PROFILING CROSS-SPECIES HUMORAL IMMUNE RESPONSES TO ARBOVIRUSES BY PROTEIN MICROARRAY

Natalie Cleton [1 2], Chantal Reusken [1], Johan Reimerink [2], Gert-Jan Godeke [2], Kees van Maanen [3], Jeroen Kortekaas [4], Richard Bowen [5], Marion Koopmans [1 2]

(1) Department of Viroscience, Erasmus MC, Rotterdam, the Netherlands; (2) Department of Virology, RIVM, Bilthoven, the Netherlands; (3) Animal Health Services, Deventer, The Netherlands; (4) Central Veterinary Institute, Lelystad, The Netherlands; (5) Colorado State University, Department of Biomedical Science and Veterinary Medicine, Fort Collins, CO, USA

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Background

- Arthropod borne viruses are transmitted by vectors and are sustained in a complex, often zoonotic transmission cycle between mammalian hosts and arthropod vectors.
- They can cause disease in humans and animals ranging from rash and incapacitating arthralgia to life threatening haemorrhagic fever and encephalitis.
- Diagnosis is based predominantly on serology, as viremia is often short-lived.
- Arboviruses co-circulate, and therefore, persons and animals can be exposed to multiple arboviruses simultaneously.

Objectives

- To develop novel strategies to identify emerging arboviral threats by developing and combining laboratory-based tools and epidemiological models that can be used for risk and exposure profiling.

Method

- We developed a cross-species protein microarray for humoral immune response profiling of antibodies to flaviviruses, alphaviruses and phleboviruses.
- Target antigens NS1 protein for flaviviruses, E1 and E2 for alphaviruses and glycoprotein n-terminus for bunyaviruses were selected, produced and spotted onto nitrocellulose pads using a PerkinElmer non-contact protein array spotter (table 1).

Table 1: *Viruses for which antigens were selected , spotted on protein microarrays and tested.*

Genus	Serogroup	Virus	Abbreviation	Species pos. serum
Alphavirus	Semliki Forest Virus	chikungunya virus	CHIKV	Human
		mayaro virus	MAYV	-
Bunyavirus	phlebovirus	Rift Valley fever virus	RVFV	Sheep
Flavivirus	dengue virus	dengue virus 1	DENV1	Human
		dengue virus 2	DENV2	Human
		dengue virus 3	DENV3	Human
		dengue virus 4	DENV4	Human
	Japanese encephalitis virus	Japanese encephalitis virus	JEV	Human, horse, birds
		West Nile virus	WNV	Human, horse, chicken
		usutu virus	USUV	Human, rabbit
		St. Louis encephalitis virus	SLEV	Human
	yellow fever virus	yellow fever virus	YFV	Human, monkey
	spondweni virus	Zika virus	ZIKV	-
	tick-borne encephalitis virus	tick-borne encephalitis virus	TBEV	Human

- Serum samples from humans, horses and sheep with virologically and/or serologically confirmed arboviral infections and control sera of non-exposed individuals were incubated in serial 2-fold dilutions followed by incubation with a species specific IgG and IgM Cy5-labeled conjugate.
- After quantifying signals using a Tecan scanner, data were analyzed in ‘R’.

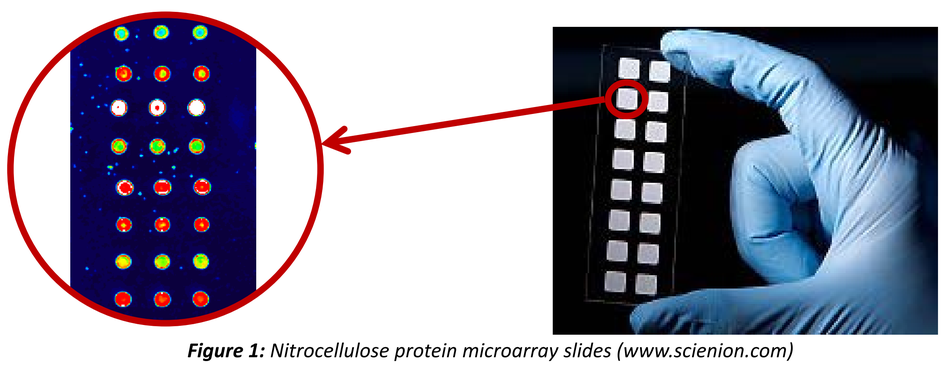
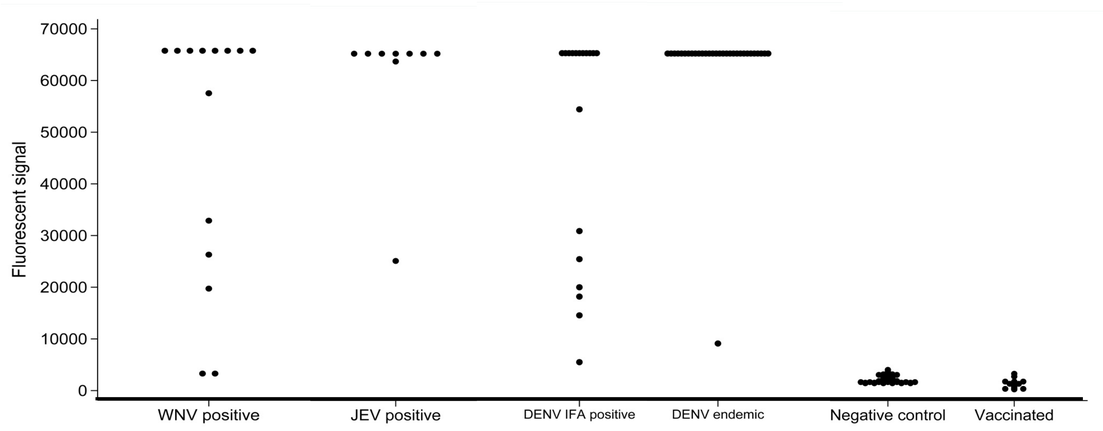


