Tonsil-Oral-Scrubbing (TOSc) as a new tool to detect PRRSV in sows and application of TOSc for PRRSV elimination in breeding herds

扁桃体口腔刮取液检测母猪蓝耳的一个新工具及其在母猪群蓝耳净化中的应用

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Oct 19th, 2029

- Epidemiology is the study of the determinants, occurrence, and distribution of health and disease in a defined population.
- 田间流行病学：运用多学科分析疾病在群体发生的空间和时间分布和规律，了解造成群体疾病的影响因素，明确疾病控制的优先级；通过科学手段（经济学，统计学等）评估干预方案；

Outline 纲要

Chapter Outlines

Assessment of TOSc to detect PRRSV in sows

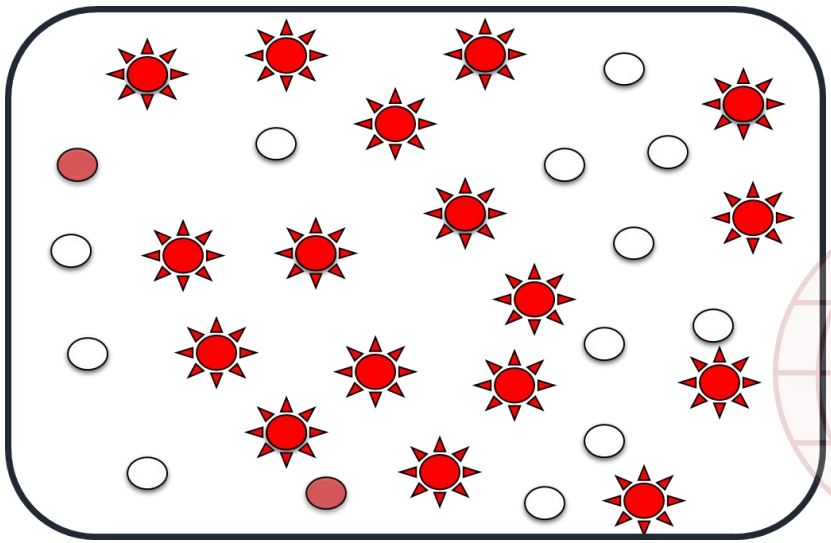
Application of TOSc for PRRSV ecology in breeding herds seeking stability

1. **Introduction 引言**
2. Comparison of TOSc to serum, oral fluid, and tonsil scraping to detect PRRSV in 60 sows over time
3. Determine the “best practice” for Tonsil-Oral-Scrubbing (TOSc) collection for sows to detect PRRSV
4. Effect of pooling and freeze-thaw of TOSc samples on the probability of PRRSV RNA detection by qPCR
5. Assessment of predictive values of TOSc and TF for live neonatal PRRSV status
6. Characterizing PRRSV dynamics in the sow population after whole-herd exposure using TOSc
7. **Discussion & Conclusion**

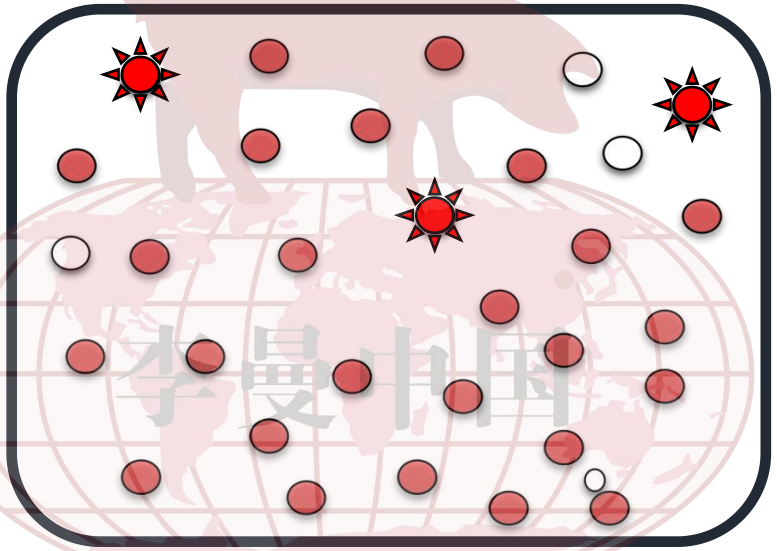
Introduction 引言

Load-Close-Homogenize for PRRSV stabilization
封群暴露净化

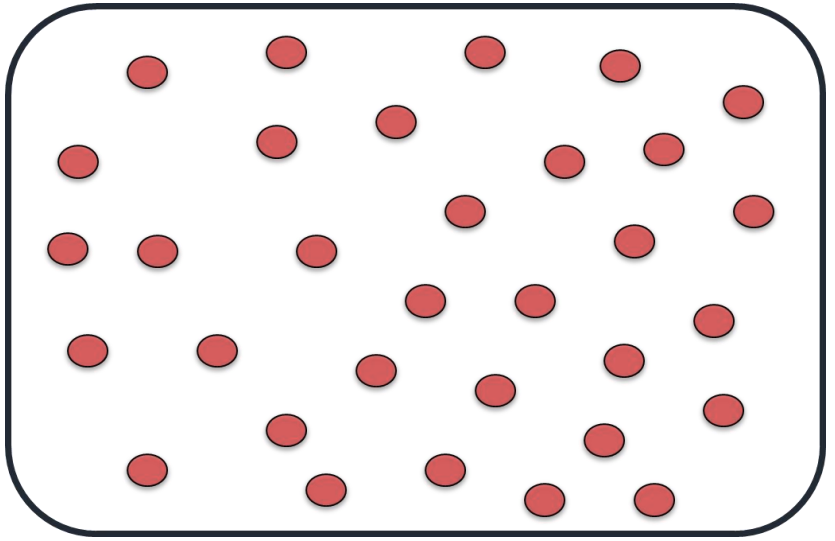
Hallmark: Consecutive PRRSV negative weaned piglets! 净化成功标志：持续生出阴性仔猪



