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THE ENGINE OF THE NEW NEW ZEALAND

Metabarcoding with MinION: Pathogenic *Leptospira* in the Environment

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Leptospira

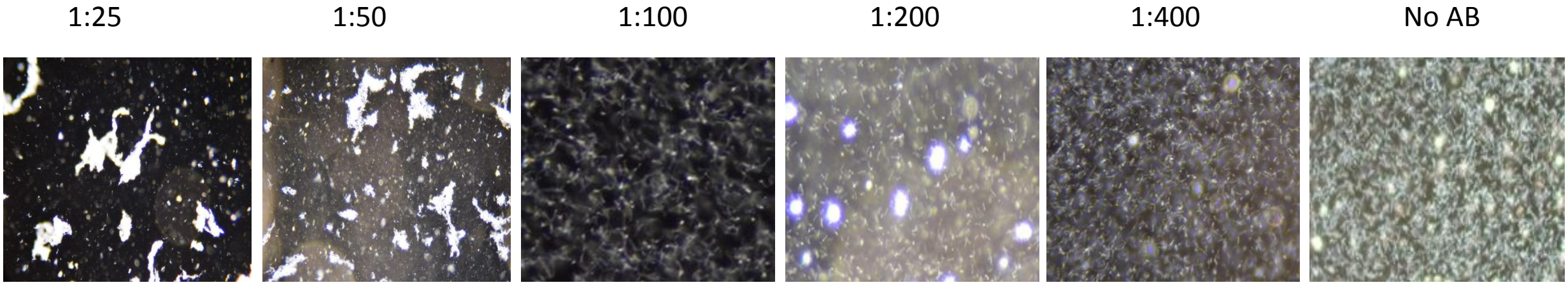
- Causes leptospirosis

- Colonises mammalian kidneys
  - secreted in their urine
  - maintained in the environment

- Diverse genus
  - >35 species (pathogenic, intermediate, saprophytic)
  - Subdivided into serogroups
    - Multiple species in a serogroup
  - >300 serovars worldwide
    - Low genetic diversity between serovars if from same species and same serogroup

