MediLabSecure Webinar
Introduction to Metagenomics Technologies
14th December 2022
10.00-13:00 CET

A Pan-virus microarray for metagenomic analysis

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Introduction

- **Metagenomic Pan-Virus microarray**
  - Developed at the Statens Serum Institute (SSI) in Denmark (MWR & AF)
  - Tests all viruses with full genome sequences at GenBank in 2018 (n=3059)
- **Why a Pan-Virus microarray?**
  - As a diagnostics/surveillance tool
  - Replaces thousands of individual PCR reactions
  - Circumvents the need for a clear clinical hypothesis
  - Relatively fast (1.5-2 days from sample preparation to result)
  - Almost identical preparation for all kinds of samples and viruses (either DNA or RNA)
  - No need for advanced bioinformatics: the analysis of results is very simple

Introduction

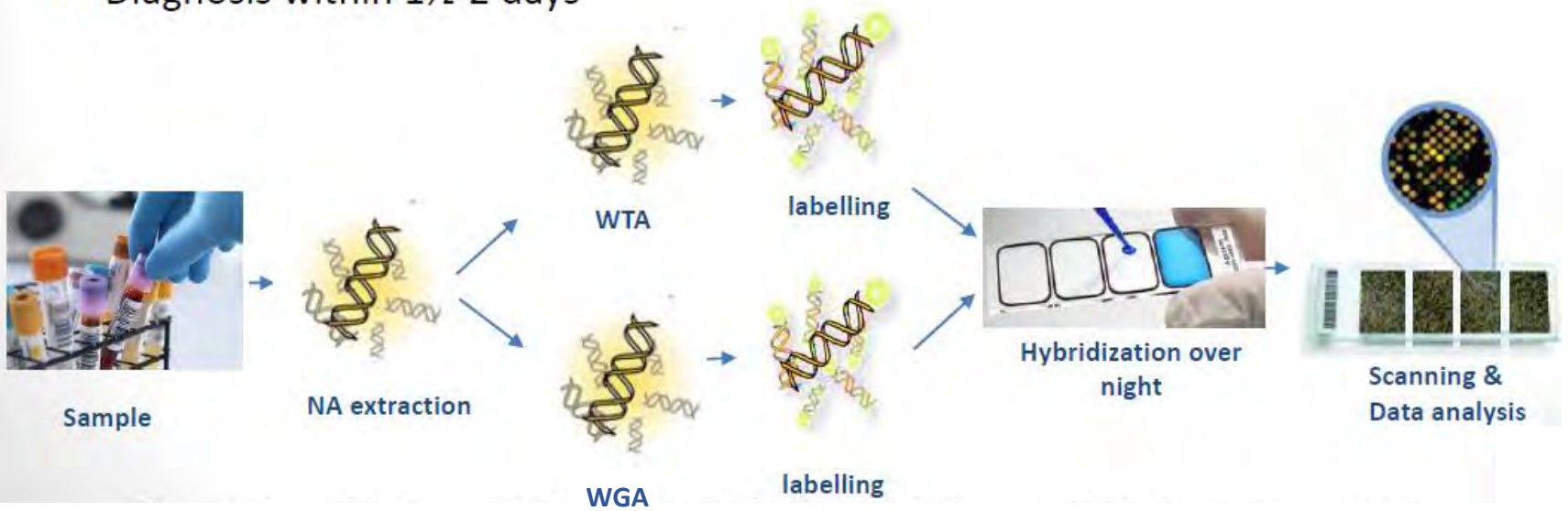
- **The SSI Pan-Virus Microarray: Design**

  - DNA Probes = 65 nt long
  - Unique and conserved virus regions
  - 30-160 probes per virus genome (depending on the size of the viral genome)
  - Ex. 1
    - EBOV genome = 19,000 nt
    - 60 x 65 nt/probe = 3,900 nt are covered by unique and specific probes (21%)
  - Ex. 2
    - CMV genome = 236,000 nt
    - 160 x 65 nt/probe = 10,400 nt are covered by unique and specific probes (4%)

