

## RESEARCH LETTER

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# Vesp v 5 and glycosylated Vesp v 1 are relevant allergens in *Vespa velutina nigrithorax* anaphylaxis

To the Editor,

*Vespa velutina nigrithorax* (VVN), commonly known as 'the yellow-legged Asian wasp', is one of the 12 colour variants of *Vespa velutina* (Lepeletier 1836).<sup>1</sup> Despite being originally from Asia, it has rapidly spread across France, reaching the Basque Country in the north of Spain and Galicia in the northwest of Spain in 2014 [as revised in<sup>2</sup>]. The presence of VVN in Great Britain was first confirmed in September 2016, and a mathematical model has predicted an invasion through the Atlantic and northern Mediterranean coasts.<sup>3</sup> *Vespa velutina* stings can provoke allergic reactions. In fact, *Vespa velutina* is the most prevalent cause of anaphylaxis in our region at the moment and almost 80% of our patients with anaphylaxis due to Hymenoptera stings are related to VVN.<sup>2</sup> The majority of these patients did not recall previous stings from this insect; however, all reported previous stings by either *Vespula* spp or *Apis mellifera*. Hence, sensitisation seems to have happened through exposure to these other species.<sup>2</sup> Recently, we have demonstrated that patients with anaphylaxis to VVN show specific IgE (slgE) against *Vespula* spp venom and its allergens, Ves v 5 and Ves v 1, and, to a lesser extent, against *Dolichovespula maculata*, *Polistes dominula*, *Vespa crabro* and *Apis mellifera* venoms.<sup>2</sup> In a small sample of patients ( $n = 15$ ), we have studied the allergenicity of VVN whole venom and its antigen 5 (nVesp v 5) and phospholipase (nVesp v 1) finding that slgE to nVesp v 5 was positive ( $>0.35$  kU<sub>A</sub>/L) in all of them while only 29% presented slgE to nVesp v 1.<sup>2</sup>

To further evaluate the allergenicity of nVesp v 5 (antigen 5 of VVN) and nVesp v 1 (phospholipase of VVN) and to investigate the role of *Vespula* spp venom as genuine sensitizer, we analysed slgE responses in 64 patients prospectively studied from September 2018 to December 2019 because of anaphylaxis to VVN. Forty-five patients attended the Allergy Department at a reference University Hospital in Santiago de Compostela, and 19 patients attended the Allergy Unit at the Hospital da Costa in Lugo, both in the northwest of Spain. All participants gave written informed consent for the study, which was approved by the Institutional Ethics Committee (code 2018/622). Nineteen patients from Santiago de Compostela were included in a previous publication<sup>2</sup> but patients with previous determination of slgE to VVN, nVesp v 1 or nVesp v 5 were not included in the present study. The median age was 56 years (range, 20–82 years), and 57 (89.1%) were men. Demographic and clinical characteristics of patients from Santiago de Compostela and Lugo were similar (data not shown).

In order to gain a better understanding of the sensitization profile of patients with VVN anaphylaxis, slgE to VVN whole-venom extract and to VVN phospholipase and antigen 5 (nVesp v 1 and nVesp v 5, respectively) were measured. For that purpose, a high-capacity immunosorbent CAP coupled to streptavidin (o212, Thermo Fisher Scientific) bounded at 15 µg protein/ml for VVN venom and 4 µg protein/ml for the single allergens was used as previously described.<sup>2,4</sup> Positive ( $>0.35$  kU<sub>A</sub>/L) slgE to VVN whole venom, nVesp v 5 and nVesp v 1, was found in 51 (79.7%), 55 (85.9%) and 26 (40.6%) of 64 patients, respectively. Levels of slgE to VVN whole venom (median 0.99 kU<sub>A</sub>/L, interquartile range [IQR] 0.38–2.08 kU<sub>A</sub>/L) tended to be lower than those of slgE to nVesp v 5 (median 1.34 kU<sub>A</sub>/L, IQR 0.57–3.05 kU<sub>A</sub>/L) but higher than those of slgE to nVesp v 1 (median 0.24 kU<sub>A</sub>/L, IQR 0.05–0.70 kU<sub>A</sub>/L). To analyse the degree of correlation between VVN allergens and *Vespula* spp allergens, slgE to *Vespula* spp, rVes v 1 and rVes v 5 was measured by means of commercially available assays (Thermo Fisher Scientific). Specific IgE to MUXF<sup>3</sup> (o214) as cross-reactive carbohydrate (CCD) marker was also measured. A significant correlation was found between slgE to VVN and slgE to *Vespula* spp (Spearman's Rho 0.781,  $P < .001$ ), between slgE to nVesp v 5 and slgE to rVes v 5 (Rho 0.780,  $P < .001$ ) and between slgE to nVesp v 1 and rVes v 1 (Rho 0.556,  $P < .001$ ) (Table S1). A strong correlation was also found between nVesp v 1 and the CCD marker (Rho 0.698,  $P < .001$ ) (Table S1). Figure 1 shows scatter plots of the relationships among VVN and its allergens and their correspondent counterparts in *Vespula* spp venom, and a scatter plot of the relationship between slgE to nVesp v 1 and slgE to the CCD marker.