Characterisation and Classification of *Leptospira*

- Gold standard test for serovar- Microscopic Agglutination Test (MAT)
  - Dilution of antibody, addition bacteria - reacts with antigens on the surface of the bacteria and causes agglutination



Caveats

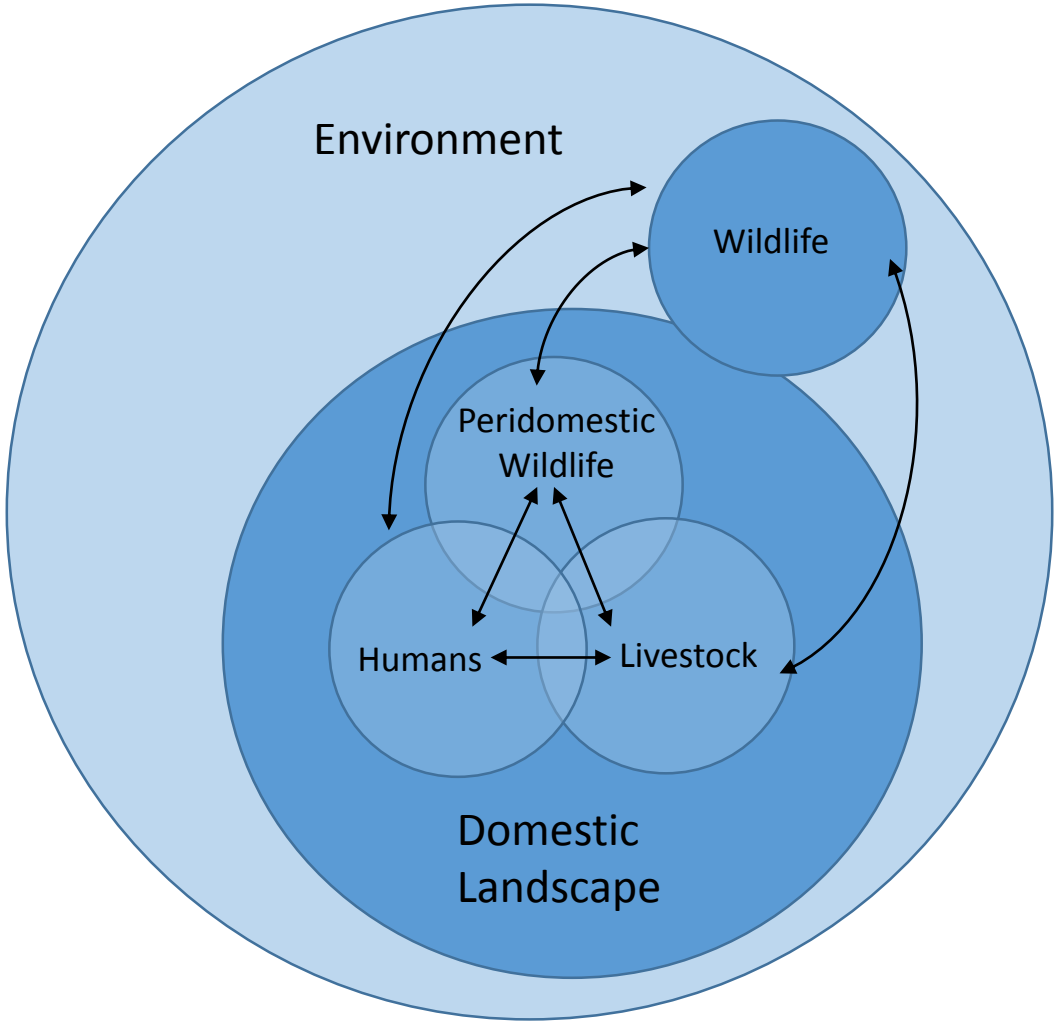
- Highly subjective
- Labour intensive
- Requires knowledge of serovars in circulation
- Cross-reactivity between serovars
  - Two serovars in one serogroup
- Cannot distinguish different species
  - Two species, one serovar
- Difficult to culture
  - maintained in liquid culture
  - Culture only ideal if infected with single type – not ideal for environmental samples (saprophytes)

