dry. Exudate is collected with a swab after pinching the skin with a sterile needle. One may also decide to take a skin biopsy by sterile means for bacterial culture (**Fig 11**), especially if chronic deep pyoderma is involved. The swab or biopsy specimen is sent to the bacteriological laboratory accompanied by a complete anamnesis to allow the laboratory to choose the correct media. The culture and the susceptibility test usually take 3–5 days.

In horses in which dermatophilosis (a true bacterial skin condition caused by *Dermatophilus congolensis*) is suspected, scabs should be collected and submitted to a laboratory capable of performing its specific culture; the test must be requested.

Bacteriological examination results should always be authorised by a veterinary microbiologist and it should be kept in mind that establishing the causal role of any bacterial isolate (except *D. congolensis*) is difficult. However, those obtained as a pure culture or as the predominant species are likely to play some role in the disease process, hence treating these may be beneficial.

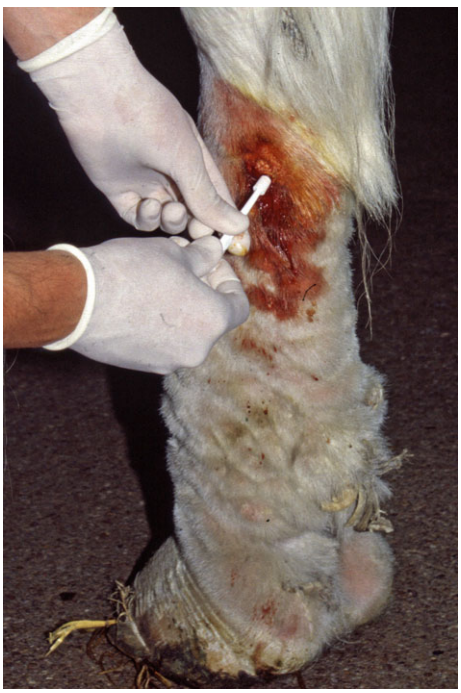

Fig 11: Technique for taking a sterile skin biopsy for bacteriological testing.

Biopsies

There are various ways of performing a tissue biopsy. The least invasive is fine needle aspiration biopsy (FNAB). A 4, 6 or 8 mm punch biopsy is somewhat more invasive as it requires local anaesthesia or sedation with an analgesic (for example, detomidine 0.01 mg/kg bwt i.v. with butorphanol 0.02 mg/kg bwt i.v.). If a larger biopsy is required, a small amount of skin and possibly underlying tissue, is removed (incision biopsy) or a lesion may be removed in its entirety (excision biopsy). When deeper defects are involved, a Trucut biopsy (core needle tissue biopsy) may be preferred.

It is important to consider carefully what issues need to be investigated and ensure that a sufficiently large, representative tissue specimen is taken. It is often advisable to take several biopsies (3 will generally suffice). There are places where this is not feasible, for instance the coronet. In that case, a single sample can best be taken from the ball side of the foot. For a biopsy in order to diagnose a skin disorder, surgical preparation (shaving, bathing, disinfecting) is not recommended (**Figs 12** and **13**). However, if an epithelial or mesenchymal neoplasm is suspected, the possible removal of a secondary epidermal change (e.g. a serocellular crust) due to cleaning of the skin is not an issue. In the case of a suspected neoplasm, the possible spread of neoplastic cells (tumour seeding) should be taken into account. One should carefully consider the pros and cons of taking a tissue biopsy beforehand in order not to hamper or complicate any subsequent treatment. One should also bear in mind that when biopsies are taken from a neoplasm, the biopsy site often bleeds more than usual on account of neovascularisation of the neoplastic tissue. When the sample is submitted to a specialist, it should be accompanied not only by the signalment of the case, but also by a detailed anamnesis, a description of the skin lesions and a list of possible differential diagnoses and previous treatment(s).