Gestation sows IA



Gestation sows IB

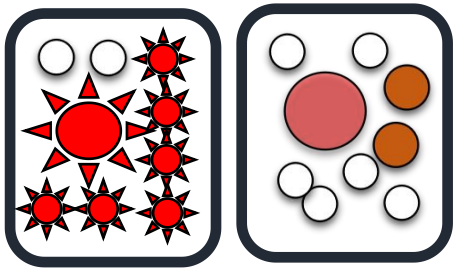


Gestation sows II

= Previously exposed and shedding 感染+排毒

= Previously exposed, not or "unknown" shedding 感染不排毒

= Not previously exposed, not shedding 未感染，未排毒



Farrowing crates

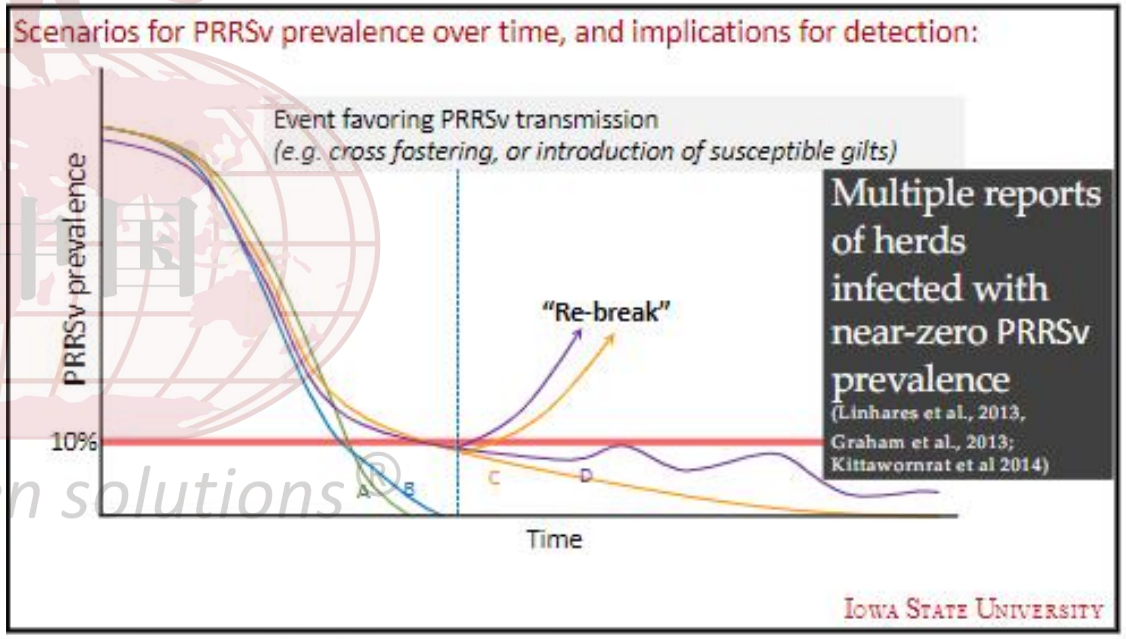
Adapted from slides of Dr. Holtkamp's courtesy

Scientific problem in breeding herds seeking stability
科学问题

1. **Extended TTS 净化时长越来越长；**
2. **PRRSV Rebreaks 蓝耳再次爆发“同一毒株”**

Time to stability (weeks)	Cohort 2011	Cohort 2021
10th percentile	15	23
25th percentile	22	29
median	**27**	**35**
75th percentile	33	49
90th percentile	46	66

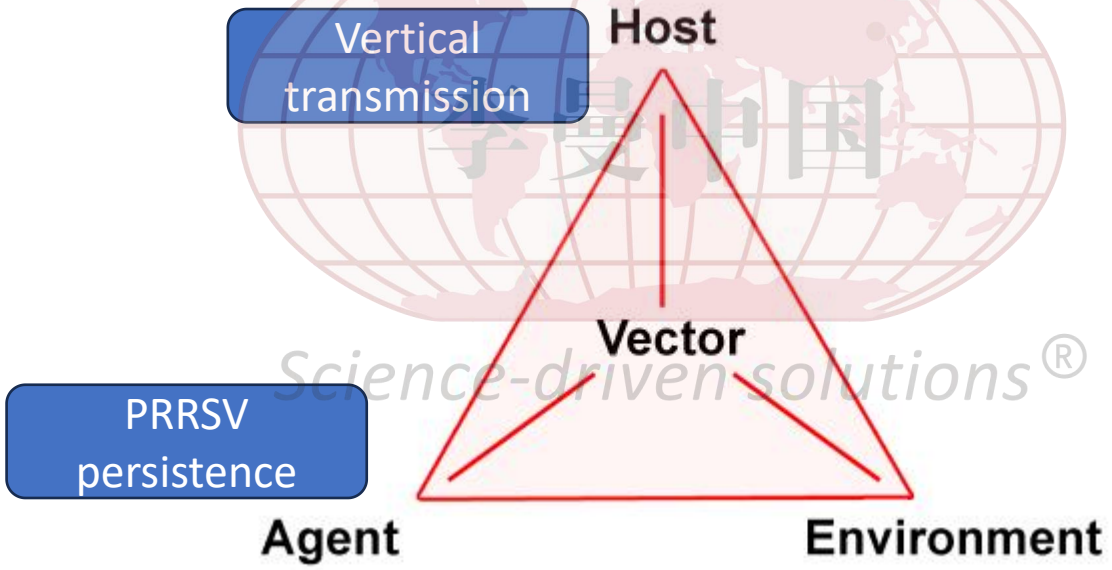
Source: NPB final research report, Swine Disease Management Information Program # 20-109



WHERE IS THE VIRUS??? 病毒在哪里循环？？

Two major PRRSV challenges 蓝耳的两大难点

1. **PRRSV can be vertically transmitted from sows to piglets.垂直传播**
2. **PRRSV persist in pigs for extended period of time. 持续感染**