To identify the genuine sensitizer in these patients, an inhibition assay of IgE reactivity to VVN whole venom, nVesp v 1 and nVesp v 5 by *Vespula* spp and the inverted inhibition of *Vespula* spp, Ves v 1 and Ves v 5 by VVN were performed in serum samples of five patients. Complete results of the inhibition assays with three different concentrations of the inhibitor (25, 50 and 100 µg/mL) in each case can be seen in Table S2. These showed a better performance of *Vespula* venom for inhibiting slgE binding to VVN, nVesp v 1 and nVesp v 5 (mean percentage of inhibition at the highest concentration of the inhibitor of 96.7%, 88.3% and 95.5%, respectively) than of VVN for *Vespula* spp, rVes v 1 and rVes v 5 (63.0%, 29.1% and 65.8%, respectively) (Table S2). The low inhibition activity of VVN over rVes v 1 could be due to the lack of glycosylation of the latter or the quantity of nVesp v 1 in the VVN extract, but these facts would deserve additional studies. Taken together, these results suggest that *Vespula*

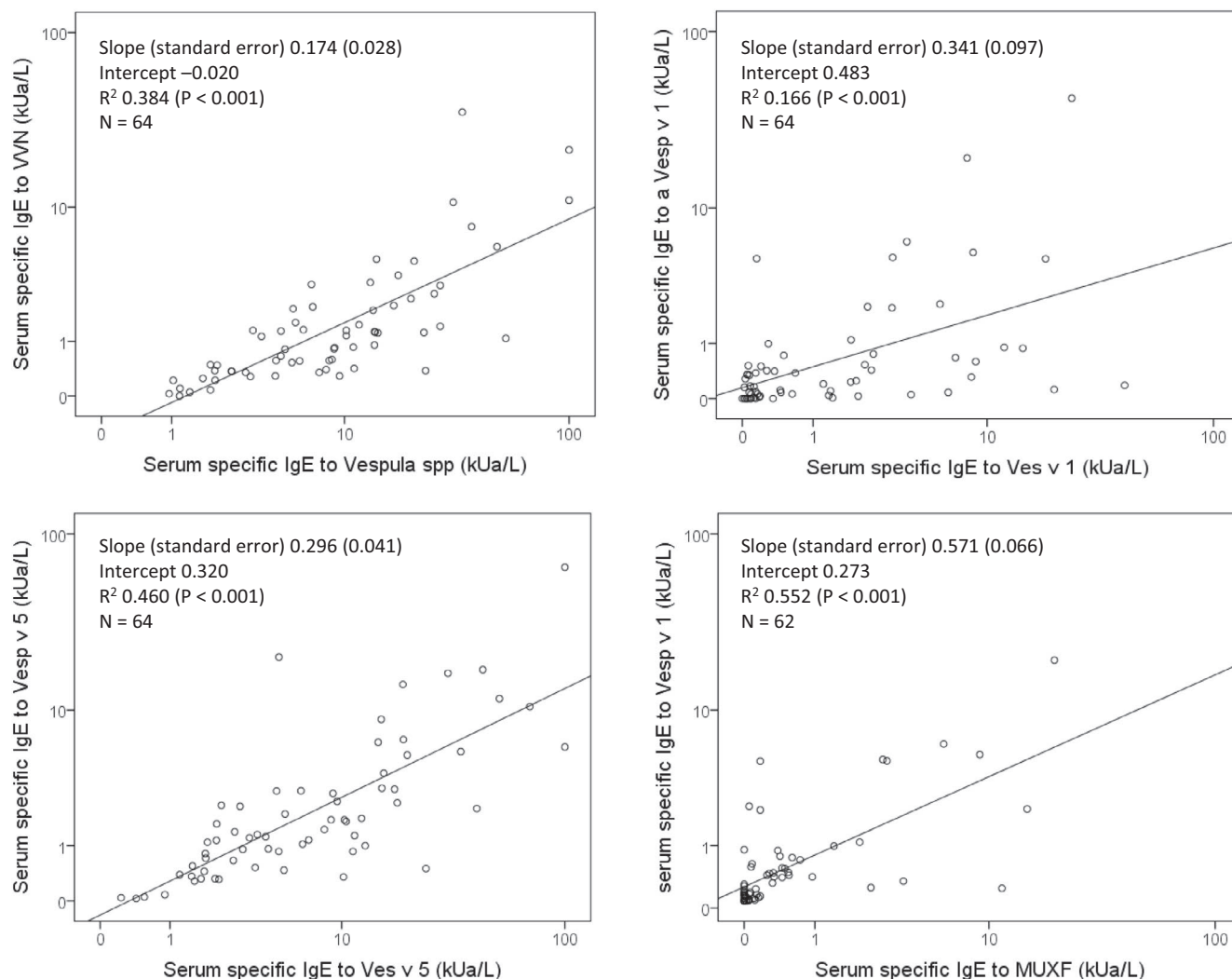


**FIGURE 1** Scatterplots representing the relationship of serum-specific IgE to *Vespa velutina nigrithorax* (VVN) venom, specific IgE to Ves v 5 vs Ves v 5, specific IgE to Ves v1 vs Ves v 1 and specific IgE to MUXF<sup>3</sup> (CCD marker, o214) vs Ves v 1. Linear regression models were developed; the corresponding lines and coefficients are represented. Note that axes were logarithmically transformed for representation

spp venom is the genuine sensitizer in these cases and support the idea that sensitisation could have occurred after having been stung by *Vespa* spp. Cross-reactivity among allergens from different vespids is well known, because genuine allergens from different species could be highly similar.