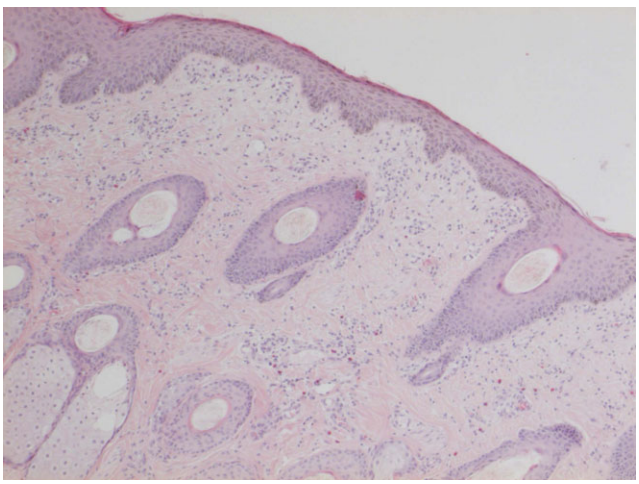

Fig 12: Section of a biopsy from a case with pemphigus foliaceus after shaving and disinfection: the characteristic changes have disappeared.

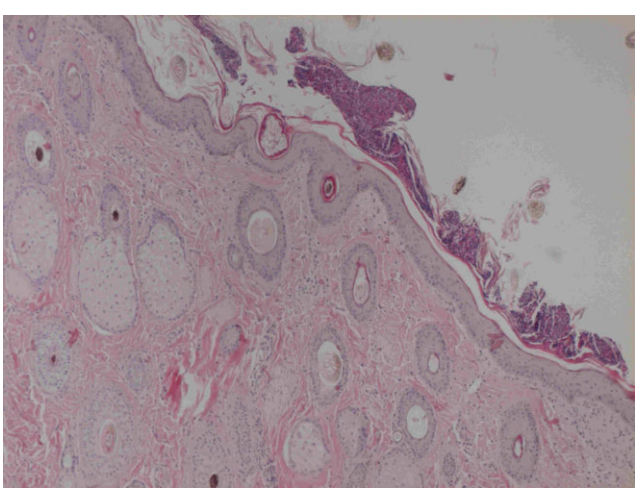

Fig 13: Section of a biopsy from a case with pemphigus foliaceus – biopsy taken without prior shaving or disinfection: the characteristic acantholytic cells in the crust are now clearly visible.

Fine needle aspiration biopsy

Biopsies of this type can best be taken with a 0.7–0.9 mm (=19–21 gauge) needle and a 10 ml hypodermic syringe. The needle is placed on the syringe and inserted into the tissue for removal (**Fig 14**). Next, material is carefully collected in various directions with a 1–2 ml vacuum. The material is not generally more than contained in the needle. The vacuum is then released and only then is the needle withdrawn from the tissue. There are several reasons for using this procedure: no neoplastic or inflammatory cells enter the needle channel, there is less chance of aspirating blood and thus 'contaminating' the sample and the collected cells do not enter too far into the needle from which it would be difficult to remove them undamaged. The collected material is then

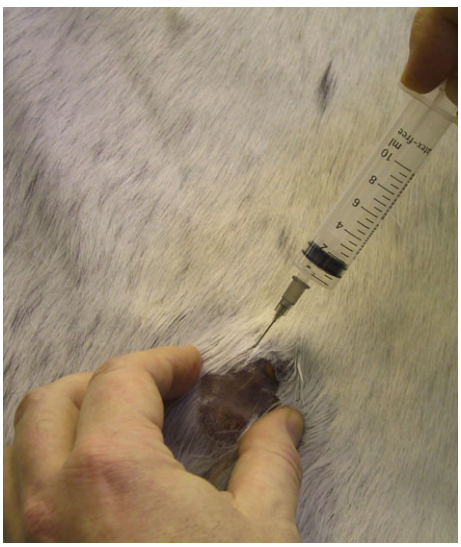

Fig 14: Taking a fine needle aspiration biopsy (FNAB).

