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## INTRODUCTION

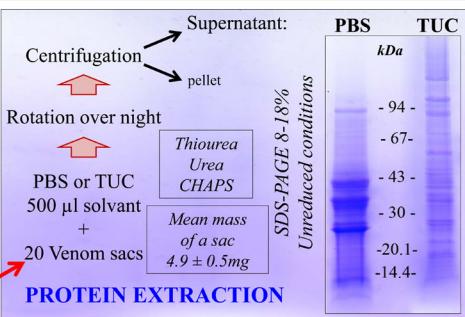
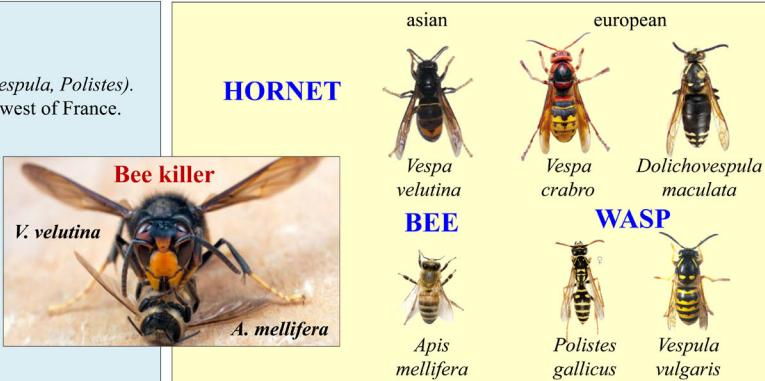
- Venoms of hymenoptera are responsible of 1/3 of anaphylactic shocks .
- 17 allergens described in the IUIS data bank (*Apis*, *Vespa*, *Vespula*, *Dolichovespula*, *Polistes*).
- In 2004, *Vespa velutina*, the yellow-leg asian hornet was introduced in south west of France.
- Now, present in more than 75% of the French territory and in expansion.
- Characteristics: bee-killers and nest close to human houses.
- risk factor for biodiversity and human health (anaphylactic shock).

## AIM

- To unravel the proteins and allergens repertoire of the venom of asian hornet using European sensitized patients' sera.

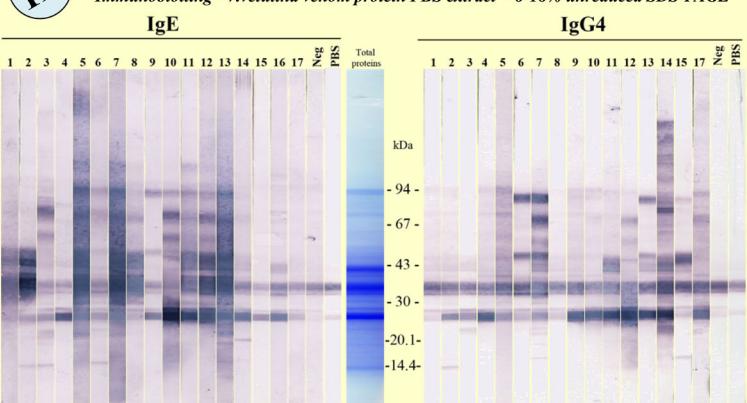
## METHOD

- Immunoproteomic study of venom proteins from asian hornet
- 1 and 2-D gel electrophoresis, Immunoblots, Mass spectrometry



## IMMUNOREACTIVITY OF 17 HYMENOPTERA VENOM ALLERGIC PATIENTS

Immunoblotting - *V. velutina* venom protein PBS extract - 8-18% unreduced SDS-PAGE      European hymenoptera venom IgE reactivities



patient	II	I3	I4	I75	i2	i5	i77
1	2.71*	13.8	10.1	1.4			
2	33.9	8.23					
3		38.6					
4	0.92	49.6					
5	51.7	25.3	2.59	22.7			
6	4.09	20.4	13.7	2.96			
7	59.3	15.3	1.75	18.5			
8	4.95	29.1	22	2.04			
9	<0.1	32.1	18.2	1.7			
10	<0.1	32	1.27	1.94			
11		6.56	0.8	5.54			
12	16.6	43.4	3.84	11.2			
13	33.4	19.8		18.5			
14		28.4		9.4			
15*	0.22‡	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
16	1.04	0.44			1.48		
17		0.56		0.23			
18	11.9	3.21		3.64			
19	18.6	>100	2.79	19.8			
20	84.8			59.7			

\*: kU/L

†: anaphylactic shock to *Vespa velutina*.

