Equine nodular skin diseases

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Although a relatively low number of aetiologies are responsible for the majority of nodular skin diseases seen in equine practice, it is worth being aware of other less common causes as prognoses and treatment can differ significantly.

When sampling these lesions, it is also important to understand the relative benefits and limitations of each diagnostic option and how these may affect the chance of a diagnostic sample or surgical cure.

Generally, nodular skin diseases in horses can be grouped into neoplastic and non-neoplastic conditions with the latter broken down into non-infectious and infectious.

Neoplastic

Sarcoids (Fibropapillomas)

- (Host adapted strain) of bovine papillomavirus 1 and 2 (Ovine papillomaviruses?).
- Genetic susceptibility with inadequate immune response.
  - Low rates of spontaneous resolution.
- Six clinical types.
  - Do not always correlate to histology.
- Local recurrence rates range from 18 to 70% for completely excised sarcoids (+/- ancillary treatment) depending on study.
- Fibroblastic sarcoids more likely to recur locally based on clinical and histological studies (mitotic rate>20/2.37m^2).

Melanocytoma/melanoma

- Non-solar associated.
- Up to 80% of grey horses older than 15yo.
  - Can start 7–8yo after greying process has finished.
  - Greying is autosomal dominant due to presence of duplication of intron 6 of STX17 gene (Grey Gene) on Chromosome 25.
    - Three alleles (N/G1=normal, G2=Grey gene duplication, G3= Grey gene triplication.
    - G2 and G3 associated with Greying.
      - Supercharges melanocyte differentiation early which exhausts stem cells causing greying.
    - G3 associated with quicker greying and higher melanoma incidence.
      - G3/G3 would be highest risk.
    - Commercial test available for Grey Gene copy number (UCDavis).
    - Melanomas can have up to nine copies of these genes.
  - Initially benign melanocytomas 90% but 66% progress to malignant melanomas within two to three years.
- Non-grey horses less frequent but more likely to be malignant earlier in disease course.
  - Often younger horses.
- Metastasis to regional lymph nodes, internal organs and unusual locations such as parotid gland, mammary glands and axillary lymph nodes.

Squamous cell carcinoma

- Can be nodular if verrucous or associated with severe dermal fibroplasia.
- Locally invasive and usually metastasise late in clinical course to regional lymph nodes.
- Usually associated with non-pigmented skin e.g. eyelids, genitals due to chronic ultraviolet light exposure.
- Complete excision difficult therefore local recurrence common.

1. Ocular
    - Usually younger horses 6–12yo.
    - Genetic component.
        - Breeds homozygous for DDB2 gene mutation have increased risk of ocular SCC. e.g. Belgians, Haflingers.
        - Screening test available (UCDavis).
2. Genital tumours.
    - Papillomavirus (EcPV-2) involvement.
    - Usually older geldings.

Squamous papillomas

- Usually EcPV1.
- Head and distal limbs of young horses.
- Often multiple.
- Spontaneously resolve within weeks.

Mast cell tumours

- Head, neck and limbs. Can be mucocutaneous.
- Usually solitary.
- Male>Female.
- Benign
- Can have eosinophils DDx eosinophilic nodular diseases.

Lymphoma

1. T cell rich B cell Lymphoma (TCRBCL) – 84% of cutaneous lymphoma in horses.
    - Mixed cell population can be confused histologically with inflammatory lesions.
    - Multiple masses.
    - Quarterhorses predisposed.
    - Low grade lymphoma.
    - Complete excision curative in 56% of cases.
    - Local recurrence poor prognostic indicator.
2. T cell lymphoma.
    - Solitary mass.
    - More frequent recurrence.
    - Higher grade.

Soft tissue sarcomas

1. Fibrosarcomas.
    - Can be confused with sarcoids.
2. Peripheral nerve sheath tumours (Schwannoma).
    - Usually benign expansile masses.
    - Eyelid predilection site.