placed carefully on a slide. To that end, the needle is detached from the syringe, and 1 ml air drawn into the syringe. Then the needle is carefully emptied on a slide. A second slide can be used to smear out the material, as done with a blood smear. The smear should be dried in the air and sent to a veterinary cytologist or pathologist. Here again, it is worth making several smears to assist reliable assessment. Lastly, when using this technique with horses, one should bear in mind that with tumours the cells are frequently firmly embedded in the tissue and often not found with FNAB. A FNAB is, for instance, generally useful for inflammatory lesions, melanoma and mast cell tumours, but may frequently be, incorrectly, 'negative' with sarcoids, lymphoma and squamous cell carcinoma. However, an advantage of FNAB is that tumour seeding resulting from the procedure is never actually a problem.

Punch biopsies

A punch biopsy can be taken when a case is thought to have an immune-mediated skin problem, a skin tumour or other disorder for which a clinical diagnosis is not possible, or to confirm a tentative clinical diagnosis. It is important to consider whether a biopsy is actually useful. For example, biopsies of skin lesions that contain changes caused by rubbing are usually not helpful for reaching a diagnosis. There are sometimes big drawbacks in taking a biopsy. Generally it is not necessary for diagnosis to obtain a biopsy of an eosinophilic granuloma (nodular necrobiosis) in the saddle region, especially since it would mean the horse could not be ridden for 1–2 months. Also, when contemplating making a biopsy in a region where dermatosparaxis (hyperelastosis cutis) is suspected, careful prior consideration is needed, because the site of the biopsy will heal with great difficulty and generally results in a large scar.

If possible, a biopsy should be taken in a primary lesion, preferably a lesion that is as fresh as possible (i.e. that has occurred recently). If, in horses, it is a matter of a possible diagnosis involving epidermal changes, it is imperative to omit shaving or bathing beforehand. Very long hairs may be carefully clipped, if appropriate.

Fig 15: Giving a subcutaneous anaesthetic.

For each procedure the horse should be carefully secured and, if necessary, sedated.

For local anaesthesia 2–4 ml lidocaine is injected under the skin (of course not in the skin) of the biopsy site using a fine needle (**Fig 15**). A regional anaesthetic block may be considered by anaesthetising the relevant nerve for the area involved. However, one should bear in mind that in horses with severe skin defects on the lower limbs, innervation often fails to follow the pattern described in anatomy books. Here again, a local anaesthetic may well be more satisfactory. When the anaesthetic has been administered, leave the needle in place (**Fig 16**) and after a few minutes, place the 4–6 mm trephine (biopsy punch) (**Fig 17**) directly adjacent to the needle. Only then should the needle be withdrawn and

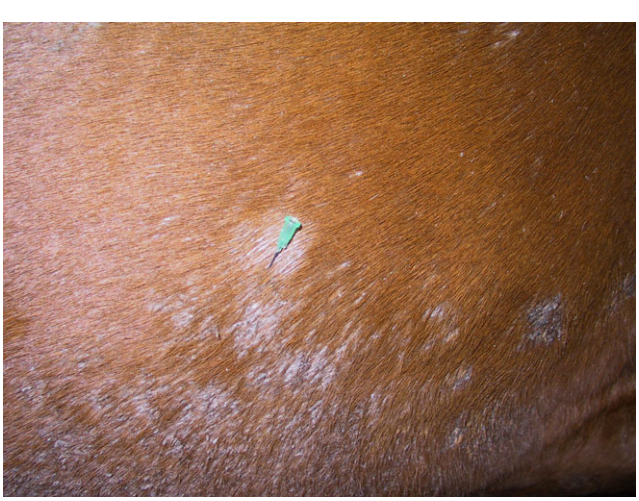

Fig 16: After giving the local anaesthetic, leave the needle in place to indicate the exact location.