Wills et al., 2003

JK Lunney · 2016

Proof of concept for TOSc for PRRSV detection
TOSc设计思路

1. Current sample type in farrowing room only target suckling piglet and might miss sow PRRSV activity;

当前主要监测样本为产房仔猪样本：睾丸液，舌尖液

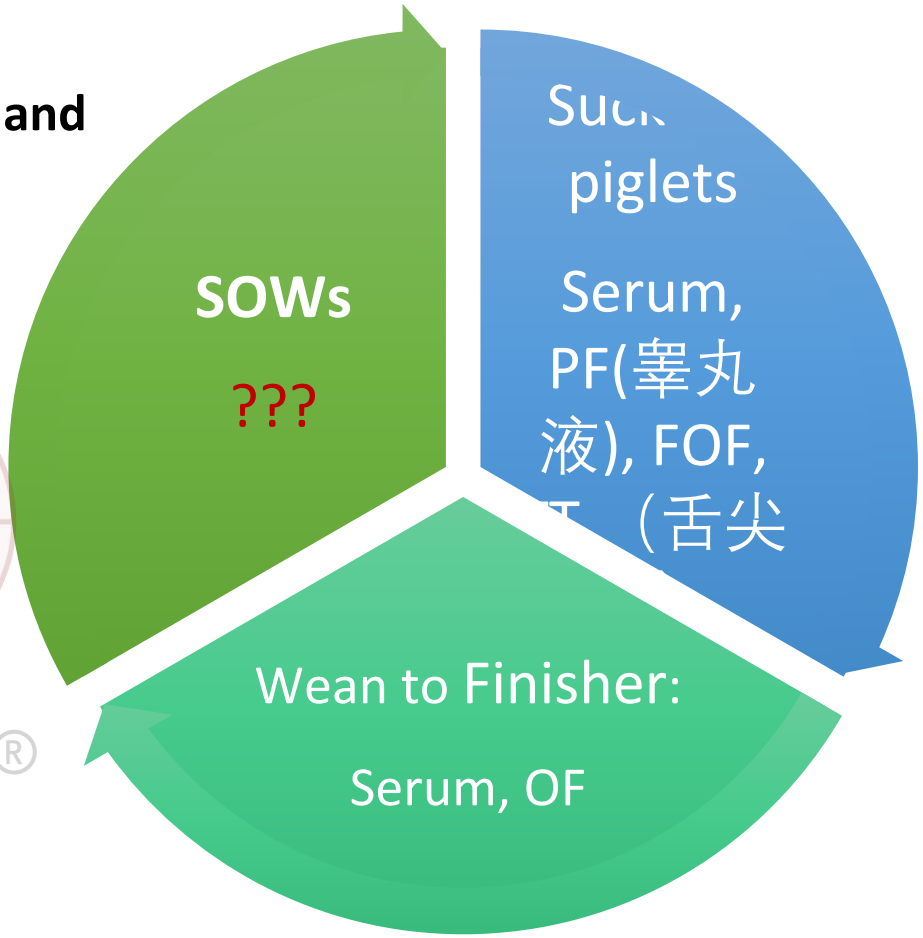
2. No easy & practical sample type for sows

Serum, tonsil scraping for sows: 母猪简单实用样本很少

- Labor intensive, 劳动力密集
- Animal offensive, 动物应激大

Oral fluid: 口腔液

- Various success rate as individual sampling method
- 成功率不能保证



Objective: an easy and practical tool to detect PRRSV in sows

Without snaring/ restraining!

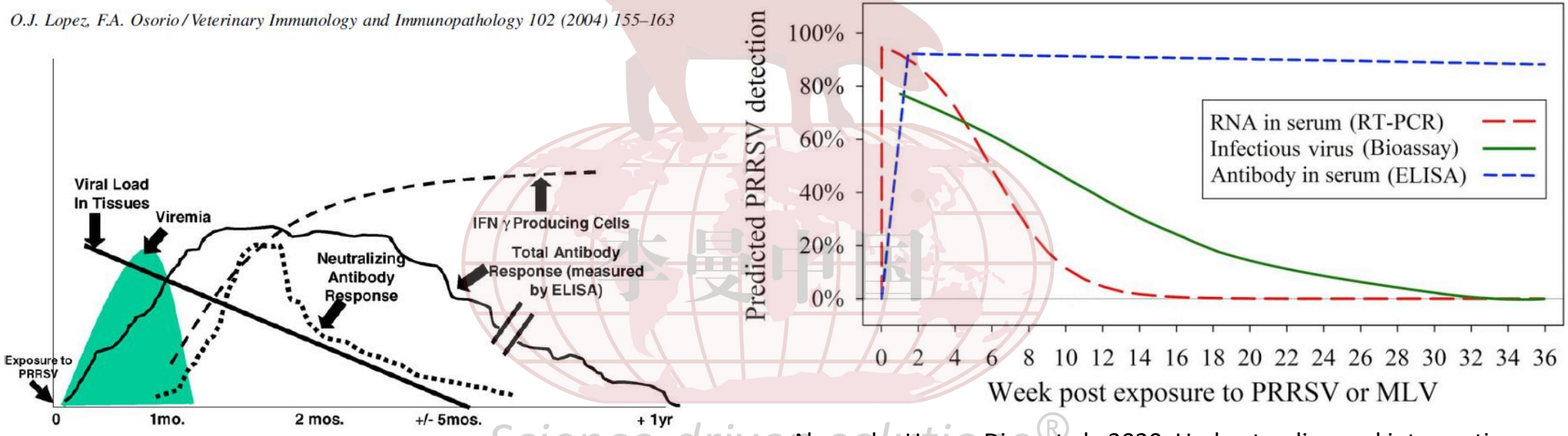
一个不用绑定母猪的用于检测蓝耳的简单实用的工具

The missing link of easy & practical sow collector

Brent Pepin, 2015

Pileri, E, 2016

Different sample type has different probabilities of PRRSV detection and changes over time
不同样本有不同的蓝耳检出概率，并且随时间变化



Alexandra Henao-Diaz, et al, 2020, Understanding and interpreting PRRSV diagnostics in the context of “disease transition stages”

