Trich-tracker: a tool to trace *Trichinella* outbreaks

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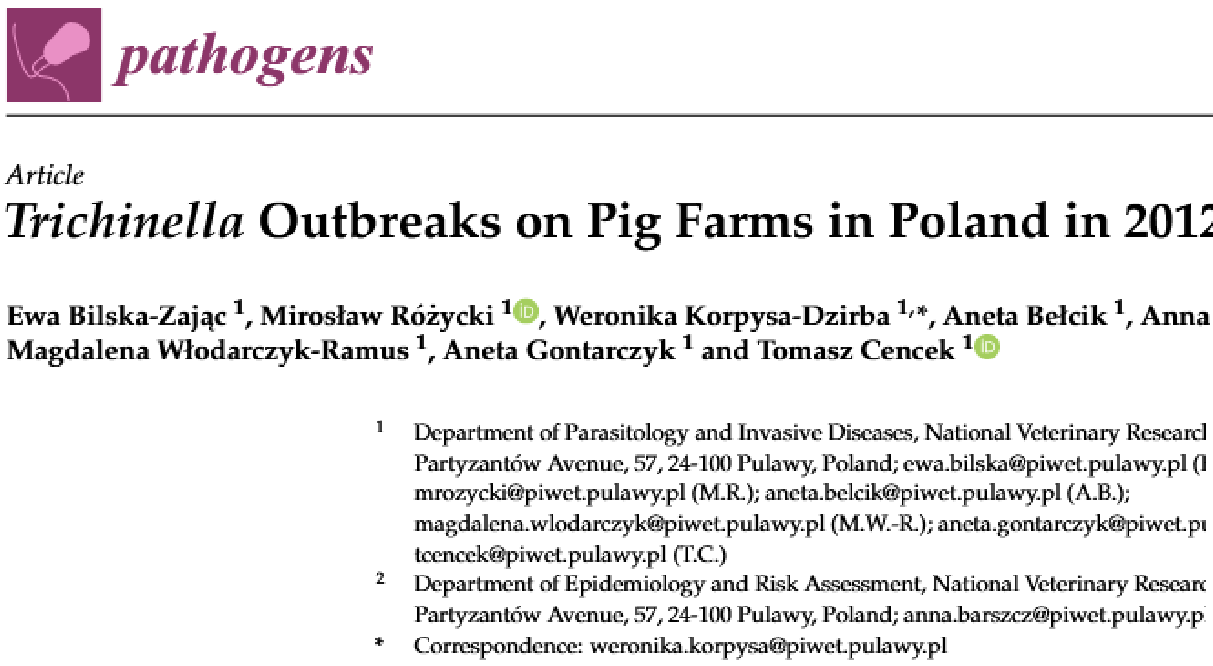

pathogens
MDPI

Article

Trichinella Outbreaks on Pig Farms in Poland in 2012–2020

Ewa Bilska-Zając [1], Mirosław Różycki [1], Weronika Korpysa-Dzirba [1,*], Aneta Bełcik [1], Anna Ziętek-Barszcz [2], Magdalena Włodarczyk-Ramus [1], Aneta Gontarczyk [1] and Tomasz Cencek [1]

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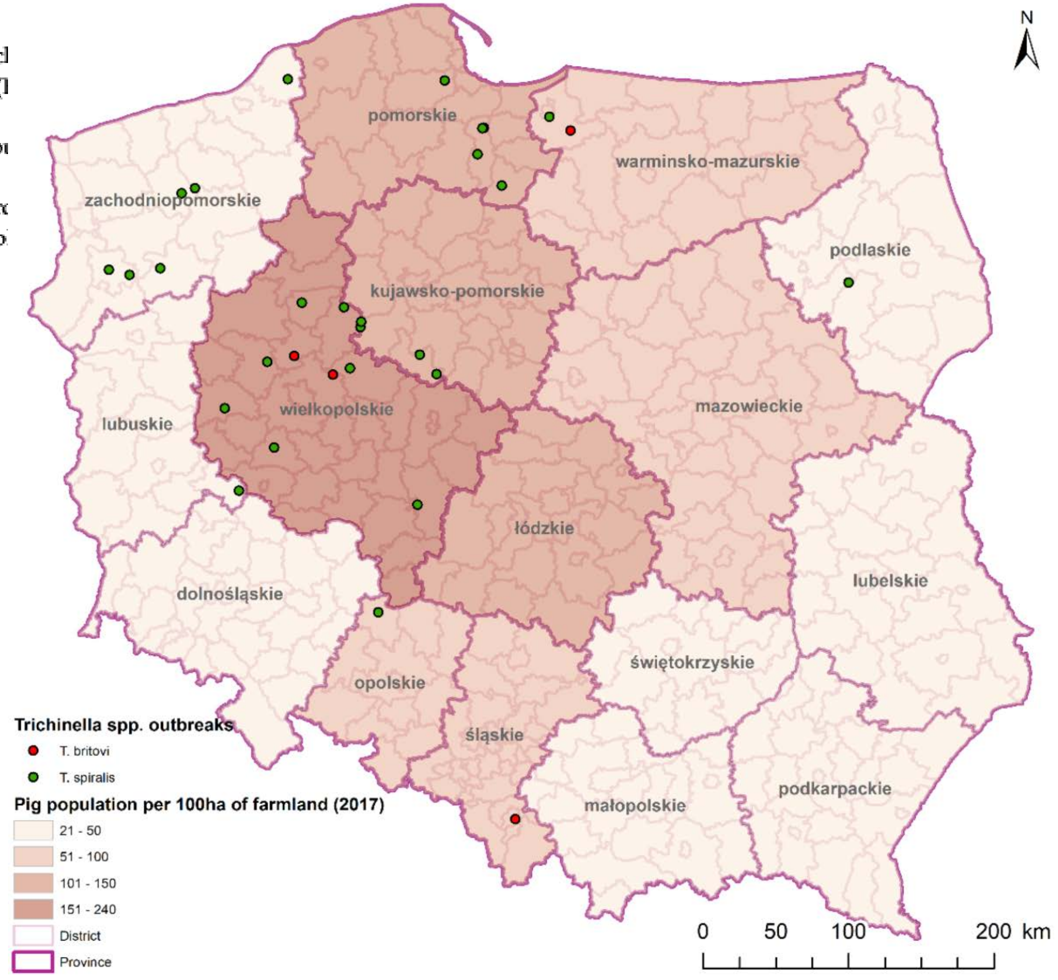

● 26 *T. spiralis*

● 4 *T.britovi*

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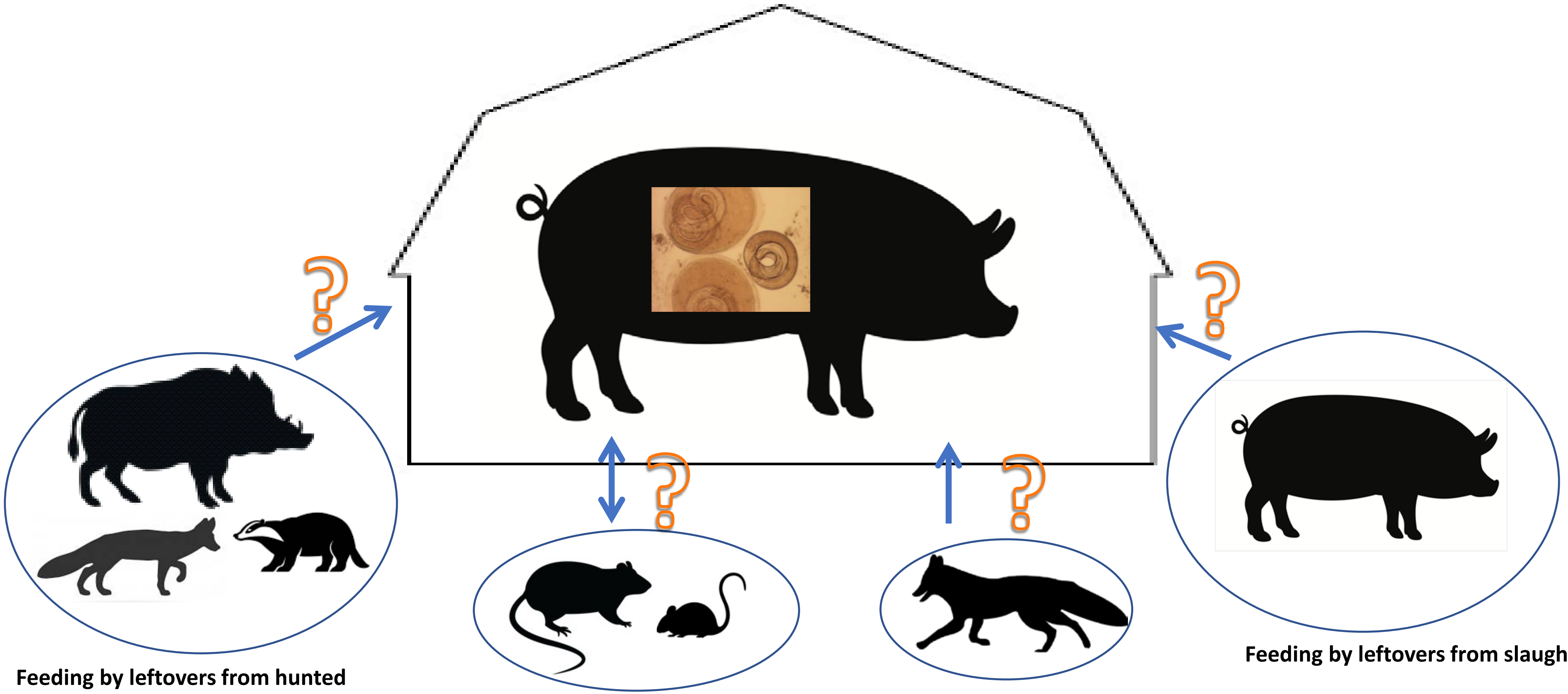
Epidemiological investigation in *Trichinella* outbreak – what is the source infection?

