

DO HONEY BEES DIE OVER WINTER FROM THE FLU?

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FACTORS AFFECTING BEE HEALTH

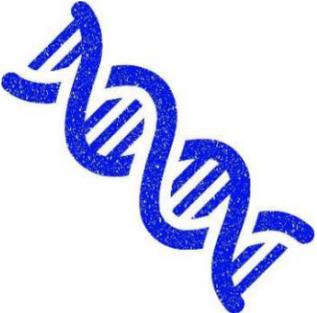
Weather



Nutrition & Habitat



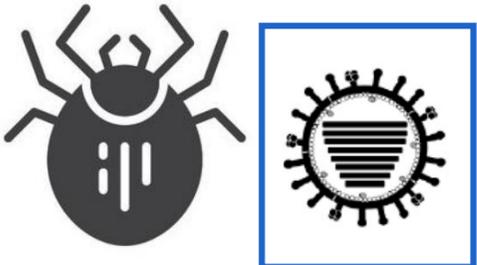
Genetic Factors



Beekeeping Practices



Viruses



Pest & Diseases



Agronomic Practices

VIRUSES IN BEES



300 colonies



Pooling
Status
Location
Subspecies

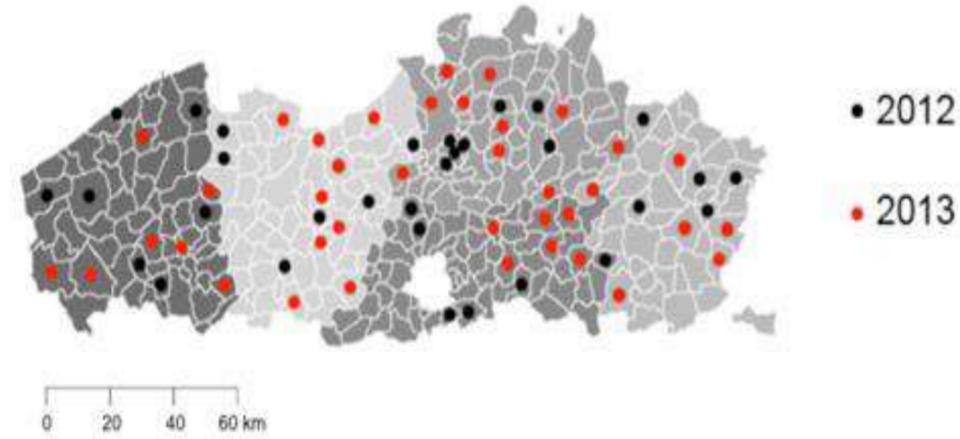


Homogenisation (beads, tissue homogeniser)

100 pools total

NetoVIR

Conceição Neto *et al.* 2015



NETOVIR

A. Selected bees, from healthy or diseased hives.



B. Homogenous suspension of bees, showing only bacteria (grey), viruses (different colors) and free floating nucleic acids (black lines).



Sample homogenization

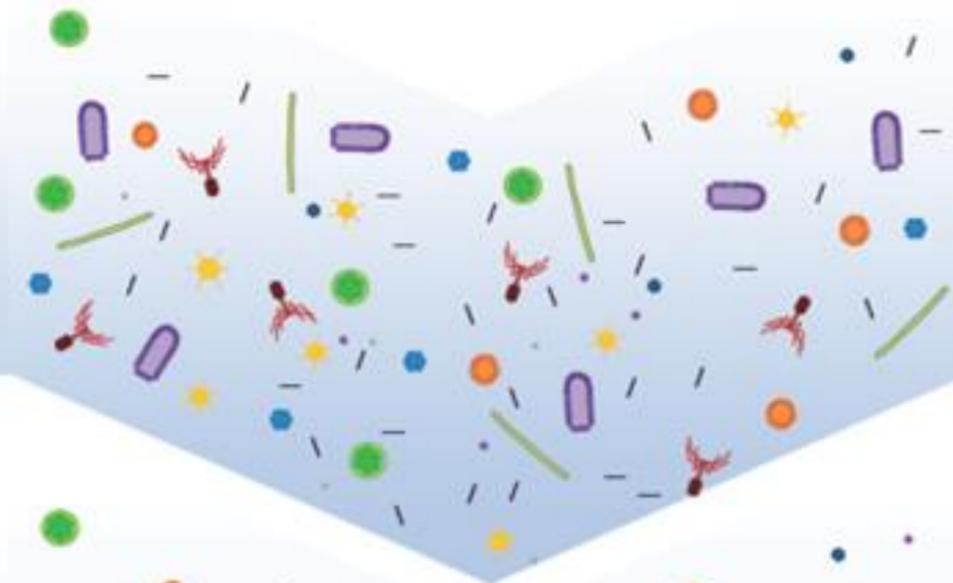
- Mixing of 1-2 bees with 1 ml of PBS and 2.8 ceramic beads
- MINILYS homogenizer for 1 min at 4000rpm

Centrifugation and filtration

- Centrifugation: 17000g for 3min
- 0.8µm centrifugal filter (PES)

NETOVIR

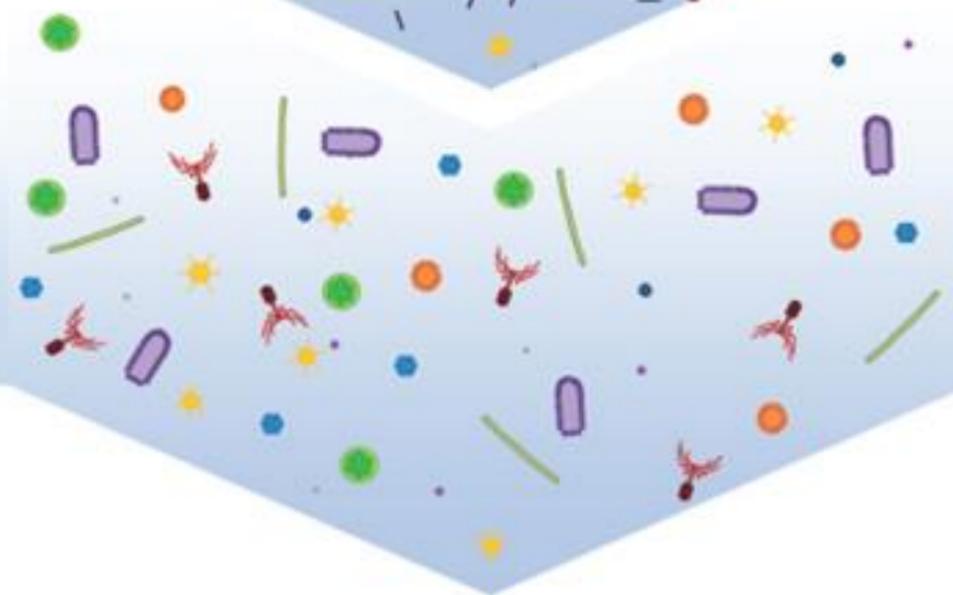
C. Sample after removal of pro- and eukaryotic organisms, ribosomes, and large organic debris.



Nuclease treatment

- Benzonase
- Micrococcal nuclease

D. Sample after removal of free floating nucleic acids.

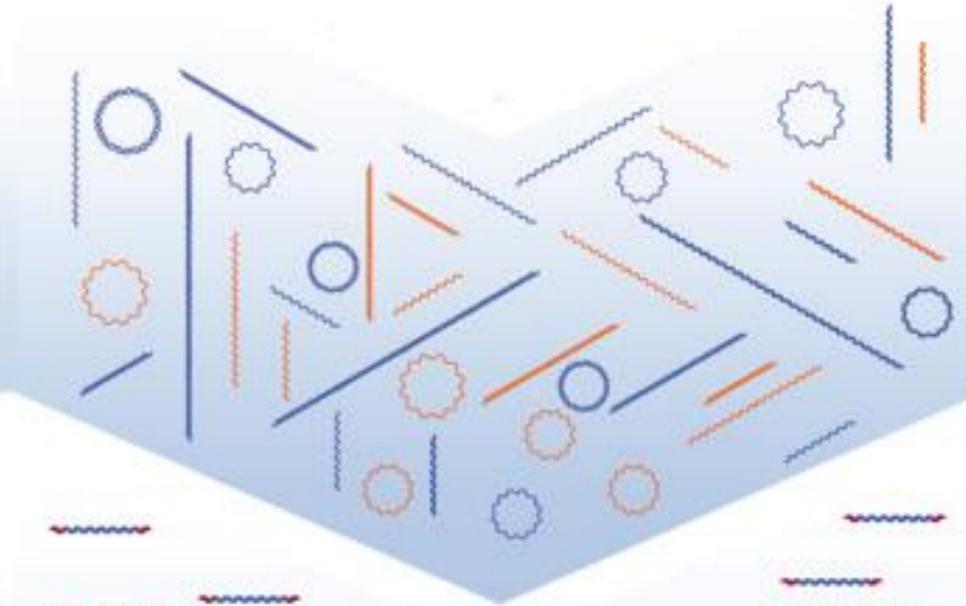


DNA/RNA extraction

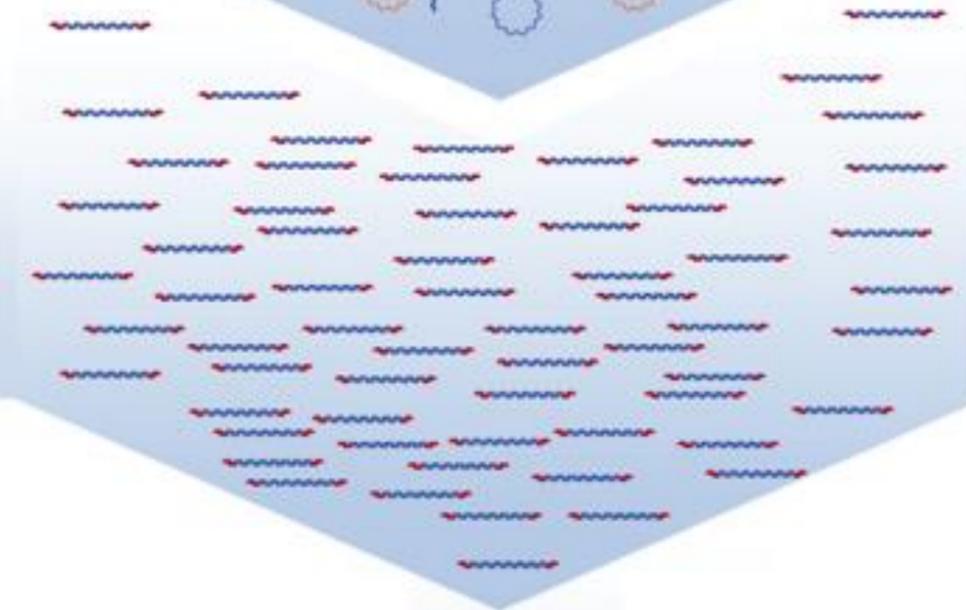
- Modified QIAamp® Viral RNA Mini kit

NETOVIR

E. Sample after extraction of various types of viral genomes composed of single and double stranded RNA (orange) and DNA (blue).