PRRSV RNA in Serum:~30 days 血液蓝耳检出：30 天

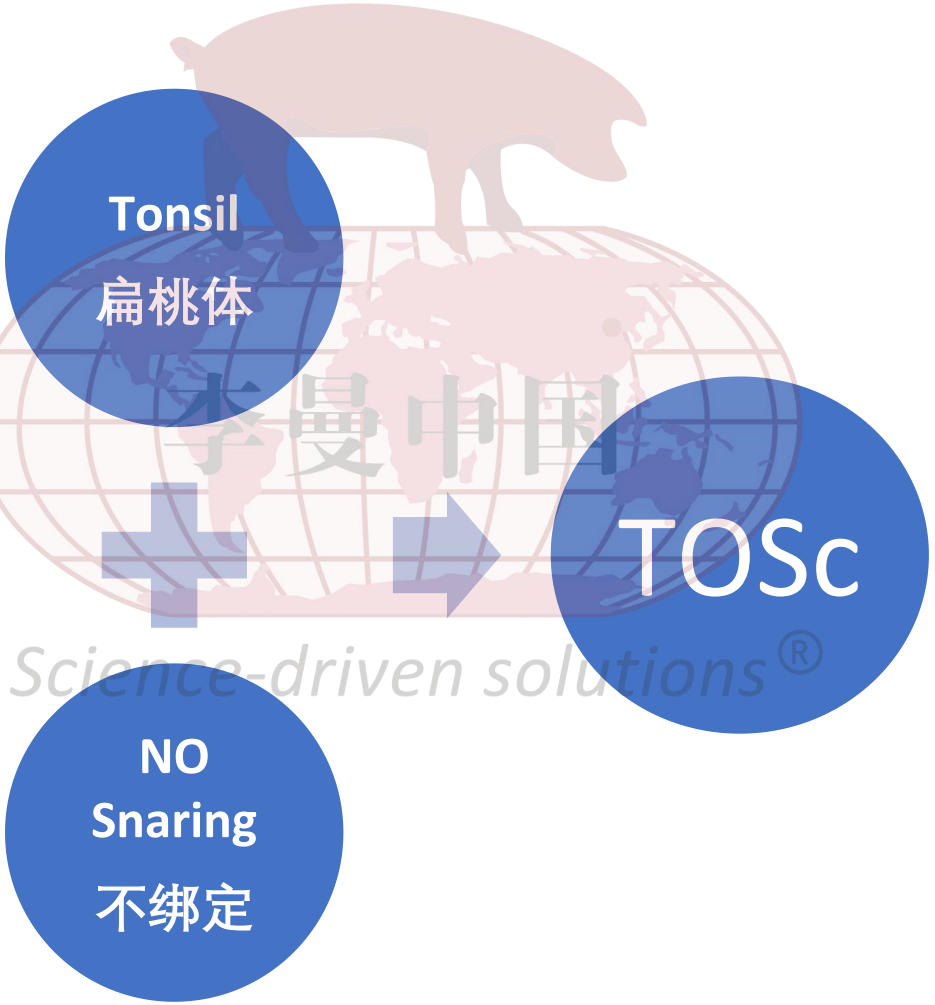
PRRSV RNA in Tonsil: up to 251 days 扁桃体：最晚检出可到251天

Tonsil Scraping（reference method） Limitations
扁桃体刮取液的局限性

- **Labor intensive 费力**
- **Potential of cross contamination 交叉污染**

source: SMEC, Clinical Skills: Tonsil Scrapings (iastate.edu)

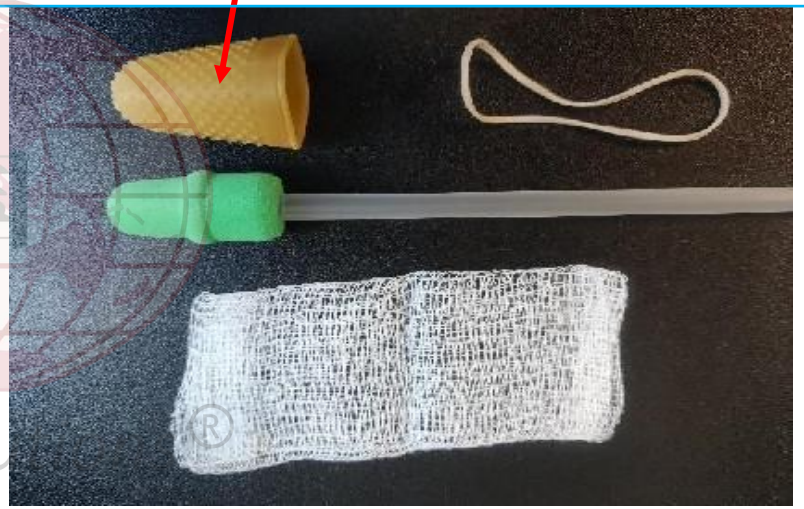
OBJECTIVE: an EASY and PRACTICAL sow samples(tonsil) Without snaring!

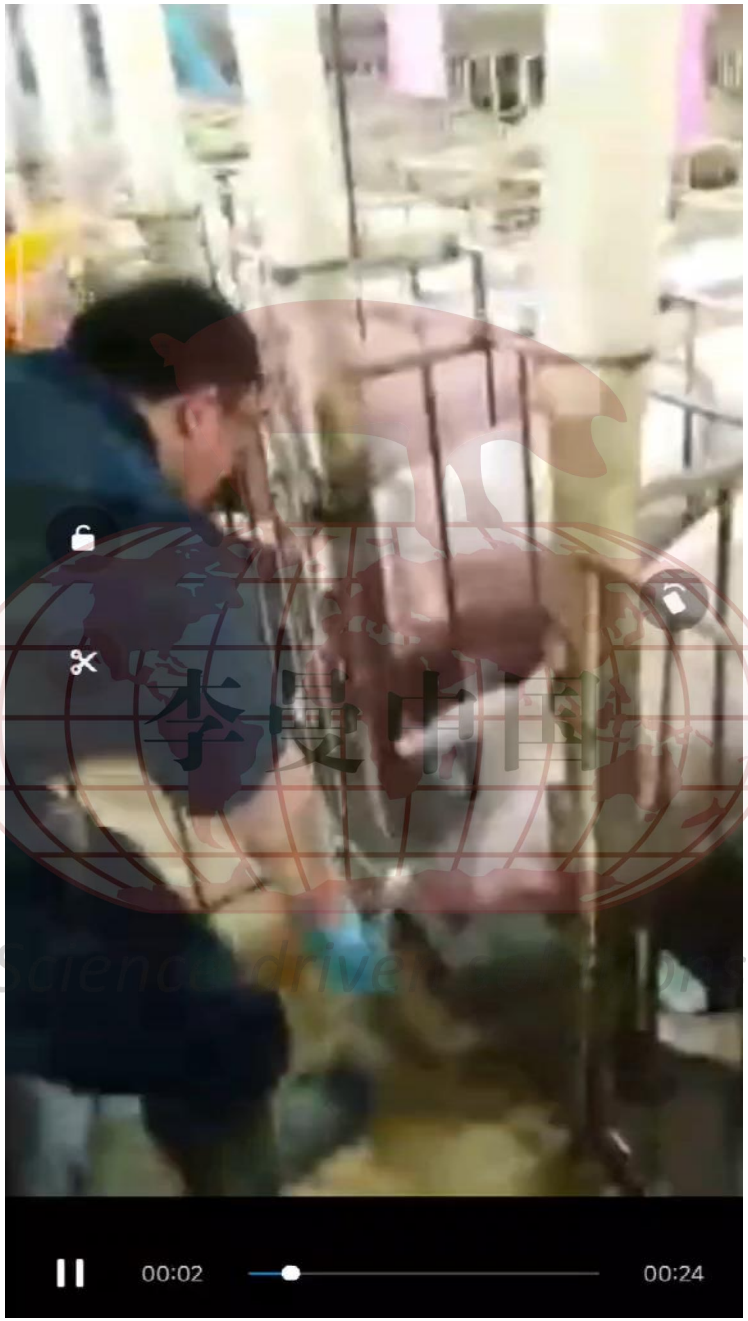


Tonsil-Oral Scrubbing (TOSc) Proof of Concept设计思路

TOSc collector-design of proof of concept

Rubber finger (thimble) to be **"ABRASIVE"** to collect tonsil tissues (**Protruding points for Scrubbing**)
指套：增加摩擦力； 刮取扁桃体组织