Cutaneous neoplasia in donkeys

1. Sarcoid = 82%.
    - M:F 2:1.
    - Males predominantly inguinal.
    - BPV1 or 2.
    - Three Donkey Papillomaviruses isolated to date.
        - Not associated with squamous papillomas or sarcoids.
2. Sarcomas = 6%
3. Fibromas/fibrolipomas = 3%
4. Donkeys generally don’t get melanomas, squamous cell carcinomas or lymphoma.

Non-neoplastic

Non-infectious

1. Collagenolytic granulomas.
    - Underlying cause unknown.
        - Hypersensitivity, trauma.
    - Necrosis with mineralisation, eosinophilic infiltrates and granulomatous inflammation.
2. Axillary nodular necrosis (Girth Galls).
    - Histologically similar to collagenolytic granulomas.
        - Deeper
        - Vascular involvement.
        - Similar pathogenesis?
3. Papular dermatitis.
    - Multiple papules.
    - Arthropod associated.
        - Midges, mosquitos, Sandflies (New Zealand).
        - Culicoides (Overseas).
    - Usually dorsocaudal but also ears and head.
    - Eosinophilic inflammation.
4. Proud flesh.
    - Trauma
    - Secondary to distal limb skin disease/wounds.
    - Common with ulcerated sarcoids.
5. Hypersensitivities
    - Urticaria
    - Pseudolymphoma
        - Chronic arthropod HST?
        - Fungal infections – Look like lymph nodes histologically.
6. Foreign body reactions.
    - Granulomas
    - Abscesses / sinuses.
    - Calcinosis circumscripta.
        - Firm subcutaneous mass – usually focal.
        - Aetiology unknown- suspect traumatic.
            - Not usually associated with systemic calcium: phosphate imbalances.
        - Often near joints.
        - Gritty on cytology.
7. Amyloidosis
    - Multiple papules, nodules or plaques.
    - Head, neck, shoulders or pectoral area.
    - Cause not fully understood.
    - Not usually associated with systemic amyloidosis, nasal amyloidosis or chronic inflammatory conditions.
8. Follicular or dermoid cysts.
    - Dermoid cysts are usually congenital in the dorsal midline of thoracolumbar regions.
    - Follicular cysts are usually acquired due to obstruction of a hair follicle which continues to produce keratin.
9. Haematomas
    - Traumatic – blunt or penetrating wounds.
    - Coagulopathy -rare in horses.
    - Clinical presentation and cytology/histology depends on age of haematoma.

Infectious

1. Fungal granulomas.
    - Dermatophytes
    - Environmental (phaeohyphomycosis/hyalohyphomycosis).

2. Dermatophytosis – Folliculitis/furunculosis.
3. Bacterial folliculitis/Furunculosis/Pseudomycetoma
    - Secondary to self trauma, dermatophytosis, dermatophilosis
    - Saddle/tack area.
        - Often coagulase negative staphs.
4. Abscesses/cellulitis
    - Usually secondary to other disease e.g. furunculosis, foreign bodies or penetrating wounds.
    - *Rhodococcus equi* (rare)
    - Strangles (fistulated lesions/lymph nodes).
5. Botryomycosis
    - *Staphylococcus*, actinomycetes
    - Rare
    - Often eosinophilic inflammation.
6. Aural plaques
    - EcPV3, 4, 6 +/- EcPV 1 and 5.
7. Penile papillomas/plaques
    - EcPV2.
8. Atypical mycobacteria
    - Secondary to penetrating wounds/foreign bodies.
9. Mycobacterium avium complex
    - Rarely skin lesions.

Exotic / notifiable nodular skin diseases

Nematodes

1. Habronemiasis – (Australia).
2. Halicocephalobus – (Europe, North America).
3. Oncocerca – (Australia,North America, Europe).
4. Parafilariasis – (Eastern Europe).

