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The diagnosis and management of the itchy horse is a process built on having an understanding of the pruritic diseases most likely to be present in your geographic area along with data provided through history taking, a thorough dermatological examination, selected diagnostics (cytology, skin scraping, fungal culture) and the assessment of response to therapy. The most common differential diagnoses for the pruritic horse include insect bite hypersensitivity (IBH), atopy, food sensitivity, pediculosis, Chorionoptes, Psoroptes, trombiculidiasis (chiggers or harvest mites), contact hypersensitivity, and those dermatoses that are variably pruritic including dermatophytosis, bacterial pyoderma, dermatophilosis, pemphigus and drug eruption.

Important historic points include management (e.g. stable vs. pasture; diet), age of onset (e.g. 2–4 years and 1–6 years for IBH and atopy, respectively) and seasonality. Seasonal pruritus problems are most commonly associated with IBH and atopy (spring, summer, fall), trombiculidiasis (fall), choriocidal mange and pediculosis (winter). Attention must be paid to whether the pruritus has created the lesions (e.g. IBH, atopy) or the lesions were noted before pruritus (e.g. dermatophytosis, bacterial pyoderma, pemphigus) - i.e. is it "an itch that rashes or a rash that itches"? History taking should include documentation of medications being taken when the pruritus/lesions developed (e.g. drug eruption, contact hypersensitivity) and response to medications (e.g. glucocorticoid responsive - IBH, atopy, food sensitivity).

On physical examination, emphasis is placed on the nature of the lesions and the distribution. Do the lesions appear to be self induced (alopecia, excoriations, skin thickening, lichenification - IBH, atopy, Choriocoptes, Psoroptes)?; are there crusted papular lesions (IBH, atopy but also must consider dermatophytosis, bacterial pyoderma, dermatophilosis, onchocerciasis, pemphigus, Psoroptes) or urticarial lesions (atopy, food sensitivity, drug eruption)? Distribution "tip offs" include: face - IBH (Culicoides; black flies), atopy, food sensitivity, trombiculidiasis; pinnae – IBH (black flies), food sensitivity, Psoroptes; mane - IBH (Culicoides), atopy, food sensitivity, Psoroptes, pediculosis; dorsum - IBH (Culicoides), pediculosis, food sensitivity; tail/rump - IBH (Culicoides), atopy, food sensitivity, Choriocoptes, pediculosis, Psoroptes, oxyuriasis, vice; legs - Choriocoptes, trombiculidiasis, contact dermatitis, IBH (stable flies), atopy, food hypersensitivity; ventrum - IBH (Culicoides, horn flies), food hypersensitivity, contact dermatitis, trombiculidiasis.

When lesions are primarily papuloscrustous +/- proceeding to focal areas of alopecia, inflammation and crust, work-up generally involves scrapings (Psoroptes), cytology, fungal culture and/or trial treatment for dermatophytosis, dermatophilosis or bacterial pyoderma. For bacterial pyoderma, this will often involve an assessment of response to systemic antibiotic therapy (e.g. TMS). Allergic horses may also have lesions that progress in this fashion. Lesions may represent secondary bacterial infections (assess response to systemic antibiotic) or may be a product of the allergy itself. Allergic individuals would usually be expected to be steroid responsive (starting at 0.5–1 mg/kg bwt/day prednisolone or 0.05–0.1 mg/kg bwt dexamethasone/day). When a diagnosis is unclear, strong consideration should be given to taking multiple skin biopsies.

Malassezia dermatitis is a controversial diagnosis in horses. It has been suggested that clinical signs are associated with greasy, waxy, foul smelling, variably pruritic dermatitis affecting the axillae, groin, udder and prepuce. It has been recently noted that Malassezia-like yeast organisms can be found in all these areas in normal horses, thereby making the significance of findings on cytological examination controversial.

