Using PRRSV classification to improve our tracking abilities

使用PRRSV分类来提高我们的跟踪能力

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14th Leman China Swine Conference
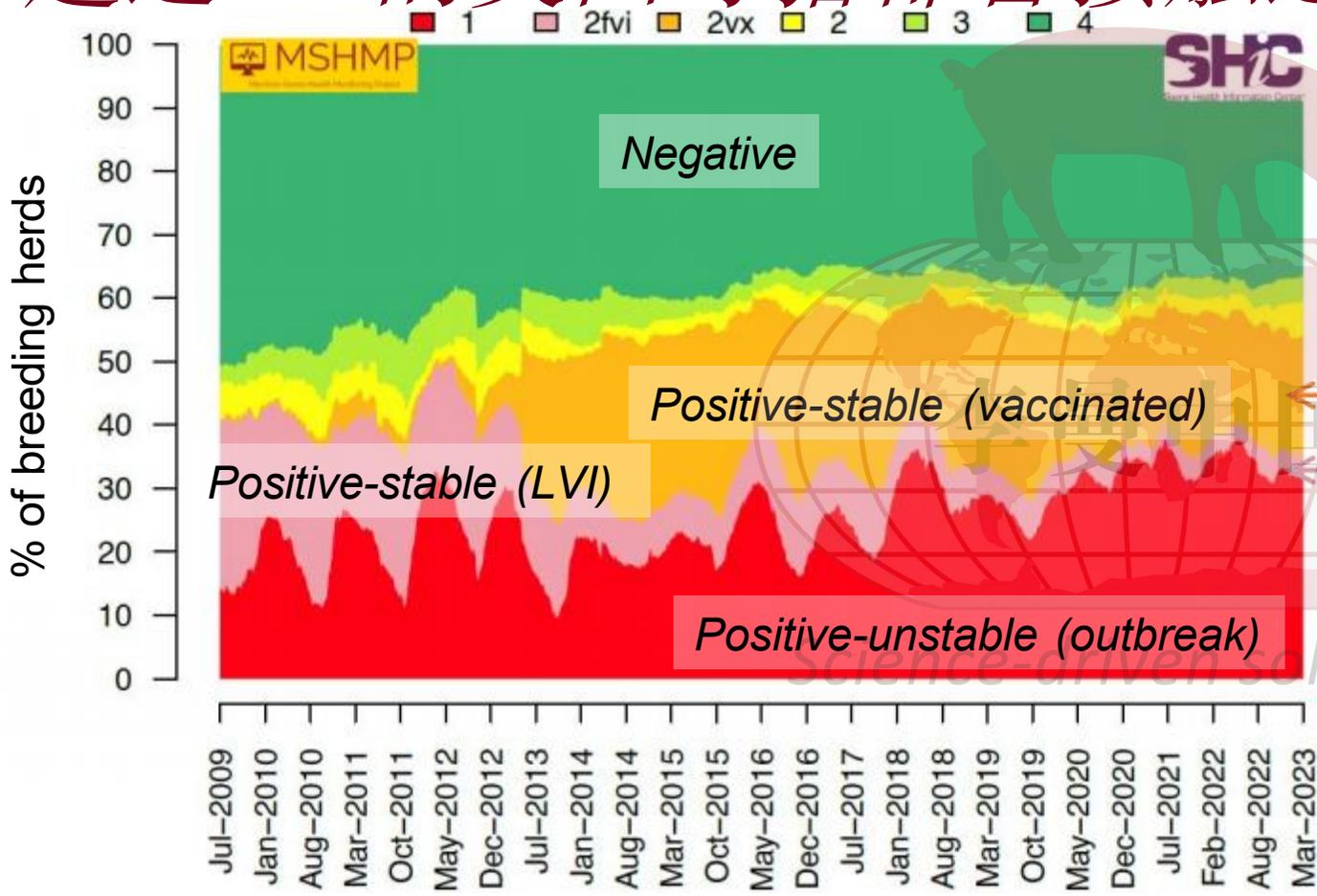
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More than 50% of the US sow herd has been exposed to PRRSV
超过50%的美国母猪都曾接触过PRRSV

免疫干预

- Immunological interventions

改良活疫苗
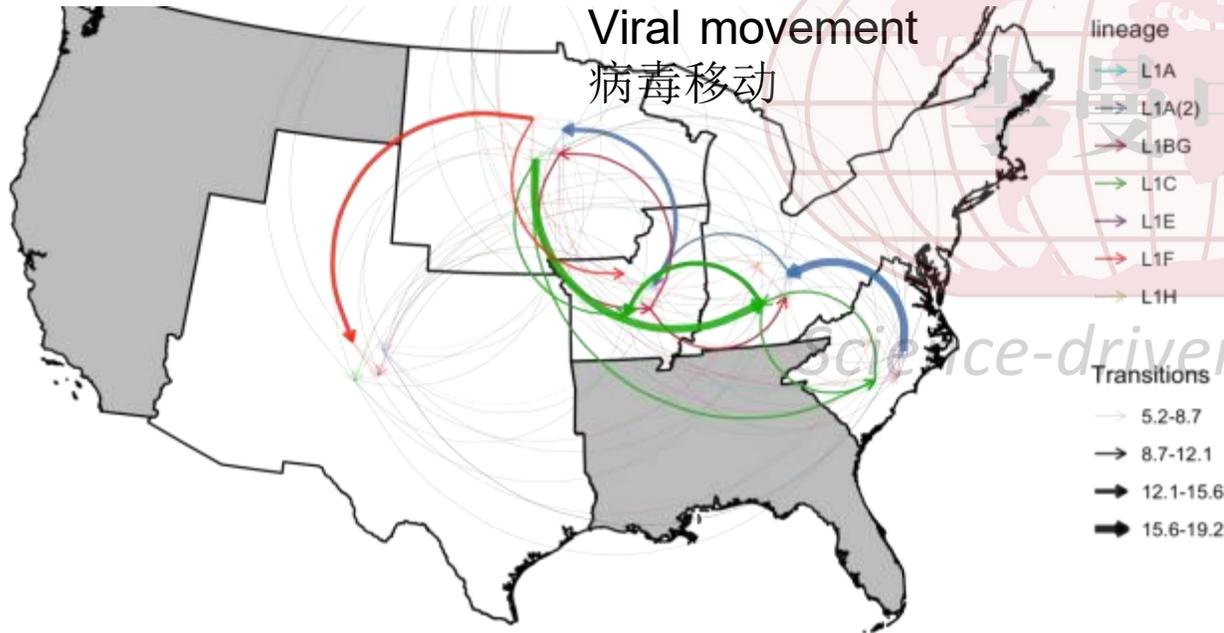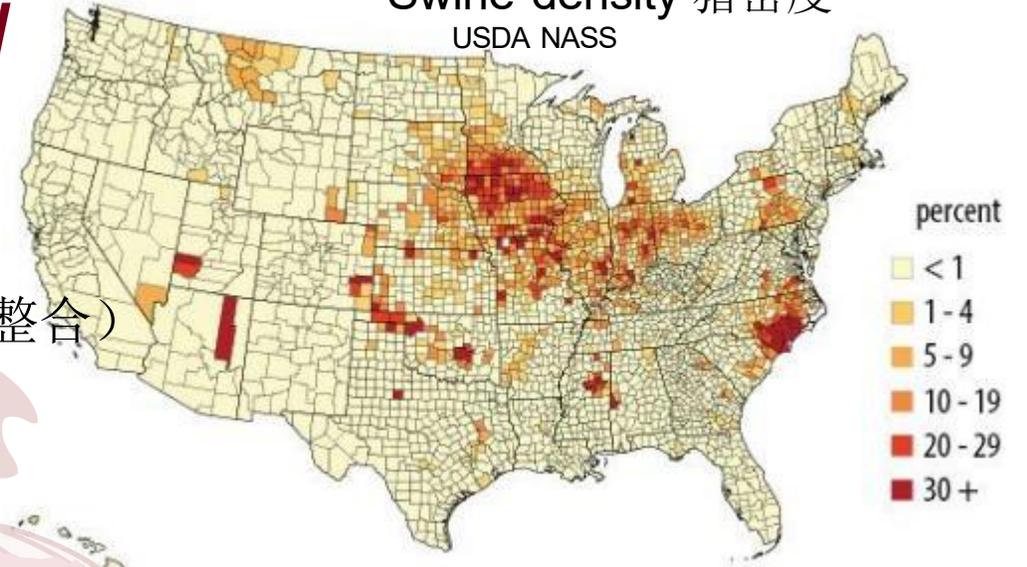Modified live vaccines