F. Sample after random amplification and library preparation of viral genomes.



Random amplification and library preparation

- Modified version of WTA2
- Modified version of Nextera XT DNA sample preparation kit

Deep sequencing

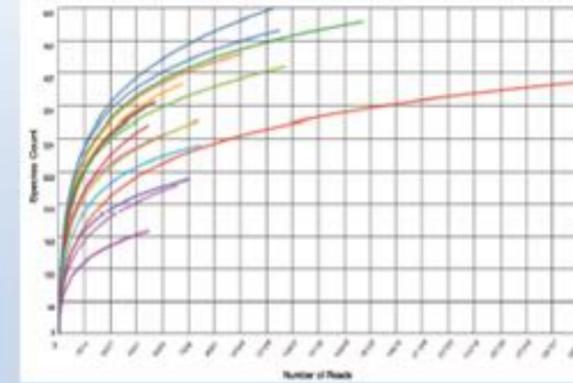
- Illumina HiSeq4000

Processing of raw sequence data

- Quality trimming
- *de novo* assembly of viral reads
- Annotation of viral reads and contigs

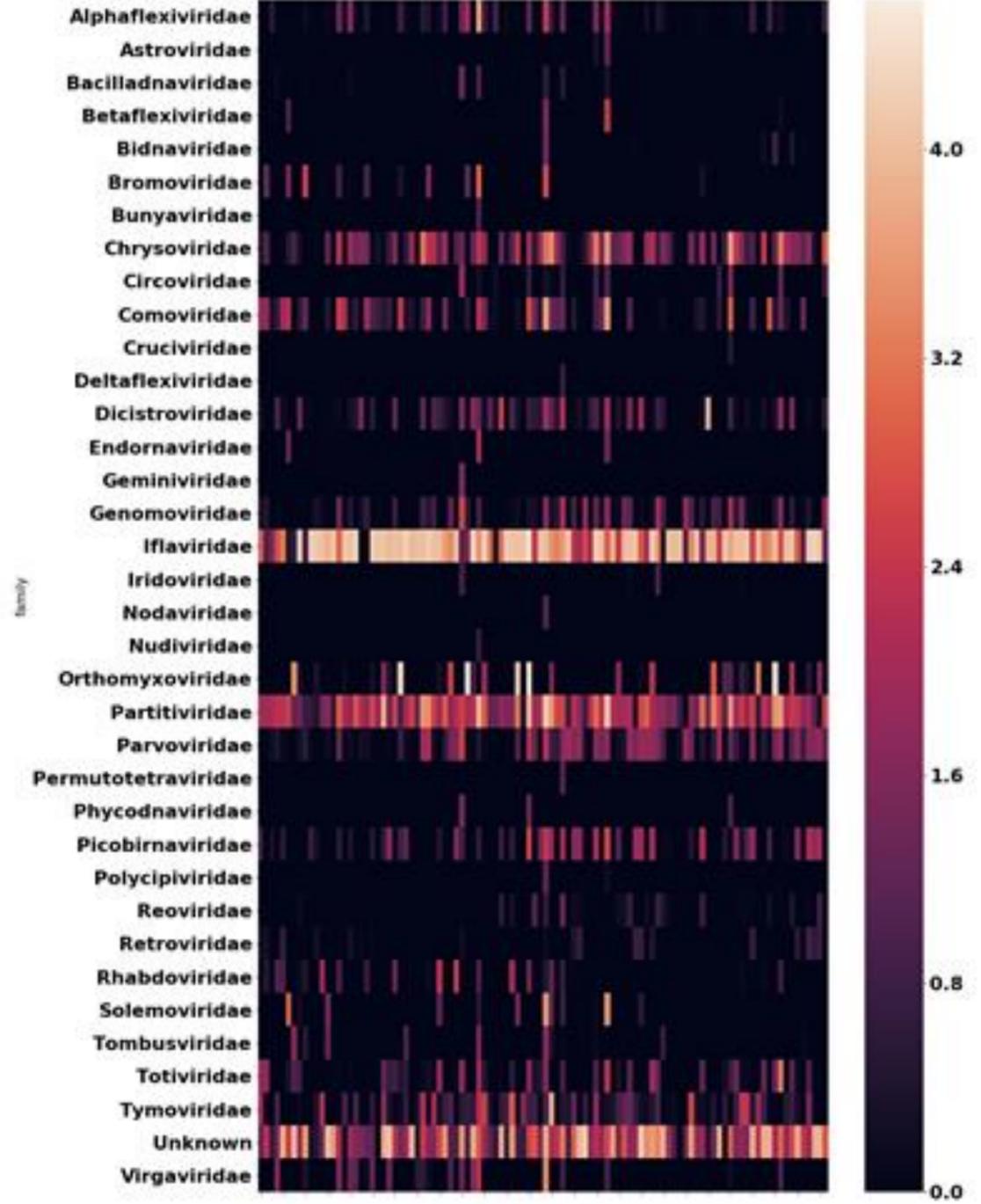
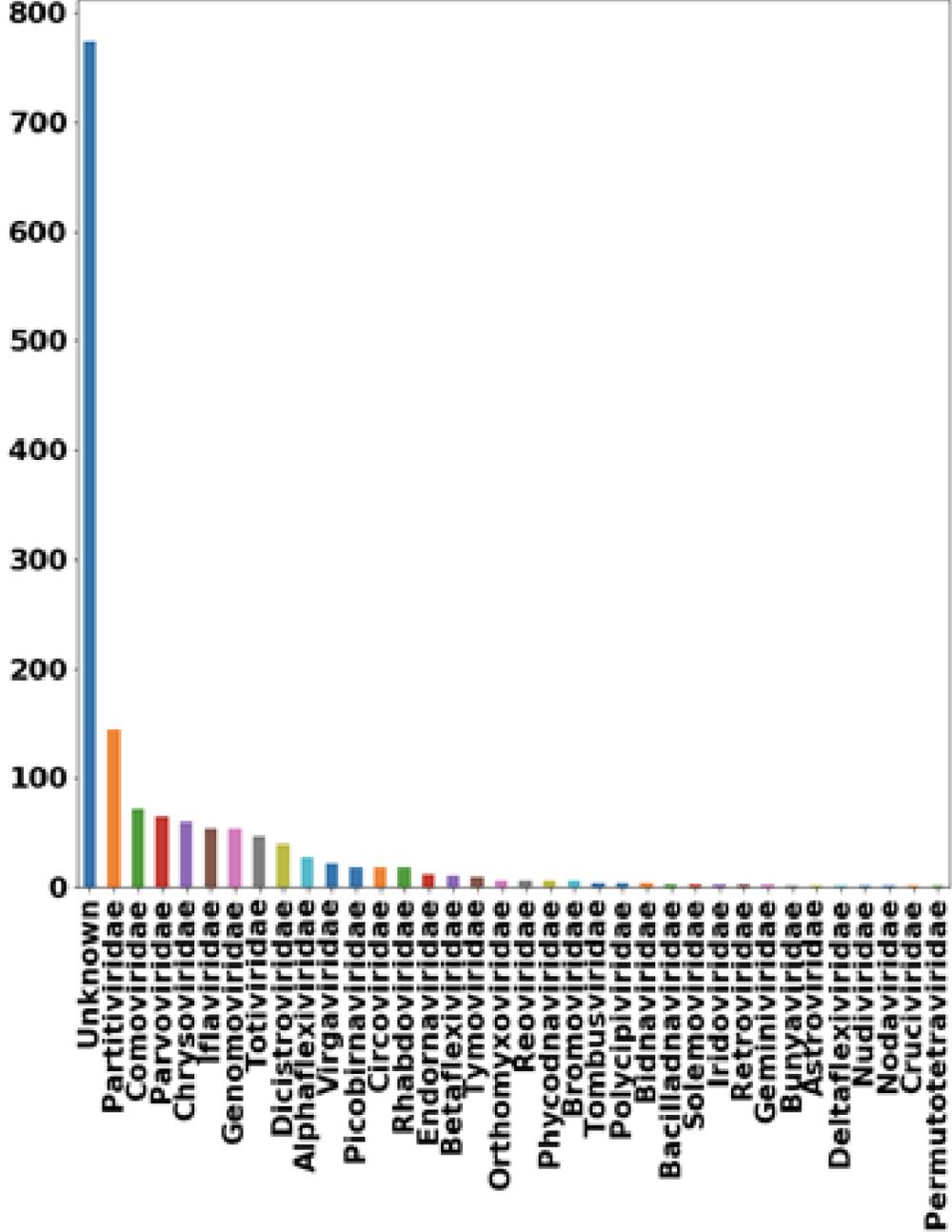
VIRUSES IN BEES

G. Bioinformatics interpretation of bee virome data.



- Read filtering and quality control (Trimmomatic / FastQC)
- De-novo Assembly (SPAdes)
- Annotation (DIAMOND)

HIGH PREVALENCE OF VIRUSES BELONGING TO MULTIPLE FAMILIES



MOST OF THE IDENTIFIED VIRUSES ARE RNA VIRUSES

Order Picornavirales

Iflaviridae

DWV, VDV, Type C

...

