



Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvj

Guest Editorial

Equine insect bite hypersensitivity: Are molecular diagnostic tests the future?

The study by Dr Nathalie van der Meide of the Cell Biology and Immunology Group at Wageningen University, and her colleagues, published in a recent issue of *The Veterinary Journal*, evaluated a diagnostic ELISA for equine insect bite hypersensitivity (IBH) using recombinant *Culicoides* Obsoletus complex allergens, which has the potential to considerably improve diagnosis of IBH (van der Meide et al., 2014). The study builds on many years of research, by different groups, who identified allergens from laboratory-bred and wild *Culicoides* spp. at the molecular level and then produced these as recombinant proteins (Langner et al., 2009; Schaffartzik et al., 2010, 2011; van der Meide et al., 2013).

In their most recent study, van der Meide et al. (2014) evaluated the performance of IgE ELISAs against seven recombinant *Culicoides* Obsoletus complex allergens, alongside, for comparison, Obsoletus complex whole body extract (WBE). A large number of sera were screened from well-characterised IBH-affected and control horses of the Icelandic breed and Shetland ponies. The individual recombinant allergens bound serum IgE from 76–85% of the IBH-affected and from 15–24% of the healthy horses. In comparison, IgE-reactivity to WBE was 86% in IBH-affected horses and 14% in the controls. Furthermore, the authors elegantly showed that a combination of three to four specific Obsoletus complex recombinant allergens allowed an IgE ELISA to be developed for in vitro diagnosis of IBH, with sensitivity and specificity of 89% during the IBH season. The use of this specific combination of recombinant Obsoletus complex allergens results in an efficient and cost effective test that performs as well as an ELISA with WBE.

Interestingly, the ELISA with Obsoletus complex WBE performed surprisingly well compared to commercial tests (see review by Schaffartzik et al., 2012). This may be due in part to the fact that *Culicoides* caught in the environment of the horses were used to prepare the WBE instead of laboratory-bred *Culicoides* species. Previous studies by van der Meide et al. (2012, 2013) have shown that allergens from local *Culicoides* spp. such as Obsoletus complex midges are more immunoreactive than those from laboratory-bred, non-indigenous species. However, few direct comparisons between allergens from different *Culicoides* sources have been undertaken (van der Meide et al., 2012, 2013). Furthermore, the study population, i.e. breed of horse, degree of exposure to *Culicoides* bites as well as the clinical definition of cases influences the results of studies on the performance of IgE ELISAs: Schaffartzik et al. 2011 found a high proportion of IBH-affected Icelandic horses with IgE against recombinant *Culicoides nubeculosus* allergens while Peeters et al. using the same recombinant *Culicoides nubeculosus* allergens but sera from Belgian Warmblood horses found a poorer performance of the ELISA.

Although the ELISA with Obsoletus complex WBE used in the studies by van der Meide et al. (2012, 2014) performed well, the use of recombinant allergens will probably be the method of choice in the future for several reasons. Firstly, midges of the Obsoletus complex have not been bred successfully in the laboratory and therefore need to be caught from the wild. Secondly, allergens prepared from insect WBE will be affected by batch to batch variation and are much less easy to standardise than those produced as recombinant proteins.

Although use of a combination of recombinant allergens in the same test is an attractive approach, in that it will reduce costs and potentially improve diagnostic performance, it has limitations in that it does not allow component-resolved diagnostics (CRD), i.e. defining the specific allergens to which an individual has been sensitised. In human allergies, CRD has become more important, because it allows discrimination between genuine sensitisation (sensitisation to the primary original sensitising molecule) and cross-reactivity of IgE against similar allergens from different sources. CRD thus allows selection of specific allergens for use in immunotherapy, resulting in higher success rates (Canonica et al., 2013). For human allergy testing, novel microarray-based tests are available that allow testing against around 100 allergens with relatively small amounts of serum, and a similar approach could be applied for veterinary species in the future.

Although our knowledge of the allergens involved in IBH has greatly improved over recent years, several issues remain to be addressed. Firstly, the recent study by Peeters et al. (2013) revealed that two new *Culicoides* Obsoletus complex allergens are relevant for IBH. There may be more unidentified allergens against which a large proportion of IBH horses are sensitised. Secondly, there is a need for standardisation of nomenclature for *Culicoides* allergens. For example, the *Culicoides* allergen homologues of an antigen-5 protein are variably named Cul n 1 (Schaffartzik et al., 2010), Cul n 12¹ or Cul o 3 (van der Meide et al., 2013), which leads to confusion. Standardisation of nomenclature could potentially be undertaken by a working party of the interest group on Comparative and Veterinary Allergy.²

Large scale, population-based multicentre studies are necessary using the same recombinant allergens and test system to