Live (field) virus inoculation

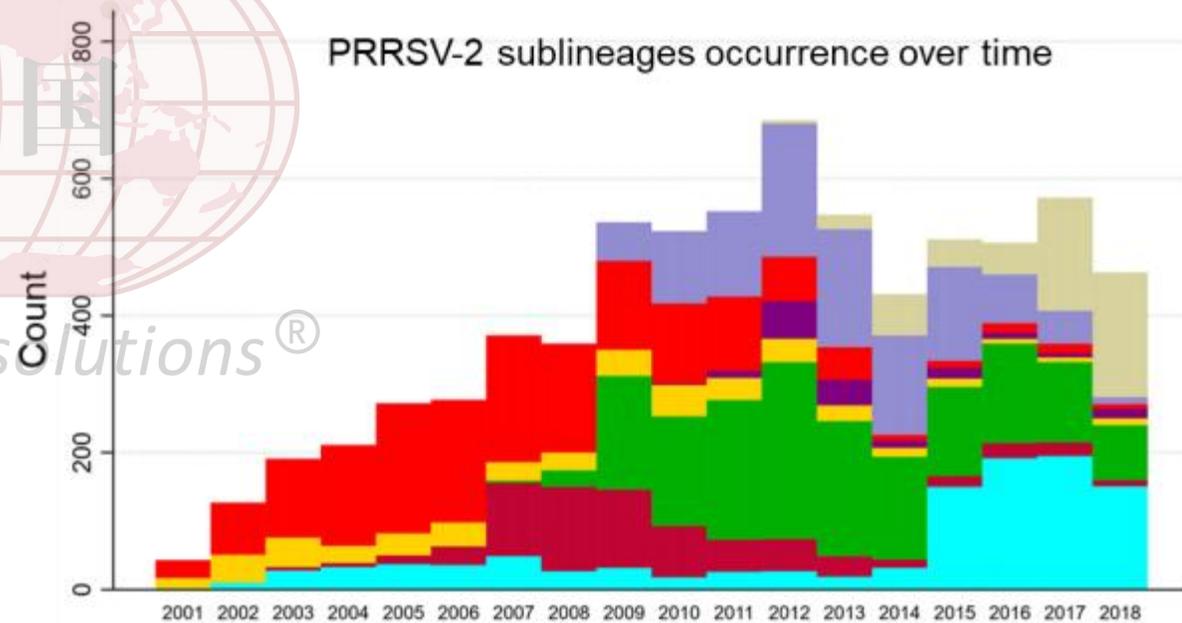活（野）病毒接种

PRRSV is challenging to control PRRSV难以控制

- High swine density 高猪群密度

- Animal movements (Vertical integration) 动物运动（垂直整合）

- Inter-regional connectivity 区域间互联互通

- Genetic and antigenic diversity 遗传和抗原多样性

Swine density 猪密度
USDA NASS

percent
	< 1
	1 - 4
	5 - 9
	10 - 19
	20 - 29
	30 +

Viral movement
病毒移动

lineage
→ L1A
→ L1A(2)
→ L1BG
→ L1C
→ L1E
→ L1F
→ L1H

Transitions
5.2-8.7
8.7-12.1
12.1-15.6
15.6-19.2

PRRSV-2 sublineages occurrence over time

Pamornchainavakul *et al.,* 2023

Paploski *et al.,* 2021

Genetic and antigenic variability of PRRSV-2
PRRSV-2的遗传和抗原变异性

Evolutionary rates of RNA viruses
RNA病毒的进化速率

Unpublished data, Paploski & VanderWaal 2023

Genetic and antigenic variability of PRRSV-2
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COMPLEX IMMUNE LANDSCAPE
复杂的免疫情形
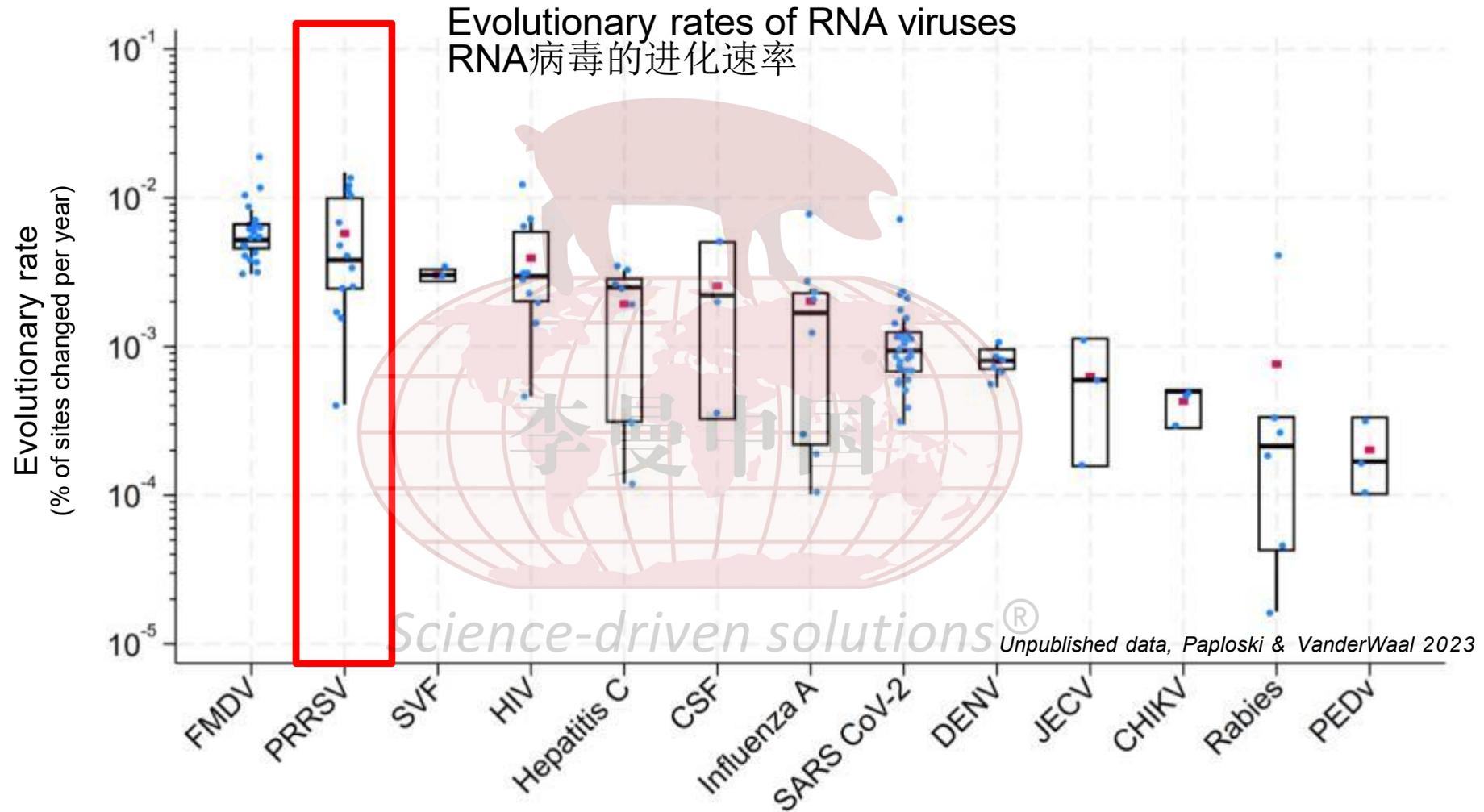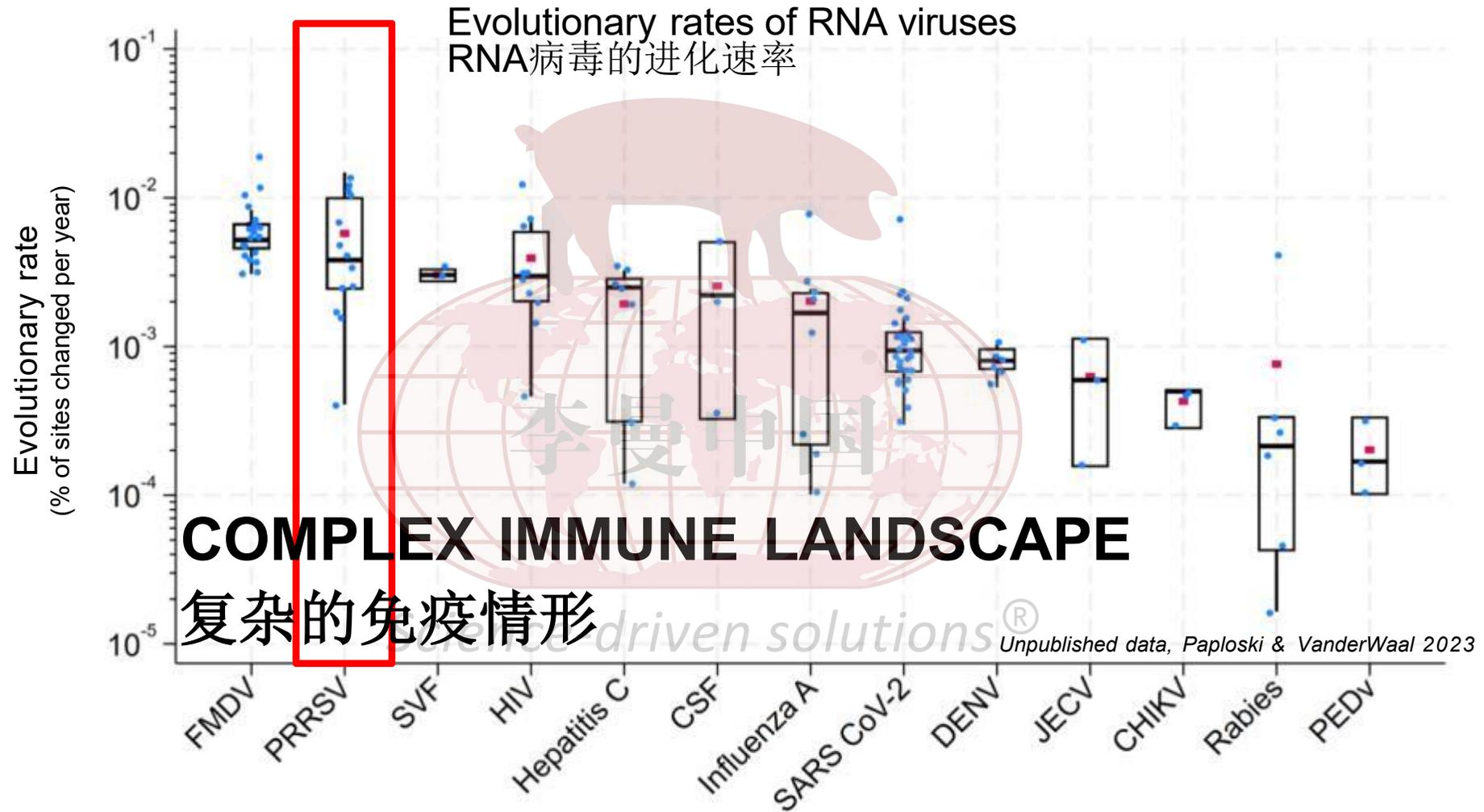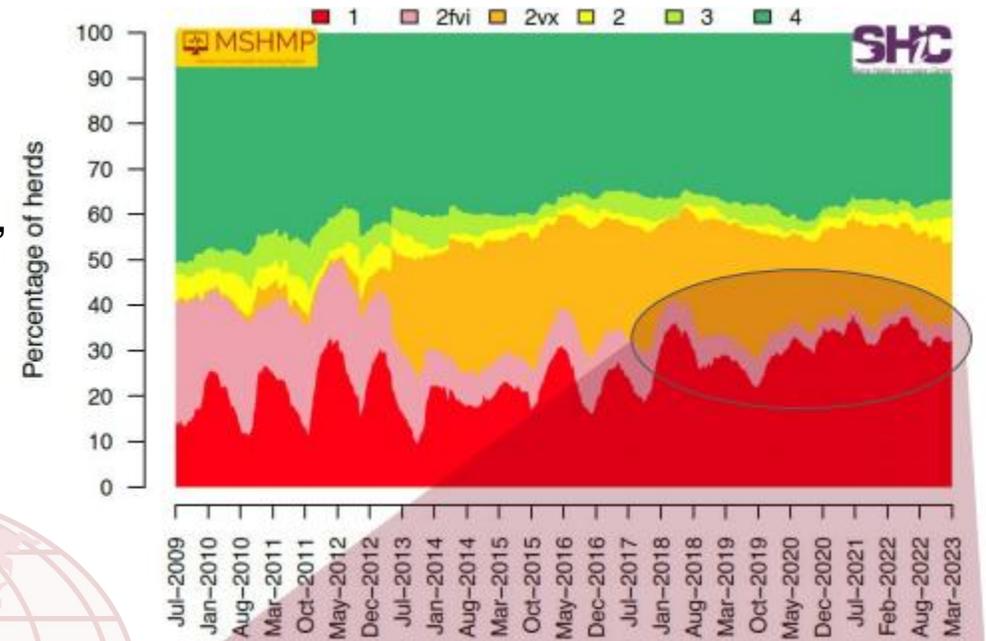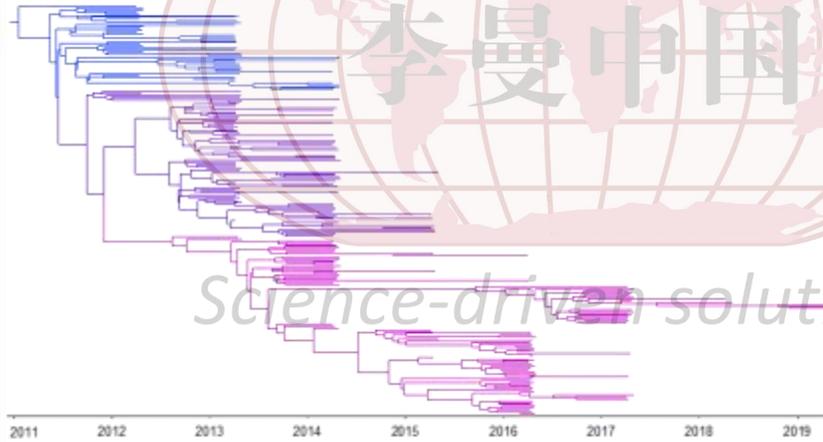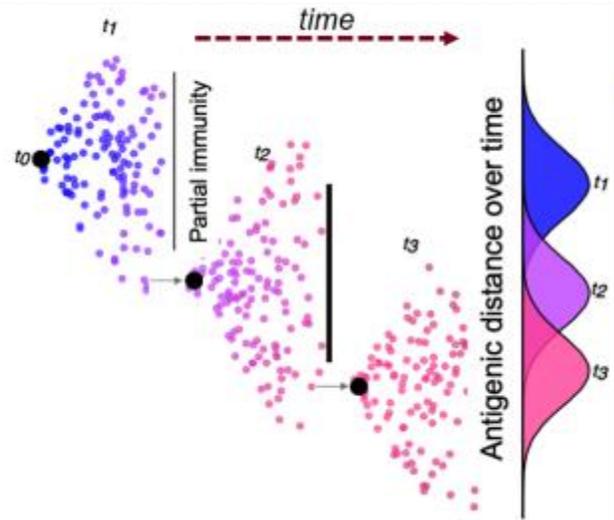
Multi-strain dynamics
多毒株动力学