Horses that are self-traumatising and may or may not have urticarial lesions generally are suffering from IBH, atopy and/or food sensitivity. It is important to recall that more than one hypersensitivity may be present in the same individual. These hypersensitivities are all expected to be steroid responsive, although with chronicity, they may require higher dosages of steroid to provide adequate pruritus/urticaria control. A diagnosis of insect bite hypersensitivity is usually made by assessing response to insect avoidance: stabling (recalling that stable flies, horse flies and deer flies feed during the daytime, black flies in the morning and evening and Culicoides spp. from dusk to dawn); mist sprayers - pyrethrins; fans; screens; at least 2% permethrin sprays (to maximise repellent activity) every 1–3 days; fly sheets; and/or permethrin impregnated fly sheets. Intradermal testing with insect antigens is associated with a significant incidence of false positives (50% or more normal horses having positive reactions, albeit lesser numbers of reactions than hypersensitive individuals). Food sensitivities are only rarely encountered in horses. Most common offending feeds include sweet feed or commercial grain mixes. The diagnosis is supported by feeding one source of hay for at least 4 weeks, then challenging to confirm a relation of the pruritus to the feed. Atopy is largely a diagnosis by exclusion. In the author’s experience, seeing a response to antihistamine therapy (e.g. hydroxyzine) is more supportive of this diagnosis than IBH. Because of the potential for false positive reactions, intradermal testing and/or in vitro serological testing should not be used to diagnose atopy. However, these tests are used to select allergens for immunotherapy and avoidance. When a diagnosis for this presentation (pruritus +/- urticaria) is unclear, consideration should be given to taking multiple skin biopsies. Within this group (IBH, atopy and food sensitivity), skin biopsies are eosinophilic and suggest hypersensitivity, but do not differentiate as to the type of hypersensitivity.
Update on clinical aspects of *Culicoides* hypersensitivity

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**Introduction**

The equine population of the Netherlands comprises about 400,000 horses and ponies of 27 different registered breeds. Insect bite hypersensitivity (IBH), also named *Culicoides* hypersensitivity, sweet itch or summer eczema, is a significant problem with 8–18% of the equine population showing signs. This suggests that at least 30,000 horses in the Netherlands alone may suffer from IBH. The prevalence of the condition varies in the rest of Europe from 2.8% in the UK to 29% in Germany, while in Australia a prevalence of 32% has been reported. Nowadays, the incidence of the disease seems to be increasing significantly, which may be related to the global warming and the reduced use of insecticides in agriculture.

Insect bite hypersensitivity is a result of type I (immediate and late phase) and type IV (delayed) hypersensitivity to antigens (presumably salivary) from numerous *Culicoides* and *Simulium* species, *Stomoxys calcitrans* and, possibly, *Haematobia irritans*. There are over 800 species of *Culicoides*, worldwide (Fig 1). The midges are 1.5–5 mm long. The females feed on both humans and animals. *Culicoides* lay eggs in damp marshy soils or in decaying organic matter near water. The midges’ dispersal capacity is low: the adults usually only fly a few hundred metres. Their life cycle can only be completed if the ambient temperature is >5°C. In the Netherlands therefore, IBH is a seasonal condition. There is much more interest in *Culicoides* species now as these midges also are the vectors of blue tongue and African horse sickness (AHS).

IBH affects many horse breeds, animals of different age, and either sex. Clinical evidence strongly suggests that the disorder has familial and genetic predispositions.

The condition typically worsens with age. Affected horses usually show one of 3 patterns of skin disease (Fig 2):

- Dorsal distribution (pruritus, with or without crusted papules, usually beginning at the mane and base of the tail).
- Ventral distribution (pruritus, with or without crusted papules, beginning on the ventral thorax and abdomen, axillae and groin).
- Combinations of the above.

Self-trauma and chronicity lead to excoriations (erosions, ulcers), variable hair loss (hypotrichosis, alopecia), lichenification and pigmented disturbances (melanoderma, melanotrichia). Secondary bacterial infections are not uncommon.

The diagnosis is based on a typical history, physical examination, elimination of other conditions and the response to insect control. However, an objective diagnostic method is urgently needed.

There is no consistently effective treatment except prevention of contact between horse and insects. Indoor confinement (no contact with insects) and pastures with electrical fences (prevention of rubbing) are effective, but have significant welfare implications.

**Studies in the Netherlands**

In 2004 in the Netherlands, horse owners, stud books, veterinarians, animal welfare groups and research institutes requested increased urgency in investigating the disease, focusing on incidence, diagnosis, genetic predisposition, therapy and prevention. Utrecht University and Wageningen University received a grant to study insect hypersensitivity, in collaboration with several studbooks. In 2008 a grant from STW (Technology Foundation of the Netherlands) was also obtained.