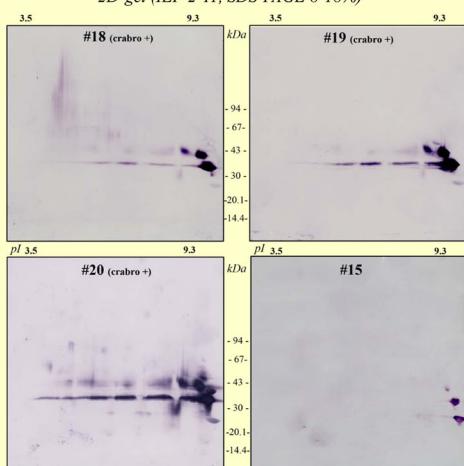
‡: ApIm <0.1

## IUIS ALLERGENS

PROTEIN NAME	kDa	pI
Vitellogenin	200	6.3-6.7
Allergen C / DPPIV	100	5.7-6.2
Carboxylesterase	70	9.33
MRJP8	65	6.53
MRJP9	60	8.7
Carboxypeptidase	60	6.65
CRP/icaripin	55	4.51
Acid phosphatase	49	5.63
Hyaluronidase	39-45	8.7-9.3
Serine protease	39	8.6
Phospholipase A1	34-38	8.3-9.0
Serine protease	31-33	6.7
Serine protease	27	8.9
Antigenic 5	23-26	8.8-9.2
Phospholipase A2	17	7.0
Protease inhibitor	8	9.8
Melittin	3	4.7

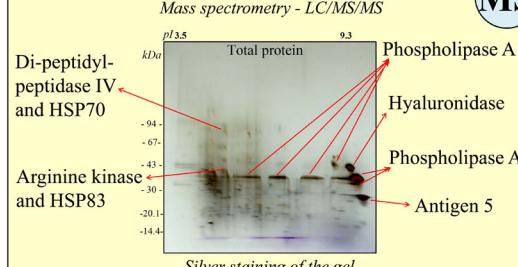
## 2D IgE MMUNOREACTIVITY OF 4 VESPA VENOM ALLERGIC PATIENTS

Immunoblot - *V. velutina* venom protein PBS extract - 2D-gel (IEF 2-11, SDS-PAGE 8-18%)



## IDENTIFIED ALLERGENS

Mass spectrometry - LC/MS/MS



## IDENTIFIED PROTEINS

Accession number	Protein name	Species
P0DAH4	Phospholipase A1	<i>Vespa affinis</i>
P0CH47	Phospholipase A1	<i>Vespa mandarinia</i>
P0CH48	Phospholipase A1	<i>Vespa crabro</i>
P0DAH5	Thioesterase A1 (venomous fragments)	<i>Vespa velutina</i>
P53357	Phospholipase A1	<i>Dolichovespula maculata</i>
Q9Q252	Phospholipase A1	<i>Polistes dominula</i>
Q9Q252	Phospholipase A1	<i>Polistes annularis</i>
A2V1C4	Phospholipase A1	<i>Polybia paulista</i>
P01657	Antigen 2 (Orientotocin)	<i>Vespa orientalis</i>
P08870	Antigen 5	<i>Vespa mandarinia</i>
P357812	Antigen 5	<i>Vespa crabro</i>
P0DAH9	Antigen 5	<i>Vespa velutina</i>
Q9H3R6	Arginine kinase	<i>Vespa velutina</i>
B1A487	Dipeptidyl-peptidase 4	<i>Vespa velutina</i>
O61367	Arginine kinase	<i>Apis mellifera</i>
Q9U639	HSP 70 cognate	<i>Mandaca sexta</i>
Q9HL55	HSP 83	<i>Bombyx mori</i>
Q9H3R6	Arginine kinase/arginine phosphate dehydrogenase [NAD(+)]	<i>Vespa velutina</i>
P29110	14-3-3 protein eta	<i>Aedes aegypti</i>
Q9V3P0	14-3-3 protein eta	<i>Drosophila melanogaster</i>
P0CV91	Peroxiredoxin-1	<i>Drosophila melanogaster</i>
	Peroxiredoxin-4	<i>Crotalus atrox</i>

## CONCLUSION

- Resection of venom sac from captured and frozen *Vespa velutina* gives rise to protein rich extracts either in aqueous solvant or in detergent.
- 1-D immunoblotting of separated proteins from PBS extracts with sera from European hymenoptera allergic patients revealed several bands of IgE as well as IgG4 reactivities.
- 2-D electrophoresis followed by mass spectrometry analysis of spotted proteins corresponding to allergens, showed that, at least, phospholipase A1, hyaluronidase, antigen 5 and dipeptidyl peptidase IV from *Vespa velutina* venom are homologous enough to European hymenoptera venom components to be cross-reactive. European hymenoptera sensitized patients are at risk upon *Vespa velutina* sting.
- Whether *Vespa velutina* venom comprises unique allergen(s) is still under investigation.