- As viruses evolve, immune responses generated against a past "strain" may become less effective 随着病毒进化，针对过去"毒株"产生的免疫反应可能会变得不那么有效

  → creates a complex system, with different antigenic variants nteracting through the cross-immunity that is generated within hosts 产生了一个复杂的系统，其中不同的抗原变体通过产生的交叉免疫相互作用
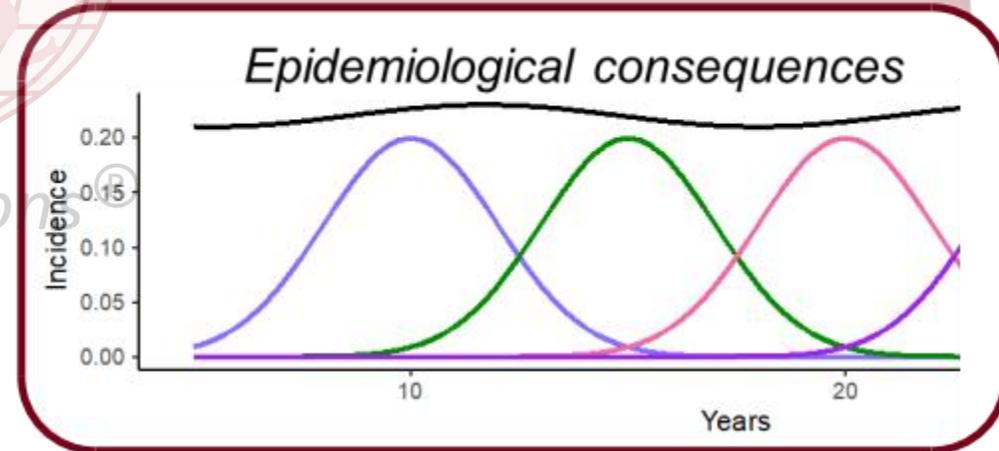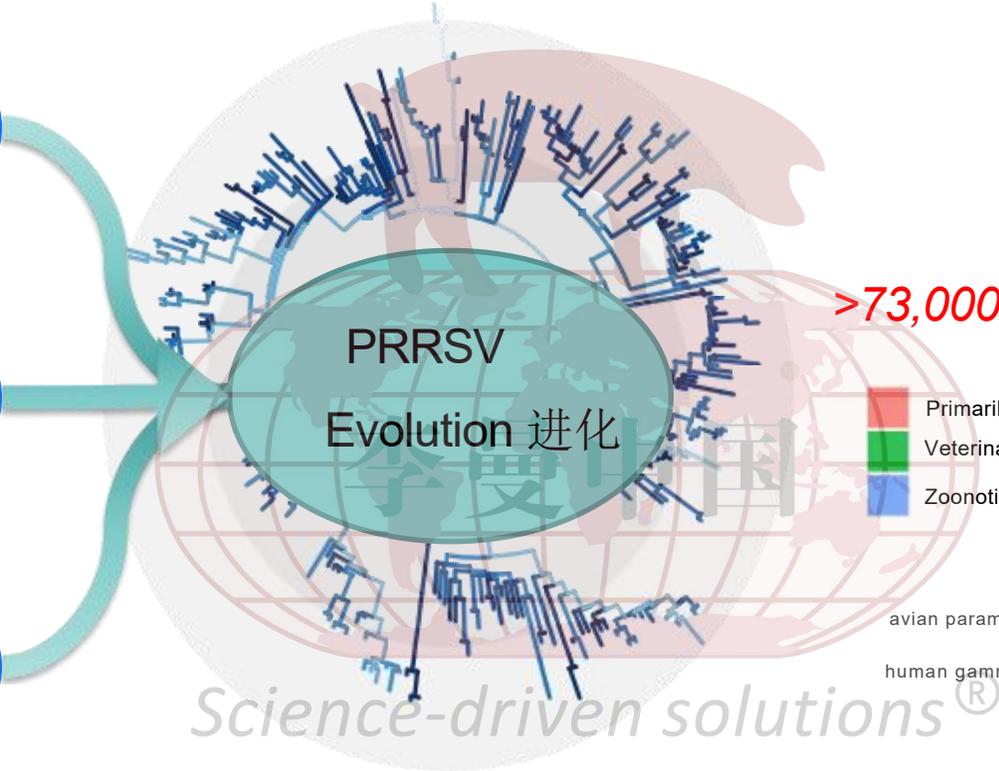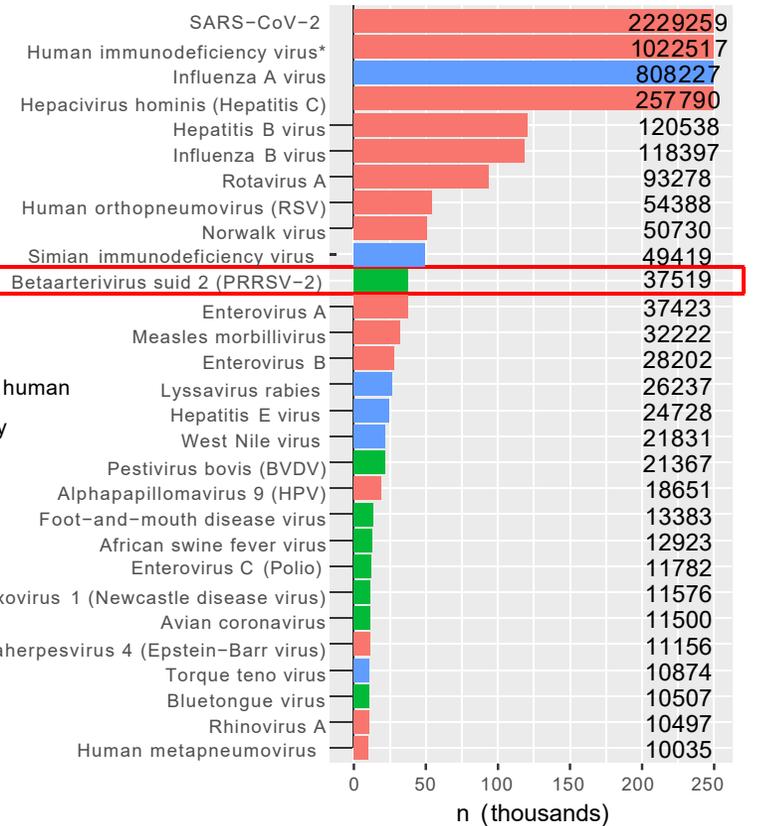
Evolutionary dynamics of PRRSV-2
PRRSV-2的进化动态