Dicistroviridae

BQCV, ABPV

...

Unclassified Picorna-like viruses

Unclassified order
Orthomyxoviridae

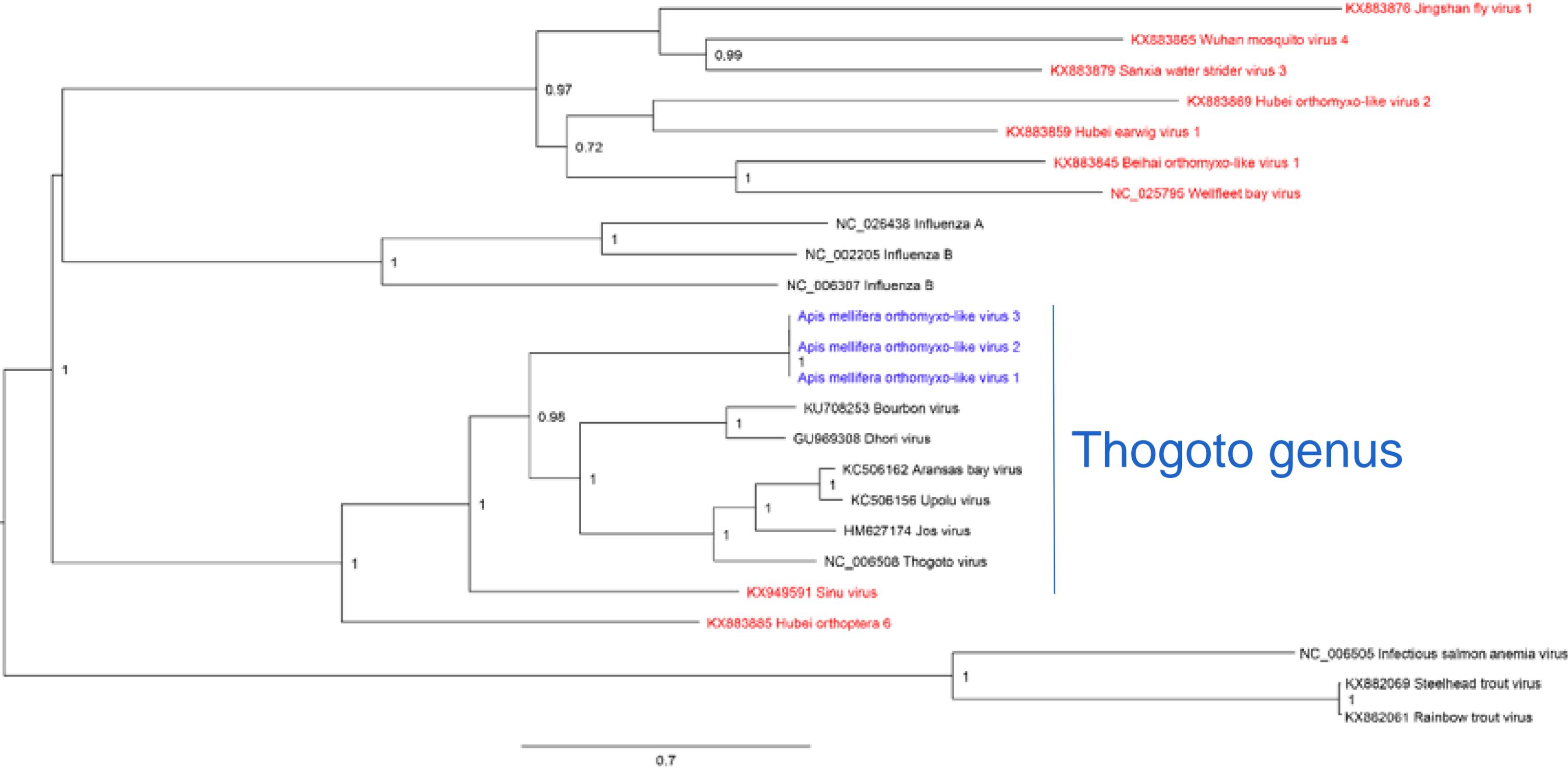
Non-
Segmented

+ssRNA

Segmented

-ssRNA

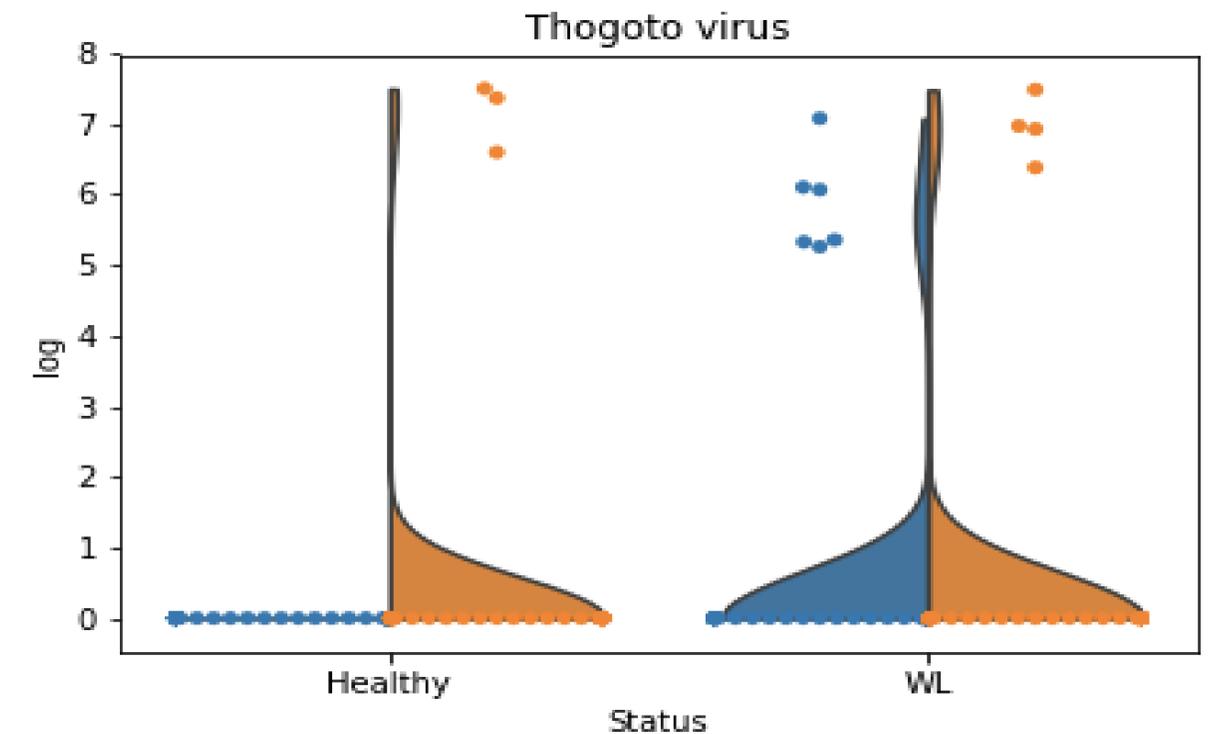
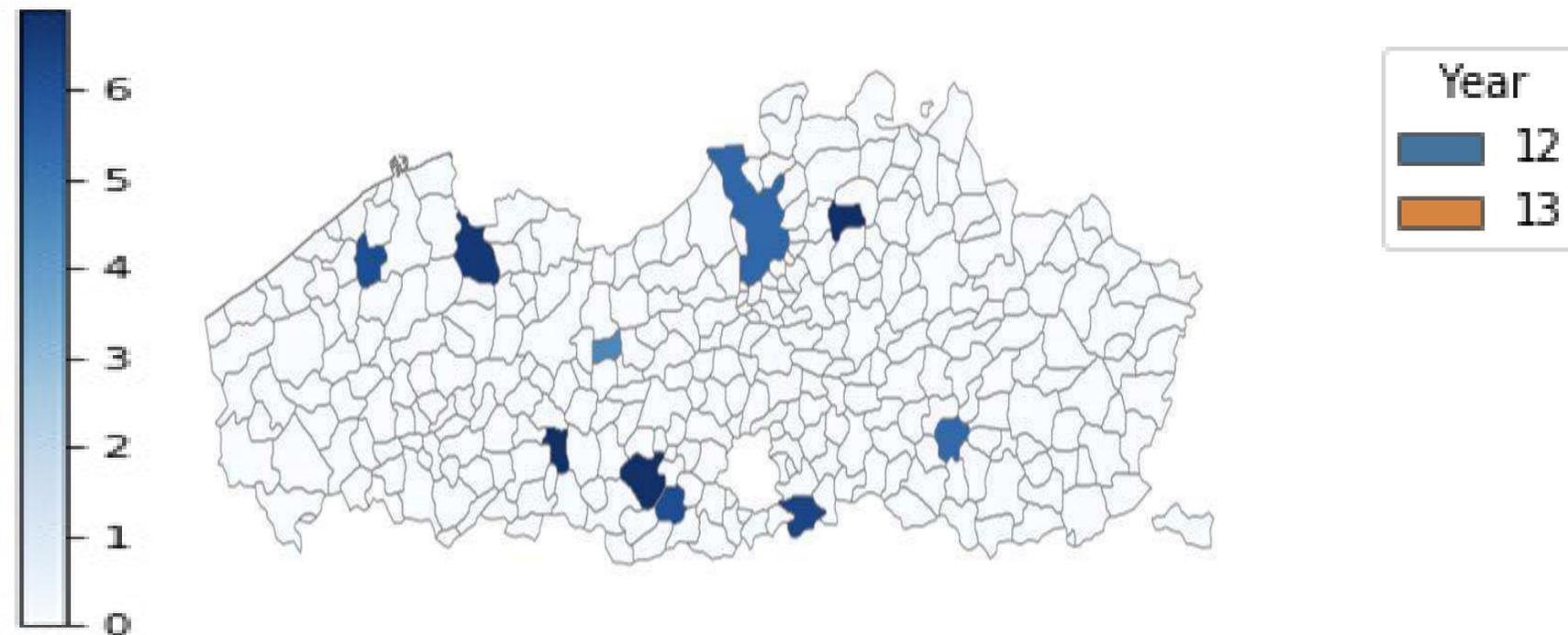
MOST OF THE IDENTIFIED VIRUSES ARE RNA VIRUSES



