**Cloning, production and characterization *Culicoides nubeculosus* allergens associated with insect bite hypersensitivity in the horse**

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Insect bite hypersensitivity (IBH) is a seasonal pruritic dermatitis in the horse resembling to human atopic dermatitis and is mediated by IgE reactions to the bites of midges of the genus *Culicoides*. To define the molecular structures involved in the pathogenesis of IBH we cloned, produced and characterized the allergen repertoire present in salivary glands of *C. nubeculosus*. mRNA from salivary glands of the midge was isolated and used to construct a cDNA library displayed on phage surface . The library was screened with solid-phase immobilized serum IgE from IBH-affected horses. Affinity enrichment of phagemids displaying putative IgE-binding proteins yielded 10 incomplete sequences coding for discrete proteins. BLAST analyses revealed sequence homology of the cloned sequences to already identified salivary gland proteins of *C. nubeculosus* not yet described as allergens. In a first step the truncated cDNAs sequences were subcloned into a high level expression vectors pet-17b, expressed as $[His]_6$-fusions in *Eschericha coli*, and purified by $Ni^{2+}$-chelate affinity chromatography. ELISA experiments demonstrated that the truncated recombinant proteins were able to specifically bind serum IgE of IBH-affected horses. In a second step, we completed the open reading frame of the cDNA sequences by using RACE-PCR, subcloned the full length sequences and expressed the corresponding proteins. The full length recombinant proteins bound IgE of horses suffering from IBH at frequencies varying between 19% and 57% and were able to induce immediate type skin reactions in IBH-affected but not in healthy control horses. These experiments demonstrate the allergenic nature of the cloned proteins both, *in vivo* and *in vitro*, and will serve as a basis for the development of standardized diagnostic tests and vaccines to improve the diagnosis and the allergen-specific immunotherapeutic treatment of IBH.

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