Mutation 突变

Immune selection
免疫选择

Multi-strain Recombination
多毒株重组

PRRSV
Evolution 进化

Primarily human
Veterinary
Zoonotic

Sequences on Genbank

	n (thousands)
SARS-CoV-2	2229259
Human immunodeficiency virus*	1022517
Influenza A virus	808227
Hepacivirus hominis (Hepatitis C)	257790
Hepatitis B virus	120538
Influenza B virus	118397
Rotavirus A	93278
Human orthopneumovirus (RSV)	54388
Norwalk virus	50730
Simian immunodeficiency virus	49419
Betaarterivirus suid 2 (PRRSV-2)	37519
Enterovirus A	37423
Measles morbillivirus	32222
Enterovirus B	28202
Lyssavirus rabies	26237
Hepatitis E virus	24728
West Nile virus	21831
Pestivirus bovis (BVDV)	21367
Alphapapillomavirus 9 (HPV)	18651
Foot-and-mouth disease virus	13383
African swine fever virus	12923
Enterovirus C (Polio)	11782
avian paramyxovirus 1 (Newcastle disease virus)	11576
Avian coronavirus	11500
human gammaherpesvirus 4 (Epstein-Barr virus)	11156
Torque teno virus	10874
Bluetongue virus	10507
Rhinovirus A	10497
Human metapneumovirus	10035

>73,000

But we still need to classify PRRSV even in this complex scenario. How do we do it?
但即使在这种复杂的情况下，我们仍然需要对PRRSV进行分类。我们如何做到这一点？

How do we classify PRRSV-2 (ORF5)?
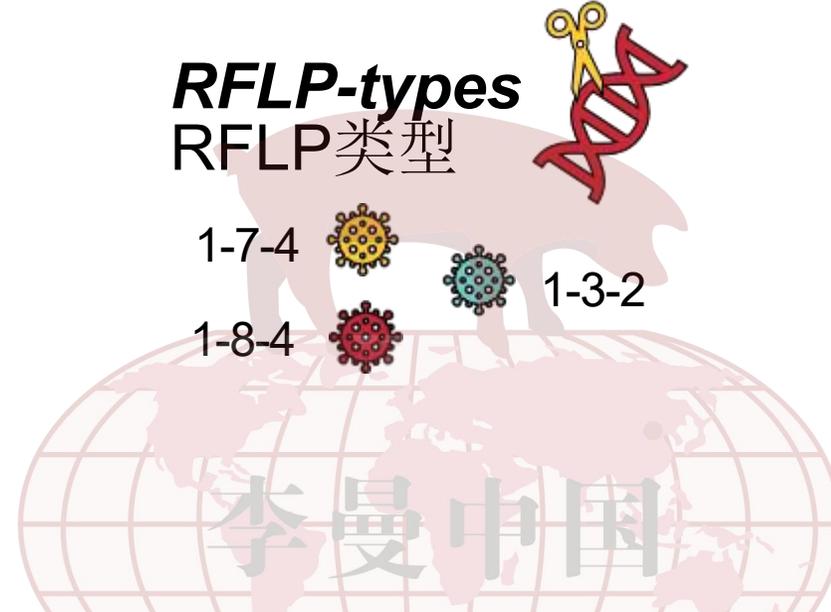## 我们如何对PRRSV-2（ORF5）进行分类？

Genetic distance
基因距离

	A	B	C
A		2.9	8.6
B	2.9		4.9
C	8.6	4.9	

RFLP-types
RFLP类型

1-7-4

1-3-2

1-8-4

L1A
L1B
L1C
L1D
L1E
L1F
L1G
L1H
L1D*
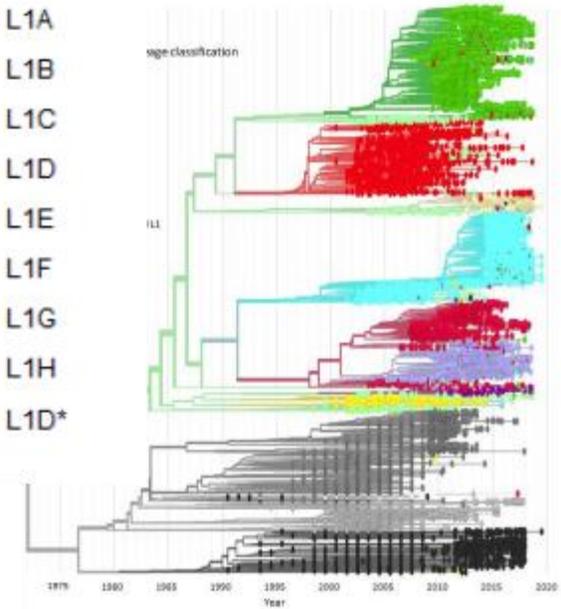
>Seq1

atgttggagaaatgcttgaccgcgggctgttgctcgcaattgctttctttgtggtgtatcgtgccgttctgtttttgctgtgctcgccaac
acaaatttgattgggcagtggagagttttgtcatctttcccgttttgactcacattgtctcctatggtgccctcactactagccatttcct
ggctgcgttgacttgcttcgtcattaggtttgcaaagaattgcatgtcctgcgctacgcgtgtaccagatataccaactttcttctg
atcgacctcaaaagagttgtgcttgatggttccgtggcaaccccctataaccagagtttcagcggaacaatggggtcgtccttag
>Seq2

atgttggggaaatgcttgaccgcgggctgttgctcgccgttgctttttttgtggtgtatcgtgccgtcttgtttttgttgcgcccgtcaac
gtagatttgactgggcagtggagtgttttgtcatttttcccgtgttgactcacattgtctcctatggtgccctcactactagccatttcct
ggctgcgttgattgcttcgtcattaggcttgcgaagaactgcatgtcctggcgctactcgtgtaccagatataccaacttccttttttc

RFLP enzymes:
Mlu1: acgcgt
HincII: gtyrac
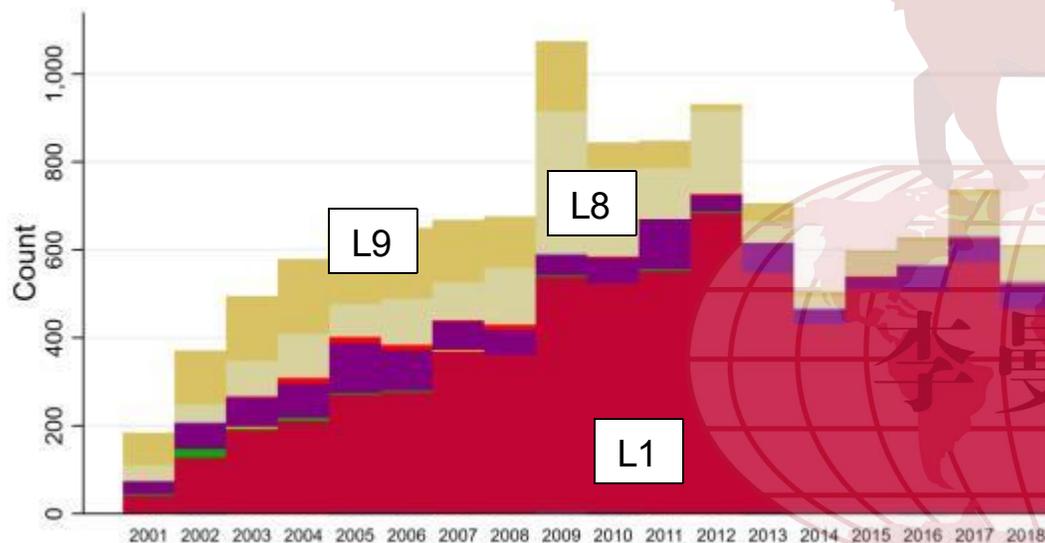SacII: ccgcgg

