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Development of new assays for diagnosis of West Nile virus

Prj: 761

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Development of rapid and reliable West Nile virus antibody assays based on pseudotyped virus

Research priority: Improved prevention of current and emerging infectious diseases by the development of more effective vaccines, diagnostic tools, biosecurity and management strategies

Postdoctoral researcher: Dr Barnabas King

West Nile virus (1)

- West Nile virus (WNV) is transmitted by mosquitoes
- The virus cycles between mosquitoes and wild birds
- Horses (and people) are a 'dead-end' host; they cannot pass the virus on

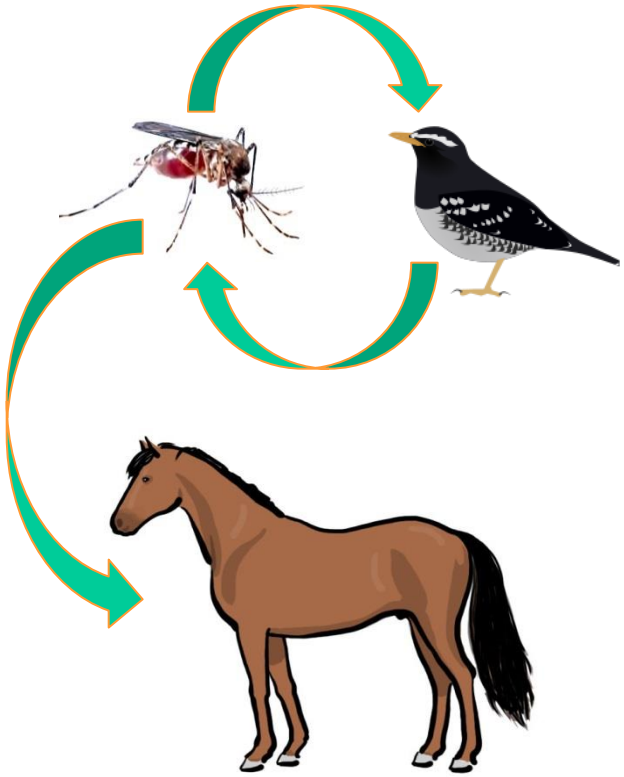


Figure: J Daly

West Nile virus (2)

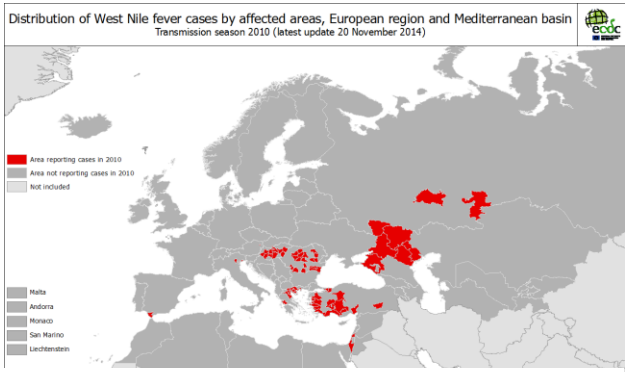
- Infection is usually mild **but**, in around 20% of cases, inflammation of the brain results in neurological damage, which may be permanent, and even fatal

Horse with weakness / paralysis of hind limbs due to WNV infection

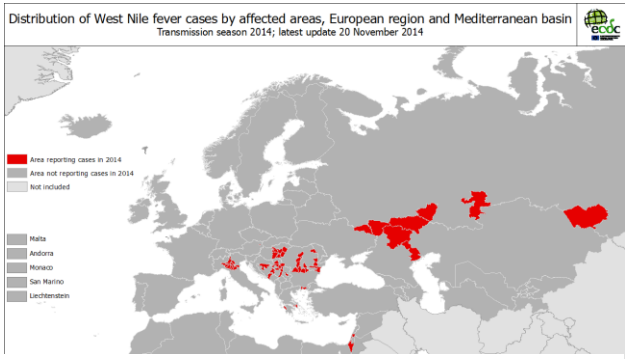
Photograph: http://arthurveterinaryclinic.blogspot.co.uk/

Relevance to the thoroughbred

- There is a constant risk that WNV could spread to the UK from America or Continental Europe
- Thoroughbred horses travelling overseas are at risk of being bitten by infected mosquitoes



Human WNV cases in 2010



Human WNV cases in 2014

Figures: ECDC
http://ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever-maps/Pages/historical-data.aspx