Veterinary Service is obliged to conduct epidemiological investigation to find source of *Trichinella* infection in pigs and to eliminate it.



Feeding by leftovers from hunted animals

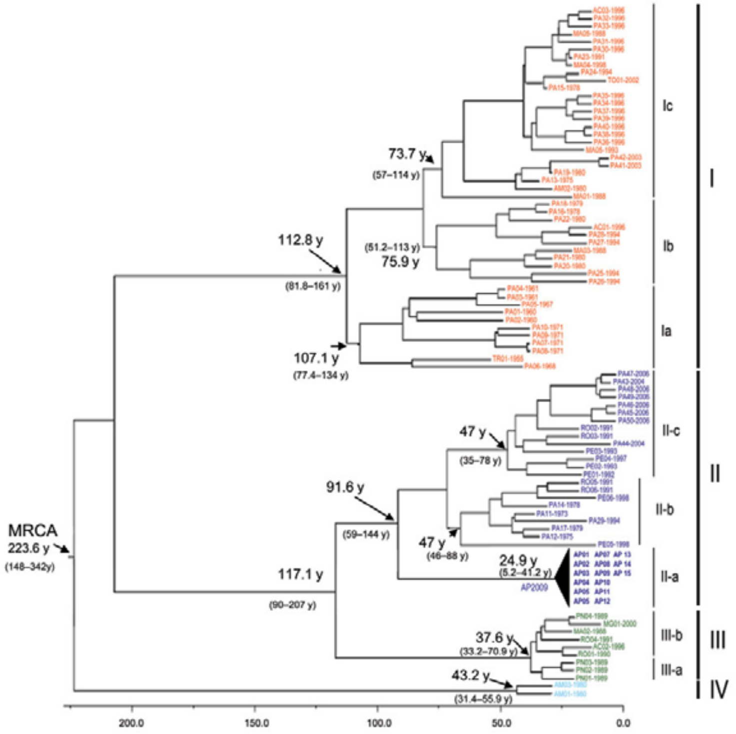
Vectors from natural environment

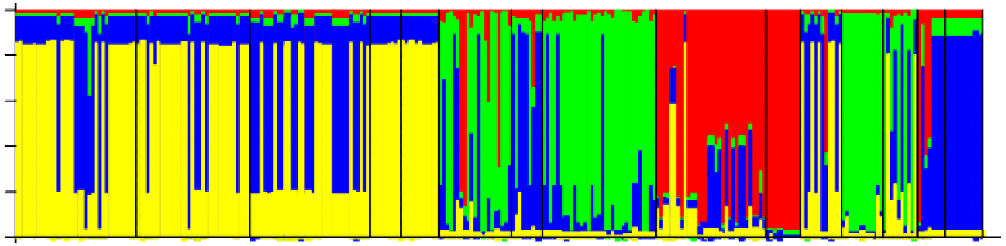
Feeding by carcasses from breeding animals

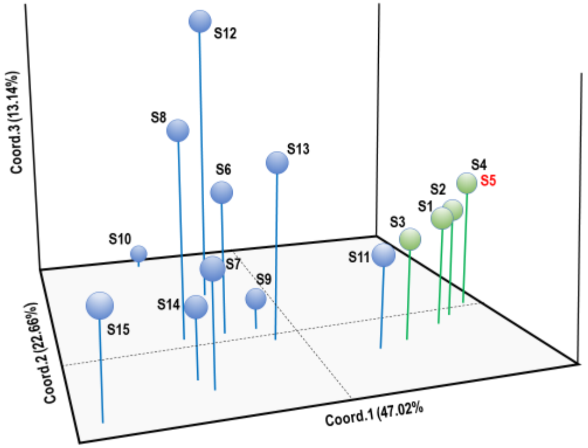
Feeding by leftovers from slaughtering

Molecular epidemiology

- Use DNA sequence to separate distinct sources





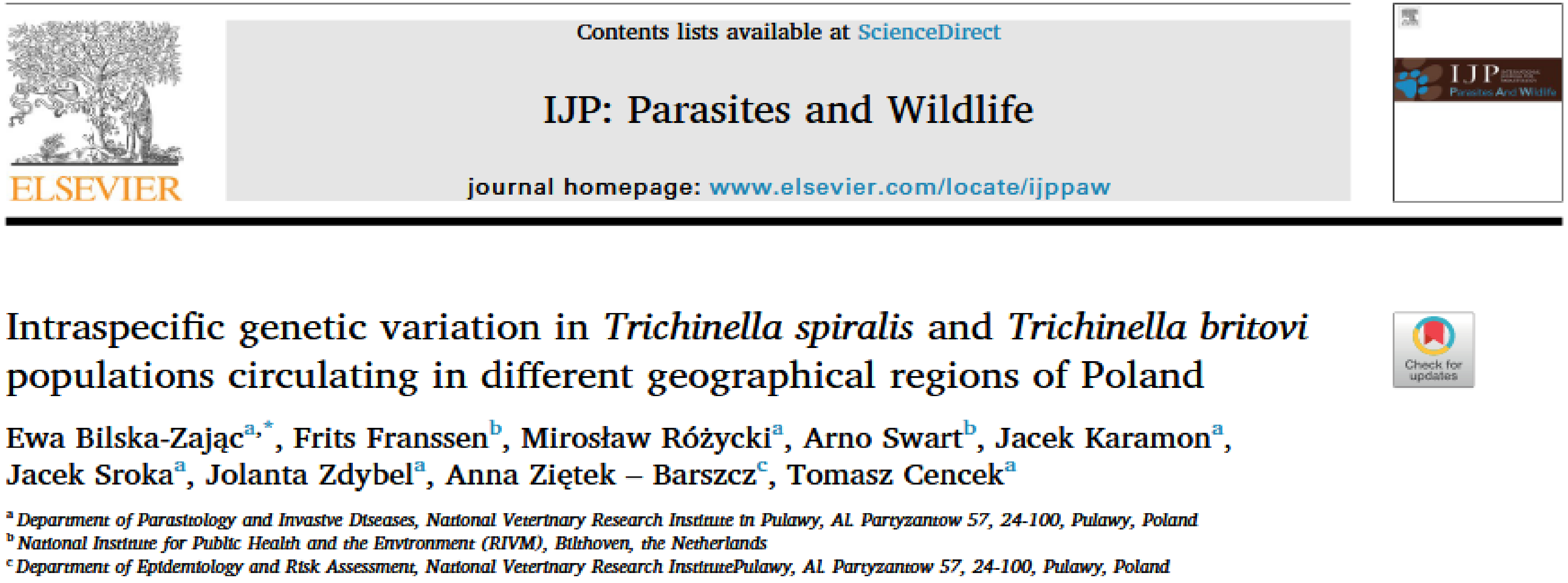


But what markers?

- Singular fragments of gene?

to less information in case of inbreed *T.spiralis*

Only two genotypes…



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ELSEVIER

Intraspecific genetic variation in *Trichinella spiralis* and *Trichinella britovi* populations circulating in different geographical regions of Poland

Check for updates

Ewa Bilska-Zając[a,*], Frits Franssen[b], Mirosław Różycki[a], Arno Swart[b], Jacek Karamon[a], Jacek Sroka[a], Jolanta Zdybel[a], Anna Ziętek – Barszcz[c], Tomasz Cencek[a]