<sup>5,6</sup> This fact, together with the lack of a commercially available VVN extract for allergen immunotherapy, led us to use *Vespa* spp venom for immunotherapy in patients with VVN anaphylaxis. This decision was also based on previous experiences in patients with anaphylaxis to *Vespa orientalis*<sup>7</sup> and patients with anaphylaxis to *Vespa crabro* who were successfully treated with *Vespa* spp extracts.<sup>8</sup> Even though we did not perform sting challenges with VVN because of the well-known toxicity of the venom,<sup>9</sup> 12 patients from Santiago de Compostela suffered new VVN stings after having started *Vespa* spp immunotherapy with no reaction (data not shown). Further studies are needed to evaluate the effect of *Vespa* spp immunotherapy on biological parameters, such as IgG4 evolution in patients with anaphylaxis to VVN.

In order to further characterize nVesp v 1 and to evaluate the possible interference of CCDs on its sIgE measurement in patients with VVN anaphylaxis, an additional CAP-inhibition assay with a CCD inhibitor (ProGlycAn CCD-Blocker -HÄMOSAN, Vienna, Austria-) at 40 µg/mL was performed in 5 representative patients with positive sIgE to nVesp v 1. In 3 patients with positive (>0.35 kU<sub>A</sub>/L) sIgE to nVesp v 1 and positive sIgE to CCD, the inhibitor showed extensive (≥90%) reduction of sIgE reactivity to nVesp v 1. In 2 patients with positive sIgE to nVesp v 1 and negative sIgE to CCD, the inhibitor induced no reduction of sIgE reactivity to nVesp v 1 (inhibition lower than 4%) (Table S3). The inhibition of sIgE reactivity by the CCDs points at the glycosylated nature of nVesp v 1, a feature that is not common among venom phospholipases. To verify its glycosylation, a specific stain of a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of nVesp v 5, nVesp v1 and nVes v1 (Novex-Tricine, Invitrogen, UK) with the Pierce Glycoprotein Staining Kit (Thermo