Introduction

- **The SSI Pan-Virus Microarray: Overview**

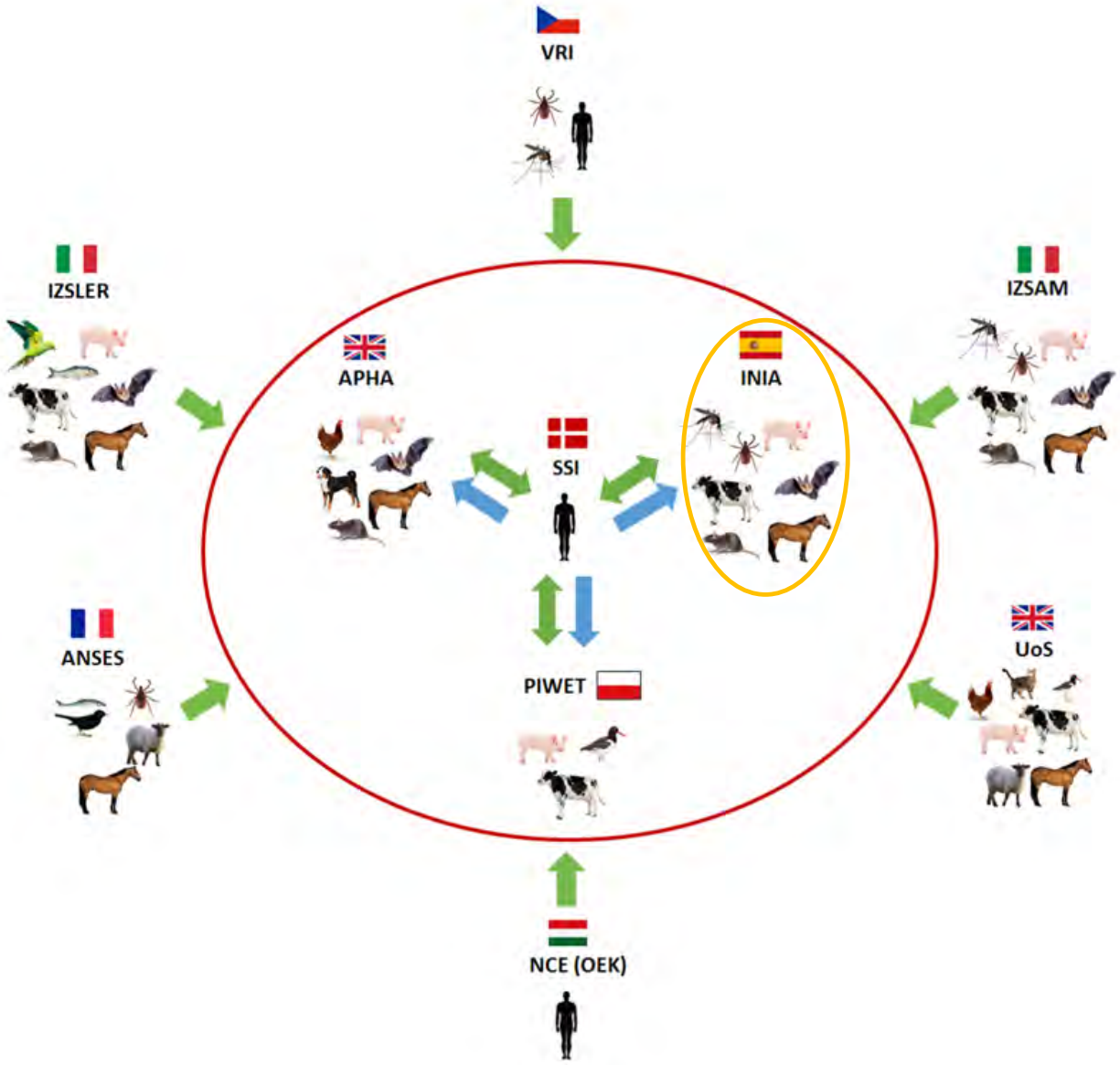
  - Blood, swaps, biopsies, urine, feces, tracheal secret etc.
  - Unbiased metagenomic detection
    - Whole Transcriptome Amplification (WTA) (RNA virus)
    - Whole Genome Amplification (WGA) (DNA virus)
  - Diagnosis within 1½-2 days



Introduction

- **MAD-Vir*: A One Health European Joint Programme (OHEJP) Project (EU-H2020)**

  - **AIM:** To use Microarray technology for unbiased Metagenomic virus surveillance and detection of emerging virus threats
  - Implementation of the Pan-Virus microarray method at INIA (ES), APHA (UK) and PIWET (PL)
  - Use Pan-Virus microarray analysis of suspicious animal, human and surveillance samples for “emerging threats and FBZ” (~1000 samples)
  - Validate and compare results to already developed diagnostic methods (e.g. pathogen specific PCRs, Microfluidic PCRs, Nanostring, NGS etc)



* “Metagenomic Array Detection of emerging Virus in EU”

Objectives

- **At INIA, we pursued two specific objectives:**

  I. To contribute to the **harmonisation and validation** of the Pan-Virus microarray developed at the Statens Serum Institute (SSI) in Denmark, using samples obtained from animals either experimentally infected with known viruses or from clinical cases occurring in the field.