Fig 17: Trephine for punch biopsies.

the trephine rotated in one direction using some pressure until it rotates freely. The trephine can then be withdrawn carefully with a 'scooping' motion (**Fig 18**), taking care the biopsy does not drop out of the device. Occasionally the biopsy is still attached to a strand of subcutis. If so, just cut it through with a small scalpel or with scissors. Always take care when using atraumatic tweezers, because 'squeeze artefacts' can easily be induced in the biopsy (**Fig 19**) hampering adequate evaluation and interpretation of the biopsy. If necessary, the biopsy can be plucked out of the trephine using a needle and then dropped into a little pot containing 10% neutral-buffered formalin (=4% neutral-buffered formaldehyde).

Many laboratories provide prefilled pots with the correct concentration of formaldehyde. For adequate transportation the local or international regulations for transport of

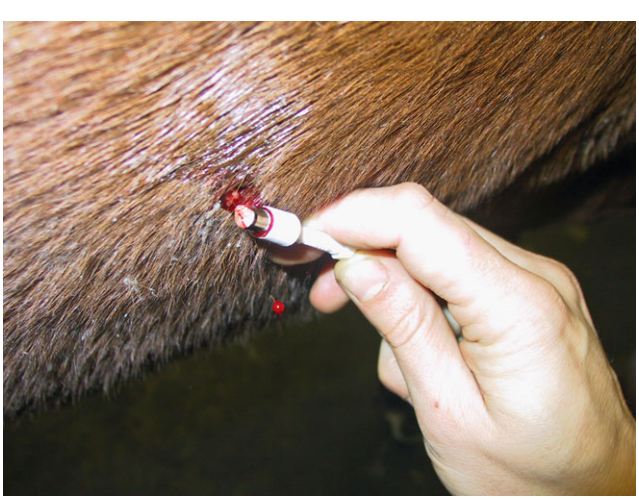

Fig 18: Withdraw the trephine with a scooping motion.

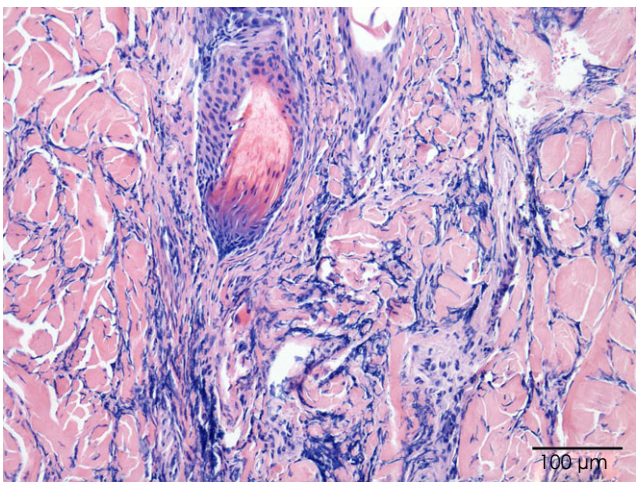

Fig 19: In the microscopic view of this skin biopsy a 'squeeze artefact' can clearly be seen in the centre.

diagnostic specimens need to be followed. Since the skin has not been thoroughly cleansed beforehand, it is sensible not to suture the wound, but leave it open (**Fig 20**). Do explain this properly to the owner. If the wound bleeds, compress it for 10 min with a tampon.

Incision biopsy

In some cases a punch biopsy is not large enough for a good diagnosis and it is preferable to take an incision biopsy (**Fig 21**). This enables the clinician to obtain distinctly more tissue as with punch biopsies. It is advisable to consider carefully in advance whether or not surgical preparation (e.g. thorough cleansing of the skin surface) is sensible. If a neoplasm is anticipated, the incision must be made in such a way that the biopsy scar can be included in its entirety during surgical intervention to remove the complete tumour at a later stage.