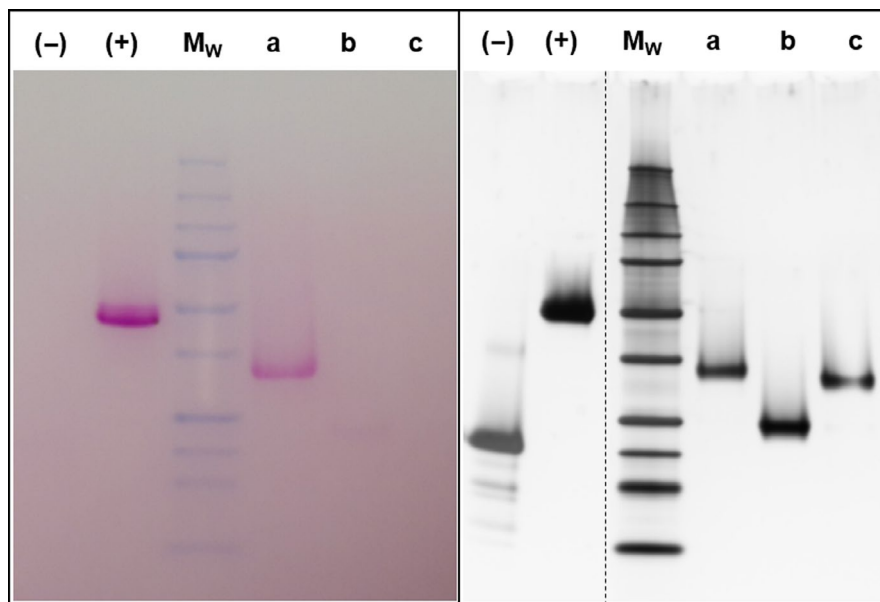


**FIGURE 2** SDS-PAGE (Novex-Tricine, Invitrogen, UK) stained by using the Pierce Glycoprotein Staining Kit (Thermo Fisher Scientific) was performed (left-coloured panel). According to the manufacturer's instructions, 5–10 µg of each protein must be applied in each lane of the SDS-PAGE, and magenta bands will indicate that the protein is glycosylated. The kit provides a 'positive control' (+), corresponding to horseradish peroxidase, as well as a 'negative control' (–) (soybean trypsin inhibitor). In parallel, an identical SDS-PAGE (with only 0.5 µg of each protein applied in each lane) was performed and silver-stained (black-and-white panel on the right). The magenta-coloured bands correspond to proteins that are glycosylated. The exact same proteins are shown in each SDS-PAGE, where 'a, b and c' correspond to (a) nVesp v 1, (b) nVesp v 5 and (c) nVes v 1 from *Vespula vulgaris*, respectively. Mw correspond to the protein markers (BioRad Precision-Plus Protein All Blue Standards) with molecular weights corresponding to 10, 15, 20, 25, 37, 50, 75, 100, 150 and 250 kDa, which are visible as light-blue bands in the left panel and clearly stained in the right panel. The dotted line shown in the right panel is used to indicate that the picture is a composition from the original SDS-PAGE, so that both panels show the same order of samples applied from left to right

Fisher Scientific) was performed (Figure 2), showing that nVesp v 1, but neither nVesp v 5 nor nVes v1, is glycosylated. Taken together, these results show, firstly, that Vesp v 1 do not reach the level to be considered a major allergen but may behave as a genuine allergen in some patients. Secondly, its glycosylated nature could represent a diagnostic problem. The contribution of such CCDs to clinical symptoms is unclear. It is generally assumed that sIgE to CCDs has no clinical relevance, but we have previously shown positive in vitro basophil activation tests by N-glycans.<sup>10</sup>

The study has some limitations. First, the identification of VVN was recalled by patients because samples of the insects were not available. However, the fact that almost all patients live or work in a rural environment where they are used to recognizing Hymenoptera decreases the risk of misidentification. Second, inhibition experiments were performed in a small number of patients. However, we did not use pooled sera, and results from individual serum samples are useful to identifying different patterns of reaction.

In conclusion, the present study demonstrates that: (a) nVesp v 1 is glycosylated and the possible interference of CCDs should be taken into account when measuring sIgE to nVesp v 1 or VVN whole venom containing nVesp v 1; (b) *Vespula* spp appears to be the genuine sensitizer in most VVN allergic patients; and (c)

nVesp v 5 is confirmed as a major allergen in patients with VVN anaphylaxis.