Bacteria

1. Corynebacterium pseudotuberculosis biovar equi.
    - Ulcerative lymphangitis.
    - Abscesses
2. Burkholderia mallei (Glanders / Farcy).
    - Re-emerging disease.
        - Asia, Africa and South America.
    - Usually multisystemic disease.
    - Farcy – Ulcerated skin nodules and lymphangitis.
3. Burkholderia pseudomallei (Melioidosis).
    - Emerging disease.
        - Asia
        - Tropical / subtropical.
    - Usually multisystemic disease.
    - Maculopapular to crusting skin lesions.
    - Lymphangitis
4. Fungal / stramenopila.
    - Systemic
        - Histoplasma capsulatum var farciminosum.
            - Epizootic lymphangitis.
        - Coccidiomycosis
    - Localised
        - Sporothrix
        - Pythiosis

Diagnostics for nodular skin diseases in horses

Cytology of masses (note different to cytology for dermatology)

- Can be useful for inflammatory vs neoplastic masses.
- Can be diagnostic for some skin neoplasms, e.g. mast cell tumours.
- Not always useful for a definitive diagnosis with inflammatory diseases.
  - E.g. Eosinophils can be seen in:
    - Collagenolytic granulomas.
    - Axillary nodular necrosis.
    - Mast cell tumours.
    - Botryomycosis.
    - Foreign body reactions.
    - Fungal granulomas.
  - Sarcoids don't exfoliate well but if they do cannot reliably differentiate from reactive fibroblasts or sarcomas.
  - TCRBCL consists of a mixed lymphocyte infiltrate which can look inflammatory.

Golden rule of cytology:

- Maximise the number of intact nucleated cells in a monolayer (INCIM).
- Non-diagnostic samples most commonly due to acellular samples.
  - Always take more than one aspirate from each mass.
  - If taken in-clinic prestain at least one slide to ensure there are enough Intact Nucleated Cells In a Monolayer (INCIM) to assess.
- If multiple masses then aspirate separately. Don't assume all part of same disease process.
- If there is a fluid filled cavity then aspirate fluid for analysis +/- culture but also aspirate the more solid margin of the cavity.
- Keep cytology slides away from formalin to prevent artefact from formalin fumes.
  - Take biopsies after cytology.
  - Keep formalin container away from cytology slides at all times.
  - Move cytology slides away from the patient prior to biopsying.
    - Not on same surface as formalin container.
  - Do not send cytology samples in courier back with formalin fixed tissue.

Biopsies

Generally, the best diagnostic technique for investigating nodular skin diseases in horses.

Goals – need to be balanced

1. Provide a surgical cure
2. Achieve a definitive diagnosis
3. Minimise trauma or risk to the patient
4. Financial constraints

Rules for skin mass biopsies

1. Use excisional or incisional/wedge biopsies
   - Excisional
     - Ideal – diagnosis and surgical cure.
     - Small masses.
     - When previous cytology or incisional biopsy is diagnostic.
       - Helps with prognosis and surgical margin requirement.
     - Lymph nodes (relatively uncommon in horses).
       - Traumatised with incisional or punch/Trucut biopsies makes interpretation nearly impossible.
       - Architecture often needed when considering lymphoproliferative disease or staging neoplasia.

- Incisional / wedge.
    - When cytology non-diagnostic.
    - When excision isn't easy or possible.
        - Size
        - Location
        - Local anaesthesia difficulties.
    - May not be enough tissue.
    - Prone to trauma when handling during and after biopsy.
- Never use skin punch or tru-cut biopsies for masses or lymph node biopsies.
    - Often don't sample enough tissue for a diagnosis.
    - Induces artefact (e.g. shearing from artefact).
    - Damages tissue.
    - May not go deep enough.