  II. To explore the ability of the SSI Pan-Virus microarray to **identify and type novel or eccentric variants** within a virus species (level of discrimination).

Methods

- **Samples analyzed (SSI and INIA)**

Sample types	Animal species*	Viruses (family/species/variant)
Blood Brain Feathers Faeces Heart Kidney Lung Lymph node	Mammals: - Horse - Mice - Swine - Sheep Birds: - Barn Own - Cinereous vulture - Golden eagle - Partridges (Red-legged & Grey)	*Flaviviridae*: - West Nile virus (lineages 1, 2, 5 & 6) - Usutu virus (Spain/2009) - Bagaza virus (Spain/2010) - Classical swine fever virus (Gt-2.3) *Asfarviridae*: - African swine fever virus (Gt –II) *Picornaviridae*: - Porcine teschovirus (PTV-12) *Reoviridae*: - Bluetongue virus (St-4) *Paramyxoviridae*: - Peste des petits ruminants virus (Gt-IV)
*Experimentally infected (black) or clinical cases (grey)		

Methods

- **Samples analysed (SSI and INIA)**

Experimental infections (n=16 samples)

Animal species	Inoculated virus	Samples
Mice	WNV	Brain
Pig	ASFV	Lung
Pig	CSFV	Serum
Pig	PTV	Faeces
Sheep	PPRV	Mesenteric ganglion
Sheep	BTV	Blood
Red-legged partridge	WNV	Blood, feather
Red-legged partridge	USUV	Blood
Red-legged partridge	BAGV	Feather
Grey partridge	USUV	Heart, kidney

Animals with clinical signs (n=8 samples)

Animal species	Virus identified (PCR methods)	Samples
Horse	WNV	Brain
Golden eagle	WNV	Kidney
Cinereous vulture	WNV	Feather
Barn owl	Not detected	Brain, kidney, heart, spleen, lung

Results (I)

- **Pan-Vir microarray identification of viruses present in each sample at the species level**

Experimental infections (n=16 samples)

Animal species	Inoculated virus	Samples	
Mice	WNV	Brain	👍
Pig	ASFV	Lung	👍
Pig	CSFV	Serum	👍
Pig	PTV	Faeces	👍
Sheep	PPRV	Mesenteric ganglion	👍
Sheep	BTV	Blood	👍
Red-legged partridge	WNV	Blood, feather	👍
Red-legged partridge	USUV	Blood	👎
Red-legged partridge	BAGV	Feather	👍
Grey partridge	USUV	Heart, kidney	👍

Animals with clinical signs (n=8 samples)