Situation in New Zealand

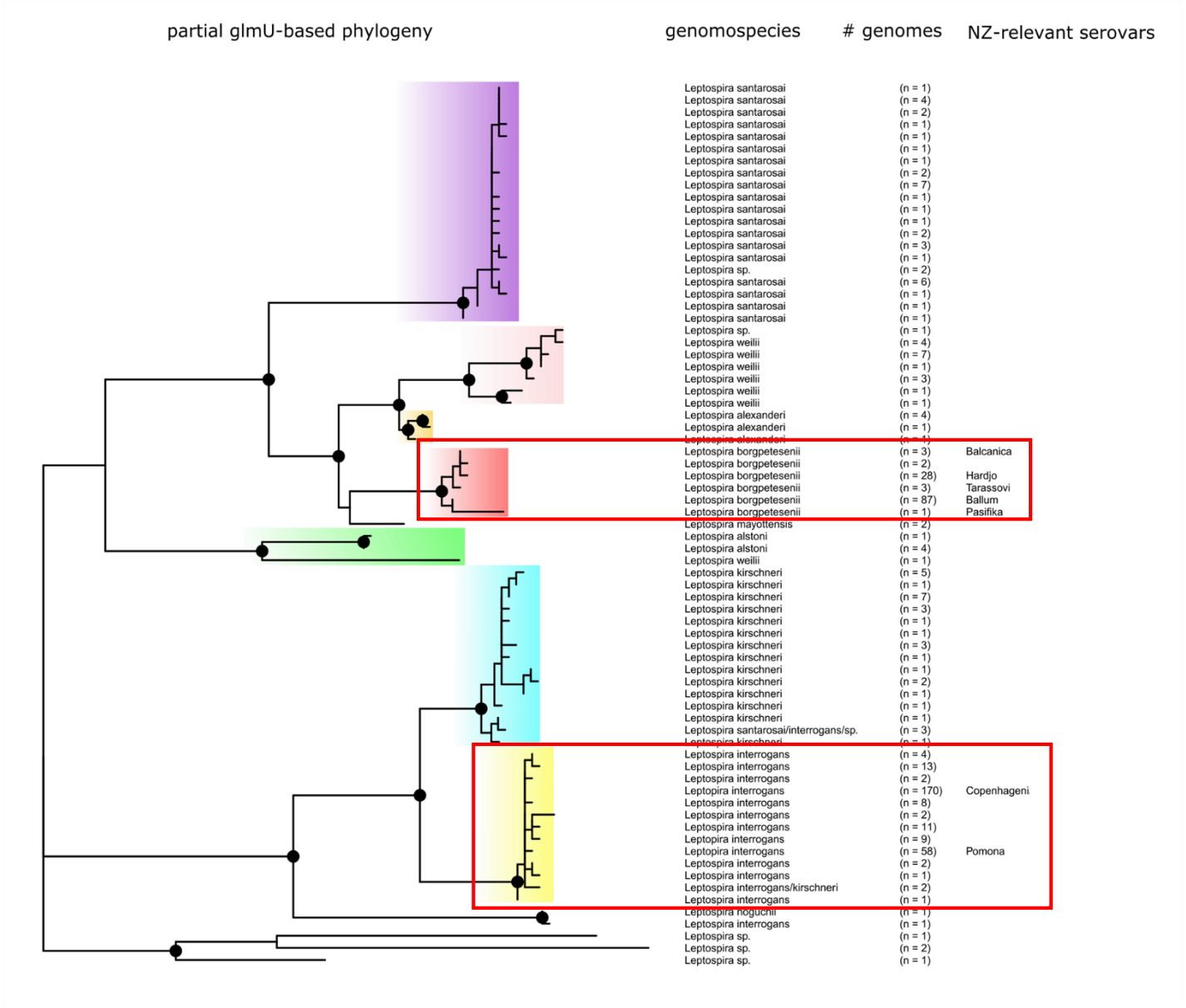
2 species, 5 serogroups, 6 serovars

Species In NZ	Serogroups in NZ	Serovars in NZ	No. of serovars worldwide
L. borgpetersenii	Sejroe	Hardjo Balcanica	21
L. interrogans	Pomona	Pomona	7
L. borgpetersenii	Ballum	Ballum Arborea?	6
L. borgpetersenii	Tarassovi	Tarassovi	26
L. interrogans	Icterohae-morrhagiae	Copenhageni	19

Farm/domestic - cattle, sheep, deer, pigs, dogs
Wild – hedgehogs, mice, rats, possums, deer



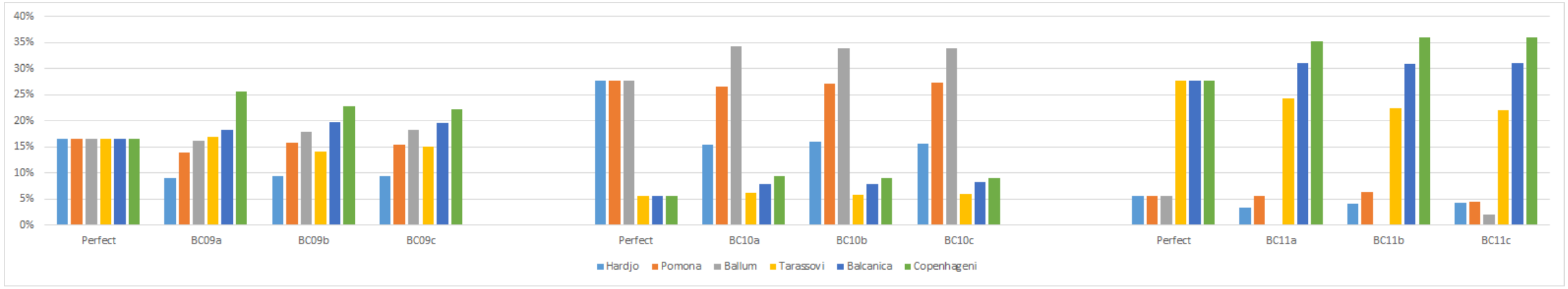
Molecular assay for detecting serovars in NZ