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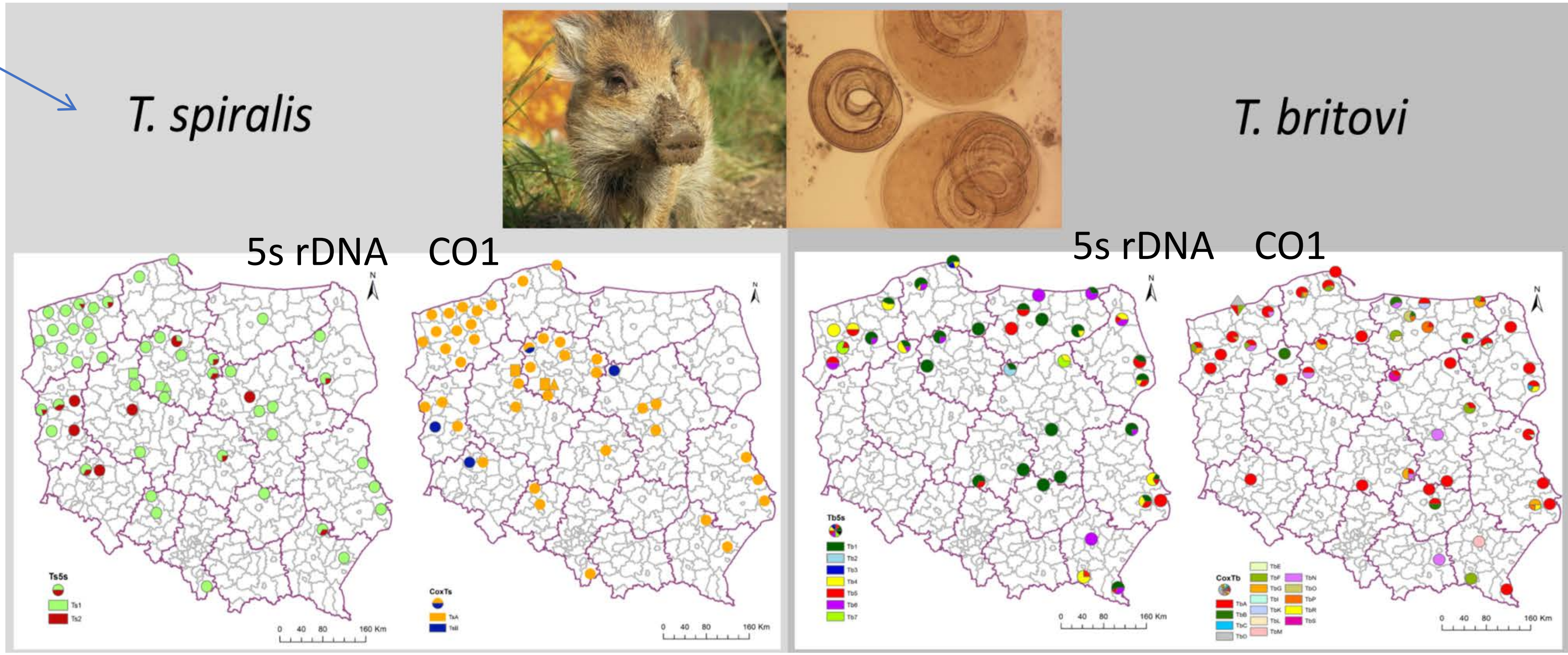
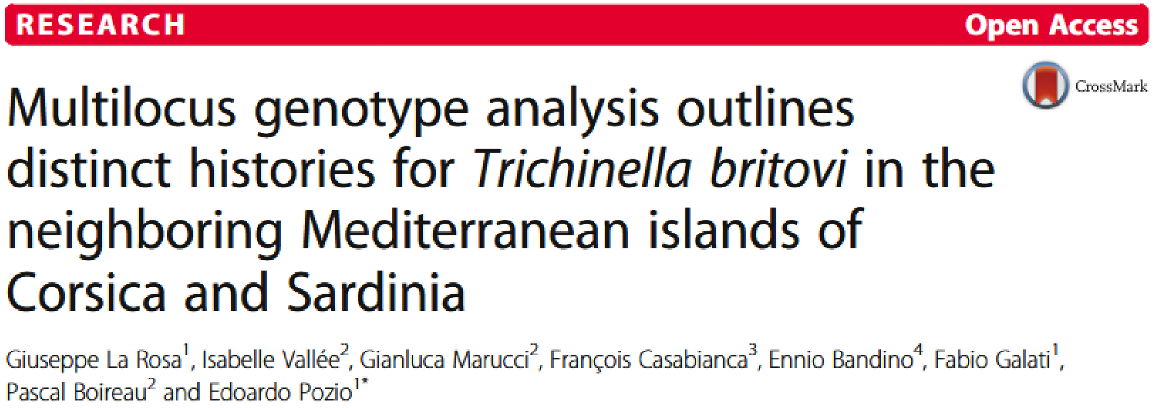


But what markers?

Microsatellites? – proved to be good in case of *T.britovi* and even homogenic *T.spiralis*
– but very time consuming !!!


RESEARCH **Open Access**

Multilocus genotype analysis outlines distinct histories for *Trichinella britovi* in the neighboring Mediterranean islands of Corsica and Sardinia

Giuseppe La Rosa[1], Isabelle Vallée[2], Gianluca Marucci[2], François Casabianca[3], Ennio Bandino[4], Fabio Galati[1], Pascal Boireau[2] and Edoardo Pozio[1*]


RESEARCH **Open Access**

Genetic evidence substantiates transmission of *Trichinella spiralis* from one swine farm to another

Ewa Bilska-Zajac[1], Daniele Tonanzi[2], Edoardo Pozio[2], Miroslaw Rozycki[1], Tomasz Cencek[1], Peter C. Thompson[3], Benjamin M. Rosenthal[3] and Giuseppe La Rosa[2*]

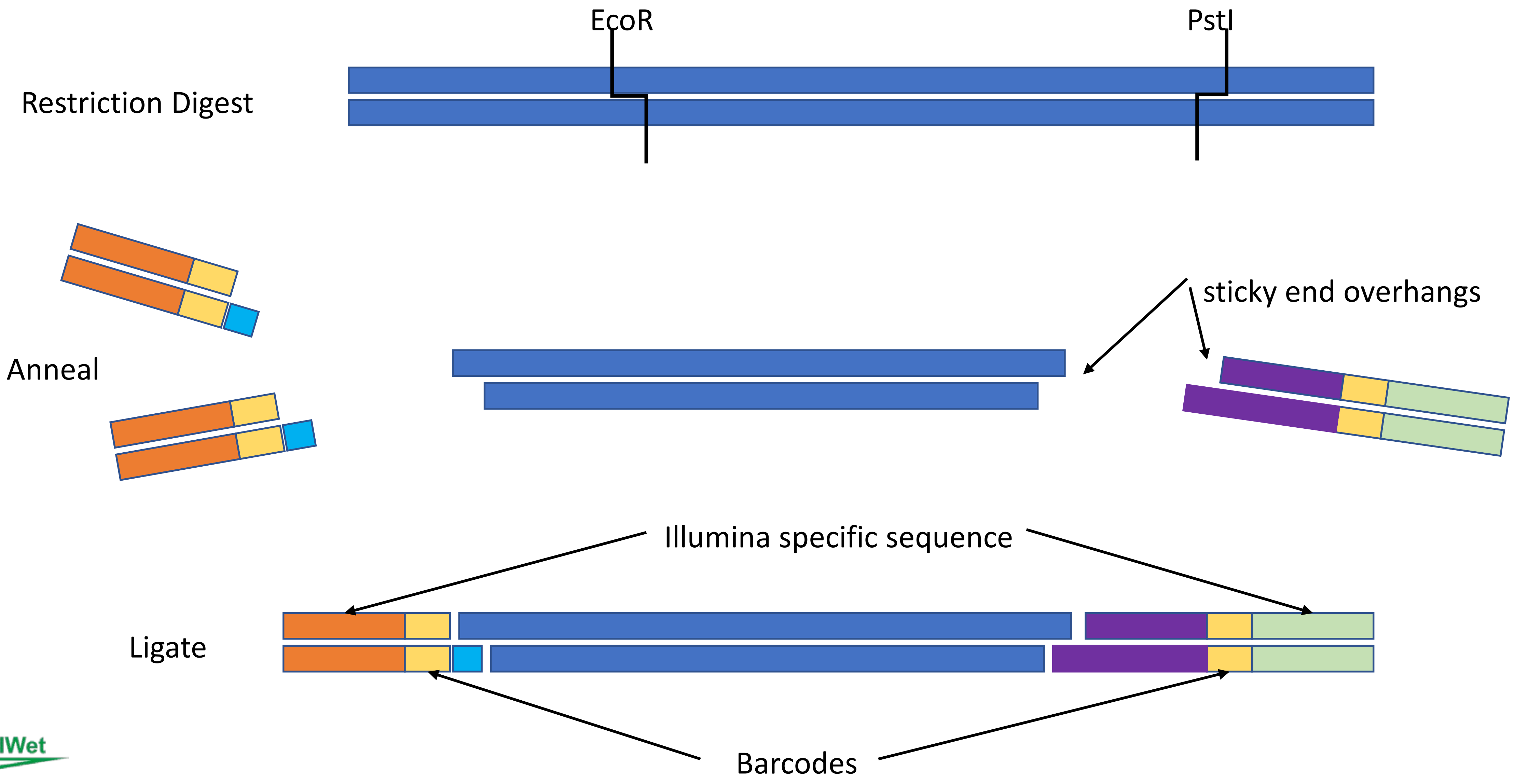
But what markers?