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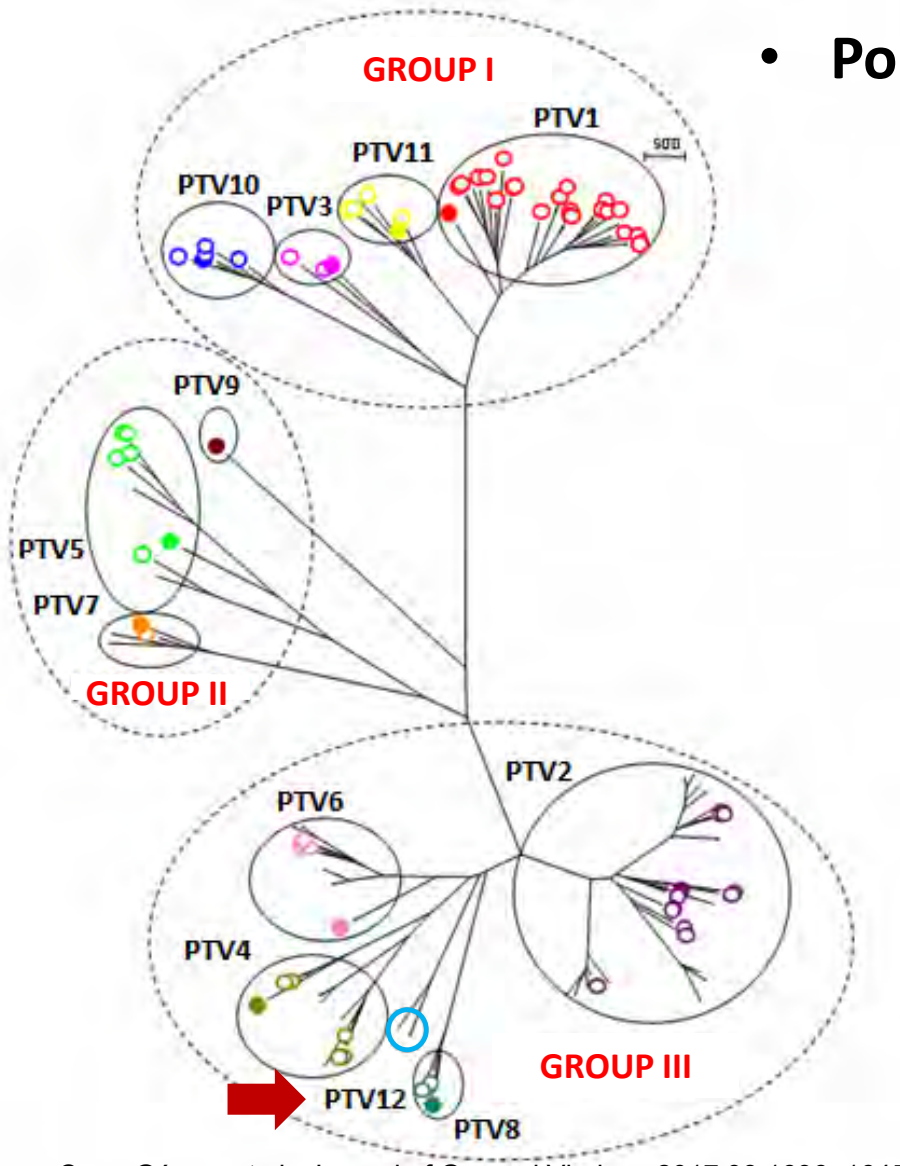
Barn owl	Not detected	Brain, kidney, heart, spleen, lung	👍

In a 3rd round of analysis (SSI) the array detected a bornavirus closely related to **canary bornavirus** in Barn owl brain.

Results (II)