MOST OF THE IDENTIFIED VIRUSES ARE RNA VIRUSES

- Thogotovirus
 - Family Orthomyxoviridae
 - Most tick-borne
 - Segmented negative-sense ssRNA virus
 - Recently described to be present in *Varroa destructor* and *Apis mellifera* (Levin 2019)

ABSOLUTE QUANTIFICATION AND ASSOCIATION WITH WINTER LOSS



pvalue=0.0246
4% positive

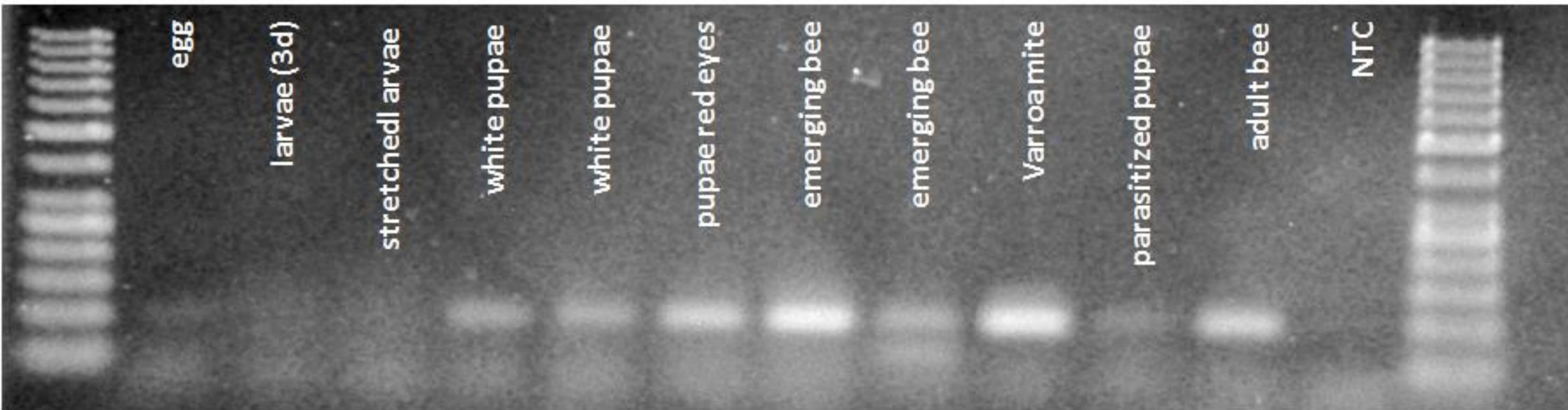
Violin plot

Two sided Mann-Withney U test

Virus	Moran I statistic	Variance	P-value
Thogoto virus	0.0348240066	0.0003270117	0.003872

THOGOTO VIRUS

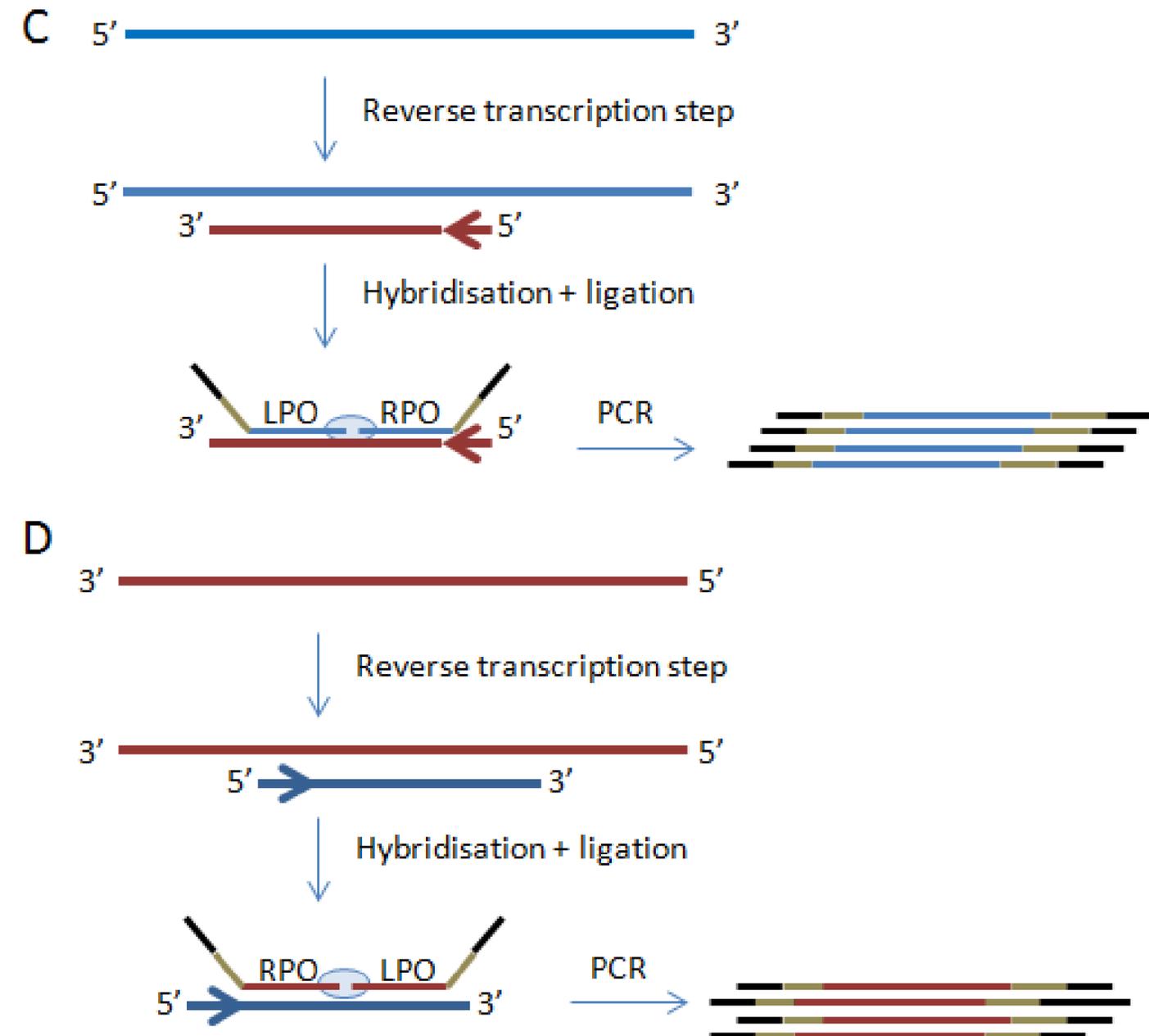
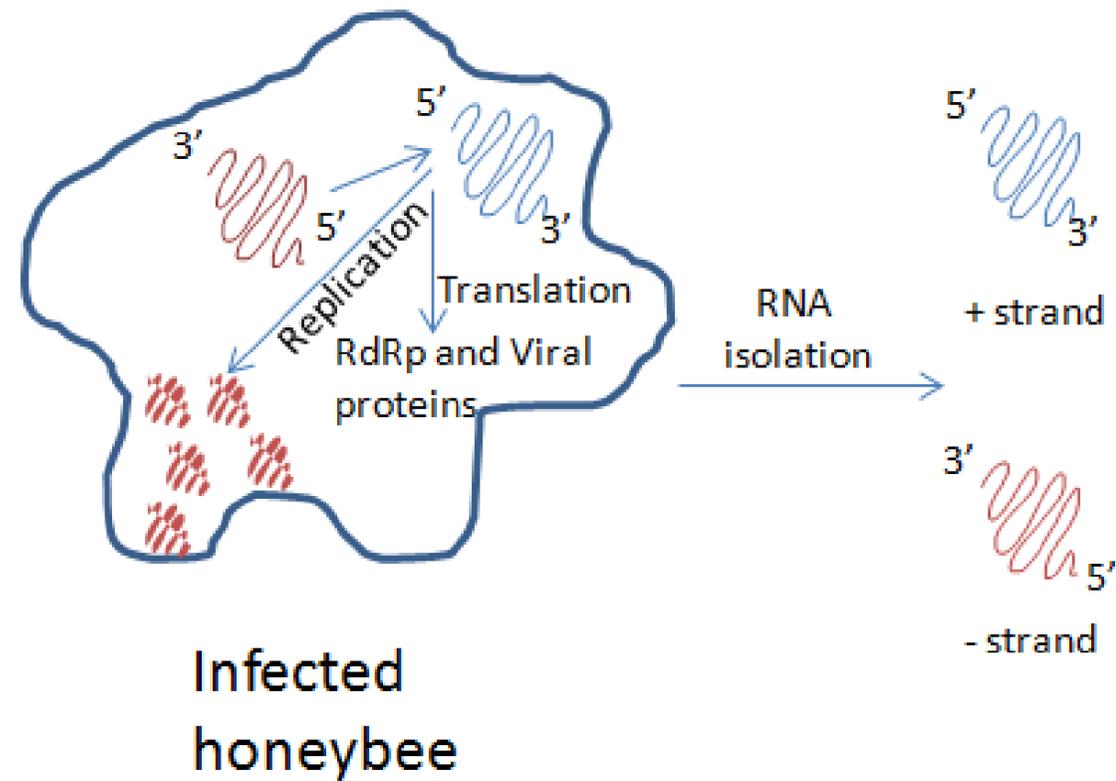
DISTRIBUTION OF VIRUS OVER DIFFERENT DEVELOPMENTAL PHASES