Lineage-based classification
基于谱系的分类

- Between 2001 and 2018, 60% of all UMN VDL sequences were classified as **Lineage 1** 2001年至2018年间，所有UMN VDL序列中有60%被归类为Lineage1
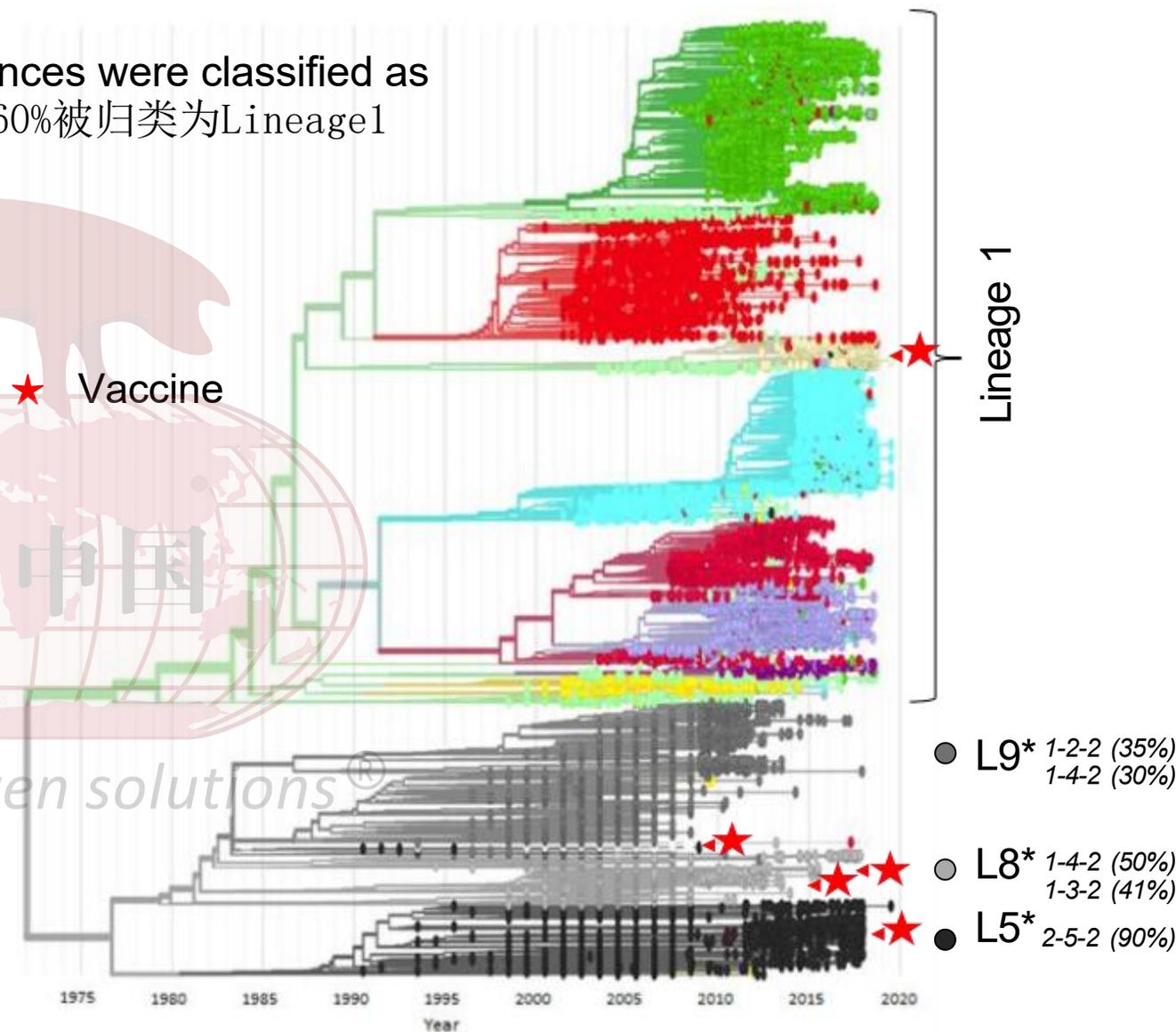 - From 2013 onwards, >80% 自2013年起，>80%

★ Vaccine

Frequency of occurrence per year, UMN VDL
每年发生频率，UMN VDL

Substructure within Lineage 1?
Lineage1内的子结构？

L9* *1-2-2 (35%)* *1-4-2 (30%)*

L8* *1-4-2 (50%)* *1-3-2 (41%)*

L5* *2-5-2 (90%)*

Shi *et al.* 2010, *J. of Virology*
Paploski et al. 2021, *Vaccines*
*Paploski et al. 2019, *Frontiers in Microbiology*
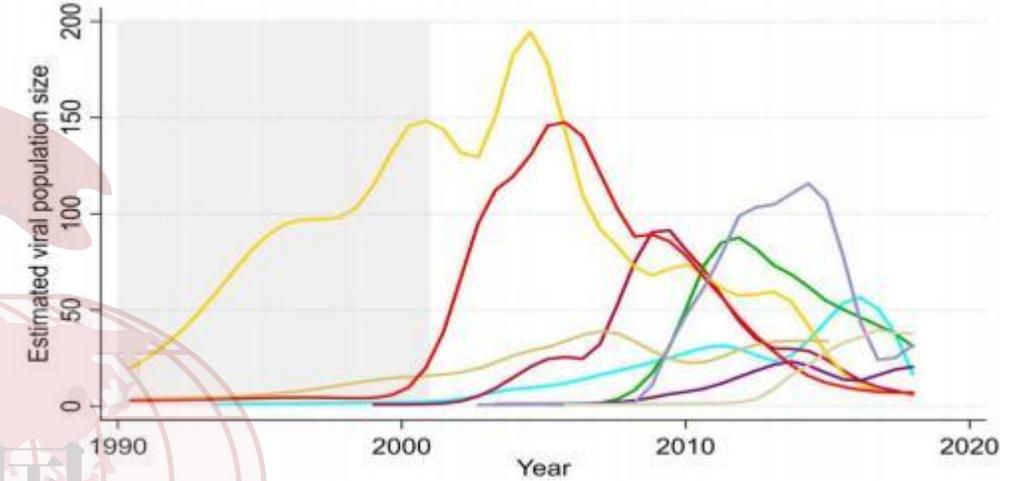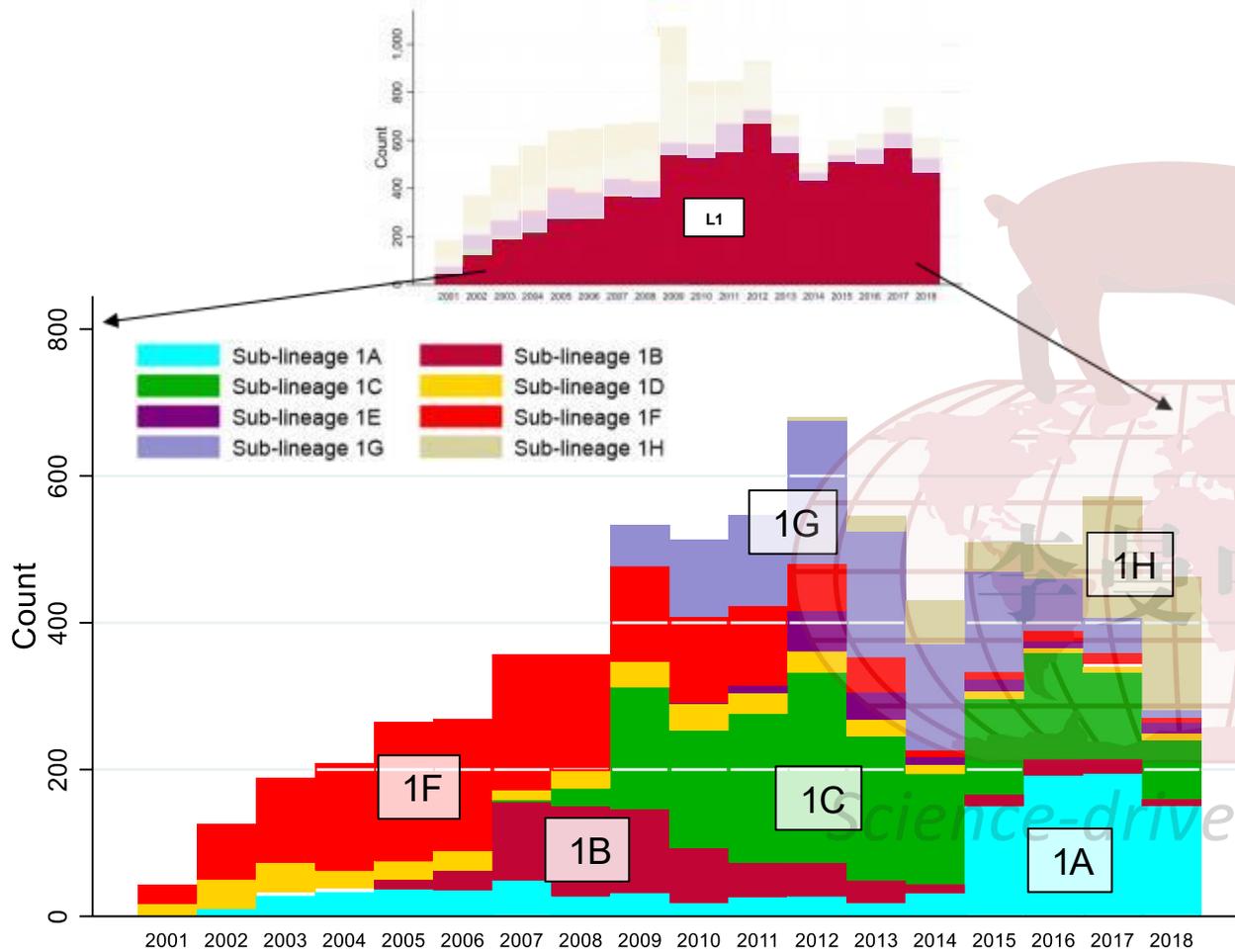
Sub-lineage emergence & Turnover
子谱系出现和更替

Estimated viral population size for different sub-lineages within Lineage 1 in the U.S. overtime
美国Lineage1中不同亚系病毒的估计病毒种群大小随时间变化