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#### CONFLICT OF INTEREST

Carmen Vidal: No conflicts of interest regarding this manuscript. Margarita Armisen: No conflicts of interest regarding this manuscript. Rafael Monsalve: ALK-Abelló employee. Jose Gómez-Rial: No conflicts of interest regarding this manuscript. Teresa González-Fernández: No conflicts of interest regarding this manuscript. Francisco Carballada: No conflicts of interest regarding this manuscript. Manuel Lombardero: ALK-Abelló employee. Arturo González-Quintela: No conflicts of interest regarding this manuscript.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## REFERENCES

1. Perrard A, Arca M, Rome Q, et al. Geographic variation of melanisation patterns in a hornet species: genetic differences, climatic pressures or aposematic constraints? *PLoS One*. 2014;9:e94162.
2. Vidal C, Armisen M, Monsalve R, et al. Anaphylaxis to *Vespa velutina nigrithorax*: pattern of sensitization for an emerging problem in Western countries. *J Invest Allergol Clin Immunol*. 2020. <https://doi.org/10.18176/jiaci.0474>. [Epub ahead of print].
3. Villemant C, Barbet-Massin M, Perrard A, et al. Predicting the invasion risk by the alien bee-hawking yellow-legged hornet *Vespa velutina nigrithorax* across Europe and other continents with niche models. *Biol Cons*. 2011;144:2142-2150.
4. Monsalve RI, Gutiérrez R, Hoof I, Lombardero M. Purification and molecular characterization of phospholipase, antigen 5 and hyaluronidases from the venom of the Asian hornet (*Vespa velutina*). *PLoS One*. 2020.
5. Monsalve RI, Vega A, Marques L, et al. Component-resolved diagnosis of vespid venom-allergic individuals: phospholipases and antigen 5s are necessary to identify *Vespula* or *Polistes* sensitization. *Allergy*. 2012;67:528-536.
6. Jakob T, Müller U, Helbling A, Spillner E. Component resolved diagnostics for hymenoptera venom allergy. *Curr Opin Allergy Clin Immunol*. 2017;17:363-372.
7. Goldberg A, Shefler I, Panasoff J, Paitan Y, Confino-Cohen R. Immunotherapy with commercial venoms is efficacious for anaphylactic reactions to *Vespa orientalis* stings. *Int Arch Allergy Immunol*. 2013;161:174-180.
8. Košnik M, Korošec P, Šilar M, Mušič E, Eren R. Wasp venom is appropriate for immunotherapy of patients with allergic reaction to the European hornet sting. *Croat Med J*. 2002;43:25-27.
9. Liu Z, Chen S, Zhou Y, et al. Deciphering the venom transcriptome of killer-wasp *Vespa velutina*. *Sci Rep*. 2015;5:9454.
10. Gonzalez-Quintela A, Gomez-Rial J, Valcarcel C, et al. Immunoglobulin-E reactivity to wine glycoproteins in heavy drinkers. *Alcohol*. 2011;45:113-122.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**Table S1. Correlation between specific IgE to *Vespa velutina nigrithorax* and *Vespula* spp venoms and their components.**

|                          |                | slgE to<br>nVesp v 1 | slgE to<br>nVesp v 5 | slgE to<br><i>Vespula</i> spp | slgE to<br>rVes v 1 | slgE to<br>rVes v 5 | slgE to<br>MUXF <sup>3</sup> |
|--------------------------|----------------|----------------------|----------------------|-------------------------------|---------------------|---------------------|------------------------------|
| <b>slgE to VVN</b>       | Spearman's Rho | 0.500                | 0.837                | 0.781                         | 0.382               | 0.650               | 0.275                        |
|                          | P-value        | <0.001               | <0.001               | <0.001                        | 0.002               | <0.001              | 0.031                        |
|                          | N              | 64                   | 64                   | 64                            | 64                  | 64                  | 62                           |
| <b>slgE to nVesp v 1</b> | Spearman's Rho |                      | 0.197                | 0.378                         | 0.556               | 0.160               | 0.698                        |
|                          | P-value        |                      | 0.118                | 0.002                         | <0.001              | 0.206               | <0.001                       |
|                          | N              |                      | 64                   | 64                            | 64                  | 64                  | 62                           |
| <b>slgE to nVesp v 5</b> | Spearman's Rho |                      |                      | 0.723                         | 0.133               | 0.780               | 0.077                        |
|                          | P-value        |                      |                      | <0.001                        | 0.295               | <0.001              | 0.550                        |
|                          | N              |                      |                      | 64                            | 64                  | 64                  | 62                           |

slgE, specific IgE; VVN, *Vespa velutina nigrithorax*; MUXF<sup>3</sup>, cross-reactive carbohydrate marker (o214).