ALL PHASES TURNED OUT DO BE INFECTED
→ VERTICAL TRANSMISSION ROUTE

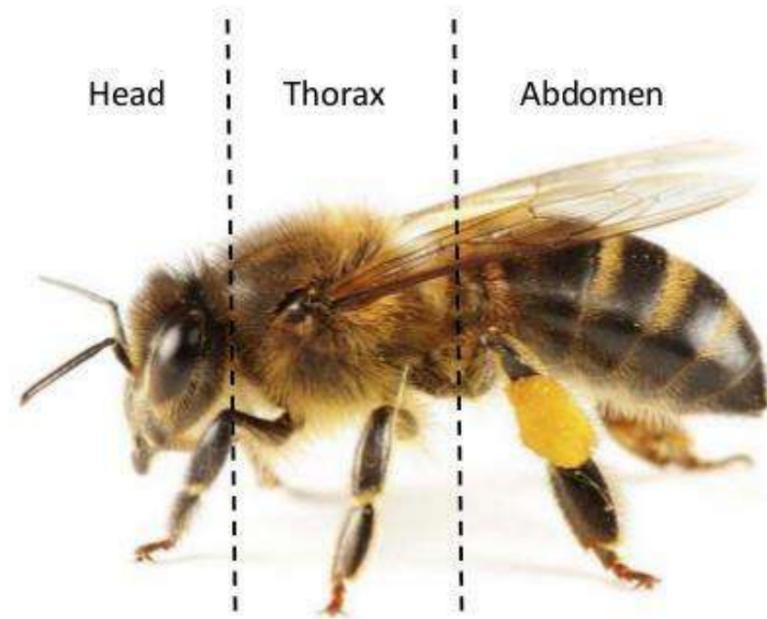
THOGOTO VIRUS : REPLICATING IN APIS MELLIFERA

STRAND SPECIFIC AMPLIFICATION → MLPA



WHICH TISSUES ARE INFECTED

TRANSMISSION ELECTRON MICROSCOPY



All tagmata
PCR positive



Hemolymph

WHICH TISSUES ARE INFECTED

TRANSMISSION ELECTRON MICROSCOPY

Collected hemolymph from 50 bees - sick and healthy bees

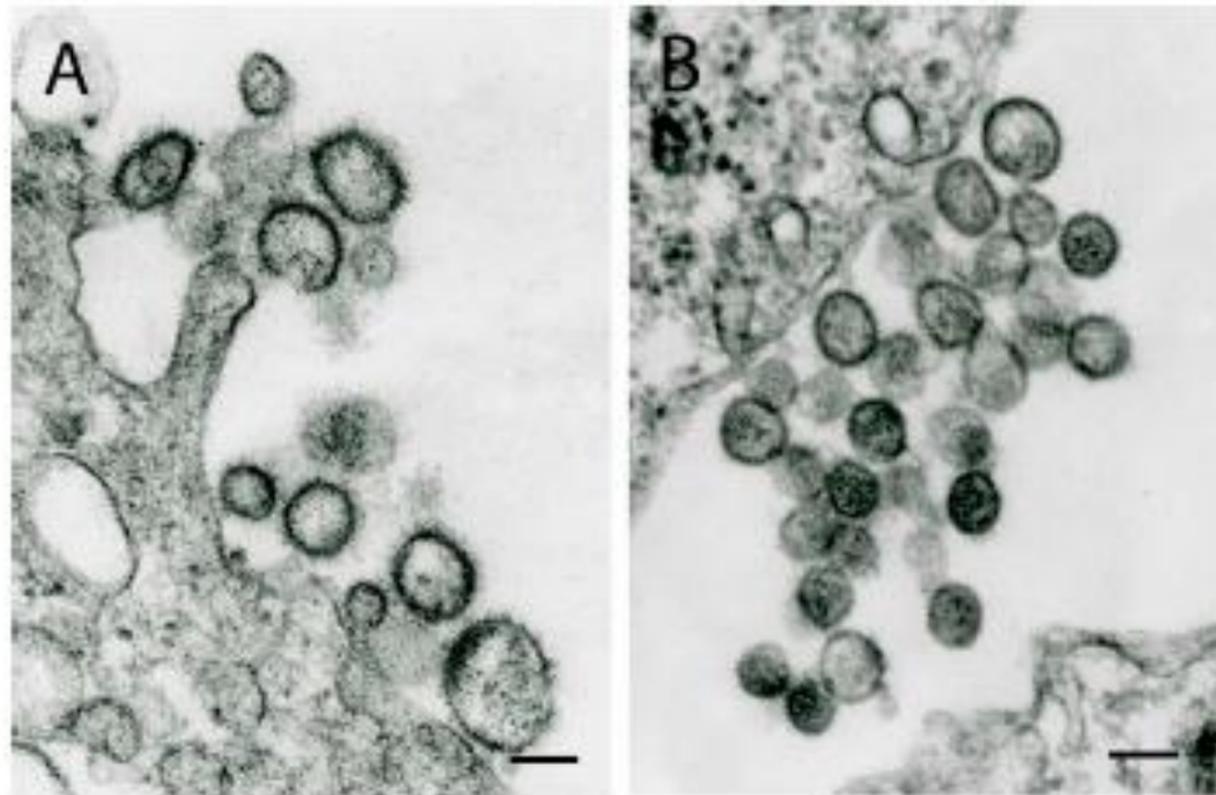
- anti clogging: citrate buffer: 1:1
- fixation in 4% FA + 2.5% GA / washing
- LMP agarose
- post fixation (1% OsO₄)/wash/1%UrAc
- wash/dehydration
- embedding/polymerization
- Ultrathin section and post staining UrAc

WHICH TISSUES ARE INFECTED

TRANSMISSION ELECTRON MICROSCOPY

Orthomyxoviridae

- virions are formed at the cell surface
- mostly spherical (70 – 120 nm), spikes from 7 nm long



Ultrastructure of tick-borne viruses that are probable members of the family Orthomyxoviridae.

(A) Aransas Bay virus (Virions 95–200 nm in diameter)