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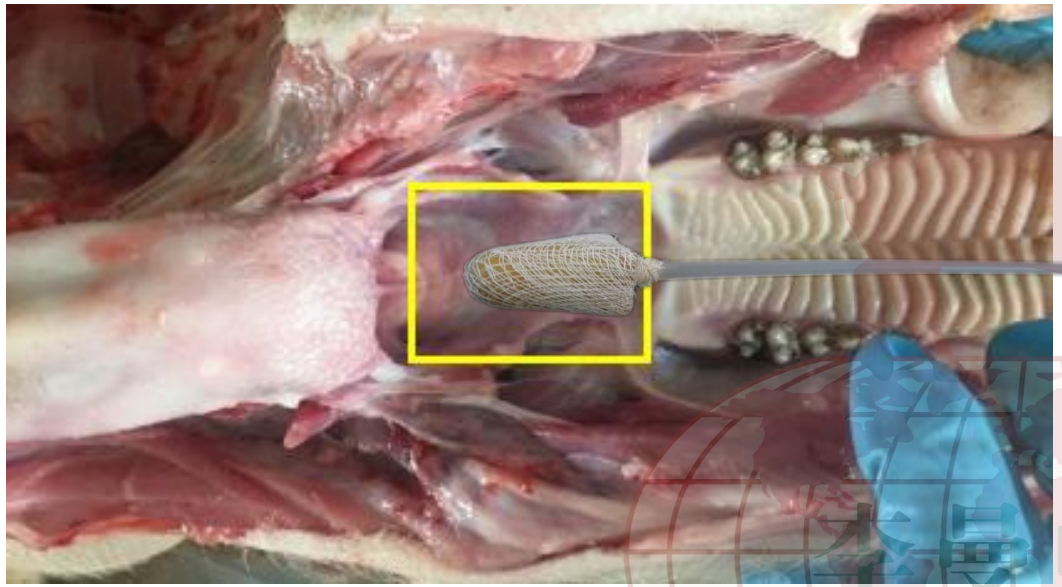
Results: Comparison of sampling types for sows in terms PRRSV PCR positivity, Ct values, and need for straining.

Sample type	Serum 血清	Tonsil Scraping 扁桃体刮液	TOSc
PCR positive rate (95%CI) 阳性率	6.3% [a] (2.1%,16.3%)	73.5% [b] (59.3%, 85.0%)	99.2% [b] (87.2%, 99.2%)
Average and range of PCR Ct values Ct值区间	35.2 (33.6-36.4)	30.7 (25.1-35.4)	29.7 (24.1-36.3)
Need for straining sows 是否要绑定	Yes	Yes	**No**

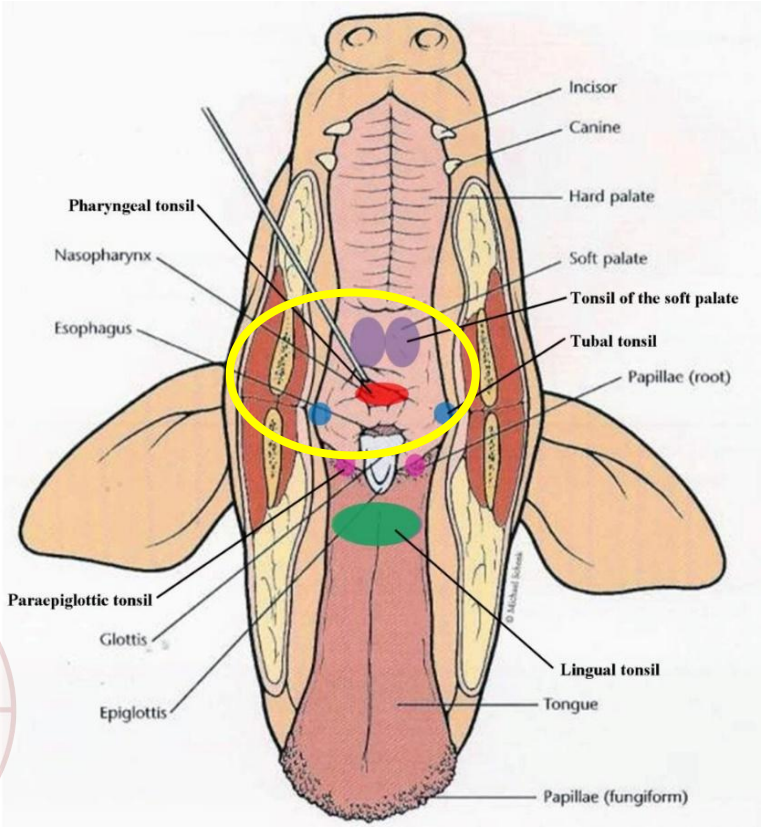
ab: different superscript letters indicate significant difference in least square means (Tukey test, $p<0.05$)

What’s TOSc-Tonsil-oral scrubbing?
TOSc 样品解析

Key points of TOSc collection process:
TOSc 采集关键点



Source: Pigs: a step-by-step guide to sampling and post-mortem | Agriculture and Food