**Table S2. Inhibition studies of specific IgE reactivity in five patients with *Vespa velutina nigrithorax* (VVN) anaphylaxis.**

| Patient | CAP-Inhibition with <i>Vespula</i> spp venom |                                |                                    |                                    |                                     | CAP-Inhibition with VVN venom |                                |                                    |                                    |                                     |
|---------|----------------------------------------------|--------------------------------|------------------------------------|------------------------------------|-------------------------------------|-------------------------------|--------------------------------|------------------------------------|------------------------------------|-------------------------------------|
|         | Allergen                                     | Baseline<br>kU <sub>A</sub> /L | 25 µg/mL<br>kU <sub>A</sub> /L (%) | 50 µg/mL<br>kU <sub>A</sub> /L (%) | 100 µg/mL<br>kU <sub>A</sub> /L (%) | Allergen                      | Baseline<br>kU <sub>A</sub> /L | 25 µg/mL<br>kU <sub>A</sub> /L (%) | 50 µg/mL<br>kU <sub>A</sub> /L (%) | 100 µg/mL<br>kU <sub>A</sub> /L (%) |
| #1      | VVN                                          | 5.20                           | 0.16 (96.9)                        | 0.07 (98.6)                        | 0.04 (99.2)                         | <i>Vespula</i> spp            | 15.6                           | 5.29 (66.0)                        | 4.72 (69.7)                        | 3.84 (75.3)                         |
|         | nVesp v 1                                    | 2.25                           | 0.15 (93.3)                        | 0.07 (96.8)                        | 0.05 (97.7)                         | rVes v 1                      | 8.72                           | 5.93 (31.9)                        | 5.86 (32.7)                        | 5.57 (36.1)                         |
|         | nVesp v 5                                    | 5.64                           | 0.30 (94.6)                        | 0.16 (97.1)                        | 0.12 (97.8)                         | rVes v 5                      | 8.51                           | 2.49 (70.7)                        | 2.16 (74.6)                        | 1.74 (79.5)                         |
| #2      | VVN                                          | 1.94                           | 1.21 (37.6)                        | 0.17 (91.2)                        | 0.11 (94.3)                         | <i>Vespula</i> spp            | 16.8                           | 7.23 (56.9)                        | 6.99 (58.3)                        | 5.33 (68.2)                         |
|         | nVesp v 1                                    | 0.01                           | ND                                 | ND                                 | ND                                  | rVes v 1                      | 0.02                           | ND                                 | ND                                 | ND                                  |
|         | nVesp v 5                                    | 8.11                           | 4.14 (48.5)                        | 0.75 (90.7)                        | 0.66 (91.8)                         | rVes v 5                      | 27.1                           | 12.0 (55.7)                        | 10.3 (61.9)                        | 9.12 (66.3)                         |
| #3      | VVN                                          | 1.58                           | 0.47 (70.2)                        | 0.20 (87.3)                        | 0.10 (93.6)                         | <i>Vespula</i> spp            | 5.44                           | 2.39 (56.0)                        | 2.21 (59.3)                        | 1.89 (34.7)                         |
|         | nVesp v 1                                    | 2.84                           | 1.36 (52.1)                        | 0.72 (74.6)                        | 0.37 (86.9)                         | rVes v 1                      | 1.13                           | 0.98 (13.2)                        | 0.99 (12.3)                        | 0.88 (22.1)                         |
|         | nVesp v 5                                    | 0.67                           | 0.15 (77.6)                        | 0.08 (88.0)                        | 0.05 (92.5)                         | rVes v 5                      | 1.32                           | 0.56 (57.5)                        | 0.55 (58.3)                        | 0.50 (62.1)                         |
| #4      | VVN                                          | 2.93                           | 0.74 (74.7)                        | 0.32 (89.1)                        | 0.23 (92.1)                         | <i>Vespula</i> spp            | 25.4                           | 14.1 (44.4)                        | 14.4 (43.3)                        | 14.5 (43.3)                         |
|         | nVesp v 1                                    | 1.83                           | 0.87 (52.4)                        | 0.51 (72.1)                        | 0.36 (80.3)                         | rVes v 1                      | 0.12                           | ND                                 | ND                                 | ND                                  |
|         | nVesp v 5                                    | 5.38                           | 1.04 (80.6)                        | 0.24 (95.5)                        | 0.22 (95.9)                         | rVes v 5                      | 37.1                           | 24.3 (34.5)                        | 24.5 (34.0)                        | 25.0 (32.6)                         |
| #5      | VVN                                          | 3.77                           | 0.02 (98.9)                        | 0.01 (98.9)                        | 0.02 (98.3)                         | <i>Vespula</i> spp            | 16.5                           | 5.95 (64.0)                        | 6.25 (62.1)                        | 6.12 (63.0)                         |
|         | nVesp v 1                                    | 0.02                           | ND                                 | ND                                 | ND                                  | rVes v 1                      | 0.04                           | ND                                 | ND                                 | ND                                  |
|         | nVesp v 5                                    | 6.47                           | 0.07 (99.4)                        | 0.07 (99.7)                        | 0.04 (98.4)                         | rVes v 5                      | 17.1                           | 7.69 (55.0)                        | 8.3 (51.4)                         | 7.9 (53.8)                          |