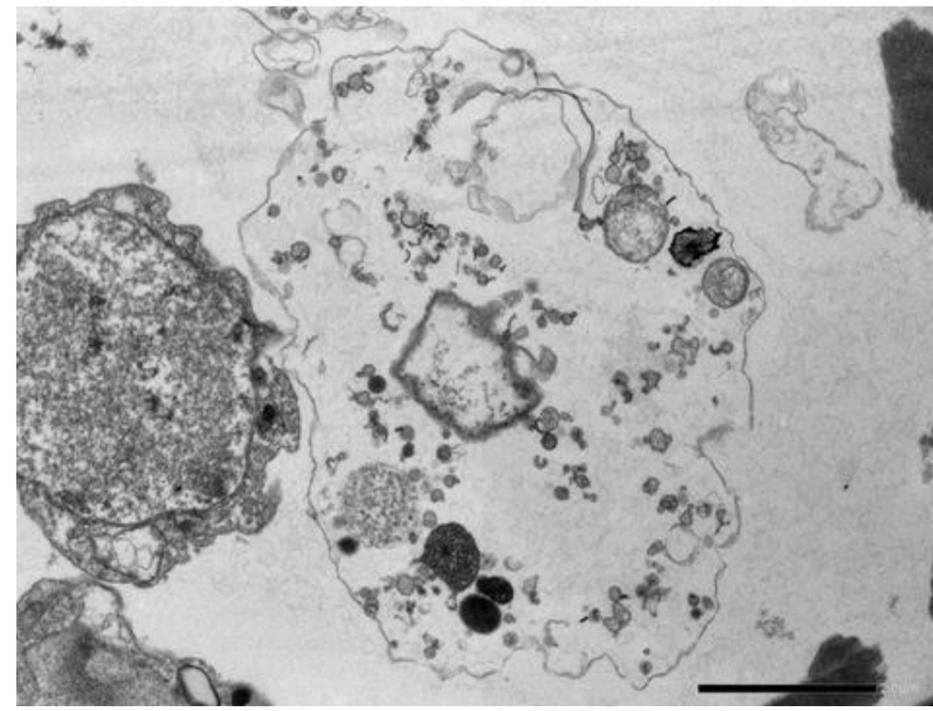
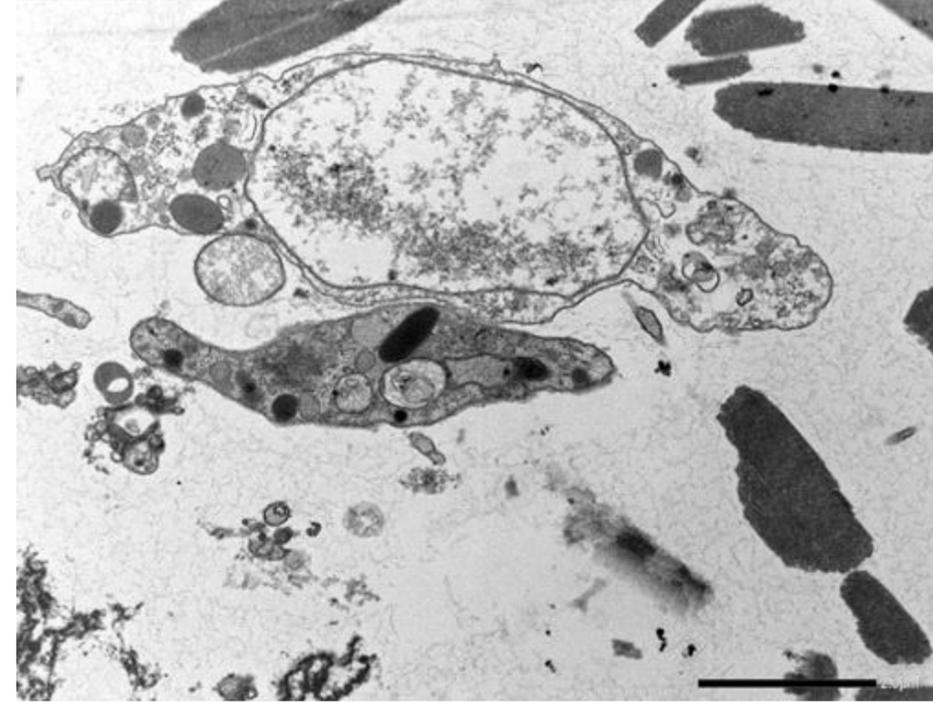
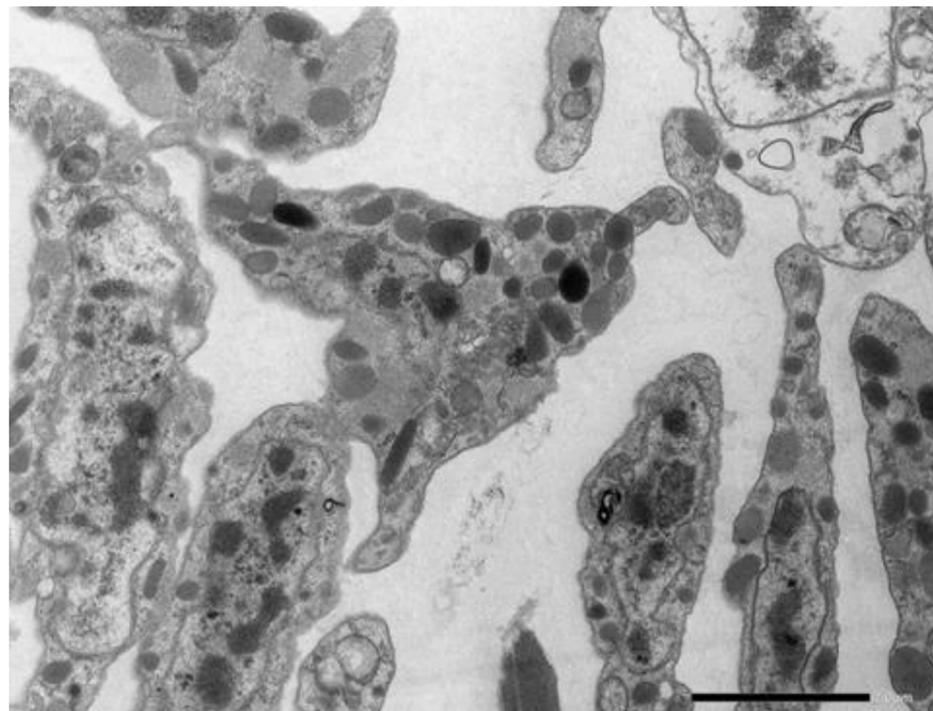
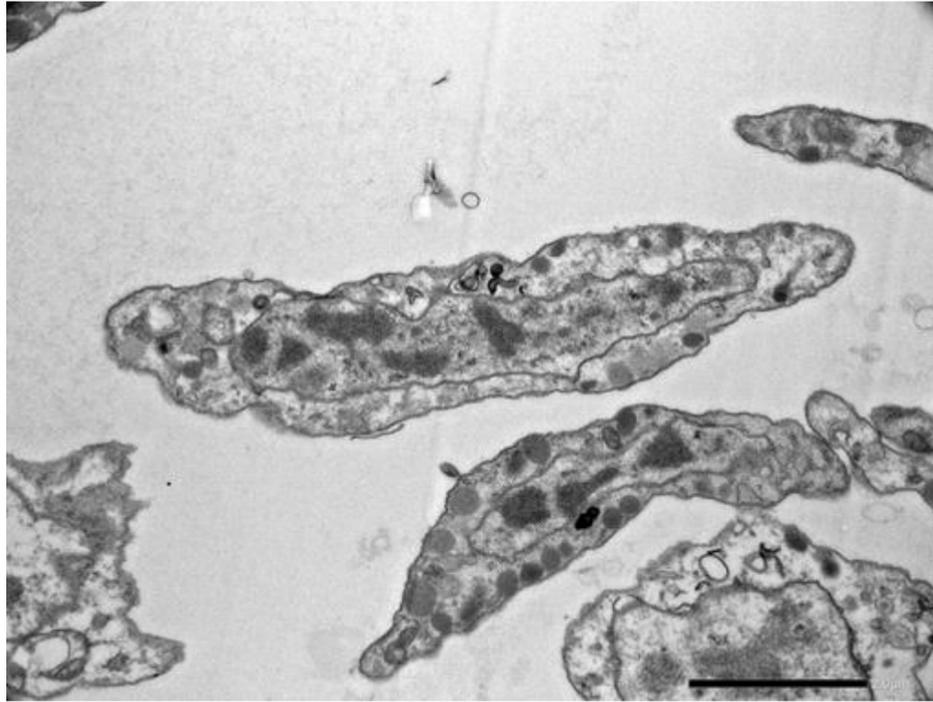
(B) Upolu virus (unclassified; probable genus Thogotovirus). A group of virions 75–95 nm in diameter at the surface of a Vero E6 cell.

(Popov, 2019 in Viruses)