Sub-lineages within Lineage 1 over time, UMN VDL
随着时间的推移，UMN VDL中的Lineage 1内的子谱系

- Sequential dominance of different sub-lineages
  不同子谱系的顺序优势

  – *Years between peaks: 峰间年数：*
    . ~3 years (median, range: 1-3) 约3年（中位数，范围：1-3）
  – *Years emergence →dominance:* ~4.5 years
    (median, range 2-6) 出现优势的年份：约4.5年（中位数，范围2-6）
  – *Years between sub-lineage emergence:*1-5 years
    (last emergence in 2014/2020) 子系出现之间的年份：1-5年
    （最后一次出现于2014/2020年）

Periodic emergence events occur in the U.S.
在美国，周期性的突发事件时有发生

Nextstrain visualization of **lineage co-circulation, turnover**, and **inter-regional spread** of PRRSV-2 (Lineage 1)
PRRSV-2（Lineage1）谱系共循环、周转和跨区域传播的Nextstrain可视化

Pamornchainavakul *et al.,* 2023, *Pathogens*
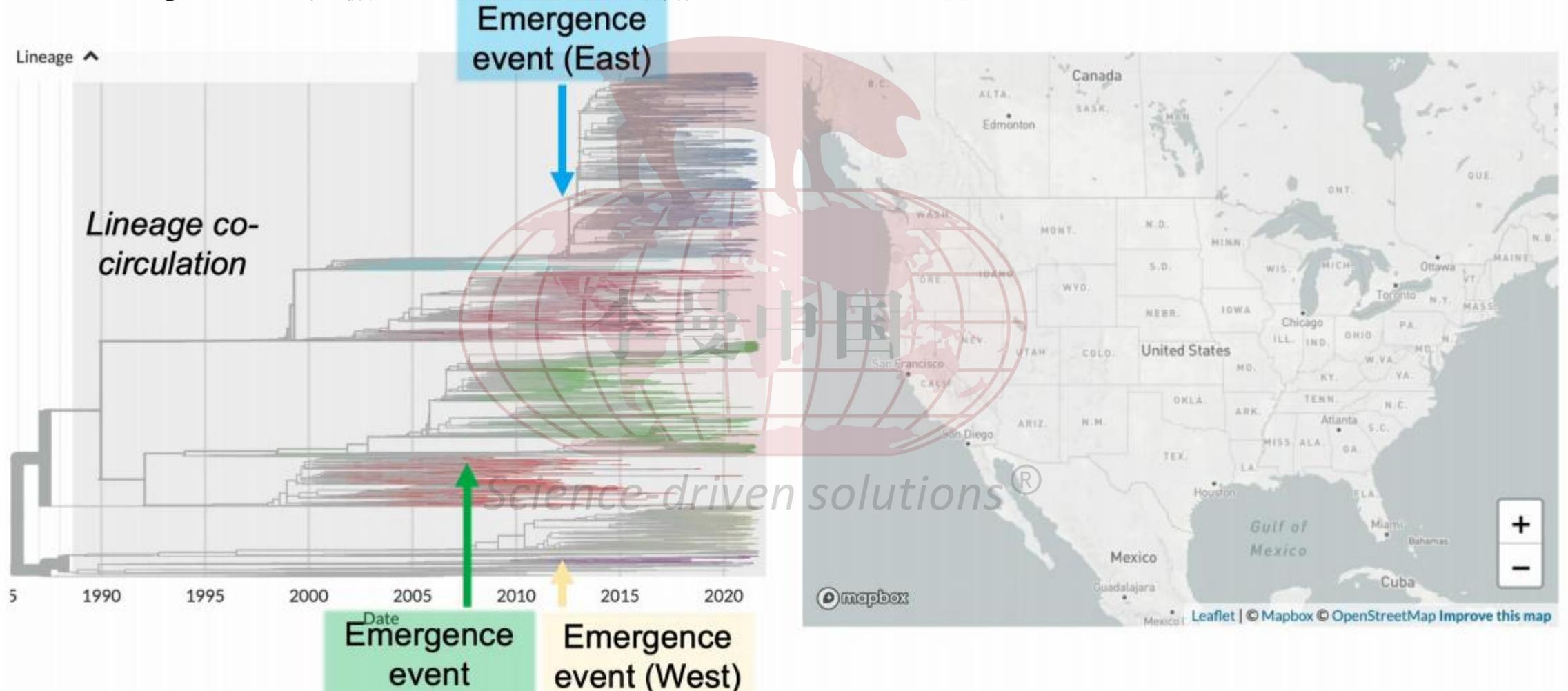
# However, issues persist 然而，问题仍然存在

- Genetic distances can be hard to calculate and interpret if you have thousands of sequences

如果有数千个序列，遗传距离的计算和解释可能会变得困难

- RFLPs are not particularly useful RFLPs不是特别有用
  - Inability to represent genetic relationships between viruses
  - 无法表示病毒之间的遗传关系
  - Distinct viruses with the same RFLP (conversely, similar viruses with different RFLP)
  - 具有相同RFLP的不同病毒（相反，具有不同RFLP的相似病毒）
  - Instability over few animal passages
  - 少数动物通道不稳定

Sub-lineages still form large groups 子谱系仍然形成大群体
  - Difficulty in performing epidemiological investigations, assessing introductions or persistence solely based on it *Science-driven solutions*®
  - 仅根据其进行流行病学调查、评估引入或持续存在的难度

- ***Variant** classification advances of these points*
  这些点的变体分类进展

What is a PRRSV-2 "variant"?
什么是PRRSV-2"变种"？

- **Subdivision within a sub-lineage, in which groups are smaller but still phylogenetically linked**

- 子谱系内的细分，其中群体较小，但仍具有系统发育联系

- Sequences belonging to the same variant have an *average genetic distance of ~2.5%, but can be as high as ~5%*

- 属于同一变种的序列平均遗传距离为~2.5%，但最高可达~5%

- Variants are *typically ~5%* different from the closest related variant
- 变异体通常与最接近的变异体存在约5%的差异

- **New variants** are assigned based on genetic distance from other variants and phylogenetic stability of the grouping on trees

- 根据与其他变种的遗传距离以及树状图上分组系统发育的稳定性，分配新的变种

- The number of active variants at any point of time is dynamic, given their frequent emergence and extinction

- 任何时间点的活跃变种数量都是动态的，因为它们经常出现和消失
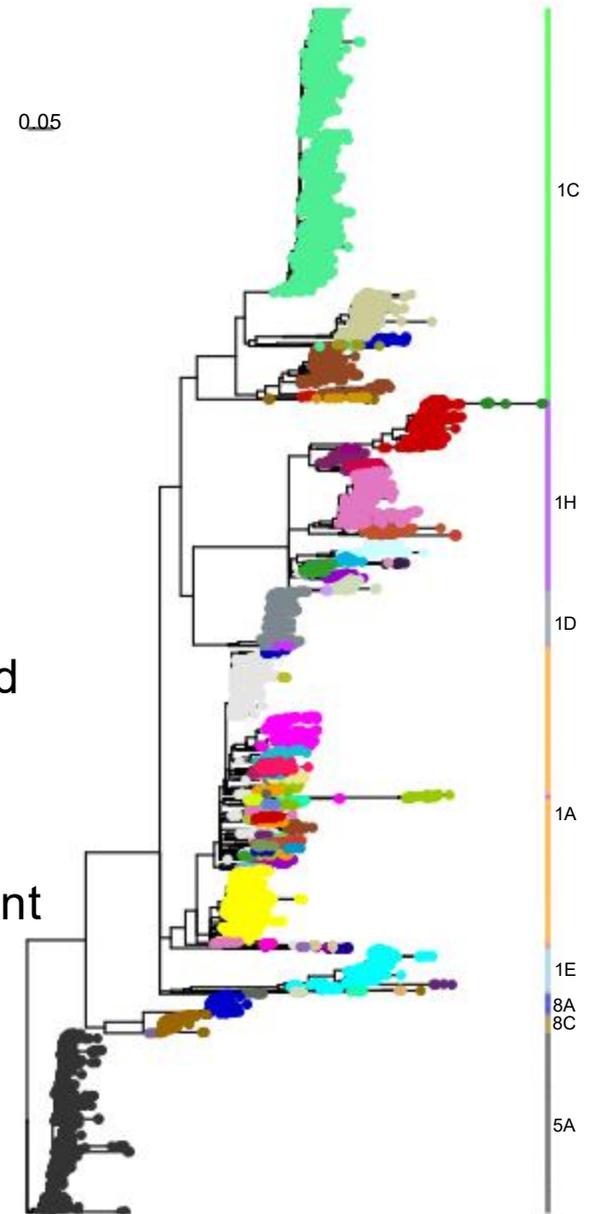
*Visualizing **variants** of PRRSV: an example*
PRRSV变体的可视化：一个示例

- L1H tree of American PRRSV-2 sequences:
- 美国PRRSV-2序列的L1H树：

Tree colored by RFLP
RFLP着色的树

- Viruses grouped by enzyme cut patterns in ORF5
- 根据ORF5中的酶切模式对病毒进行分组
- This sub-lineage 1H tree contains only two major RFLP types, which are inter-mixed on the tree
- 这个亚系1H树只包含两种主要的RFLP类型，它们在树上相互混合
- Because of this, these groupings lack significant meaning
- 因此，这些分组缺乏显著意义
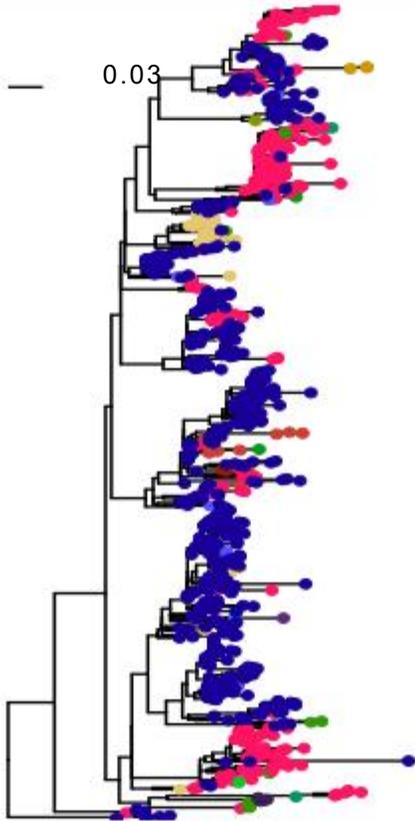
Tree colored by variant
树按变体着色