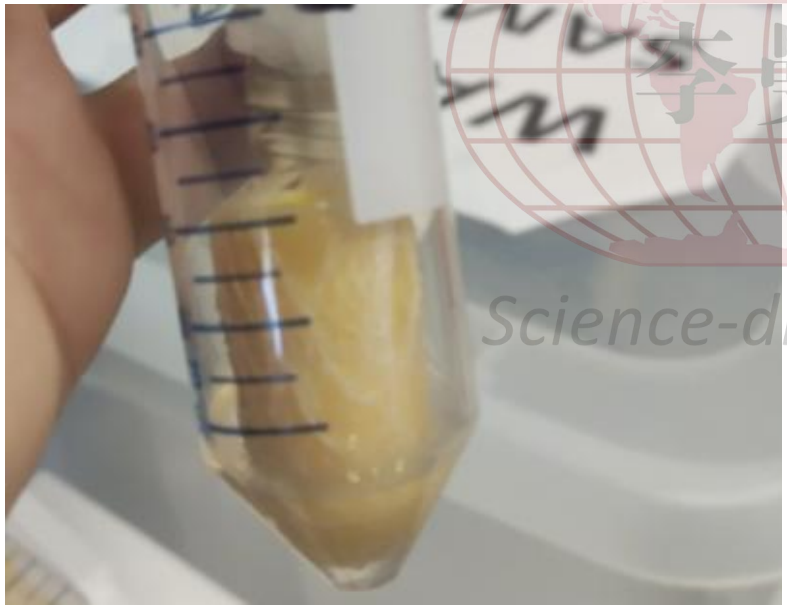


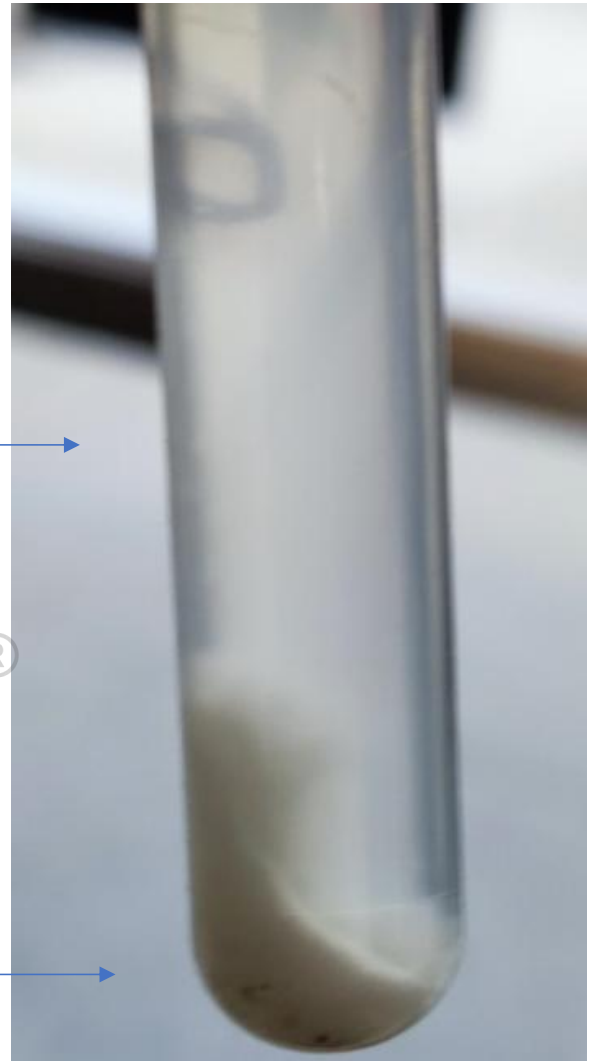
Source: Adina Rachel Bujold, Role of Actinobacillus suis adhesins in host-pathogen interactions in the tonsils of the soft palate of swine

1. In parallel with the vertical axis of mouth; (move) 与嘴中线平行
2. Depth with 5 inches of handle outside the mouth 输精管在外8-10公分
3. Upwards angle 采集器向上
4. Reaching soft palatine tonsil, No resistance when scraping (hard palatine or bitten by the sow) 穿过硬腭，达到软腭扁桃体（采集时应感受不到非常硬的阻力）
5. Helpful when some sow is pushed frontwards especially for gilts.

TOSc作为与口腔液和扁桃体刮除不同样本类型的支持证据
Supporting evidences of TOSc as a different sample type from Oral fluid and tonsil scraping