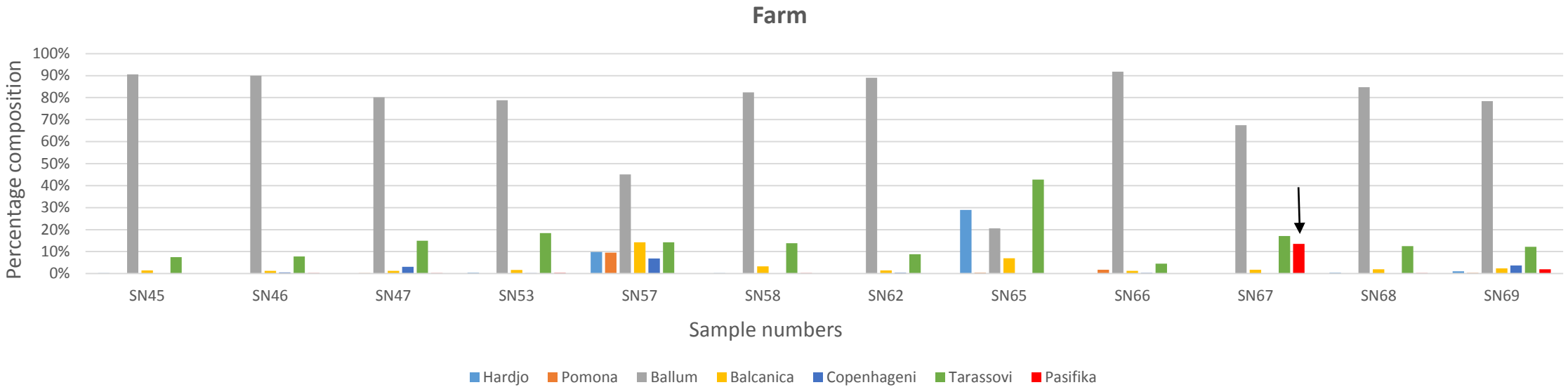
Source: Dr David Wilkinson

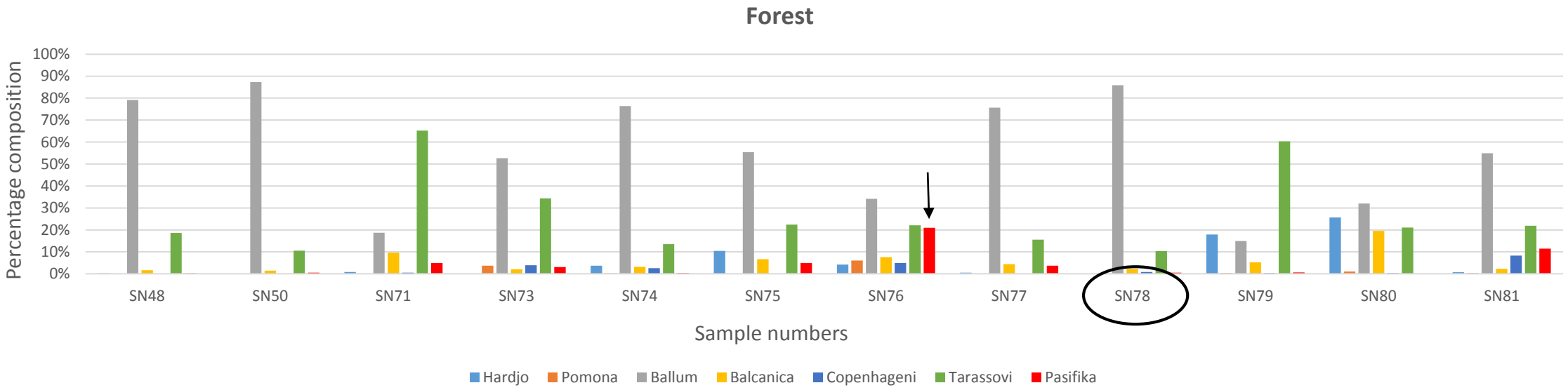
Metabarcoding with MinION

	Hardjo	Pomona	Ballum	Tarassovi	Balcanica	Copenhageni
BC01	70	3	2	11	10	3
BC02	0	92	0	0	0	8
BC03	0	0	95	3	1	0
BC04	0	7	0	0	0	93
BC05	1	0	0	97	1	0
BC06	5	0	1	12	83	0



Pathogenic *Leptospira* in the Environment





Summary

- Established a marker for serotyping using a single locus
  - Effective for known and unknown serovars in New Zealand
  - Could standardise diagnostics across medical, veterinary and environmental field New Zealand - useful tool for One Health research
  - Can be used on mixed population with barcoding
    - with varying levels of specificity when using MinION
    - recommend Illumina
- First study to culture and discriminate pathogenic *Leptospira* in New Zealand ground water

Pathogenic species in the NZ environment	Serovars
Leptospira borgpetersenii	Hardjo, Ballum, Tarassovi, “Pasifika”
Leptospira interrogans	Pomona? Copenhageni?
Leptospira santarosai	?
Leptospira alstoni	?

- Other species

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Thank you

Massey University Leptospirosis Research Group