Diagnosis of WNV

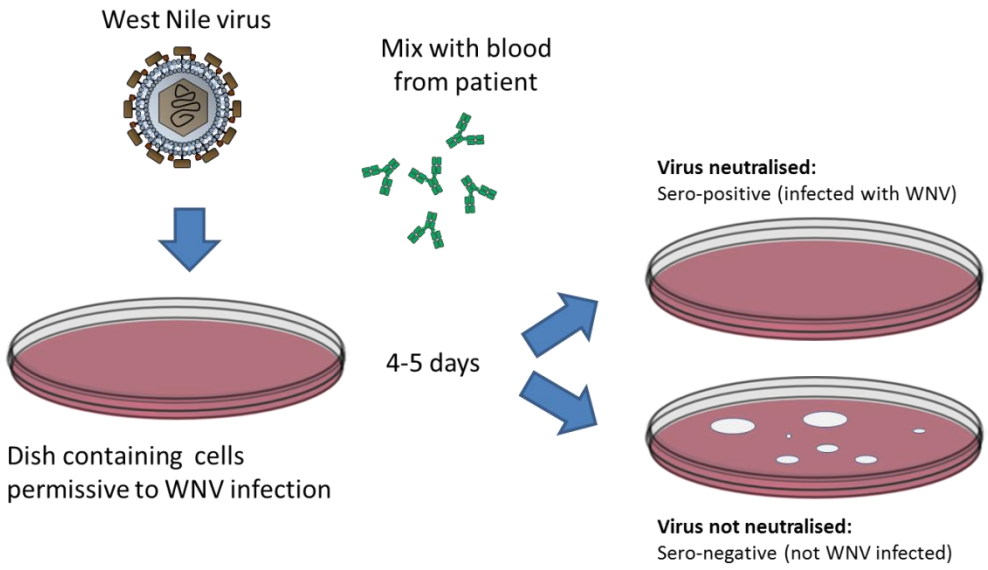
- Narrow window of opportunity to detect virus in horse's bloodstream means that diagnosis relies on detection of antibodies
- Initial diagnosis can be made by ELISA (rapid & relatively cheap)
- However, antibodies to related viruses that can also cause neurological signs in horses can cross-react in ELISA
- Therefore, a positive ELISA test should be confirmed by a virus neutralisation test (PRNT)

Plaque reduction neutralisation test (PRNT)

Live WNV is mixed with blood from patient

If no antibodies are present, the virus infects the cells, causing damage (plaques)

If antibodies to the surface (E) protein are present, they bind to it and neutralise the virus, preventing it from entering the cells and the number of plaques is reduced



Disadvantages

- Takes several days to obtain a result
- The plaques are counted by eye
- The live virus is dangerous to people as well as horses and therefore has to be handled in a specialised laboratory with a high level of biosafety containment (BSL-3)
- As a result, the test is very expensive to perform

Figure: B King

Project aims and objectives

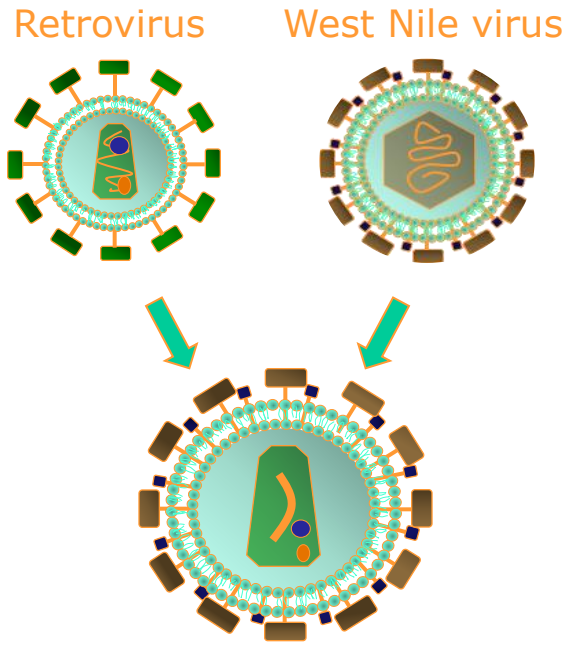
The aim of the project was to develop a pseudotyped virus assay for detection of antibodies to West Nile virus

Objective 1: Generate a West Nile virus prME pseudotyped virus (WNVprME PV)

Objective 2: Develop and validate WNV pseudotype neutralisation assay (PNA) and rapid entry assay

What makes a pseudotyped virus?