The studies performed up to the present time include:

**Intradermal test**

In 2004 an intradermal test with a *Culicoides nubeculosus* extract (Greer Laboratories, Lenoir, USA) was performed in April in 9 pairs of horses (each pair consisting of one animal with IH and one control). All horses were injected with different concentrations of the allergen (*Culicoides nubeculosus*) and positive and negative control solutions (histamine and buffer respectively). The resulting wheals were measured at 30 min, 4, 24 and 48 h. There was no statistical significant difference between the affected cases and the control horses. There were several possible explanations for these results. They may reflect the fact that the *Culicoides* species represented in the antigen solution was not the correct one for the Netherlands, as different species may be involved. In 2002 results were published from an intradermal skin test with *Culicoides variipennis* in Austria, and these results were also disappointing.

**Harvesting indigenous ‘midges’**

In 2005–2006 a new study was instigated in which we trapped midges by placing horses/ponies under a large mosquito tent (Fig 3) for 1 h. The insects remaining in the tent were harvested and identified. The large majority of *Culicoides* were found to be...
C. obsoletus (Meigen) (94.1%), in addition a smaller number was C. pulicaris (Linnaeus) (5.81%), C. stigma (Meigen) (0.06%) and a single C. vexans (Staeger) (0.03%) were identified.

Formulation of 'local' Culicoides mixture
A whole body extract of the local (collected) Culicoides species was made and this was used at a 1:1000 w/v concentration in an intradermal test. Reliable results were obtained in 10 pairs of horses (each pair: one IBH horse and one control horse). This test therefore supports the clinical diagnosis of equine insect hypersensitivity.

Dutch Culicoides extracts of different species
Using around 50,000 midges 3 different whole body extracts (C. obsoletus nonblood-fed, C. obsoletus blood-fed and C. pulicaris) were formulated in 2 different concentrations (1:1000 w/v and 1:5000 w/v). These were used to establish whether there is a difference in response in the intradermal test between horses suffering from IBH and unaffected. Again 20 horses were used in the same fashion as previously described. All horses were required to have remained at their current location for at least one year. Horses were not sedated for the test. The wheal diameter (in mm) and firmness of the injection sites were evaluated 30 min, 1 h, 4 h and 24 h after the injection. The most reliable responses were achieved at 1 and 4 h; at these times there was a significant difference in reaction between the IBH horses and the control horses to all of the extracts. However, there was no clear difference between the individual different extracts (C. obsoletus nonblood-fed, C. obsoletus blood-fed and C. pulicaris).

IBH as sentinel for African horse sickness (AHS)
As Culicoides spp. are also vectors for AHS, we performed a study for the Dutch Government to investigate geographically where the highest Culicoides spp. population were to be found. The aim of this study was to identify by mapping the appearance of IBH throughout the country patterns of spread of Culicoides species throughout the Netherlands. This study showed that our coastal areas were identified as low risk areas for IBH and Noord-Brabant and Limburg were at higher risk for both horses and ponies. The Achterhoek and Twente regions were a higher risk area for ponies only.

Control of IBH by dietary supplement
The effects on the severity of IBH of a dietary powder supplement containing vitamins and amino acids with a solution containing polypeptides and vitamins in natural oil (Hypo-ex-cena®) was evaluated in a double blind placebo controlled study. In the initial trial in September–November 2008 we found no significant difference between the treated and placebo groups (n = 50). The study has been repeated from April–June 2009. The results were not available on submission of this abstract.

Future
At present several studies on IBH are underway. We are investigating the genetic background of individual horses with IBH to establish any genetic basis that might be used to limit the disease through breeding programmes. We are carrying out further studies on the sensitivity and specificity of intradermal skin testing. We are also attempting to develop a reliable ‘blood test’ for IB. We are trying to assess the effectiveness of various therapeutic interventions under field conditions and researching the specific behavioural and feeding activities of the various species of Culicoides.

Conclusion
Insect bite hypersensitivity is a serious welfare problem worldwide and our primary aim is to develop reliable blood and skin tests and to find a usable genetic marker. We hope that the positive results of the intradermal test will make future experiments with desensitisation of horses and ponies with IBH more successful.