- Viruses grouped by ORF5 sequence similarity (*typically ~2% genetic distance to each other*)

按ORF5分组的病毒序列相似性（通常彼此之间有约2%的遗传距离）

- The same sub-lineage 1H tree has numerous variants that are well-defined in three (limited inter-mixing of colors)
- 同一亚系1H树有许多变种在三个维度上都有明确的定义（颜色间混合有限）

- **Variant clusters: smaller than sub-lineages, but still phylogenetically meaningful**
- 变体集群：规模较小 虽然与亚系相比，其亲缘关系较为模糊，但仍具有系统发育意义
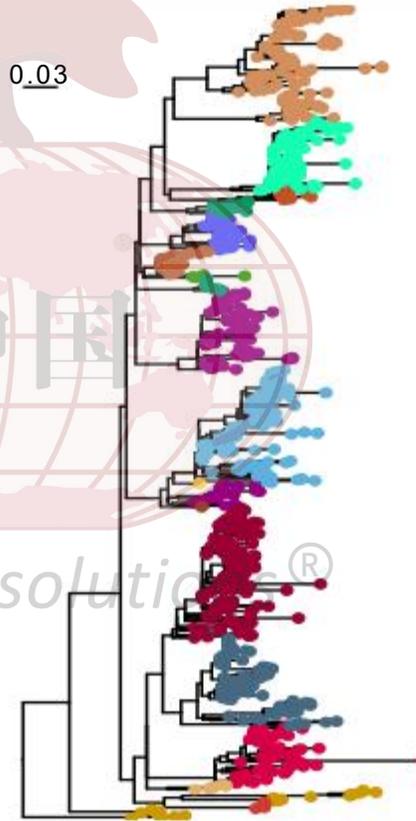
*What variant classifications **can** and **can not** tell you ...*
变体分类能告诉您什么，以及不能告诉您什么…

✓More reliable than RFLPs at determining relatedness, and whether virus A is the same or different than virus B 在确定遗传多样性方面比RFLPs更可靠相关性和病毒A是否与病毒B相同或不同

✓Discriminate between new and previous wild-type viruses in a farm (based on ORF5 gene) 区分农场中新的和以前的野生型病毒（基于ORF5基因）

✓More useful for epidemiological investigations, such as determining possible sources of introduction and tracking between-farm spread. 对于流行病学调查，如确定可能的感染源，这更有用农场间介绍和跟踪传播。

✗ No classification system reliably provides information on virulence or clinical picture (apparent virulence likely influenced by co-infections and other external factors)
✗没有分类系统可靠地提供关于毒力或临床表现的信息（明显的毒力可能受到共感染的影响）其他外部因素）

✗ Classifications do not directly translate to immunological cross-protection, although viruses labeled as the same variant are more genetically homologous
✗分类不能直接翻译为尽管标记为同一变体的病毒在基因上更为同源，但它们在免疫交叉保护方面可能存在差异

Other benefits: 其他好处：
- *Supports organization & findability of additional information about a particular variant*
- *支持组织并查找特定变体的附加信息*
- *Supports improved synergy & communication between research labs, diagnostic labs, monitoring programs, and the field*
- *支持研究实验室、诊断实验室、监测计划和现场之间更好的协同和沟通*

Can I classify my sequences into variants?
我可以将我的序列分类为变体吗？

- YES, online classifier (model based), see link on the right
- 是，在线分类器（基于模型），请点击右侧链接
- Model trained **exclusively on American sequences**
- 仅使用美国序列进行模型训练
- **Chinese sequences: can be classified if we can train the model with Chinese PRRSV-2 diversity**
- 中国序列：如果可以用中国**PRRSV-2**多样性训练模型，则可以对其进行分类
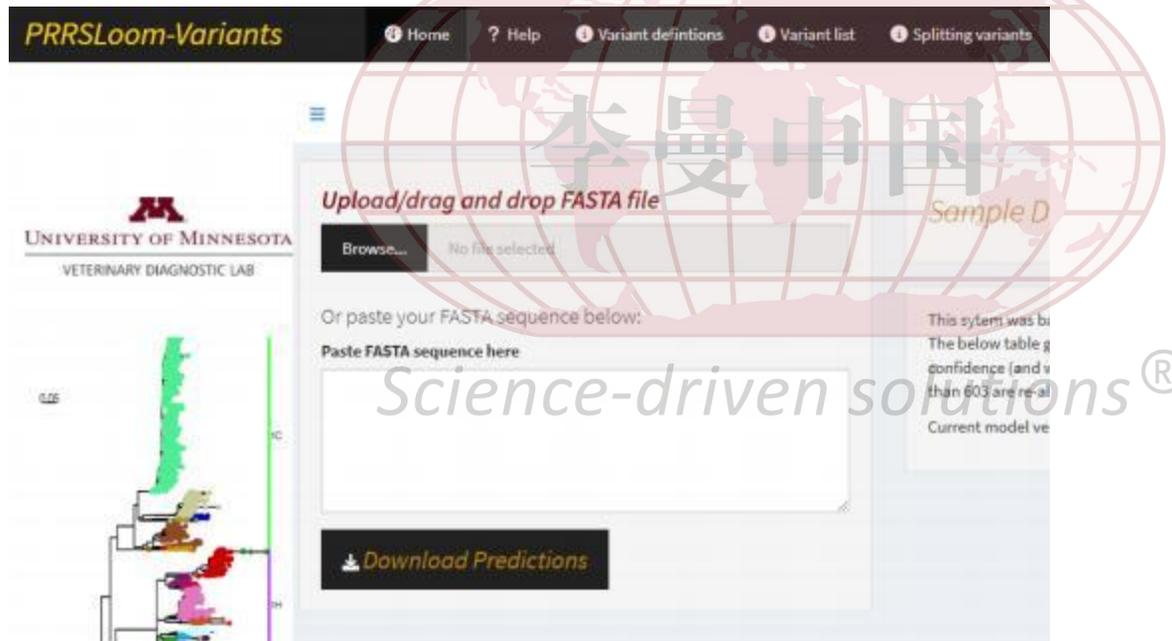
Link to variant
classifier: PRRSLoom

Can I classify my sequences into variants?

- YES, online classifier (model based), see link on the right
- Model trained **exclusively on American sequences**
- **Chinese sequences: can be classified if we can train the model with Chinese PRRSV-2 diversity**

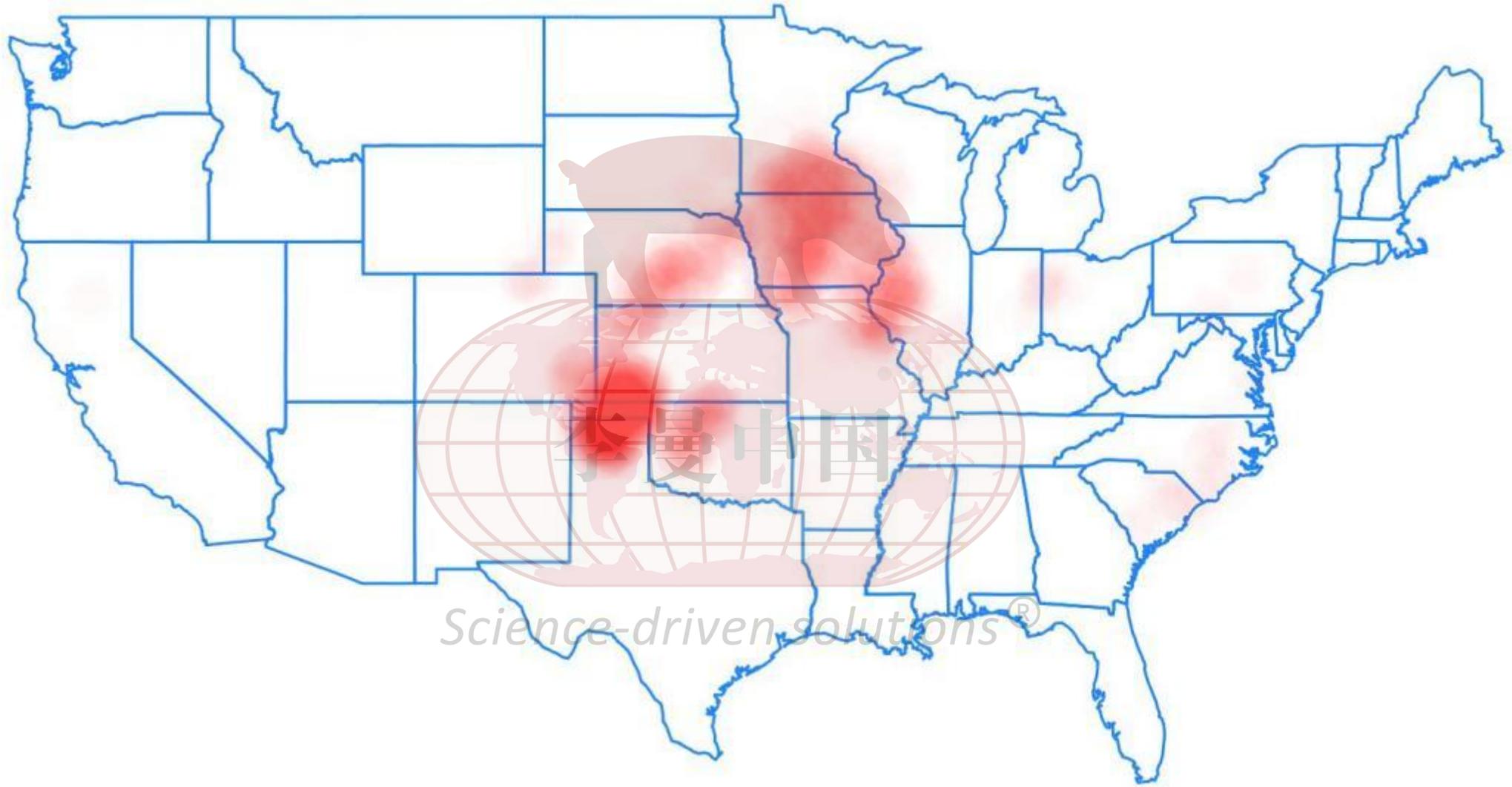**How can the PRRSV-2 variant classification improve our tracking abilities?**