Cano-Gómez et al., Journal of General Virology 2017;98:1636–1645

- **Porcine teschovirus new serotype**

PTV12

Nucleotide homology:
Intragroup: 81-87%
Intergroup: 75-79%

Faecal sample
Experimentally inoculated pig
Viral load: Ct 26

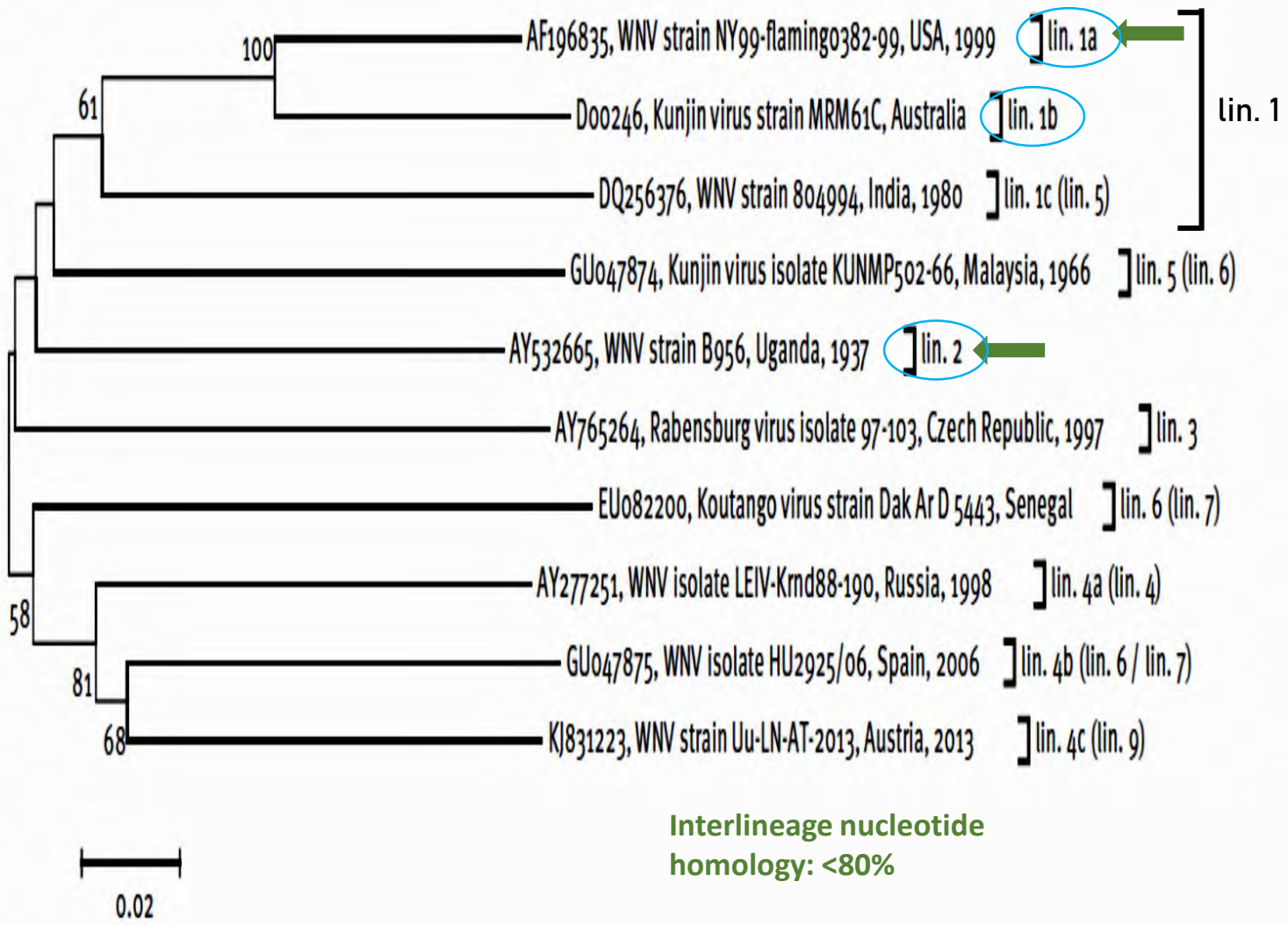
Pan-Vir array results (SSI):

Porcine teschovirus 4
Porcine teschovirus 8
Porcine teschovirus 2
Porcine teschovirus 1
Porcine enterovirus,
Porcine bocavirus,
(Porcine adenovirus type 3)

Correct detection and group assignation

Results (III)