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Immunology of Culicoides hypersensitivity: Prospects for immunotherapy?

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Insect bite hypersensitivity (IBH), more commonly known as ‘sweet itch’ in the UK, is most frequently an allergy to the bites of midges (Culicoides [Diptera: Ceratopogonidae]) although other biting insects can be implicated in some cases. The reported incidence of IBH in native horses across UK and Europe is about 3–5% but the incidence can reach over 30% in horses first exposed to Culicoides as adults; such as those exported from Iceland (where Culicoides do not occur) to mainland Europe (Björnsdóttir et al. 2006).

Once established, IBH recurs annually during periods of high insect activity; clinical signs include severe pruritus leading to self trauma, hair loss, serous effusion, haemorrhage and secondary infection. Affected horses develop an acute phase inflammatory response to intradermal challenge with Culicoides salivary gland proteins, followed by a late phase response in which skin biopsies show increased numbers of infiltrating T cells, mast cells, eosinophils and IgE producing B cells. Persistent exposure to insect bites leads to a chronic inflammatory response characterised by dermal fibrosis, epidermal hyperplasia and hyperkeratosis.

Although all horses exposed to Culicoides bites have IgG antibodies to insect salivary proteins, only horses with IBH have Culicoides-specific IgE antibodies. Horses in Iceland that have never been exposed to Culicoides bites have no antibodies to Culicoides proteins (Wilson et al. 2001; Helberg et al. 2006). Similarly peripheral blood mononuclear cells (PBMC) from both allergic and healthy horses exposed to Culicoides proliferate in vitro when stimulated by Culicoides antigens, but those from allergic horses produce higher levels of interferon-γ, which promotes IgE synthesis, and less interferon gamma, which inhibits IgE synthesis (Hamza et al. 2007). Cultures of lymphocytes from healthy horses also have higher levels of the cytokines IL10 and TGF-beta which suggests that regulatory T cells may be important in maintaining a tolerant immune response to midge proteins.

Some genetic predisposition for IBH has been demonstrated in several breeds including Icelandic horses, but there is little evidence that the very high incidence (>30%) seen in exported Icelandic horses is solely due to a genetic susceptibility. In a study of 1200 second generation Icelandic horses born in Germany the overall incidence was 4.6%, close to the average incidence for other breeds. When both parents were affected by IBH the prevalence in offspring was slightly higher (12.2%) compared to offspring with one affected parent (6.5%) or offspring where neither parent was affected (2.9%) (Marti et al. 2008).

Surprisingly the reduced prevalence of IBH in second generation Icelandic horses occurs despite the transfer of maternal Culicoides specific IgE via colostrum which might be expected to prime an allergic response (Wilson et al. 2001). However maternal IgE in foal serum wanes rapidly, becoming undetectable by age 6 weeks while significant IgE synthesis is not detectable until about 6 months. In indigenous European horses which develop IBH, the peak age of disease onset is between 2 and 4 years coinciding with the maturation of IgE responses and it has been proposed that early exposure to midge bites prior to onset of IgE synthesis facilitates the development of immune tolerance in the majority of foals born in areas where midges are endemic (Wilson et al. 2008).

No current treatment for IBH is fully effective, therefore immunotherapy has been proposed as a potential alternative for this condition, but if this approach is to be successfully preparations of the allergens will be required. Accordingly, our recent work at Bristol Veterinary School has concentrated on identifying the allergens in Culicoides saliva. We have constructed a cDNA library from Culicoides nubeculosus salivary gland messenger RNA from which over 50 novel sequences of putative secretory proteins have been generated (Russell et al. 2009). In addition we have analysed the most abundant proteins in C. nubeculosus saliva using a combination of 2D electrophoresis and mass-spectrometry. These data were combined to identify the respective clones from the cDNA library, including several novel proteins that we have identified as major allergens on the basis of their reactivity with IgE from allergic horses. Currently, we are building a representative library of all the abundant proteins from Culicoides saliva expressed as recombinant proteins in insect cell culture using baculovirus.