- Whole Genome Sequencing???
- Expensive, time consuming, too much data to process…

It is best to design the method according to your needs

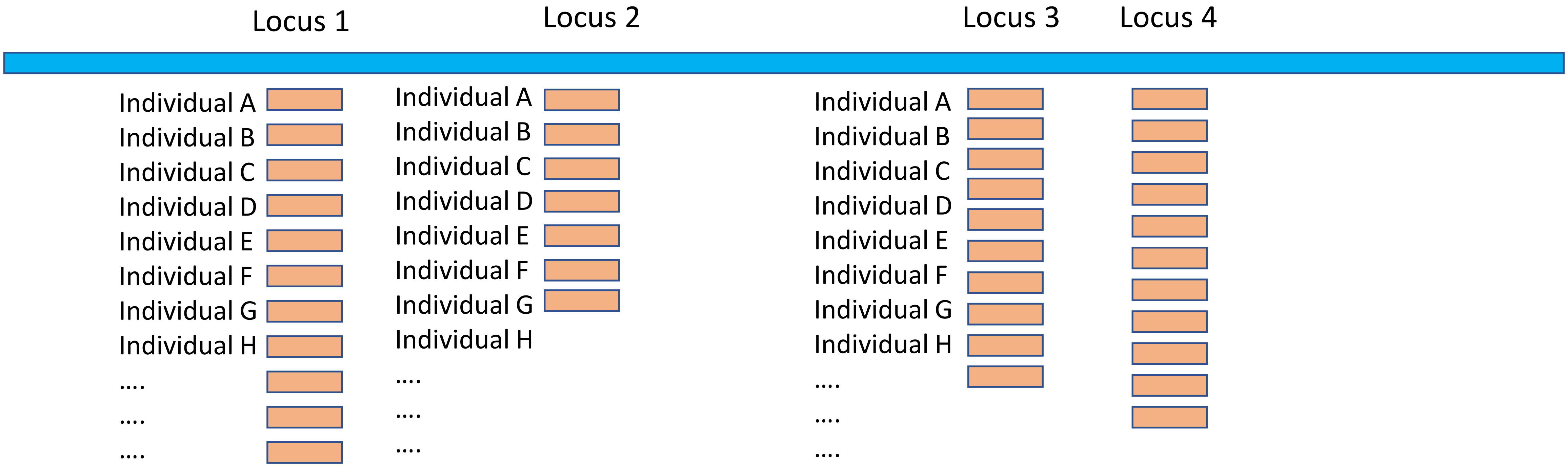
- Reduced representation libraries
  - Sample limited amounts of DNA from multiple places in the genome
  - Restriction-site Associated DNA sequencing - **RADseq**
  - It is method which was tested and working properly on non-model organisms, therefore we thought it would be an adequate technique for *Trichinella spiralis.*

Method – double digest RADseq



Method – double digest RADseq

- Individual reads will line up with specific locus in a genome
- Loci should be distributed randomly across the chromosomes



Locus 1

Individual A
Individual B
Individual C
Individual D
Individual E
Individual F
Individual G
Individual H
....
....
....

Locus 2

Individual A
Individual B
Individual C
Individual D
Individual E
Individual F
Individual G
Individual H
....
....
....

Locus 3

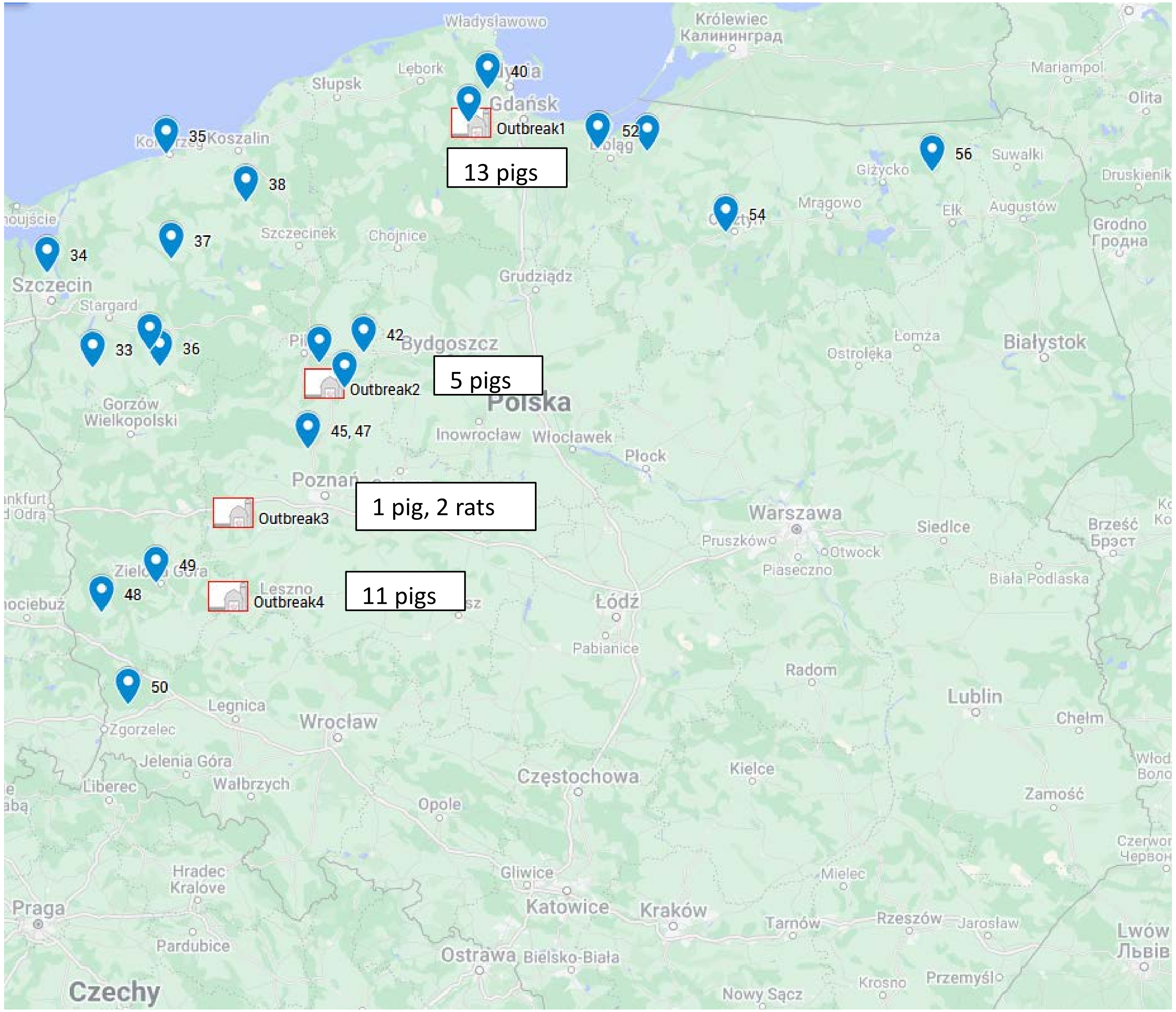
Locus 4

Individual A
Individual B
Individual C
Individual D
Individual E
Individual F
Individual G
Individual H
....
....
....

Material - *Trichinella spiralis* isolates

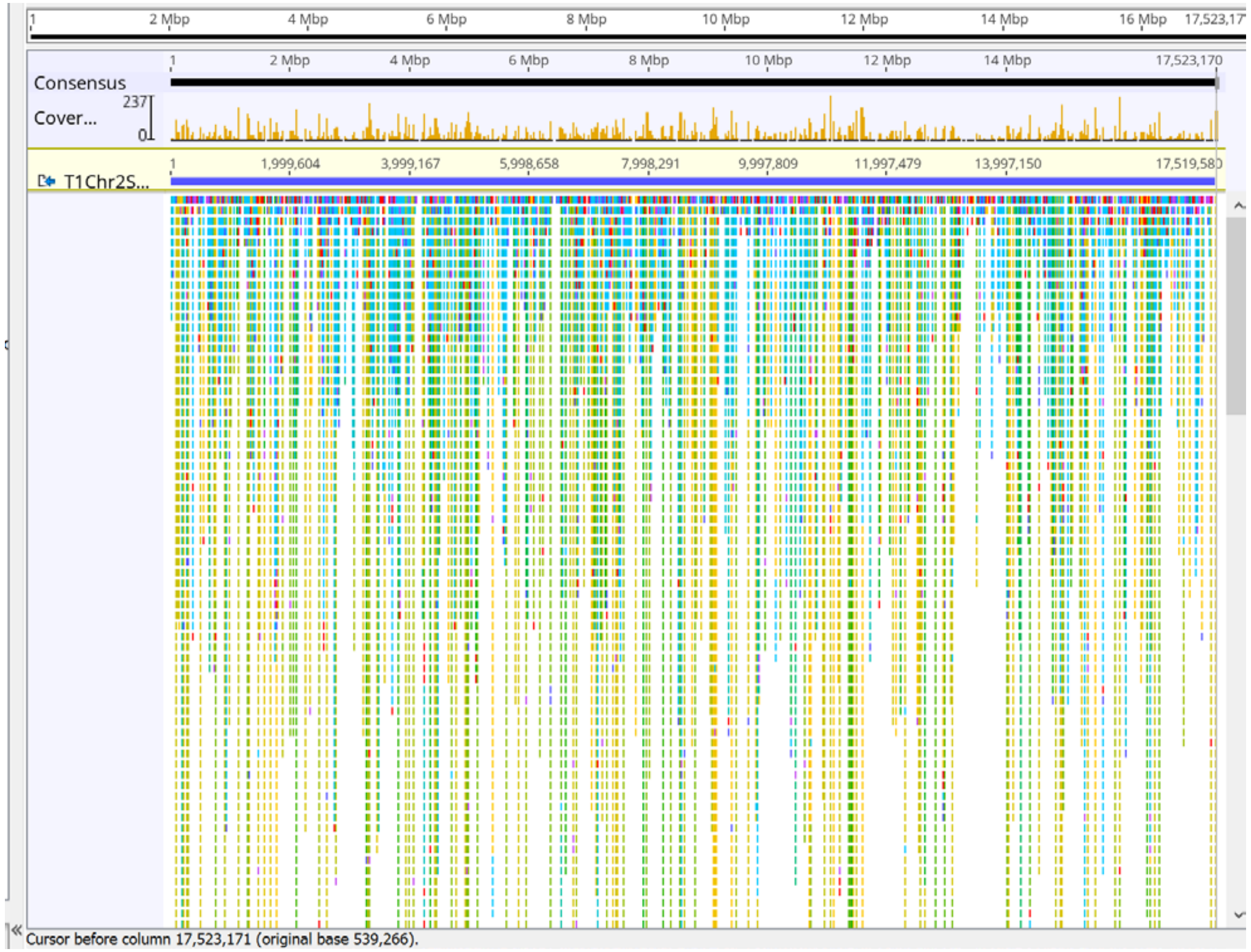
4 outbreaks
(30 pigs and 2 rats)

