Emerging arthropod-borne Orthoflavivirus: Tembusu Virus

Amber Martyn [1,3] *, Denise Marston [2], Giovanni Lo Iacono [3], Marine Petite [4], Nicholas Johnson[1]

[1]Vector borne diseases research group, Animal and Plant Health Agency

[2]APH Science Strategy And Planning, Animal and Plant Health Agency

[3] University of Surrey, School of Veterinary Medicine and Science

[4] University of Surrey, School of Bioscience

*Corresponding author

*Amber.martyn@apha.gov.uk or Amber.martyn@surrey.ac.uk

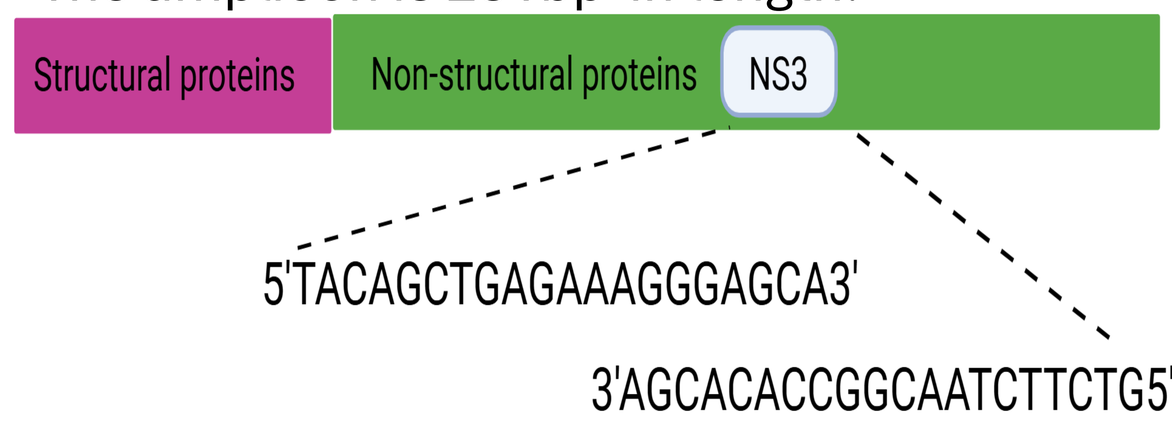
Arthropod-borne Orthoflaviviruses are an emerging threat to the UK. Tembusu Virus is an emerging arthropod-borne Orthoflavivirus first identified in Malaysia in 1955 which has remained largely undetected until 2010. In recent years it has spread dramatically through Malaysia, Thailand, China and Taiwan causing devastating disease in the duck farming economy. The virus is epornitic with the transmission cycle between avian species and *Culex tritaeniorhynchus*. The zoonotic potential of this virus is uncertain [1-2].

Aims and Objectives:

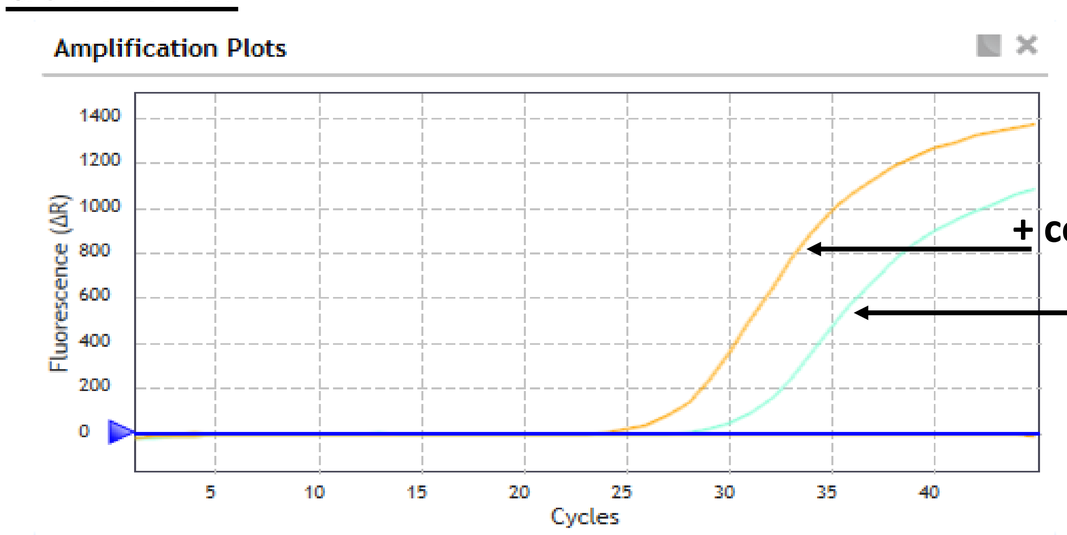
- Use established Pan-flaviviral RT-PCR to confirm presence of TMUV RNA in virus stock.
- Design specific primers to enable rapid and differential diagnosis of TMUV.
- Optimise PCR conditions to ensure sensitivity and specificity.