**Inhibition with *Vespula* spp venom.** Briefly, 100 µL of serum were preincubated with 100 µL of the phosphate-buffered saline *Vespula* spp solutions (25, 50, and 100 µL/mL). After overnight incubation at 4°C, CAP assays with customised VVN, nVesp v 1 and nVesp v 5 activated ImmunoCAPs were performed as usual.

**Inhibition with VVN venom.** Briefly, 100 µL of serum were preincubated with 100 µL of the phosphate-buffered saline VVN solutions (25, 50, and 100 µL/mL). After overnight incubation at 4°C, CAP assays with commercially available *Vespula* spp, rVes v 1 and rVes v 5 ImmunoCAPs were performed as usual.

Bovine serum albumin (BSA) was used as negative control in every case. The degree of inhibition was expressed as percentage with respect to the control value (1-sIgE after inhibition/sIgE before inhibition) X 100. Figures represent specific IgE reactivity and (percentage of inhibition at a given concentration of inhibitor). ND, Not Determined.

**Table S3. Results of CAP-inhibition assays of sIgE to nVesp v 1 with CCD inhibitor in five representative patients with a positive sIgE to nVesp v 1 and different levels of sIgE to the CCD marker (MUXF<sup>3</sup>).**

| Patient | Baseline sIgE (kU <sub>A</sub> /L) |           | CAP-inhibition with CCD-blocker (40 µg/mL) |
|---------|------------------------------------|-----------|--------------------------------------------|
|         | MUXF <sup>3</sup>                  | nVesp v 1 | nVesp v 1 kU <sub>A</sub> /L (%)           |
| #6      | 59.9                               | 27.12     | 0.82 (96.9)                                |
| #7      | 19.8                               | 19.6      | 0.15 (99.2)                                |
| #8      | 3.05                               | 4.81      | 0.07 (98.5)                                |
| #9      | 0.17                               | 4.79      | 4.62 (3.5)                                 |
| #10     | 0.17                               | 2.13      | 2.06 (3.2)                                 |

**Inhibition with CCD.** A neoglycoprotein consisting of a CCD (MUXF<sup>3</sup>) coupled to human albumin (ProGlycAn CCD-Blocker, HÄMOSAN, Vienna, Austria) was used as inhibitor. Briefly, 100 µL of serum were preincubated with 100 µL of the CCD-blocker solution (40 µg/mL). After overnight incubation at 4°C, CAP assays with customised nVesp v 1 were performed as usual. Bovine serum albumin was used as negative control. The degree of inhibition was expressed as percentage with respect to the control value (1-sIgE after inhibition/sIgE before inhibition) x 100. Figures represent specific IgE reactivity and (percentage of inhibition).