24 wild boars



Data generation

- Double-digest RADseq
- Libraries sequenced on Illumina MiSeq platform
- 150 bp paired-end reads
- Average: 750,000 reads/sample



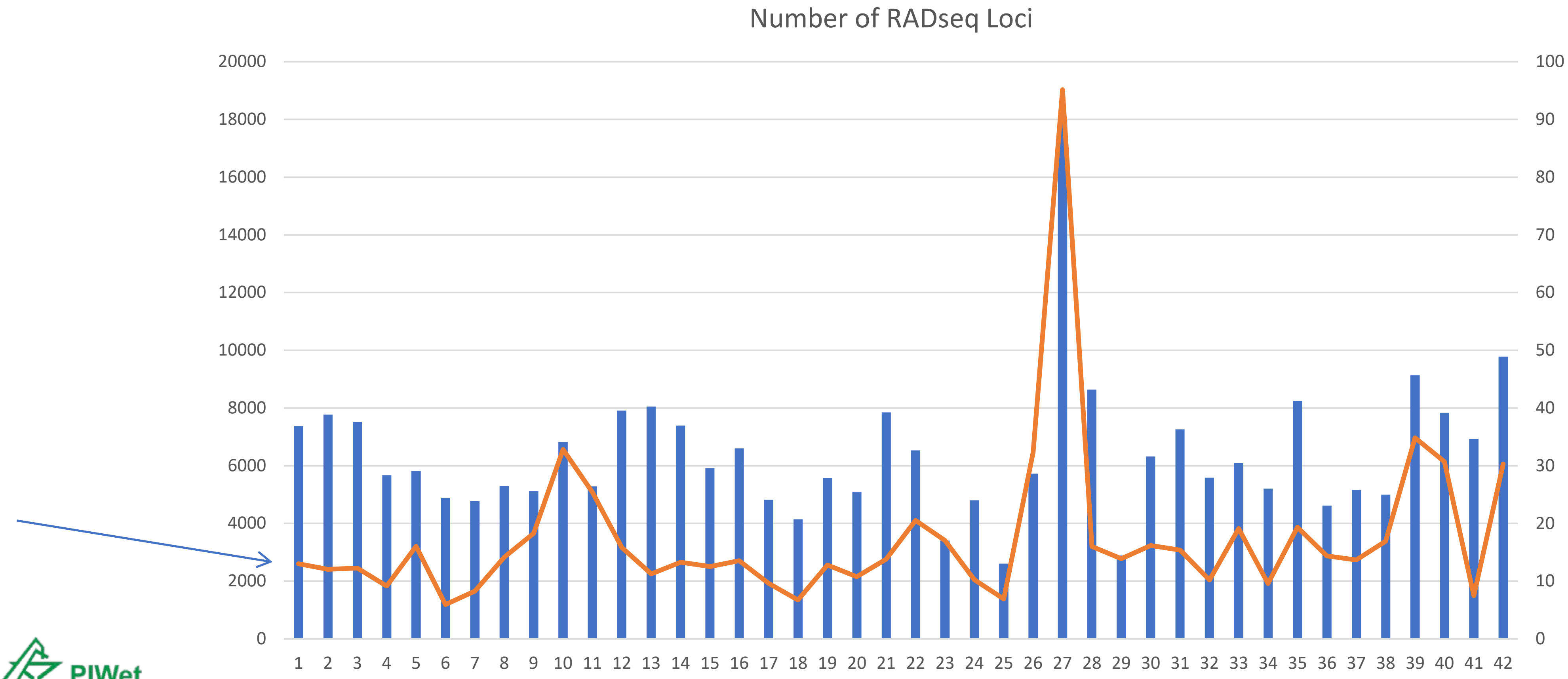
Cursor before column 17,523,171 (original base 539,266).

Distribution of RADseq reads mapped to reference sequence

Results

Good number of loci and depth of coverage

- 2000 loci across the genome in each sample - 7x coverage.



Results

Final dataset after bioinformatic processing

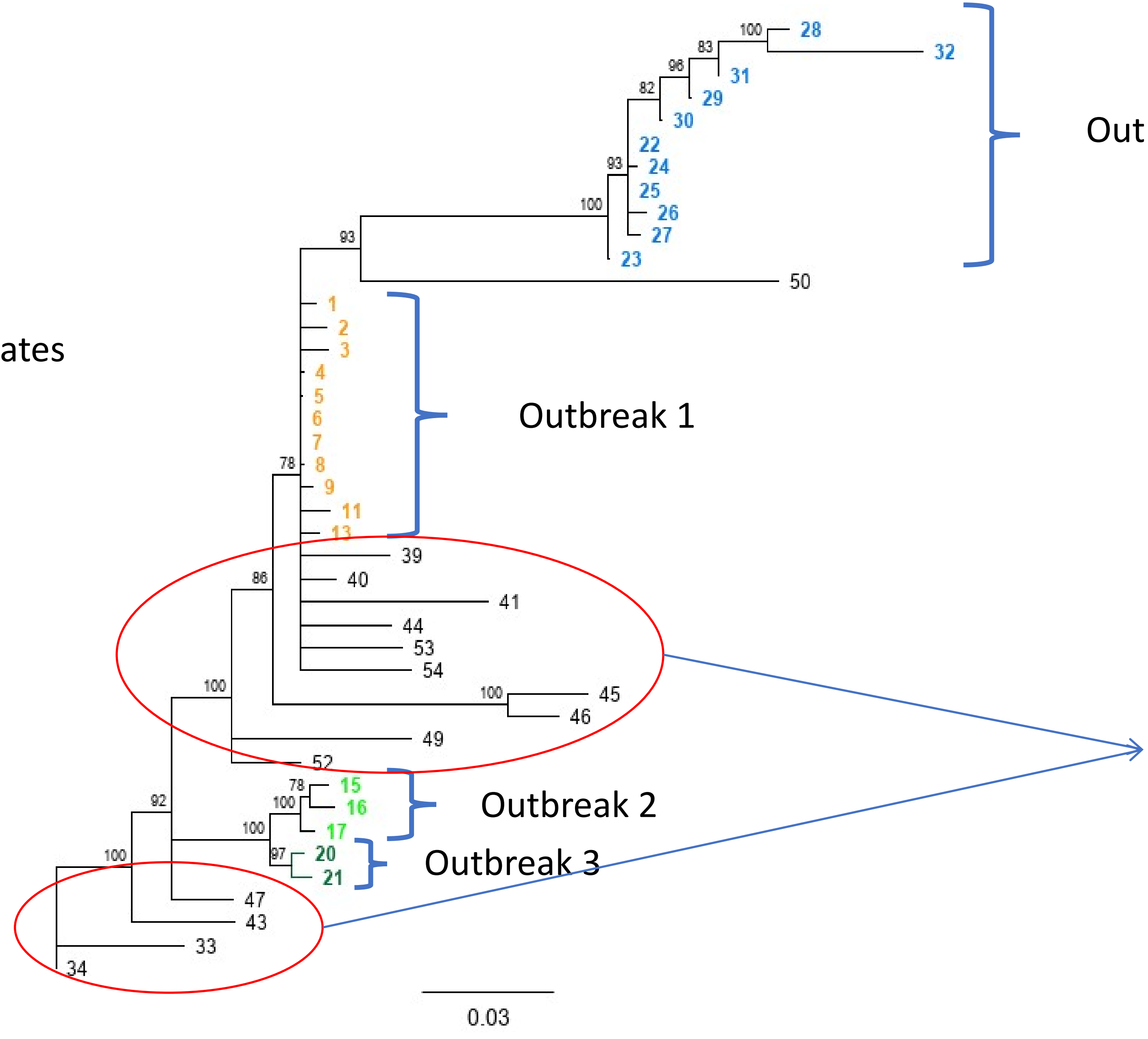
- RADseq data of *T. spiralis* from 42 hosts
    - 26 pigs
    - 1 rat
    - 15 wild boars