如何对PRRSV-2变异株进行分类提高我们的追踪能力？

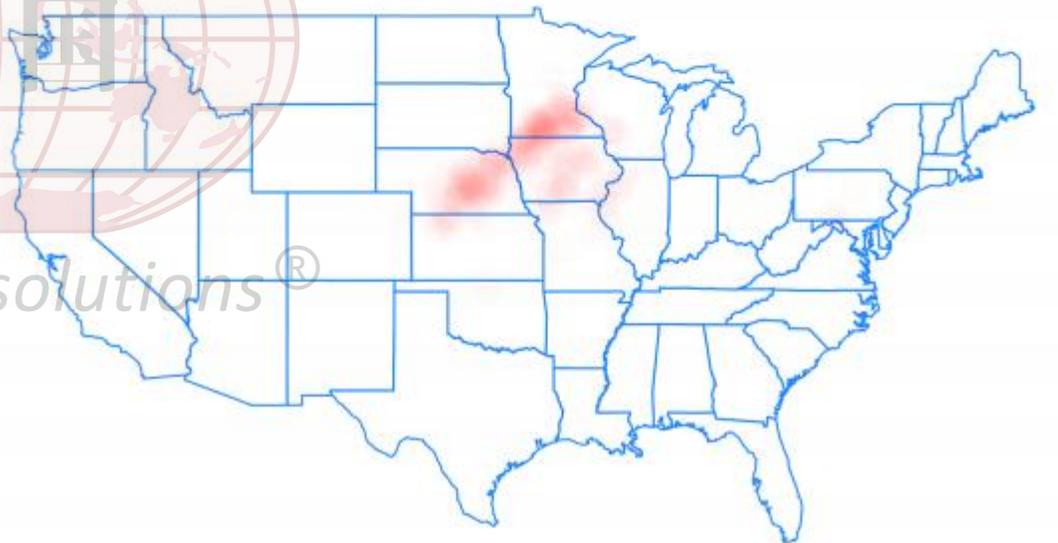*Science-driven solutions®*

Link to variant classifier: PRRSLoom

RFLP 1-8-4

RFLP 1-8-4

Variant 1H.11

Variant 1H.9

```
. tab variants_other if RFLP_others == "1_8_4"

variants_other |   Freq.    Percent      Cum.
----------------+-----------------------------------
1A-unclassified |      17       0.54       0.54
           1A.2 |       2       0.06       0.61
           1C.3 |       3       0.10       0.70
           1C.5 |       6       0.19       0.89
           1D.2 |     754      24.03      24.92
1H-unclassified |     268       8.54      33.46
           1H.1 |     113       3.60      37.06
          1H.10 |     112       3.57      40.63
          1H.11 |     575      18.32      58.95
          1H.17 |      71       2.26      61.22
          1H.23 |     107       3.41      64.63
           1H.5 |     190       6.05      70.68
           1H.7 |      33       1.05      71.73
           1H.9 |     536      17.08      88.81
         others |     351      11.19     100.00
----------------+-----------------------------------
          Total |   3,138     100.00
```

Noise has been added to all coordinates - they do not accurately represent farm locations

A lot changes when sequences are classified using the variant scheme

当使用变体方案对序列进行分类时，会发生很多变化

- Geographic distribution
- 地理分布
- Systems (companies) infected
- 受感染的系统（公司）
- Farms infected •农场感染
- Epidemiological investigations
- 流行病学调查
  - Is this a new introduction? 这是新的引入吗？
  - Movement of animals? 动物迁徙？
  - Routes of transmission? 传播途径？
- Management choices 管理选择
  - Flow of animals •动物流动
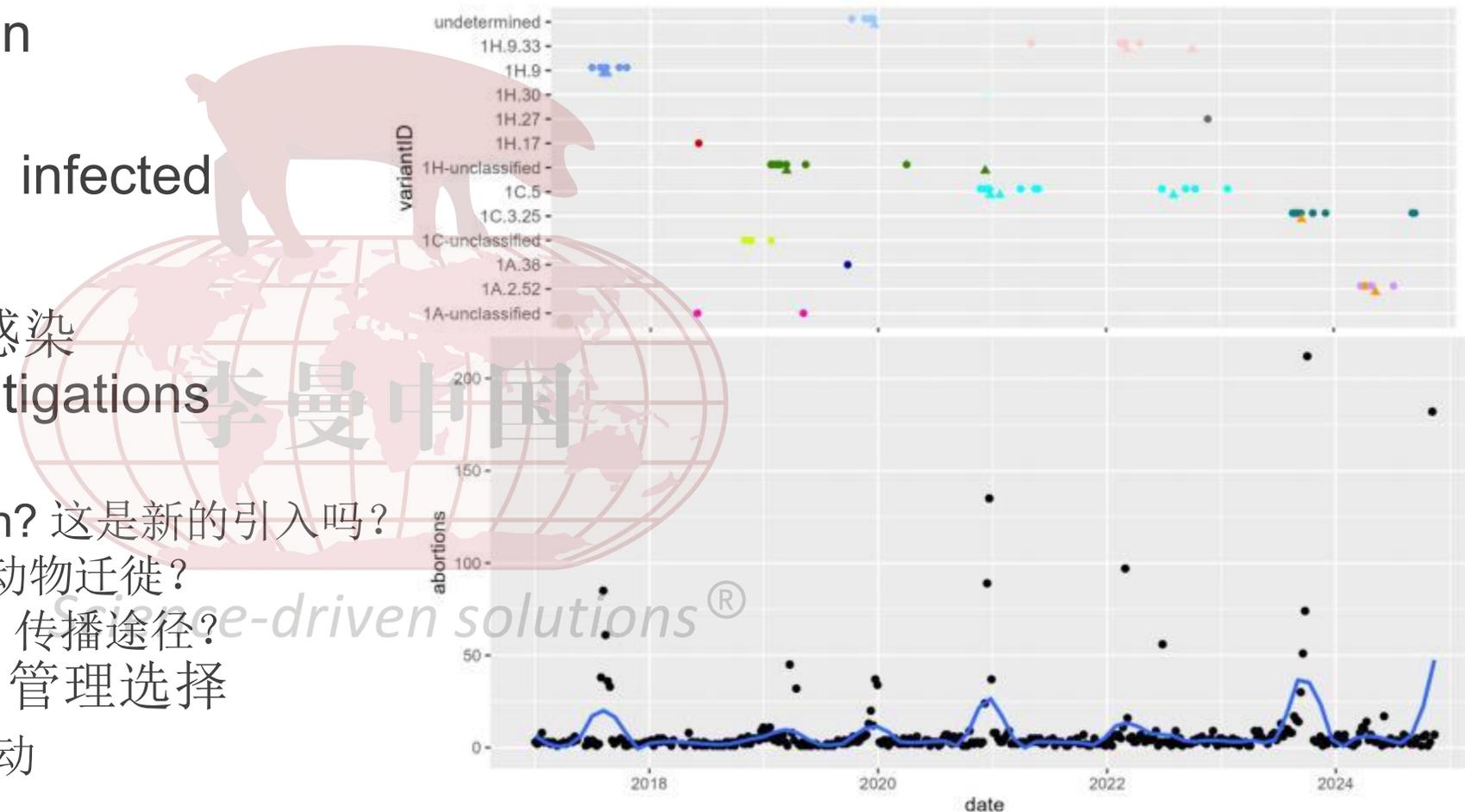
Take home messages 总结

. RFLP classification for PRRSV-2 often creates cluster of sequences that are not epidemiologically meaningful

PRRSV-2的RFLP分类通常会产生一系列在流行病学上无意义的序列簇

– Lineages and sub-lineages are an advance, but yield too large groups
– 谱系和子谱系是一种进步，但会产生大量群体

. PRRSV-2 variant classification yields clusters of sequences

PRRSV-2变体分类产生序列簇

that have a much stronger genetic meaning 具有更强的遗传意义

– But: not designed to predict virulence or antigenicity
– 但是：并非旨在预测毒力或抗原性

. PRRSV-2 variant classification is available for users .PRRSV-2变异株分类可供用户使用

– Chinese PRRSV-2 diversity is needed to train the model to adequately classify Chinese sequences
– 需要中国PRRSV-2多样性来训练模型，以充分分类中国序列

Thank you!
谢谢！

UMN Vet. Diagnostic Laboratory

ISU Vet. Diagnostic Laboratory

MSHMP Participants & Team

Veterinarians and Industry partners

EEID PRRS team
- Declan Schroeder
- Albert Rovira
- Maxim Cheeran
- Cesar Corzo
- Samantha Lycett
- Rowland Kao
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SWINE DISEASE ERADICATION CENTER
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USDA

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United States Department of Agriculture
National Institute of Food and Agriculture

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