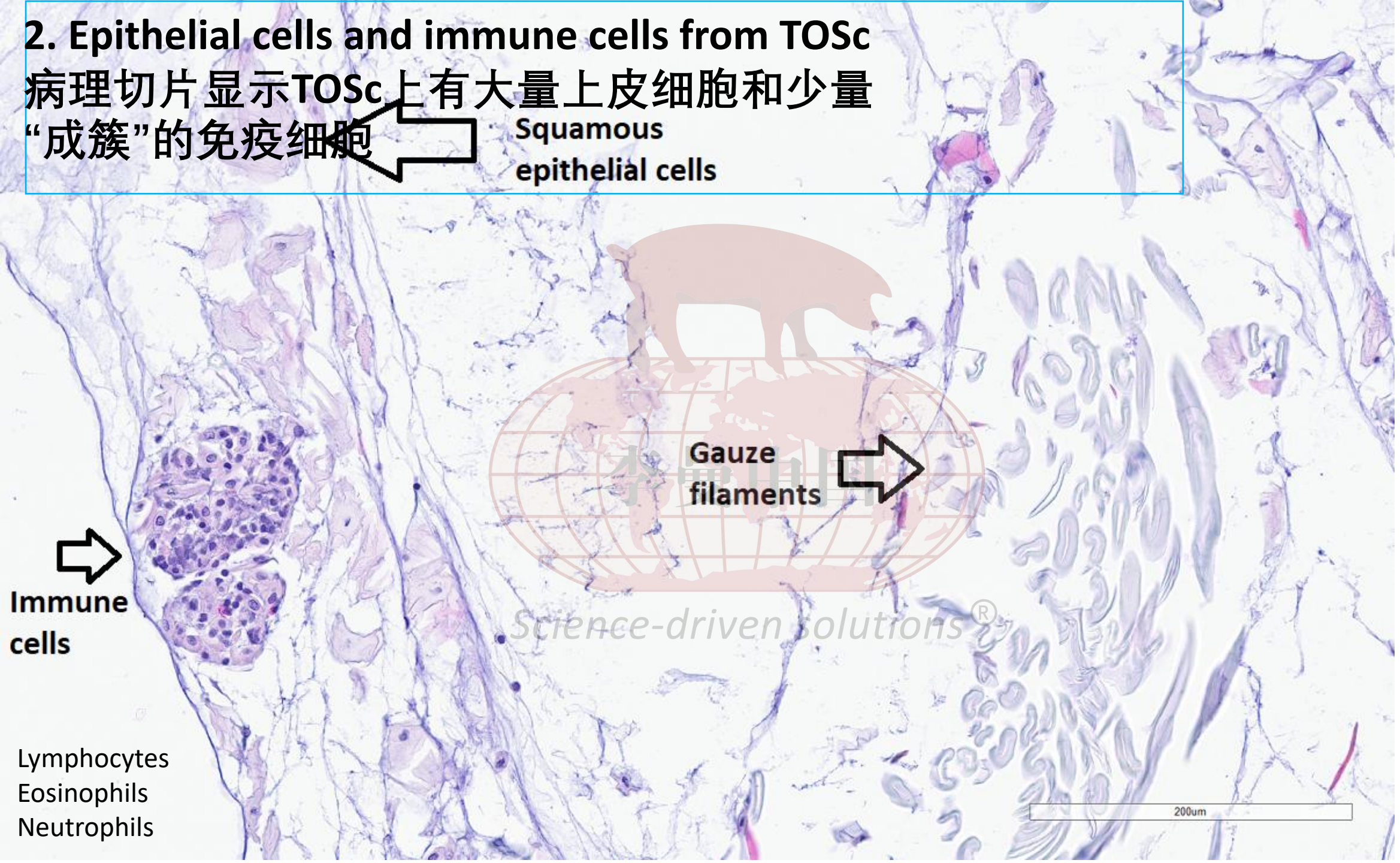
1. Increased cell deposit
细胞沉淀物





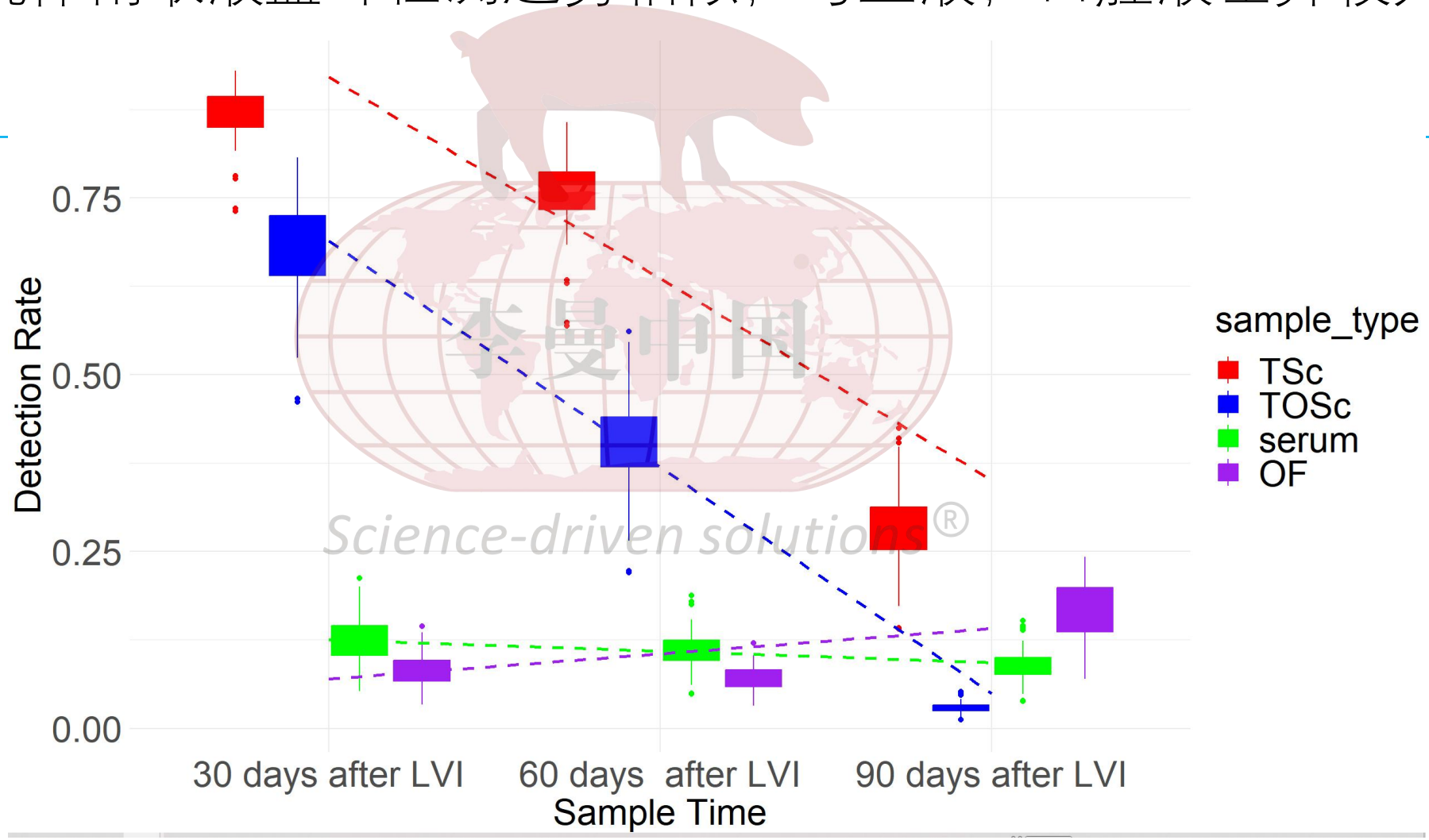
2. Epithelial cells and immune cells from TOSc

病理切片显示TOSc上有大量上皮细胞和少量“成簇”的免疫细胞



3.Similar PRRSV detection pattern with tonsil scraping and distinct mode from serum and OF over time