After a good local anaesthetic, generally administered in a diamond pattern round the biopsy site, a scalpel is used to remove a slice resembling a tangerine segment. Incision biopsies are usually sutured (**Fig 22**) because they tend to

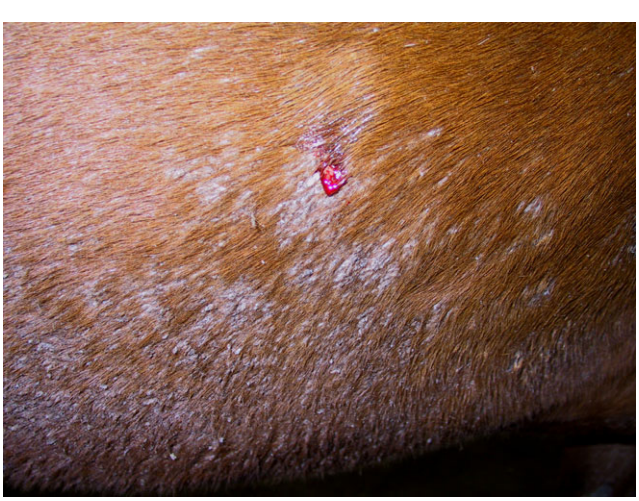

Fig 20: After a punch biopsy has been taken, it is usually better not to suture the biopsy site.

Fig 21: Large incision biopsy of skin with subcutis and a fragment of muscle layer from a horse with Streifenurtikaria (linear urticaria).

bleed and an open wound remains. Before undertaking the procedure, the owner must be informed that some reaction at the biopsy site is normal, but if abnormal swelling at the site of the incision occurs one or more stitches often have to be removed.

Excision biopsy

In some cases, it is preferable to remove the complete lesion, generally a small lump, in one go and submit it in its entirety to the pathology laboratory. If a neoplasm is suspected, the edge of the excision should, if possible, contain 2–3 cm of normal, non-neoplastic tissue and ideally be marked using surgical ink to mark the excision margins to enable the pathologist to determine whether the neoplasm has been completely removed. When different colours of surgical ink are used, the clinician can provide the pathologist with more detailed information with respect to the orientation of the excision biopsy in the animal. This additional information can be useful in identifying the exact location in case the neoplasm appears not to be completely removed and additional surgery is necessary. Because the epidermis is almost always normal in cases where the clinician decides that an excision biopsy is preferable, careful surgical preparation is carried out beforehand and the skin closed after excision.

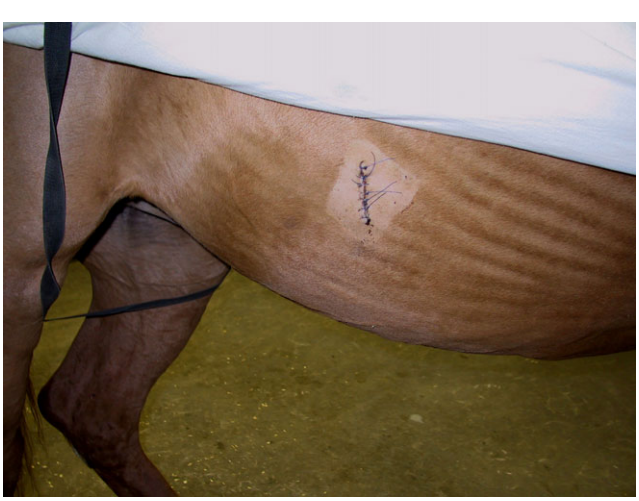

Fig 22: Horse with Streifenurtikaria (linear urticaria) after an incision biopsy was taken.