Analyzed only the loci present in every sample

- 1331 loci distributed across genome
- ~150000 bp of sequence per sample
- Total **299 Single Nucleotide Polymorphisms (SNPs)**

Results

Neighbor-joining tree
Tamura-Nei distance
1000 bootstrap replicates



Outbreak 4

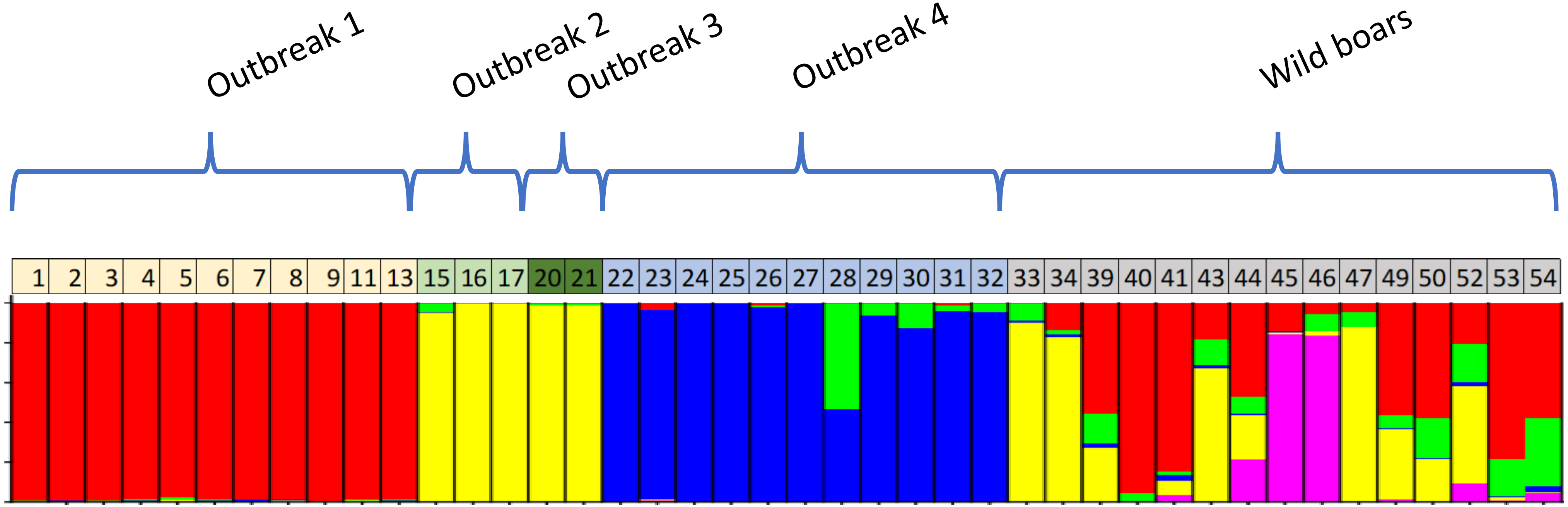
Outbreak 1

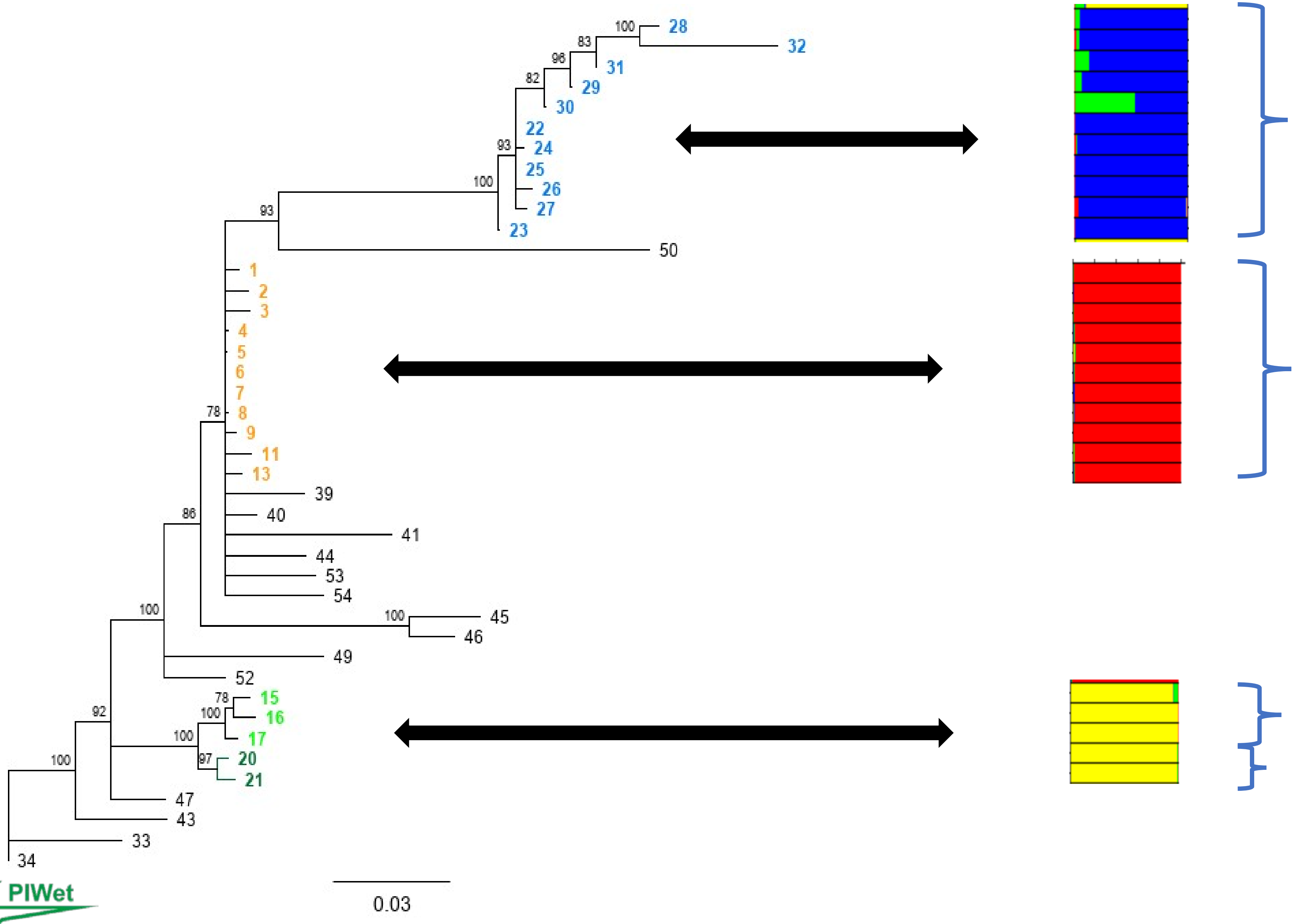
Outbreak 2

Outbreak 3

Isolates from **wild boars**

Results


Outbreak 1
Outbreak 2
Outbreak 3
Outbreak 4
Wild boars
1 2 3 4 5 6 7 8 9 11 13 15 16 17 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 39 40 41 43 44 45 46 47 49 50 52 53 54