WHICH TISSUES ARE INFECTED

TRANSMISSION ELECTRON MICROSCOPY

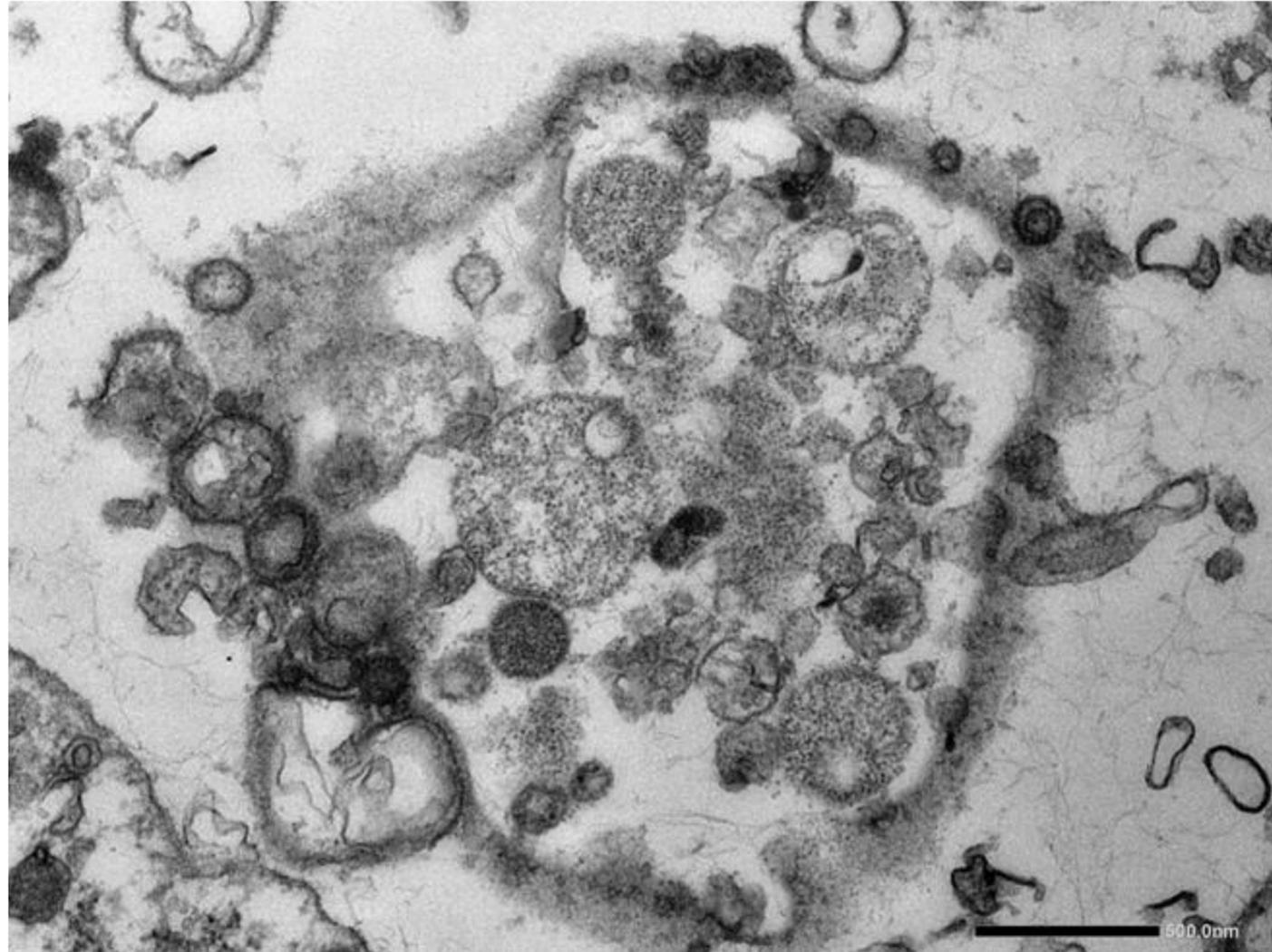
Healthy



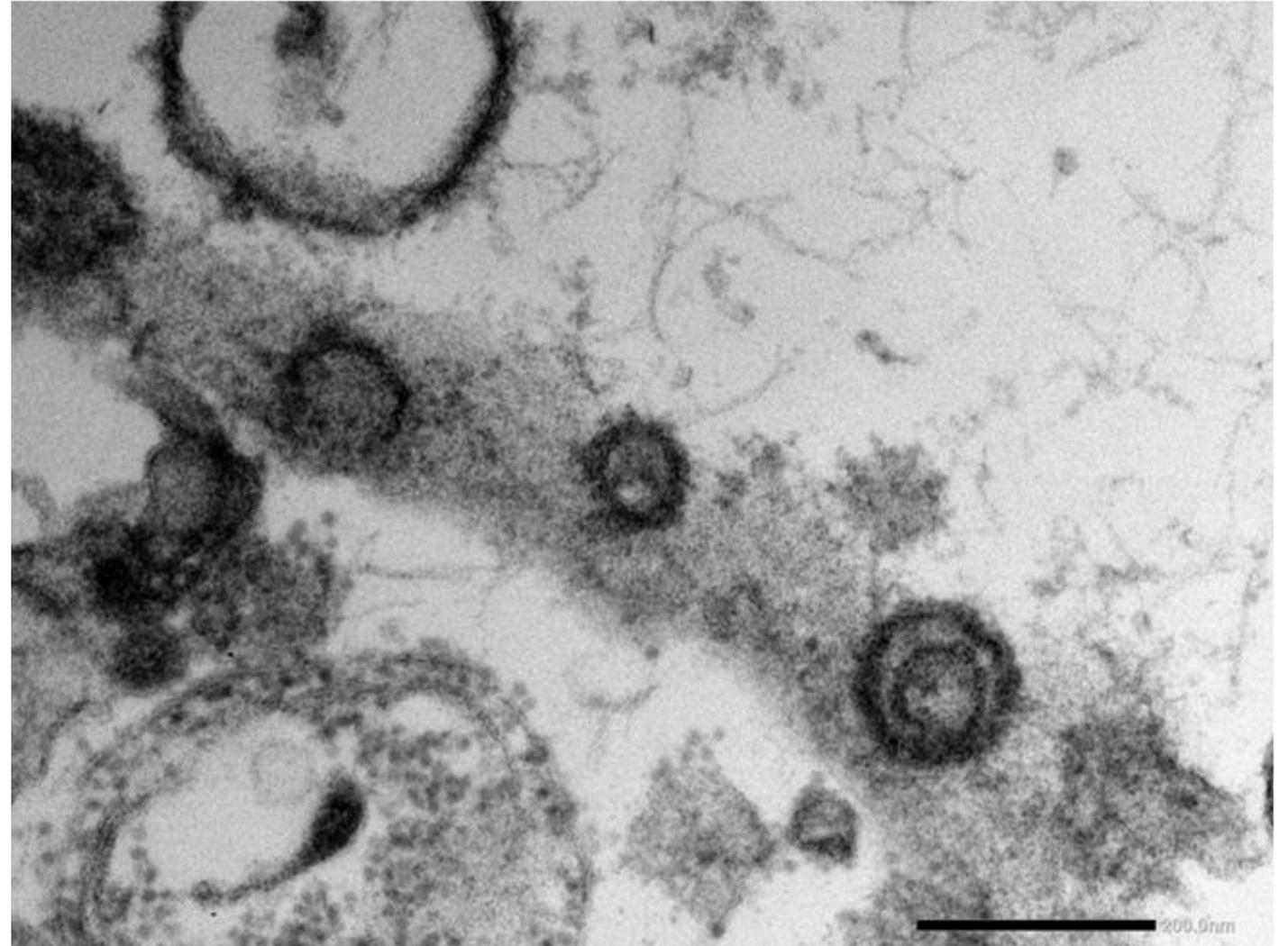
Virus infected

WHICH TISSUES ARE INFECTED

TRANSMISSION ELECTRON MICROSCOPY



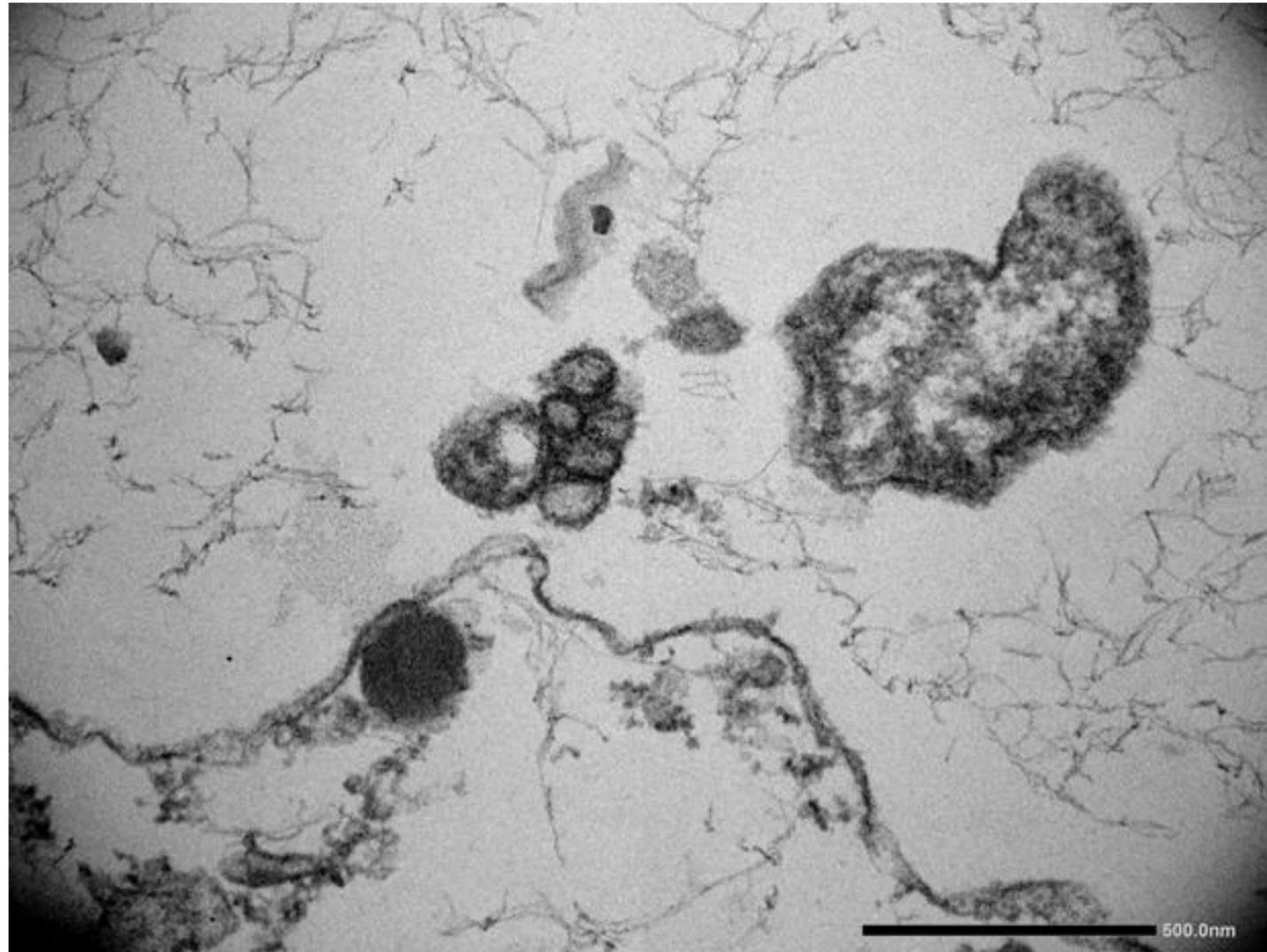
500nm



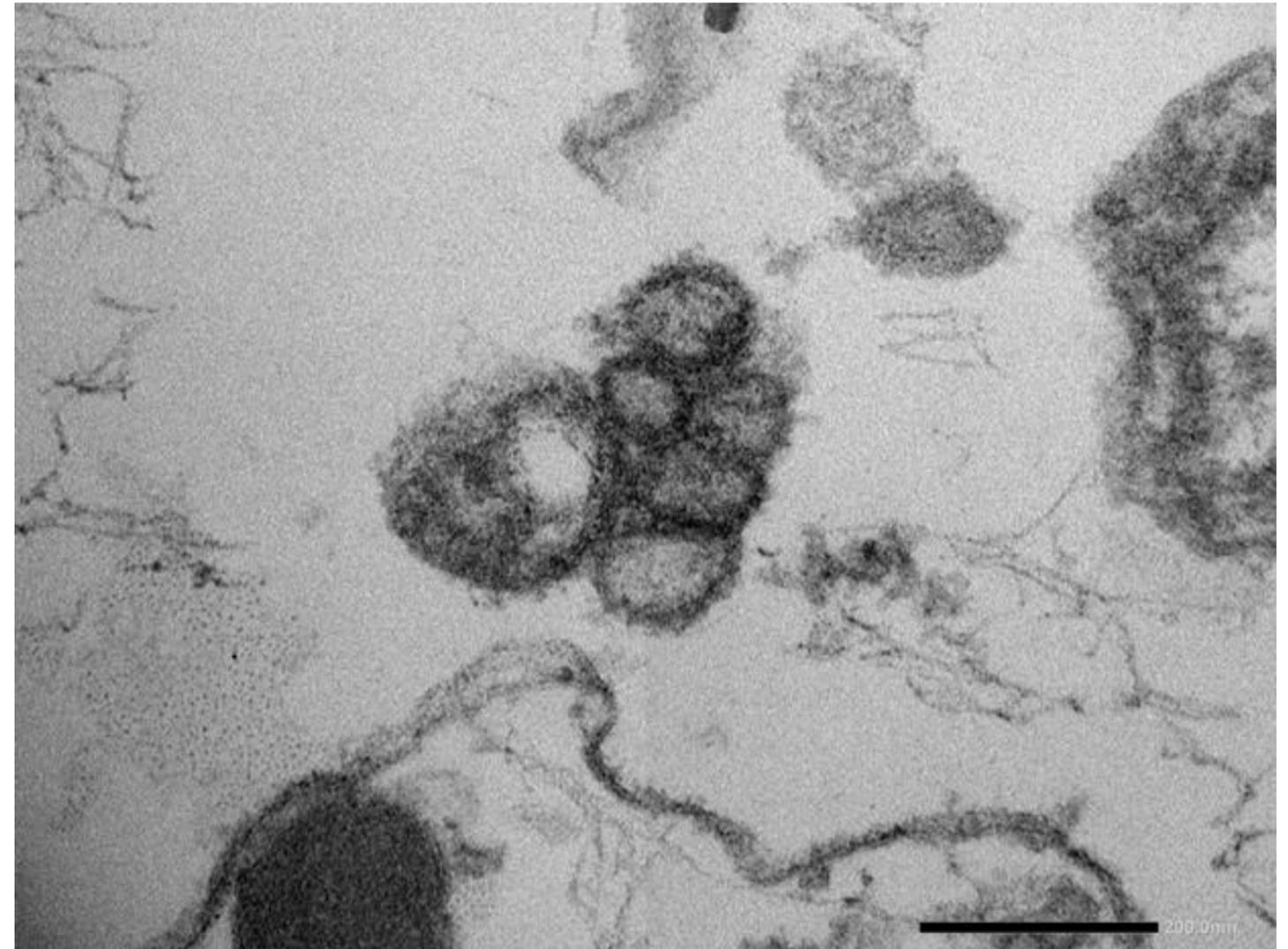
200nm

WHICH TISSUES ARE INFECTED

TRANSMISSION ELECTRON MICROSCOPY