Figure 1: *Nitrocellulose protein microarray slides (www.scienion.com)*

Results

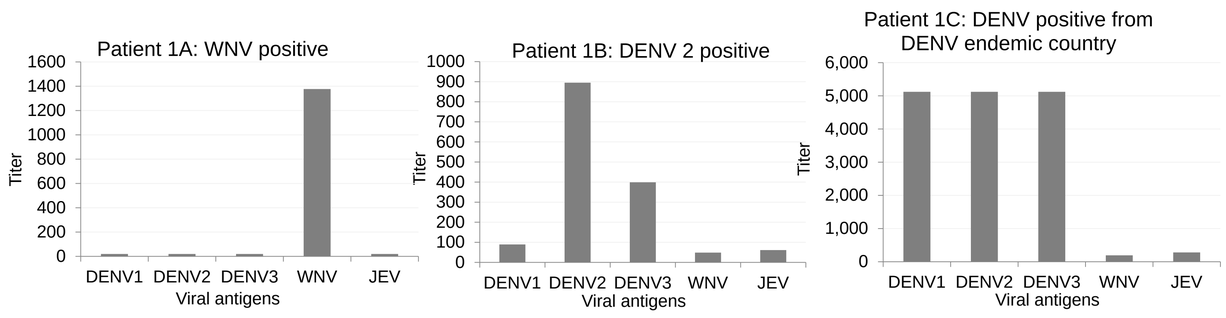
- Profiling of antibodies in human patients exposed to flaviviruses showed highly discriminatory patterns of reactivity to their corresponding flavivirus antigens with sensitivities and specificities ranging from 87%-100 (P<0,01) (figure 2).
- YFV, JEV and TBEV vaccinated individuals produced no titers to all flavivirus antigens comparable to negative controls and therefore could be distinguished from non-vaccinated individuals that had acquired a flavivirus infection (figure 2).
- The CHIKV antigens had a sensitivity and specificity of 100% for serological diagnosis of CHIKV infected patients, with no cross reactivity when testing serum from closely related sindbis virus infected patients.

Figure 2: *A dot distribution graph displaying the distribution of the fluorescent signal strength (y axis) of confirmed positive serum samples and negative and vaccinated controls by individual virus (x axis) for IgG positive serum samples in a 1:20 dilution*



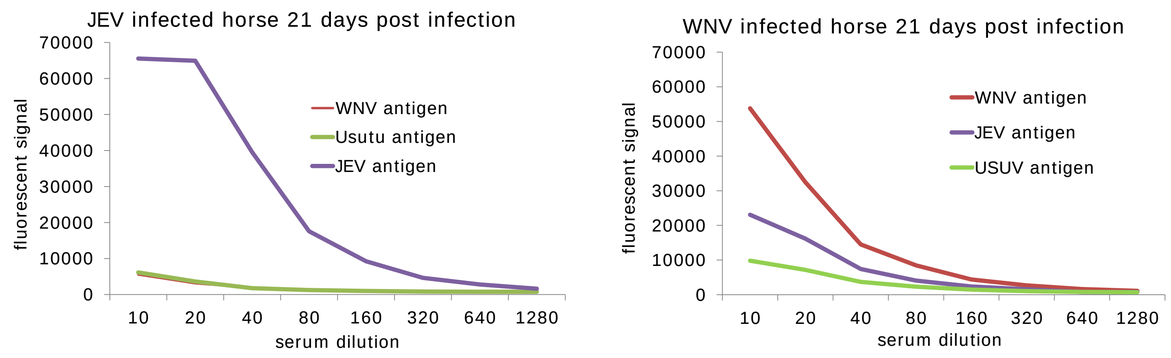
WNV positive: Patients with VNT and ELISA confirmed WNV infections
JEV positive: Patients with VNT and ELISA confirmed JEV infections
DENV IFA positive: Patients with only DENV IFA positive infections
DENV endemic: Patients with PCR and ELISA confirmed DENV infections from DENV endemic countries
Negative controls: Blood donation patients with no WNV, DENV and TBEV infections confirmed by IFA and ELSA
Vaccinated: Patients vaccinated against YFV or JEV with VNT and IFA confirmed titers.

Figure 3: *Bar graphs representing patients’ antibody responses in titers towards one specific virus (patient A and B) or multiple viruses (patient C)*



- Initial results showed high sensitivity and specificity of RVFV serology for sheep, and JEV serology for horses. WNV infected horses produced cross-reactive antibodies to JEV and USUV antigens on the protein microarray, but the highest titer was produced by the WNV antigens (figure 4).

Figure 4: *Serial dilutions of serum from JEV and WNV infected horses displaying a specific response to JEV antigens and a more cross-reactive response to WNV antigens.*



Conclusion

The first generation protein microarray can be used to determine exposure to flaviviruses, alphaviruses and bunayvirus in humans and animals.

Erasmus MC Universitair Medisch Centrum Rotterdam
GGD
Rijksinstituut voor Volksgezondheid en Milieu
Ministerie van Volksgezondheid, Welzijn en Sport
Colorado State University
CVI (LELYSTAD) WAGENINGEN UR