- A backbone virus that has been modified so that it does not cause disease and a key part of its genetic material is replaced by a reporter gene
- The surface envelope protein (prME or E) from the virus of interest (WNV)



WNV pseudotyped virus

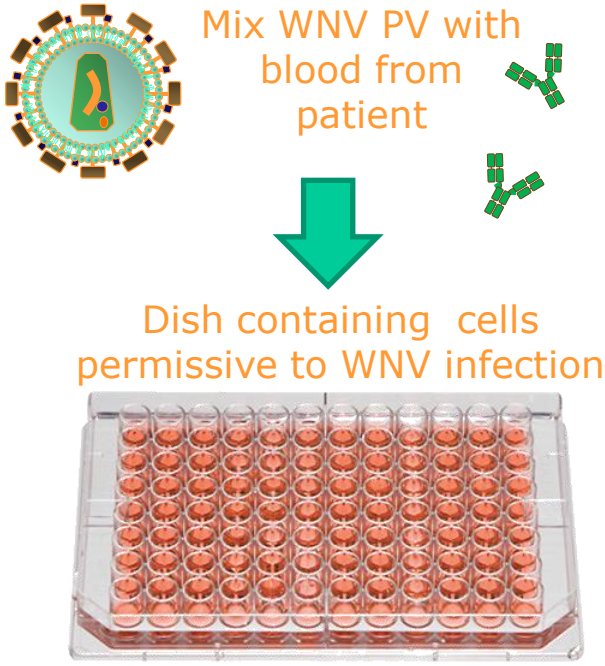
Figure: B King / J Daly

WNV pseudotyped virus neutralisation assay

WNV PV is mixed with blood from patient

If no antibodies are present, the PV enters the cells and the reporter gene is expressed, giving a signal (e.g. luminescence or fluorescence) that can be detected by a machine

If antibodies to the WNV E protein are present, the PV is neutralised and the reporter gene signal is reduced



Advantages

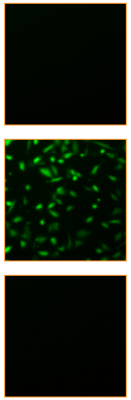
- Results obtained in 48 hours or less
- The WNV PV is safe to handle at lower levels of containment (BSL-1)
- As a result, the test is less expensive to perform
- The readout of the assay can be done by a machine enabling high throughput

Figure: B King / J Daly

Main results and conclusions (1)

- Attempts to generate WNV PVs by standard methods not successful

HIV backbone with green fluorescent protein reporter gene



Negative control

Positive control

WNV PV

MuLV backbone with luciferase reporter gene

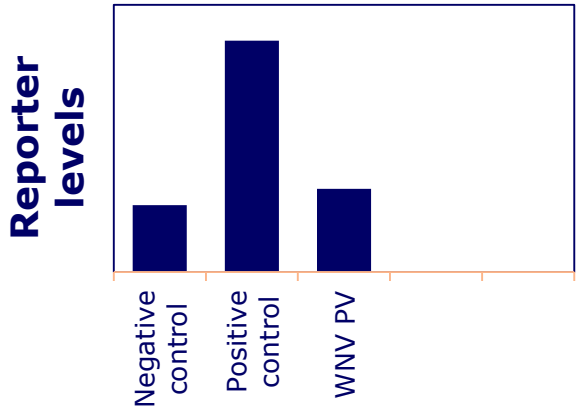


Figure: B King / J Daly

Main results and conclusions (2)

- Lots of tweaks were tried to make the system work

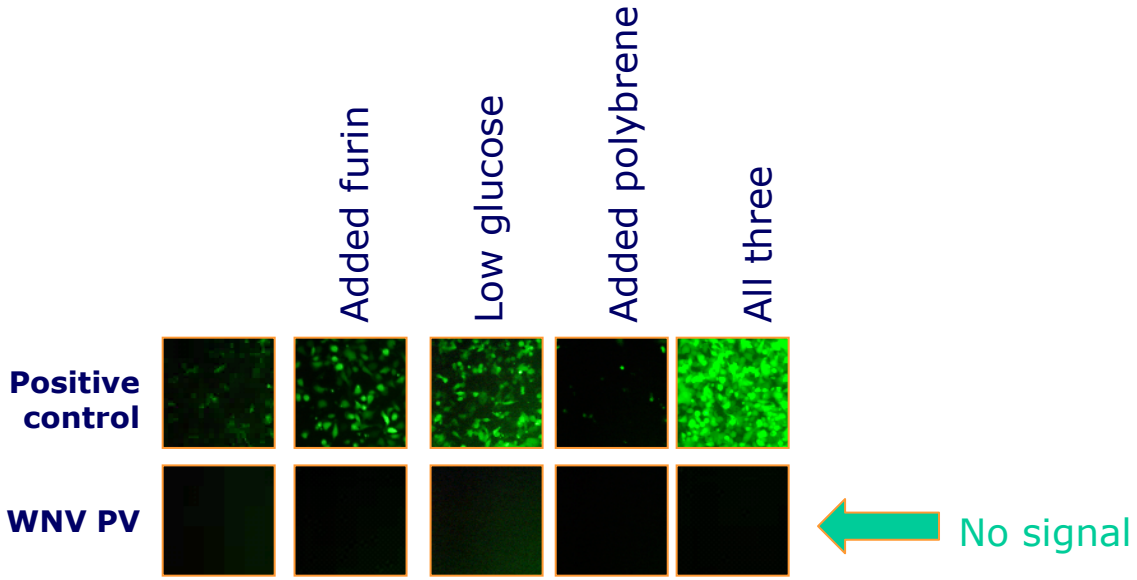
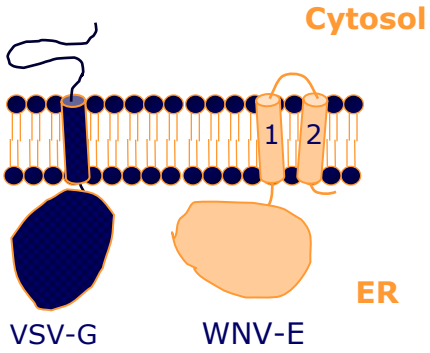