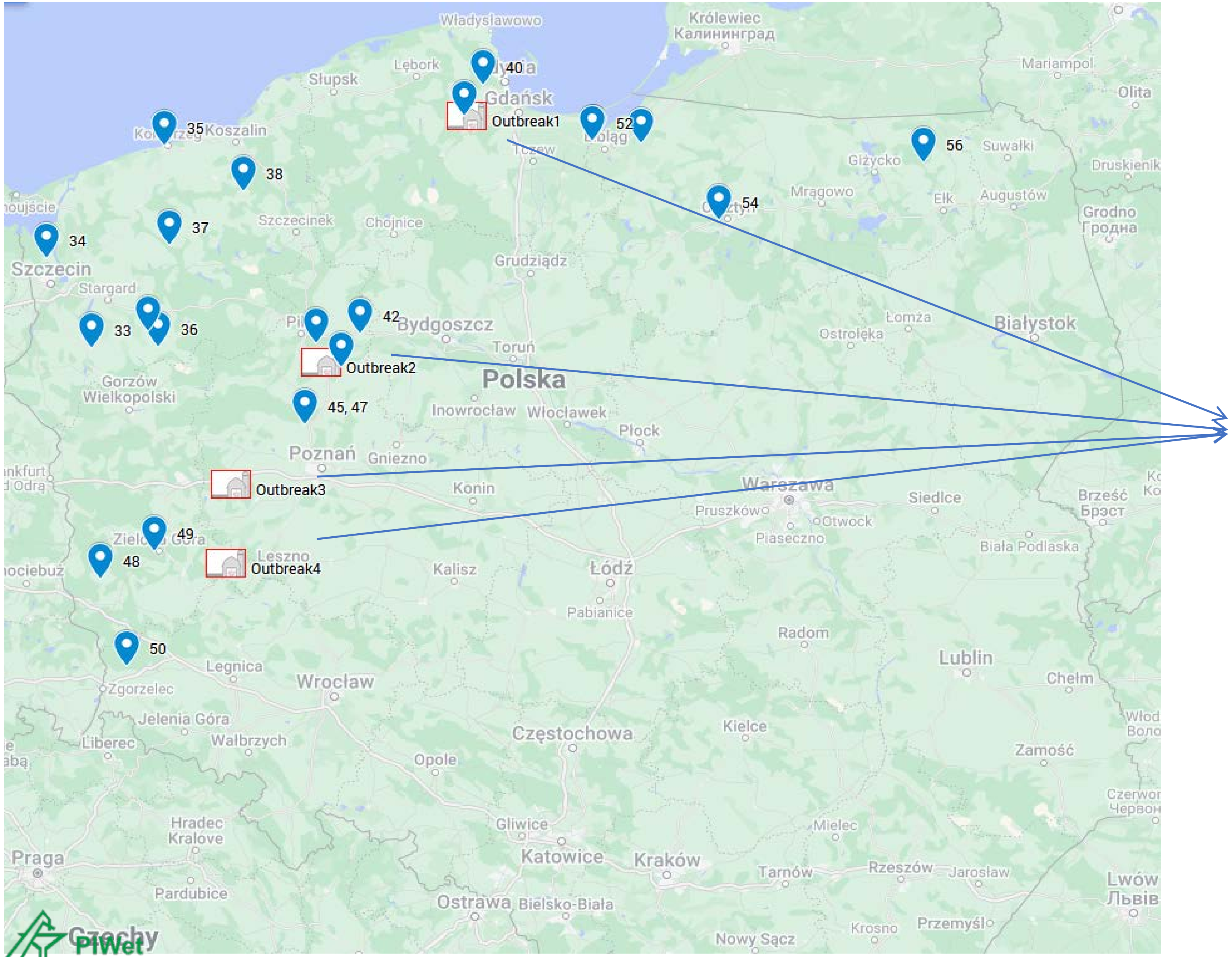
Outbreak 4

Outbreak 1

Outbreak 2

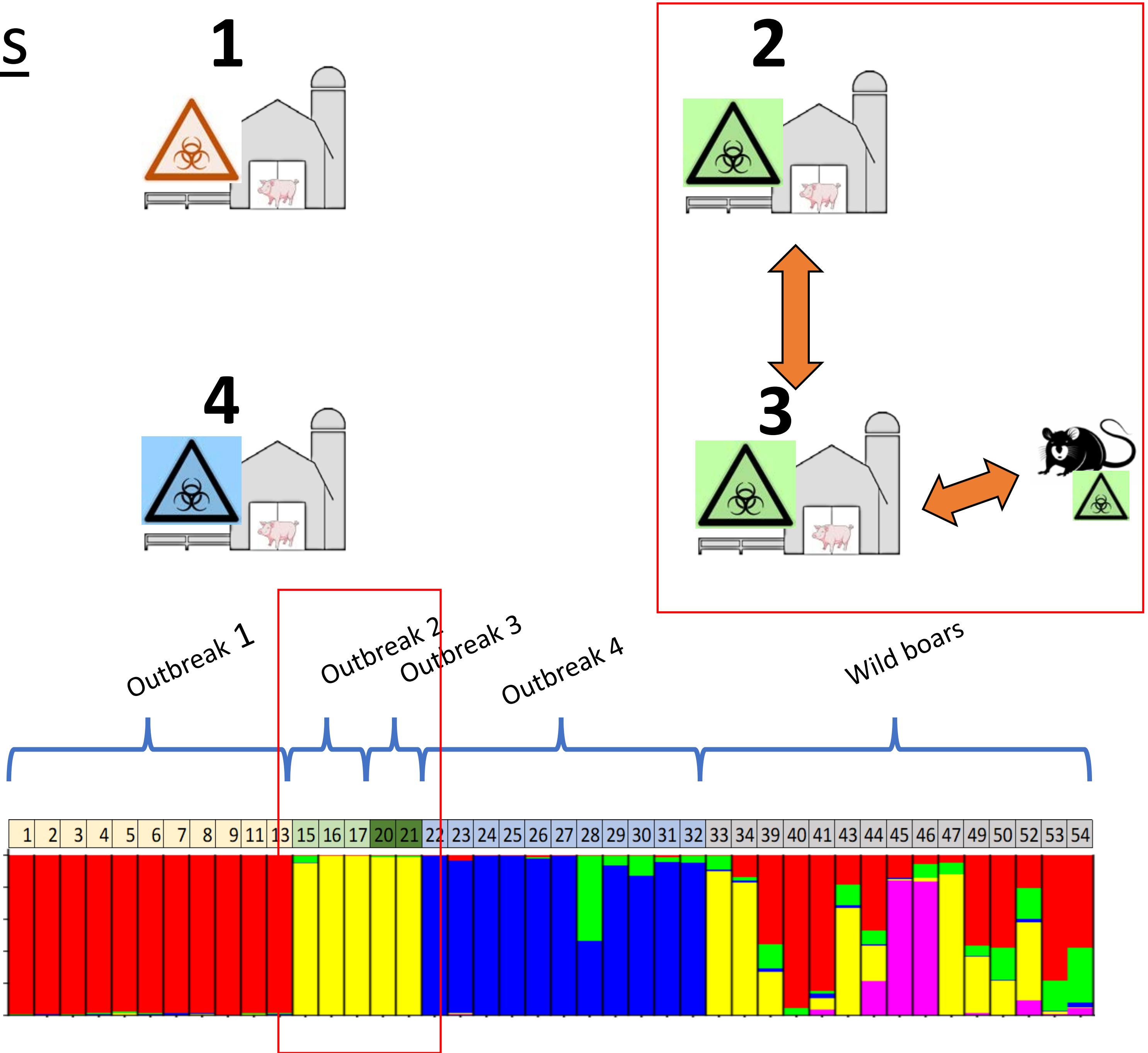
Outbreak 3

PIG outbreaks

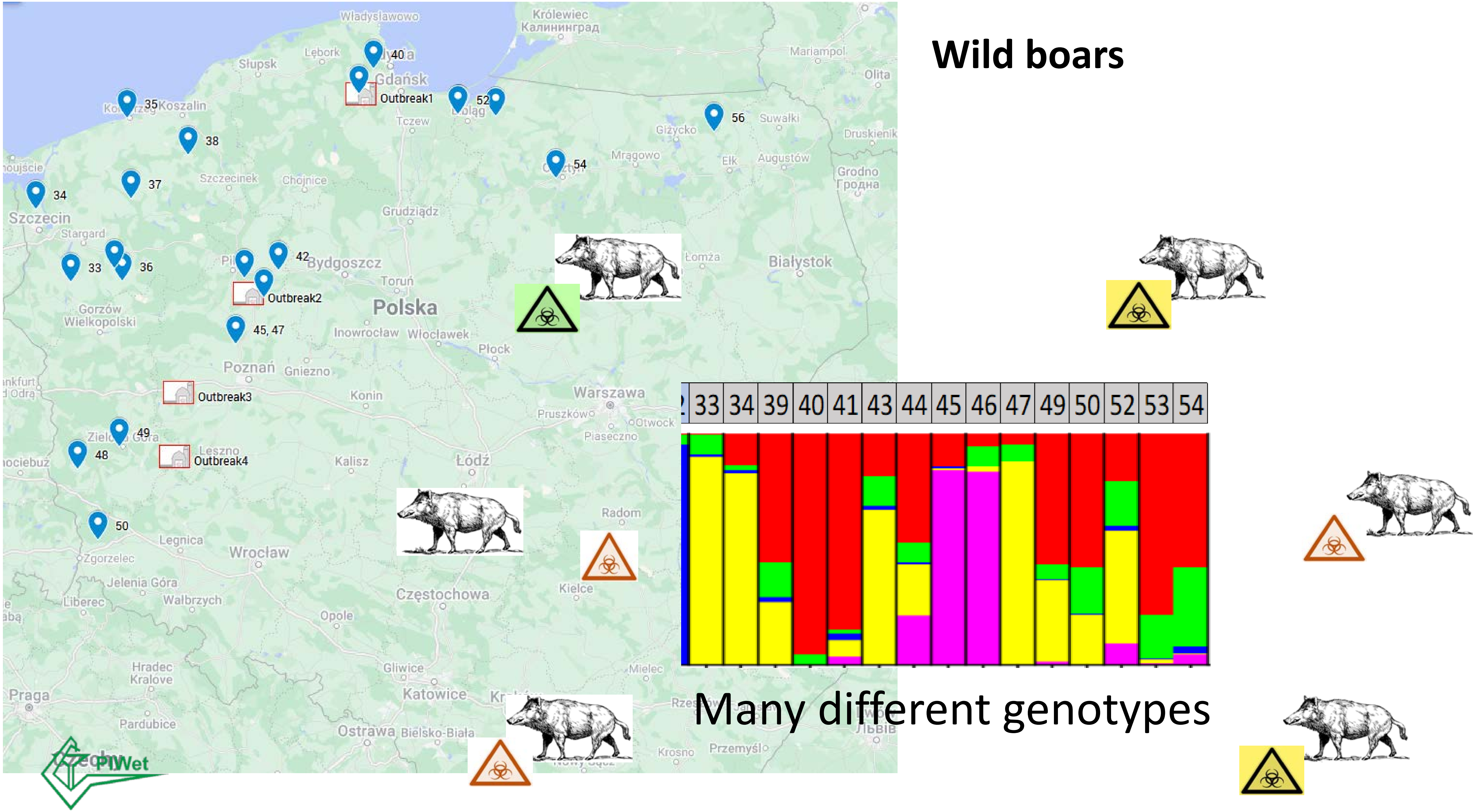


Is there a connection between analyzed outbreaks?