Having achieved this intermediate goal, our proposed future research aim will be to utilise the recombinant Culicoides allergens as diagnostic and therapeutic agents in a pilot study of immunotherapy. Traditional methods of allergic desensitisation use multiple (daily) injections of allergens starting at minute doses and incrementally raising the dose over time. Such a protocol is unlikely to be well tolerated by horses and carries a risk of potentially serious side effects including anaphylactic shock. Oral immunotherapy is increasingly used as a simpler and safer alternative to traditional injections allowing larger doses of allergen to be safely administered right from the outset. Firstly the immune responses of horses to each recombinant midge protein will be measured, only those to which it has a positive allergic reaction will be included in the therapy. The antigens/placebo will be presented as a disintegrating tablet administered within a specially adapted bit, held in place by a standard bridle, to allow the release of antigen into the oral cavity over a 15–30 min period.

We recognise that oral desensitisation in IBH is unlikely to cause the complete remission of symptoms in the first instance, but even a modest reduction in the severity of disease symptoms or in the magnitude of the allergic immune response would provide a starting point to refine and improve the treatment.

References


Intradermal and *in vitro* serological testing - do they tell us anything in the horse?

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Intradermal testing (IDT) and *in vitro* serological testing are intended to identify antigen specific IgE, the major mediator of immediate hypersensitivity reactions associated with equine atopic dermatitis and respiratory disease. The tests are performed to choose antigens for purposes of allergen specific immunotherapy (ASIT) and/or to avoid allergen exposure. Significant questions exist regarding test sensitivity, specificity and overall utility. This is in large part due to the lack of an appropriate "gold standard" for test assessment (e.g. challenge with individual allergens and assess response).

Intradermal testing is available for a wide variety of pollens, moulds, mites, insects and environmental allergens. IDT is considered to produce more specific data than *in vitro* serological testing. Performing intradermal injection, evaluation of reaction (wheal formation) is done at 15–30 min. Delayed reactions (wheal formation) may be seen at 4 h and 24 h; some not preceded by immediate reactions. Because a significant number may be seen at 4 h, it has been suggested that IDT also be read at 4 h (Lorch et al. 2001a) but because of the erratic responses seen at 4 h, others recommend only the 15–30 min reading (Baxter and Vogelnest 2008). It has been shown that horses with atopic dermatitis, recurrent pruritic urticaria (Lorch et al. 2001a; Jose-Cunilleras et al. 2001) and chronic obstructive pulmonary disease (Jose-Cunilleras et al. 2001) have a significantly greater number of positive reactions compared to horses without atopy. However, it is important to emphasise that apparently normal horses may have a significant number of positive reactions. 'False' positives may be associated with: nonspecific irritant reactions and/or the use of higher concentrations of allergens (e.g. concentrations of dust mite antigens should be lower than those conventionally used for IDT) (Lorch et al. 2001a). None of the 3 serum allergy tests reliably detected allergen hypersensitivity, compared with the ID test. The FcεRI-based ELISA performed significantly better overall than the other 2 tests. Low sensitivity for all 3 assays indicates the need for continued study to elucidate a more sensitive test for the determination of potentially pathogenic allergens in horses. There are few data on the relative success associated with hyposensitisation utilising *in vitro* serological testing. A small, uncontrolled study of horses with atopic urticaria reported a poor response to ASIT based on results of an ELISA test, but good response with allergens selected on the basis of IDT (Loewenstein and Mueller 2009).

The relative value of *in vitro* serological testing is much more controversial and there are very few studies examining its utility. Various enzyme linked immunosorbent assays and a radioallergosorbent test are available. On study looked at 3 serological tests and compared them with intradermal skin testing in horses with and without atopic disease (Lorch et al. 2001b). None of the 3 serum allergy tests reliably detected allergen hypersensitivity, compared with the ID test. The FcεRI-based ELISA performed significantly better overall than the other 2 tests. Low sensitivity for all 3 assays indicates the need for continued study to elucidate a more sensitive test for the determination of potentially pathogenic allergens in horses. There are few data on the relative success associated with hyposensitisation utilising *in vitro* serological testing. A small, uncontrolled study of horses with atopic urticaria reported a poor response to ASIT based on results of an ELISA test, but good response with allergens selected on the basis of IDT (Loewenstein and Mueller 2009).

References


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