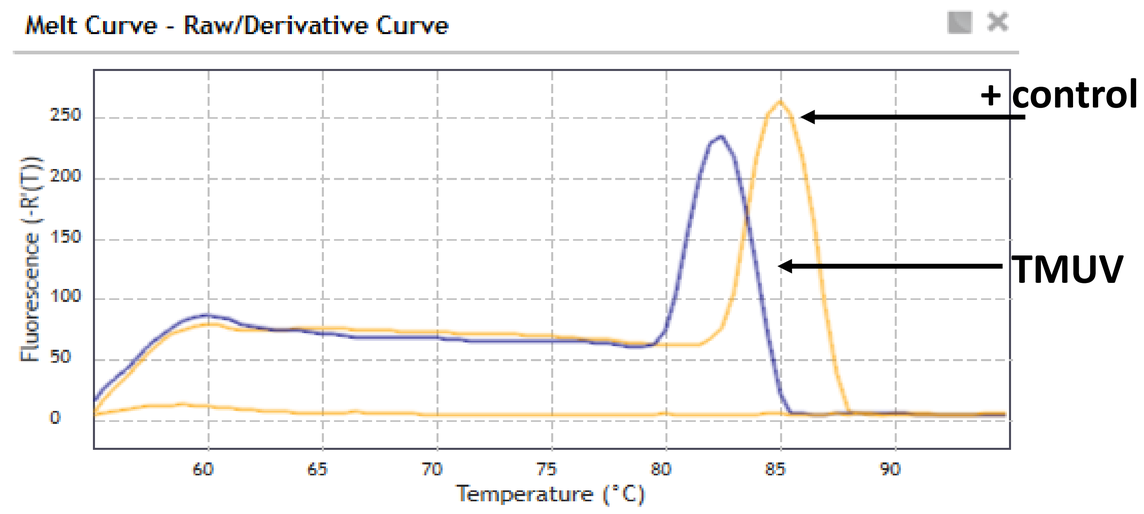
Primer Design:

- TMUV primer pair designed for the NS3 region of the genome.
- The amplicon is 231bp in length.