2. Don't biopsy ulcerated area of masses where possible.
    - Can miss primary epidermal changes.
        - Can't reliably differentiate granulation tissue from an ulcerated sarcoid.
            - Diagnosis of sarcoids often depends on the interaction of the tumour with the epidermis.
            - Ulcerated sarcoids also have granulation tissue that blends in with the tumour.
    - Secondary infection can mask the underlying primary process.
3. Avoid artefacts.
    - Fixation
        - Fix immediately.
            - Carry formalin with you in your vehicle.
                - Secure it safely and store it in damage proof containers especially if in the cab with you.
        - Formalin:tissue ratio 10:1 even if large masses/proud flesh.
            - Have 10L buckets in clinic for fixing large specimens.
                - Can also be used for companion animal (large mammary masses, spleens) and production animal cases (whole brains).
        - Don't cut biopsies into smaller pieces or partially incise excisional biopsies to reduce fixation time or for curiosity reasons.
            - Affects margin assessment.
                - Tissue deforms during fixation therefore postoperative incisions will gape open and be interpreted as incomplete excision.
                - Can introduce neoplastic cells, microbes or foreign material to the real surgical margins.
            - Can cause artefacts.
    - Crush / shearing.
        - Commonly induced during/after surgery by forceps manipulating biopsies or squashing a biopsy into too small a container.
            - Avoid rat-tooth forceps for extraction of biopsied tissue from the site.
                - Teeth creates "pseudocysts" in the tissue.
                    - Toothless Adsons type forceps or needle-nose forceps.
                    - Elevate biopsies from underneath with tips of closed forceps or hypodermic needles
            - Carry a range of different sized containers or just a few large ones.
                - Urine specimen containers are great but avoid larger rigid plastic containers as they are prone to cracking.
                    - Softer plastic containers 'honey pots' are more resilient.
                - Remove the tamper-proof ring as can sometimes affect the seal resulting in formalin leakage.
    - Local anaesthetic.
        - Generally OK for incisional/wedge and excisional biopsies of nodular skin diseases if applied deep and wide to the biopsied area and not into the mass itself.
        - OK for skin punch biopsies (dermatology cases only) if applied deep to the biopsy area.
            - Can cause artefact in primary subcutaneous dermatological diseases but not usually.
    - Margin assessment for excisional biopsies.
        - Ink margins – can be done immediately or back in clinic after fixation.
            - Tissue dyes – different colours for different margins.

        - Indian ink – cheap but doesn't stick as well to formalin fixed tissue.
    - Sutures to mark margins of specific interest e.g. marginal excision or help with orientation, e.g. Cranial/caudal, dorsal/ventral.

Biopsies for dermatological conditions

Biopsies for histology

1. Don't clip or surgically scrub.
    - Removes crusts and can cause artefact to epidermis.
2. Identify primary lesions – don't biopsy ulcers or fistulae.
    - Misses primary epidermal changes.
    - Secondary infection can mask the underlying primary process.
        - Do cytology first and if superficial pyoderma present then pretreat with antibiotics and biopsy just before finishing the course.
3. Biopsy the centre of the lesions, not the margins especially in alopecia cases.
4. 8mm skin punch biopsies.
    - Need to be able to bisect biopsy parallel with the direction of hair growth so we can visualise the entire length of hair follicles histologically.
    - Direction of hair growth difficult in the lab especially if alopecic.
    - Draw a line on skin with narrow nibbed permanent marker (Sharpie) before biopsying to show direction of hair growth.
5. Always take multiple biopsies and identify sites.
6. Drill biopsy punch in one direction only to minimise shearing artefact.
7. Use a new biopsy punch for each patient.
8. Ensure adequate formalin:tissue ratio.
    - Don't stick biopsies to cardboard or tongue depressor.
9. Don't grab biopsy with rat tooth forceps.
    - Elevate from underneath.

Biopsies for tissue culture

1. Surgically scrub to minimise skin commensal contamination.
2. Avoid ulcerated/fistulated lesions.
    - biopsy adjacent to them or find non-ulcerated primary lesions.
3. Use new sterile 8mm punches or incisional biopsies.
4. After biopsy is removed lay it on its side on a sterile surface and cut off epidermis with scalpel.
    - Minimises skin commensal contamination.
5. Place biopsy in the liquid media from an E-Swab or a sterile saline soaked surgical swa inside a sterile container.

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