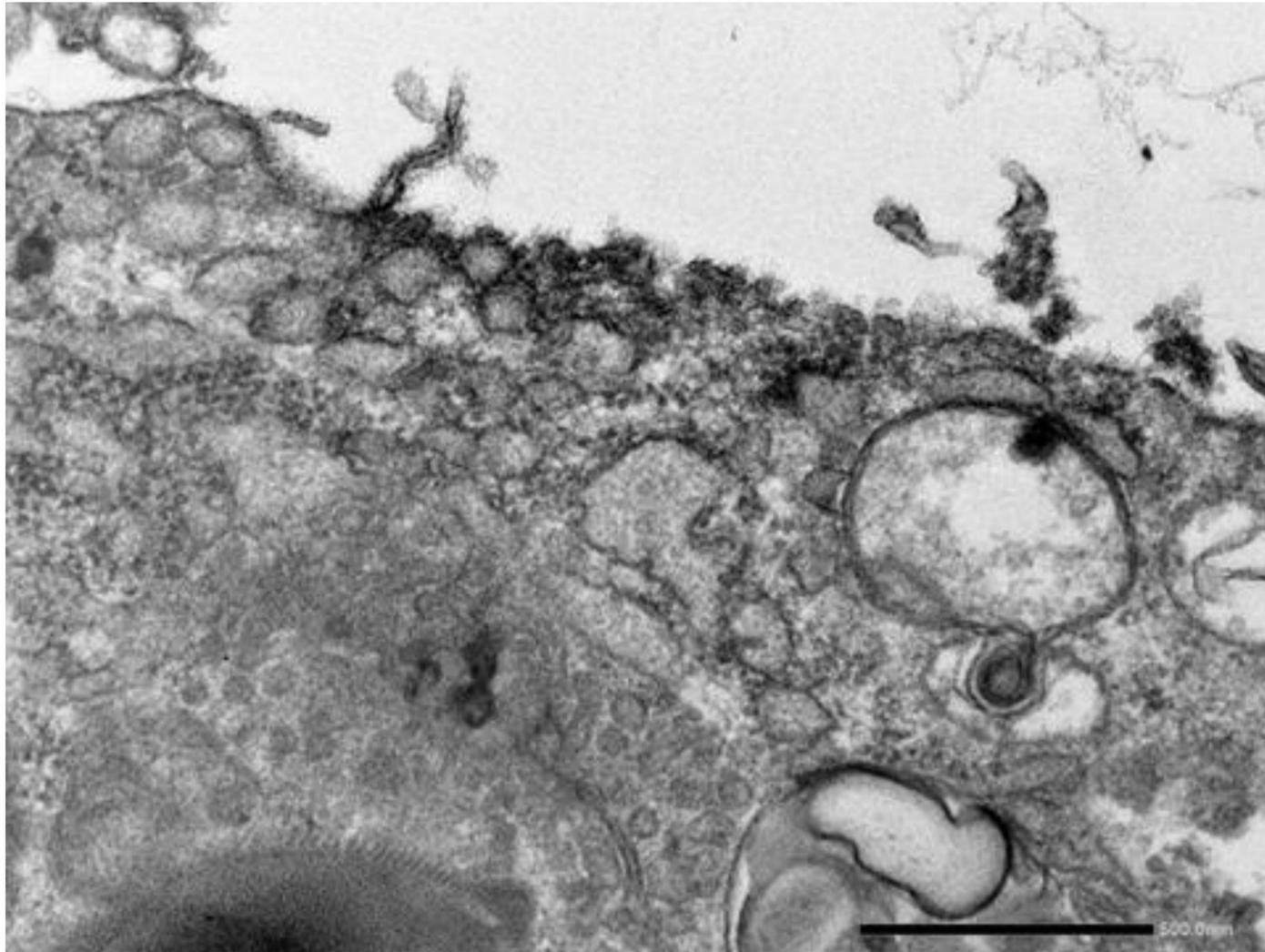
500nm



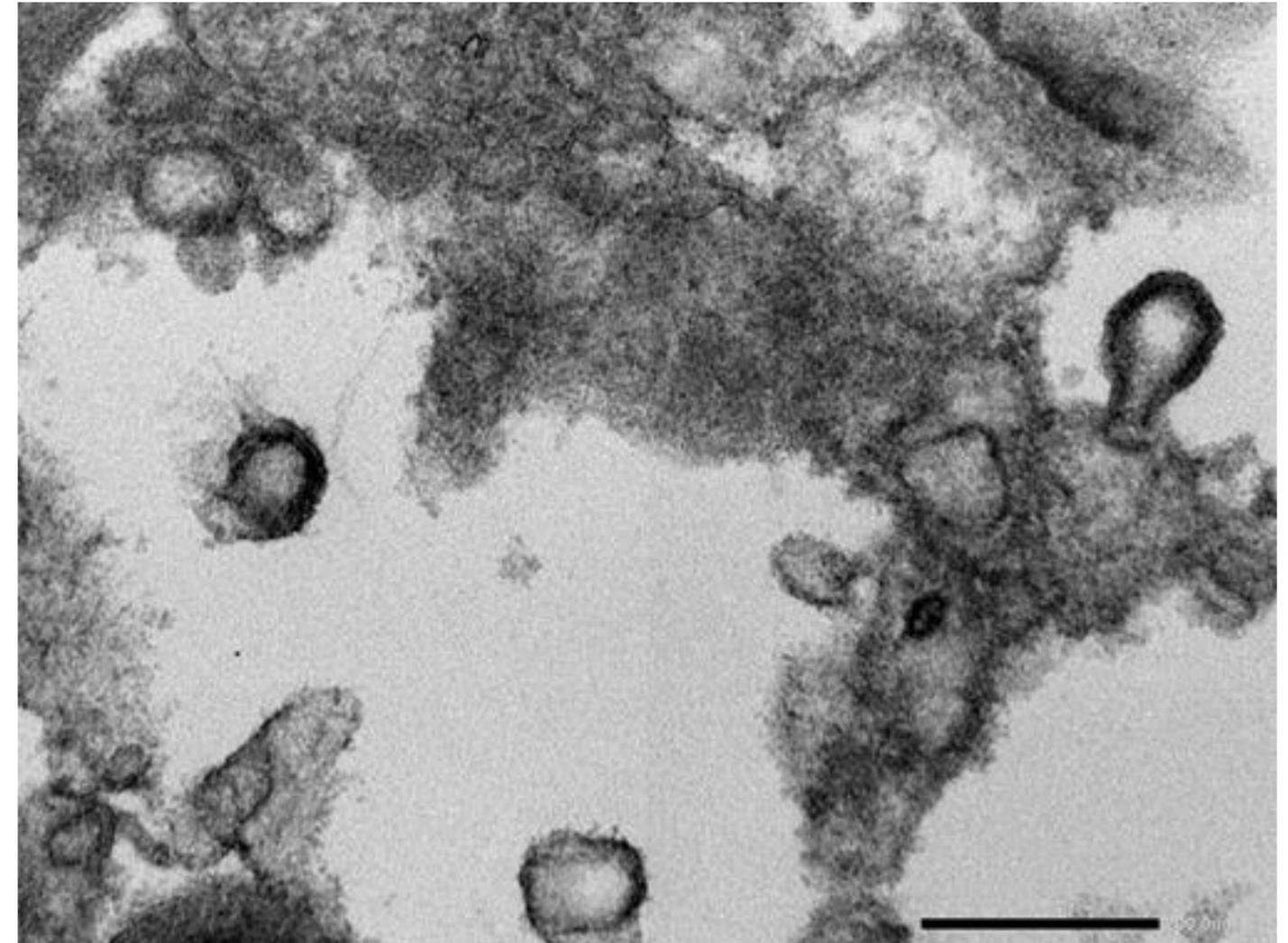
200nm

WHICH TISSUES ARE INFECTED

TRANSMISSION ELECTRON MICROSCOPY



500nm



200nm

CONCLUSION

NGS : Thogoto virus identified

Thogoto virus:

- new bee virus
replication
hemolymph as reservoir

Acknowledgements



KU Leuven
Ward Deboutte
Prof. Dr. Jelle Matthijssens

VIB – UGent: microscopy
Riet De Rycke