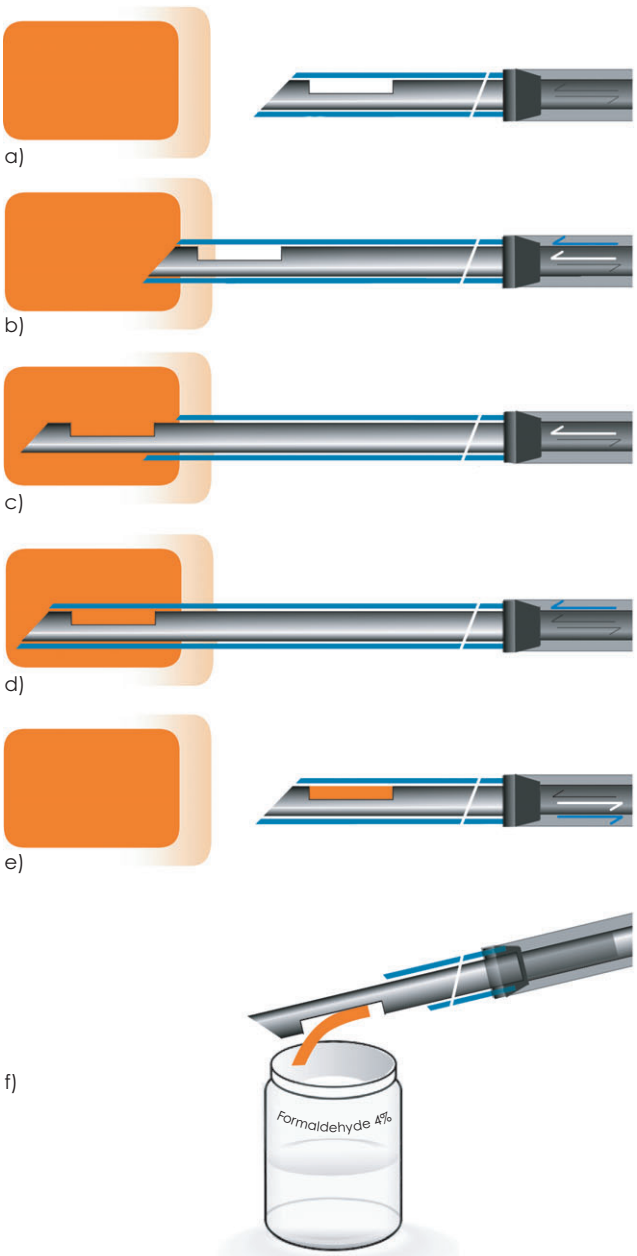

Fig 23: Trucut biopsy principles. a) Incongruous tissue skin shaft needle with chamber for tissue sample. b) Make an incision in the skin with a scalpel and insert the needle plus shaft. c) Push the needle further into the tissue for biopsy whilst holding the shaft. d) Now slide the shaft over the needle to cut out the tissue sample. e) Simultaneously withdraw the needle, shaft and tissue sample and f) Put the tissue sample in formaldehyde 4%.

Trucut biopsy

The Trucut technique (core needle tissue biopsy) is particularly suitable for the easy collection of tissue from a deeper-sited disorder with which epidermis and dermis

Fig 24: Samples of tissue obtained with a Trucut needle.

appear intact and normal. It is important to realise that only a narrow piece of tissue is obtained with this technique and therefore it is possible that the sampled tissue is not representative of the underlying lesion. A Trucut needle is also known as a liver biopsy needle. These needles consist of a stylet with a chamber and a sharp shaft (cannula) around the stylet (**Fig 23**). After the location of the biopsy has been surgically prepared, a small incision is made with a sharp-pointed scalpel. The Trucut needle is inserted through the incision. The stylet is extended into the swelling, fixed and then the cannula slides over the shaft of the stylet causing a small piece of tissue to be 'captured' in the chamber. The whole needle is then withdrawn and the piece of tissue placed in a pot with formalin (**Fig 24**). If the needle is intended for further use, the piece of tissue can be carefully prised off the stylet chamber with a small hypodermic needle. If the Trucut needle is not to be used again, the stylet chamber can be inserted into the formalin and the tissue sample 'knocked off'.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

Ethical review not applicable for this review article.

Source of funding

None.

Authorship

Both authors prepared the manuscript.