- **Detection range of West Nile virus lineages (I): Lineages 1 and 2**



Interlineage nucleotide homology: <80%

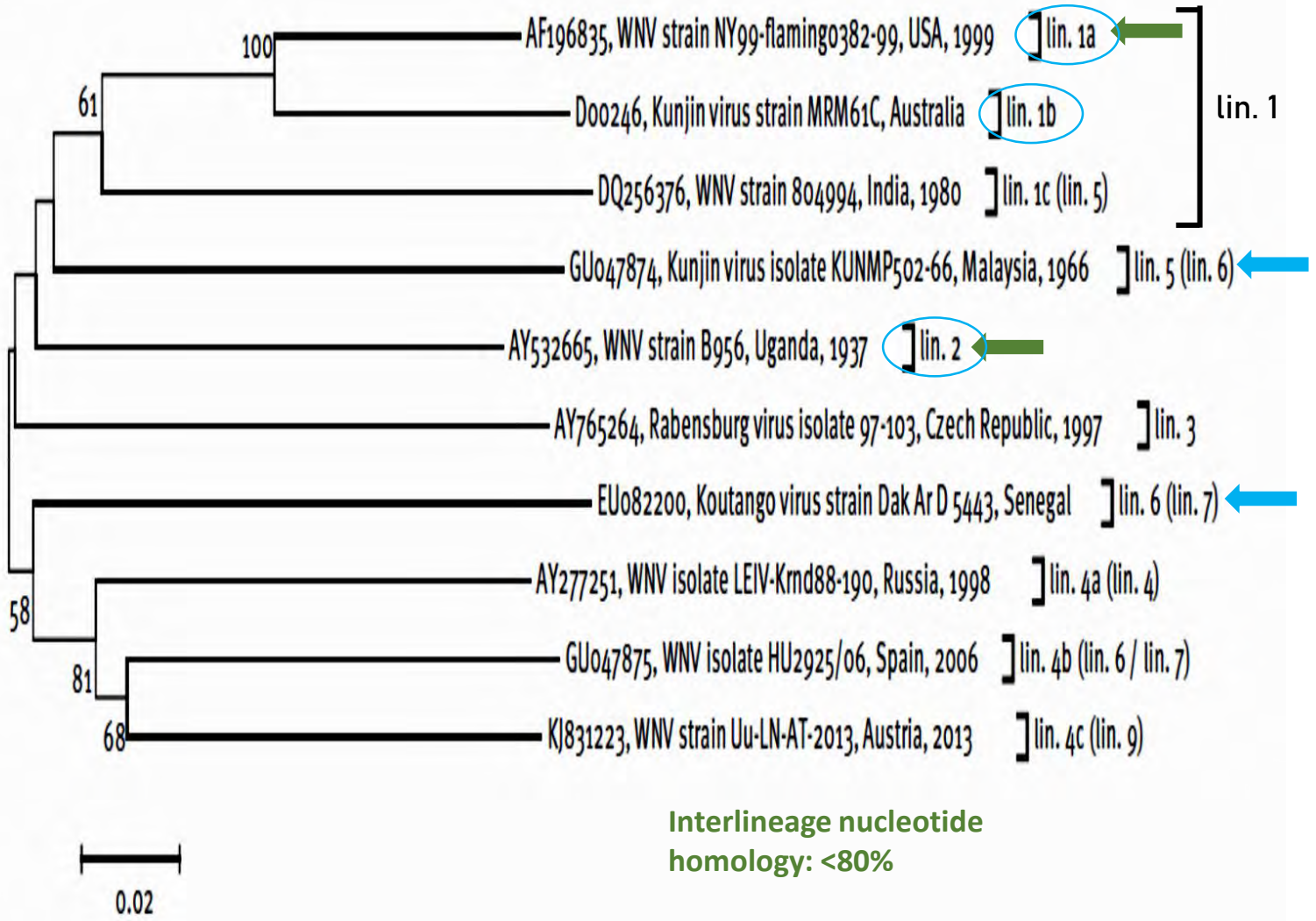
Red-legged partridge

Inoculated virus	Sample	Viral load (Ct)	Array results
Lineage 1a (Isr98)	Blood	27	WNV lin 1b (Kunjin)
Lineage 2 (Aust08)	Blood	25	WNV lin 2
Lineage 2 (Aust08)	Feathers	25	WNV lin 2

Correct discrimination of WNV lineages 1 & 2

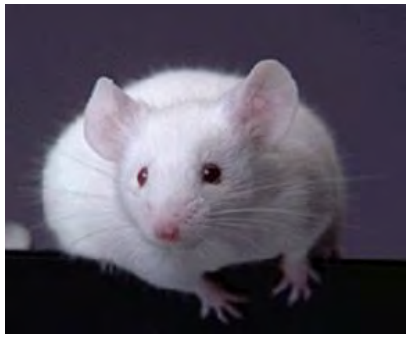
Results (IV)

- **Detection range of West Nile virus lineages (II): Lineages 5 and 6**



Interlineage nucleotide homology: <80%

Mice



Inoculated virus	Sample	Viral load Ct	Array results
Lineage 1a (Isr98)	Brain	28	WNV lin 1, Kunjin
Lineage 2 (Aust08)	Brain	24	WNV lin 2
Lineage 5 (Malaysia)	Brain	26	Negative
Lineage 6 (Koutango)	Brain	26	Negative

Failure to detect WNV lineages 5 & 6

Results (V)

- Barn owl found sick (neurological signs)
- Died after 7 days of veterinary care provided in a wildlife recovery center in Badajoz province, Spain
- After RT-PCR analysis, WNV and other suspects were discarded

Barn owl (*Tyto alba*)