¹ See: <http://www.allergome.org/www.allergome.org/>

² See: <http://www.eaaci.org/sections-a-igs/> and <http://www.eaaci.org/sections-a-igs/>

define more precisely which allergens are important for IBH, using well-characterised patients and healthy controls of different breeds and from different geographical regions. Studies are also needed to distinguish genuine from cross-reactive allergens for IBH. Only one cross-reactive allergen has been identified to date (Schaffartzik et al., 2010) and it is likely that a number of other insect and possibly mite allergens may be cross-reactive. Finally, there is a strong need for improved allergen-specific immunotherapy for equine IBH, as discussed in a recent study by Ginel et al. (2014). The method of choice should be the use of pure r-allergens, as in WBE the relevant allergenic molecules are present in too low a concentration. Ideally, individualised immunotherapy based on results of CRD should be developed. It may not be feasible to provide bespoke immunotherapy for each animal, in which case a generic vaccine containing a panel of the most common allergens might be more commercially viable. CRD could then help to predict the likely success of immunotherapy with this vaccine by matching its components against the animal's IgE reactivity.

The study by van der Meide et al. (2014) illustrates how a molecular approach will probably be applied in the development of future diagnostic tests for IBH in horses. However, clinicians should bear in mind the sensitivity and specificity of such serological assays and that a thorough clinical examination and the patient's history remain the most important components in the diagnosis of IBH. Detection of allergen-specific circulating IgE demonstrates that the animal has been immunologically sensitised, but it does not necessarily indicate that this is the cause of clinical signs of allergic disease.

Eliane Marti
Vetsuisse Faculty
University of Bern
Länggass-str 124
CH-3001 Bern, Switzerland

E-mail address: eliane.marti@vetsuisse.unibe.ch

References

- Canonica, G.W., Ansotegui, I.J., Pawankar, R., Schmid-Grendelmeier, P., van Hage, M., Baena-Cagnani, C.E., Melioli, G., Nunes, C., Passalacqua, G., Rosenwasser, L., et al., 2013. A WAO-ARIA-GA²LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organization Journal* 6, 17–34.
- Ginel, P.J., Hernández, E., Lucena, R., Blanco, B., Novales, M., Mozos, E., 2014. Allergen-specific immunotherapy in horses with insect bite hypersensitivity: A double-blind, randomized, placebo-controlled study. *Veterinary Dermatology* 25, 29–e10.
- Langner, K.F., Jarvis, D.L., Nimtz, M., Heselhaus, J.E., McHolland, L.E., Leibold, W., Drolet, B.S., 2009. Identification, expression and characterisation of a major salivary allergen (Cul s 1) of the biting midge *Culicoides sonorensis* relevant for summer eczema in horses. *International Journal for Parasitology* 39, 243–250.
- Peeters, L.M., Janssens, S., Goddeeris, B.M., De Keyser, K., Wilson, A.D., Kaufmann, C., Schaffartzik, A., Marti, E., Buys, N., 2013. Evaluation of an IgE ELISA with *Culicoides* spp. extracts and recombinant salivary antigens for diagnosis of insect bite hypersensitivity in Warmblood horses. *The Veterinary Journal* 198, 141–147.
- Schaffartzik, A., Hamza, E., Janda, J., Cramer, R., Marti, E., Rhyner, C., 2012. Equine insect bite hypersensitivity: what do we know? *Veterinary Immunology and Immunopathology* 147, 113–126.
- Schaffartzik, A., Marti, E., Cramer, R., Rhyner, C., 2010. Cloning, production and characterization of antigen 5 like proteins from *Simulium vittatum* and *Culicoides nubeculosus*, the first cross-reactive allergen associated with equine insect bite hypersensitivity. *Veterinary Immunology and Immunopathology* 137, 76–83.
- Schaffartzik, A., Marti, E., Torsteinsdottir, S., Mellor, P.S., Cramer, R., Rhyner, C., 2011. Selective cloning, characterization, and production of the *Culicoides nubeculosus* salivary gland allergen repertoire associated with equine insect bite hypersensitivity. *Veterinary Immunology and Immunopathology* 139, 200–209.
- van der Meide, N.M., Meulenbroeks, C., van Altena, C., Schurink, A., Ducro, B.J., Wagner, B., Leibold, W., Rohwer, J., Jacobs, F., van Oldruitenborgh-Oosterbaan, M.M., et al., 2012. *Culicoides obsoletus* extract relevant for diagnostics of insect bite hypersensitivity in horses. *Veterinary Immunology and Immunopathology* 149, 245–254.
- van der Meide, N.M., Roders, N., Sloet van Oldruitenborgh-Oosterbaan, M.M., Schaap, P.J., van Oers, M.M., Leibold, W., Savelkoul, H.F., Tijhaar, E., 2013. Cloning and expression of candidate allergens from *Culicoides obsoletus* for diagnosis of insect bite hypersensitivity in horses. *Veterinary Immunology and Immunopathology* 153, 227–239.
- van der Meide, N.M.A., Savelkoul, H.F.J., Meulenbroeks, C., Ducro, B.J., Tijhaar, E., 2014. Evaluation of a diagnostic ELISA for insect bite hypersensitivity in horses using recombinant *Obsoletus* complex allergens. *The Veterinary Journal*. <http://dx.doi.org/10.1016/j.tvjl.2013.12.004>.