Figure: B King / J Daly

Main results and conclusions (3)



The envelope protein of WNV was fused with parts of the envelope protein used as a positive control (VSV-G) in an attempt to confer the ability of the positive control envelope protein to form pseudotyped viruses on WNV – without success

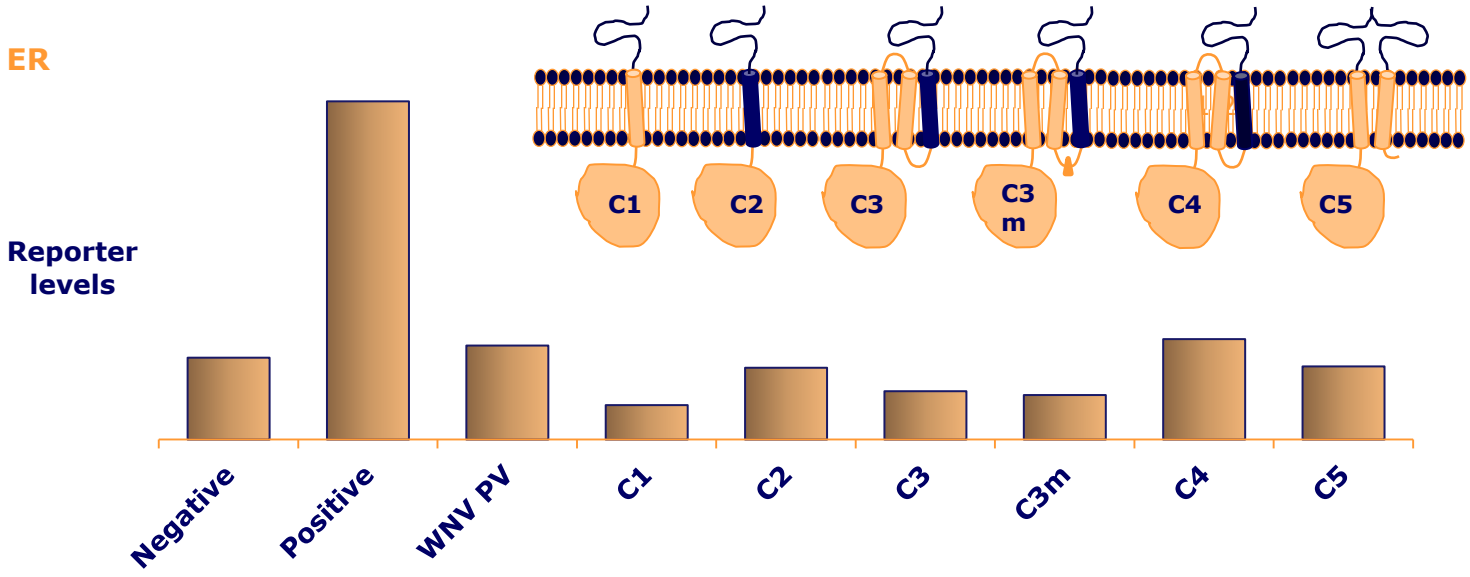


Figure: B King / J Daly

Main results and conclusions (4)

- It was concluded that the standard approach could not be applied to develop WNV PVs
- An alternative approach was therefore adopted
- Before work could commence, approval that the approach was safe had to be obtained from a national committee on genetic manipulation
- This was obtained with insufficient time left to do more than acquire and generate stocks of all the components required

Impact on the thoroughbred

- When the PV neutralisation assay is established, it will allow more rapid and cheaper screening for antibodies against WNV
- This will improve surveillance for WNV infection in horses and allow responses to vaccination against WNV to be checked

Potential next steps

- The project is ongoing to finalise development of the PV neutralisation assay with funding from the University of Nottingham (Vice-Chancellor's Scholarship for Research Excellence)