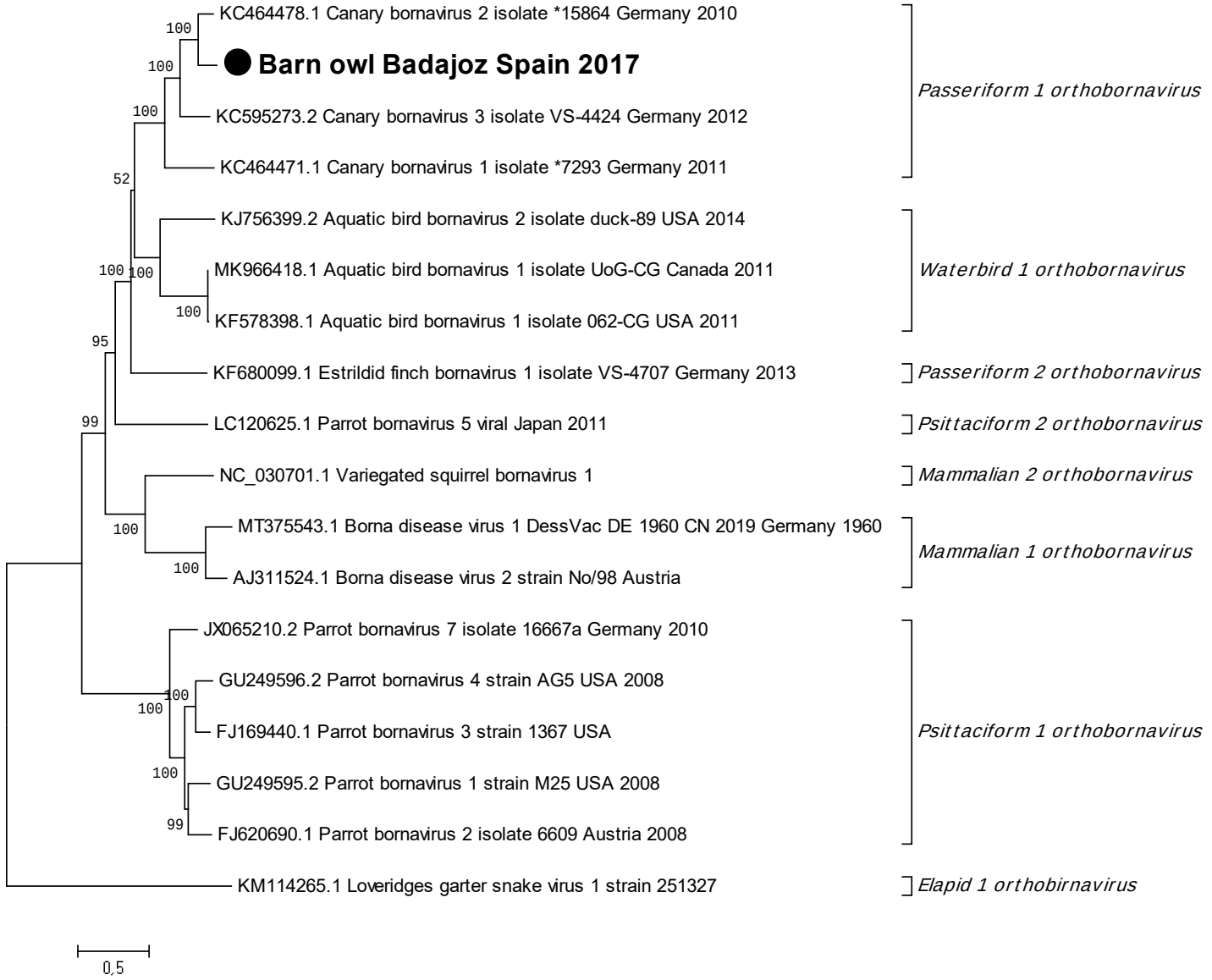


Fig. 1: Phylogenetic analysis of the genus *Orthobornavirus* (Family *Bornaviridae*) based on complete genome sequences. GenBank accession number, country and year were given for each strain. Sequence emphasized in bold and with a circle (●) was generated during this study.