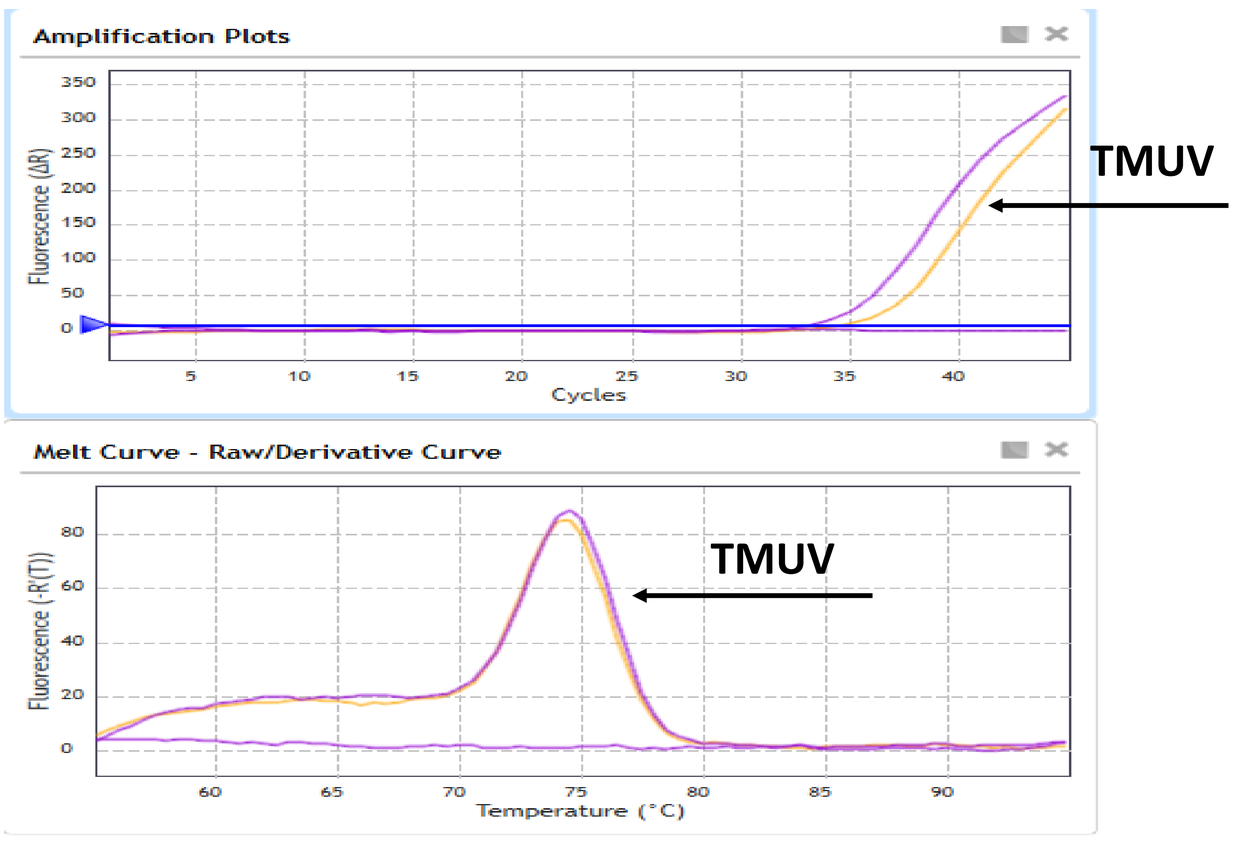
Pan-Flavivirus SYBR GREEN PCR using Pan-Flavivirus primers from validated tests used at APHA.





Results indicate, TMUV RNA was detected producing an amplification curve and specific melting temperature of 82.50°c. The positive control is differentiated by the melting temperature of 85°c.

SYBR Green PCR using primers designed in the NS3 region of the TMUV genome:



Results indicate that the primers designed in the NS3 region of the genome have amplified TMUV RNA with a good amplification curve and specific melting temperature of 74.5°c.

Future plans for the PCR are to investigate growth and dissemination of TMUV in cell culture and vector competency studies.

Conclusions:

- The validated Pan-flaviviral RT-PCR successfully amplified TMUV in the virus stocks confirming presence of a flavivirus.
- The NS3 TMUV specific PCR has yielded specific amplification confirming presence of a flavivirus.

References:

[1] Hamel, R., et al. Identification of the Tembusu Virus in Mosquitoes in Northern Thailand. Viruses **15** (7) (2023).

[2] Yan, L.. *et al.* Establishing a TaqMan-Based Real-Time PCR Assay for the rapid detection and quantification of the newly emerged duck Tembusu virus. *Virol J* **8**, 464 (2011)