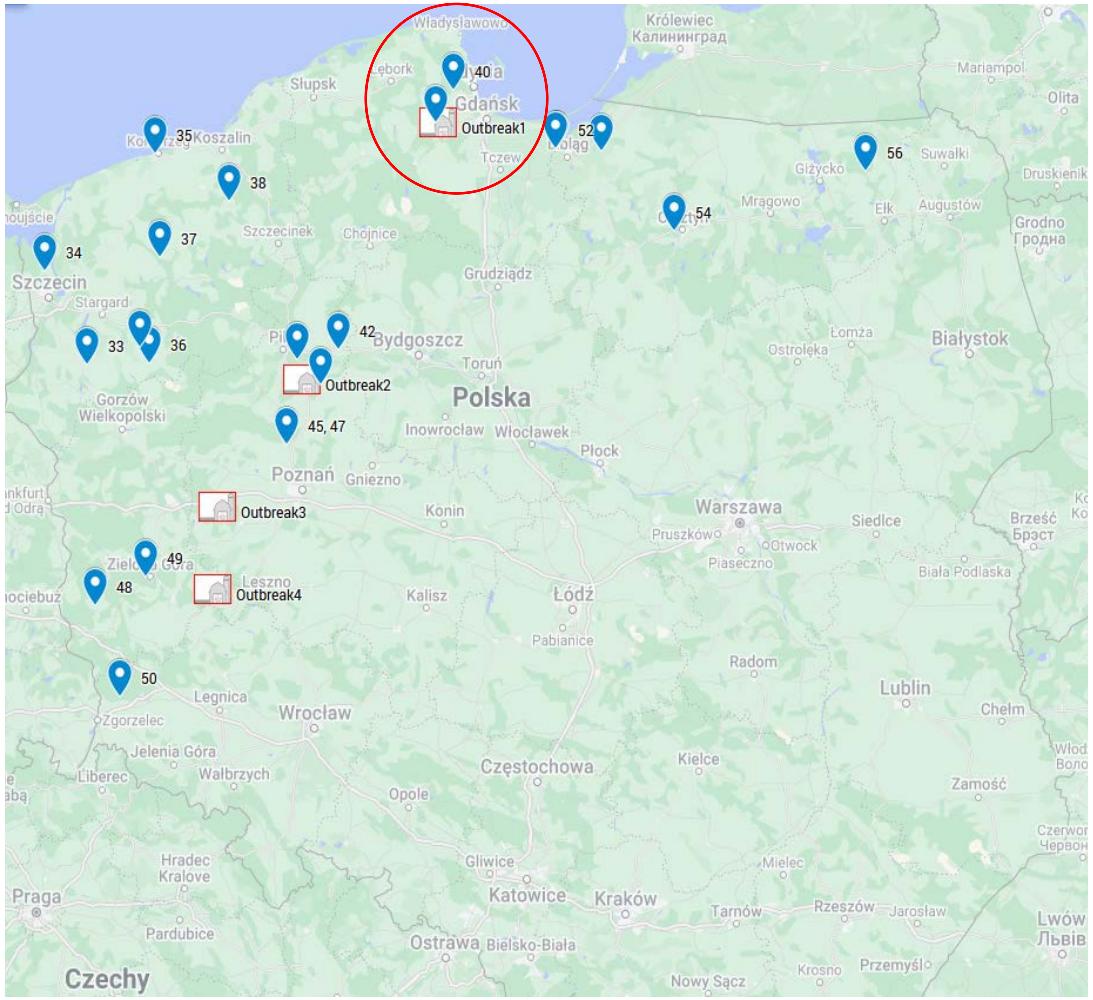
PIG outbreaks

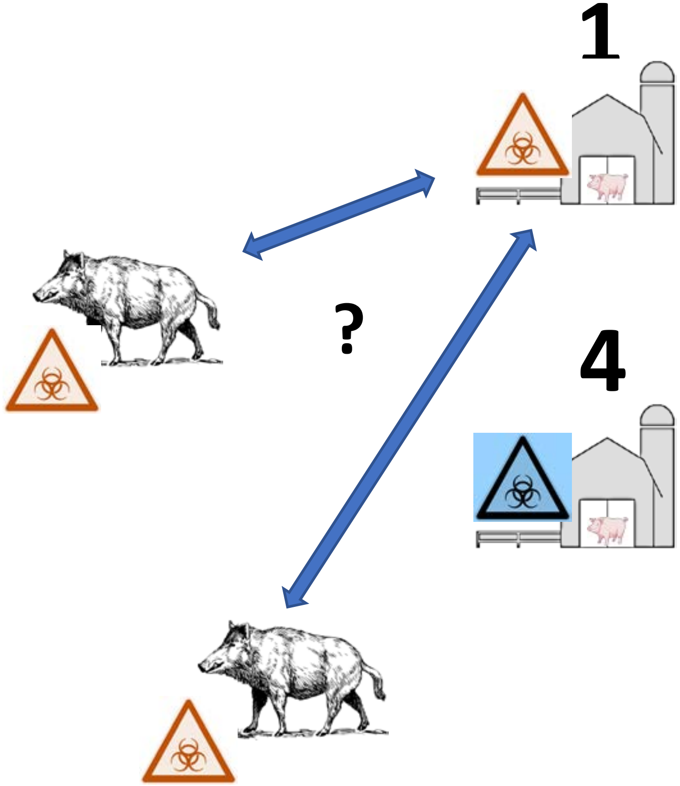


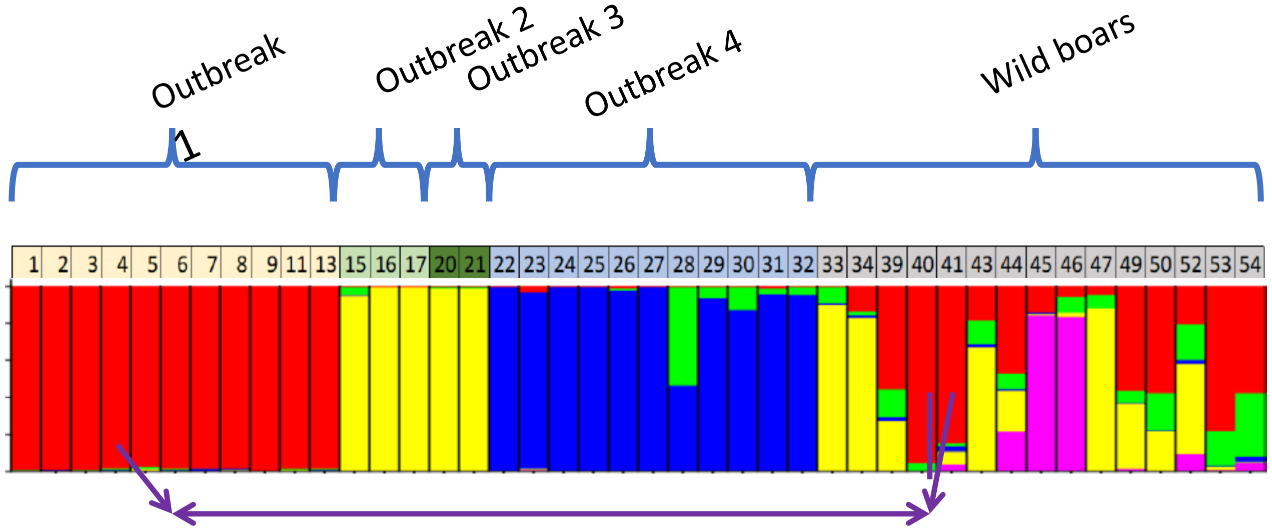
Wild boars



Many different genotypes


40
Outbreak1
35
52
56
38
54
37
34
33
36
42
Outbreak2
45, 47
Outbreak3
49
48
Outbreak4
50
Gdańsk
Bydgoszcz
Polska
Poznań
Warszawa
Szczecin
Wrocław
Łódź
Leszno


1
?
4


Outbreak 1
Outbreak 2
Outbreak 3
Outbreak 4
Wild boars
1 2 3 4 5 6 7 8 9 11 13 15 16 17 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 39 40 41 43 44 45 46 47 49 50 52 53 54

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“Trich-tracker” possible to use in ongoing outbreak

Extract DNA from Samples **1 Day**

ddRADseq Library preparation **2 Days**

Library sequencing **2 Days**

Data analysis **3-4 Days**

Conclusions

- Trich-tracker is a useful tool for tracing *T.spiralis* outbreaks
- This method balances the need for more sequence with the cost of sequencing entire genomes.

Aplications

- Outbreak investigation, source identification, isolates differentiation, tracking parasite…
- ***The method is flexible and can be used in any parasite system***

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Trich-tracker – a practical tool to trace *Trichinella spiralis* transmission based on rapid, cost-effective sampling of genome-wide genetic variation ☆

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USDA United States Department of Agriculture

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