Results (V)

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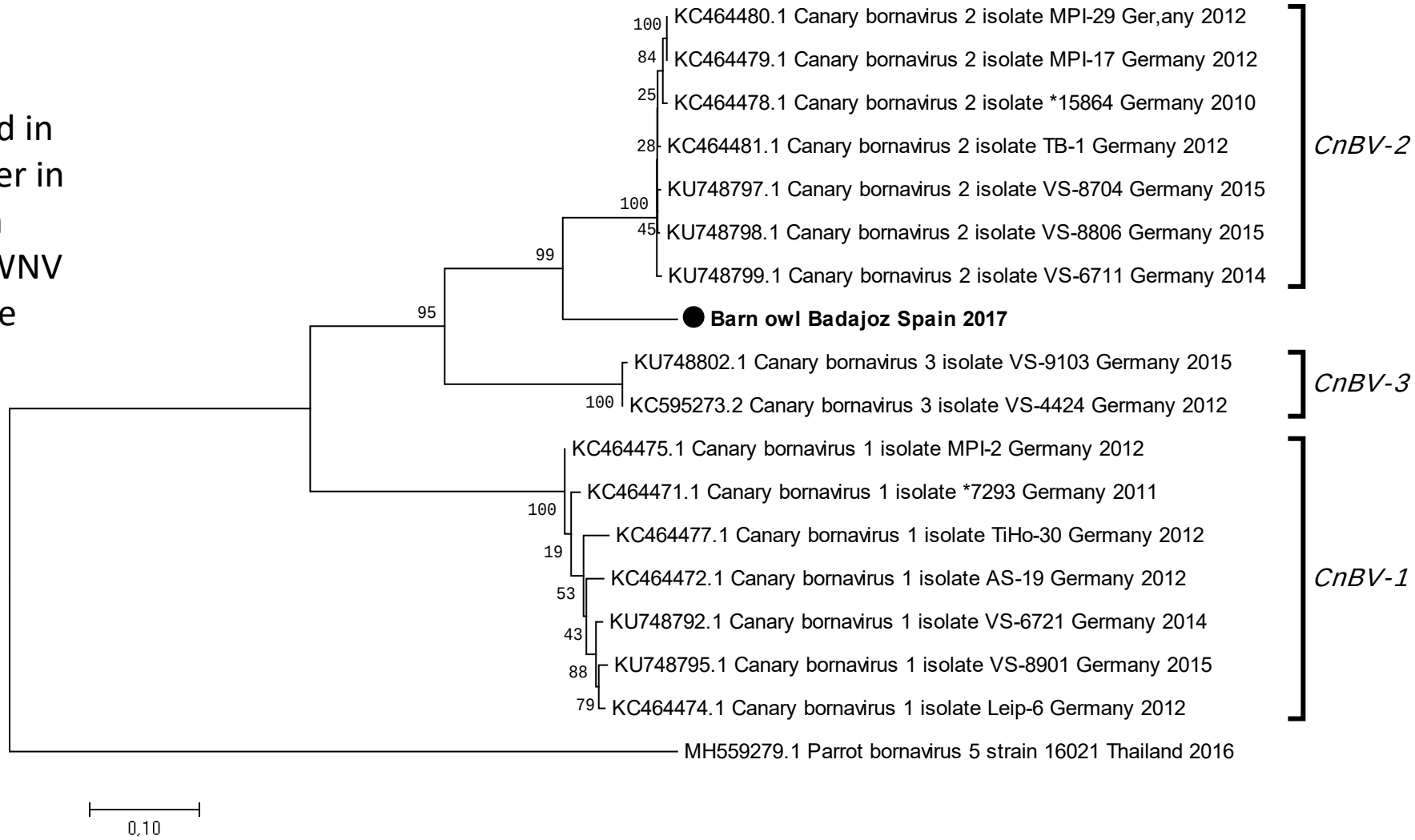

Fig. 2: Phylogenetic analysis based on a 2250 nt fragment (corresponding to N, X, P and M proteins coding regions) of *Passeriform 1 orthobornavirus* family. Each Canary Bornavirus genotype (CnBV-1, CnBV-2, CnBV-3) was indicated. GenBank accession number, country and year were given for each strain. Sequence emphasized in bold and with a circle (●) was generated during this study.

Conclusions

The Pan-virus microarray developed by SSI:

- Was able to successfully identify all viruses expected from a collection of samples of a wide range of types and animal origins, including both experimental infections and field cases.
- Exhibits enough sensitivity for application in diagnosis of clinical samples.
- Was able to detect successfully a wide range of viruses, either DNA or RNA.
- Appears to detect viruses bearing up to % 80% homology with those included in the design. As the % homology decreases, the detection is less likely.
- Demonstrated its utility as last resort technique for molecular diagnostics when other techniques fail.
- Was successfully applied to the identification of a new avian pathogen related to canary bornavirus.

The inclusion of a wider range of sequences already available in the next microarray version (in progress) will likely improve its virus detection range.

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