TOSc与扁桃体刮取液蓝耳检测趋势相似，与血液，口腔液差异较大

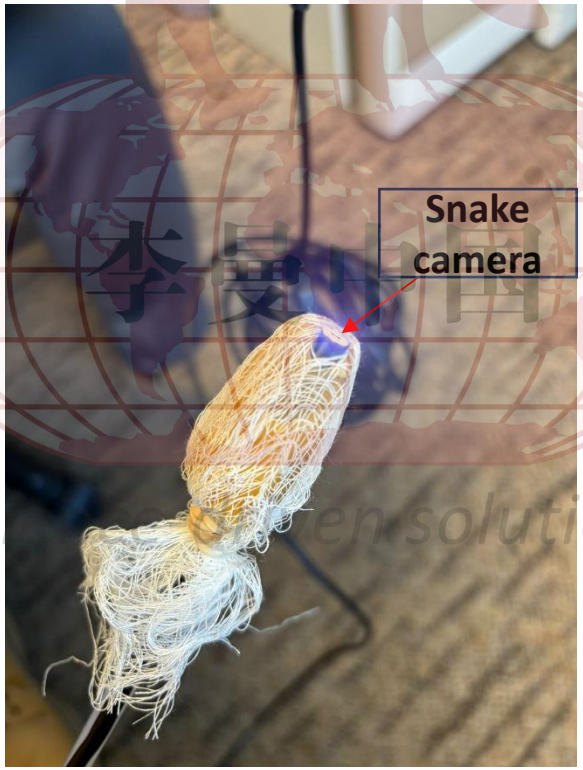


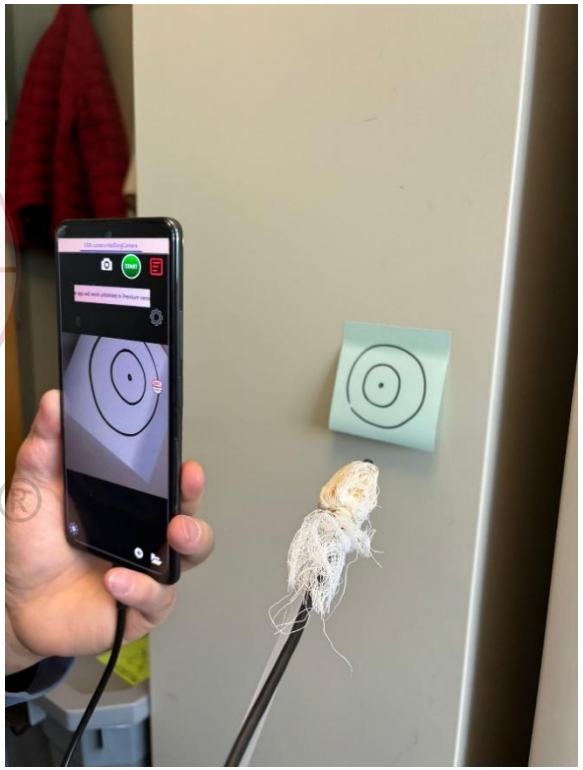
Characterize TOSc collection process by visualization
实用蛇形摄像头对采集过程进行可视化

- Research question: Does TOSc collection really target the tonsil area? Can we visualize TOSc collection process?
- 是否真的采集到扁桃体区域

- Study design: 实验设计
  - Visualize TOSc collection process by a snake-camera 蛇形摄像头

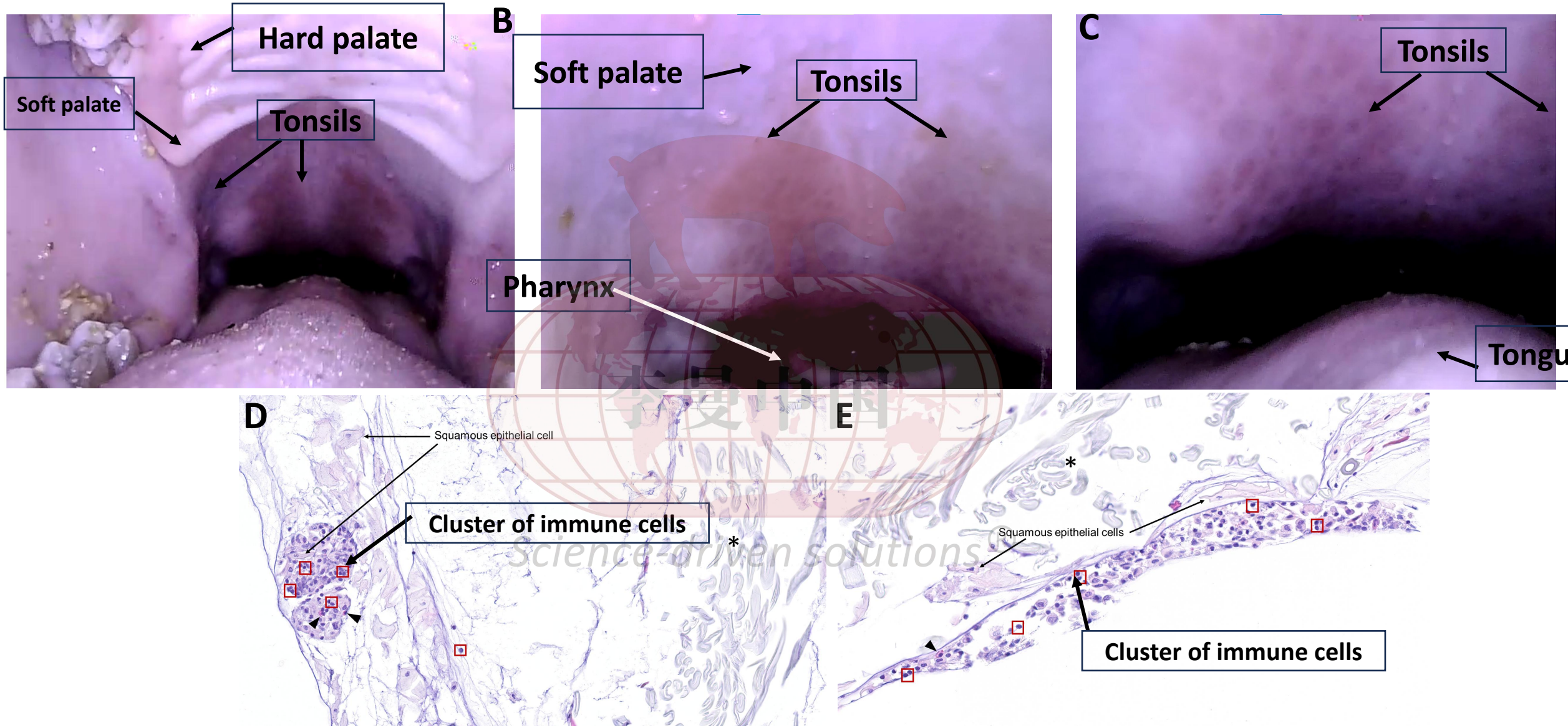
Assembly of TOSc with snake-camera 组装蛇形摄像头+TOSc 采集器





A

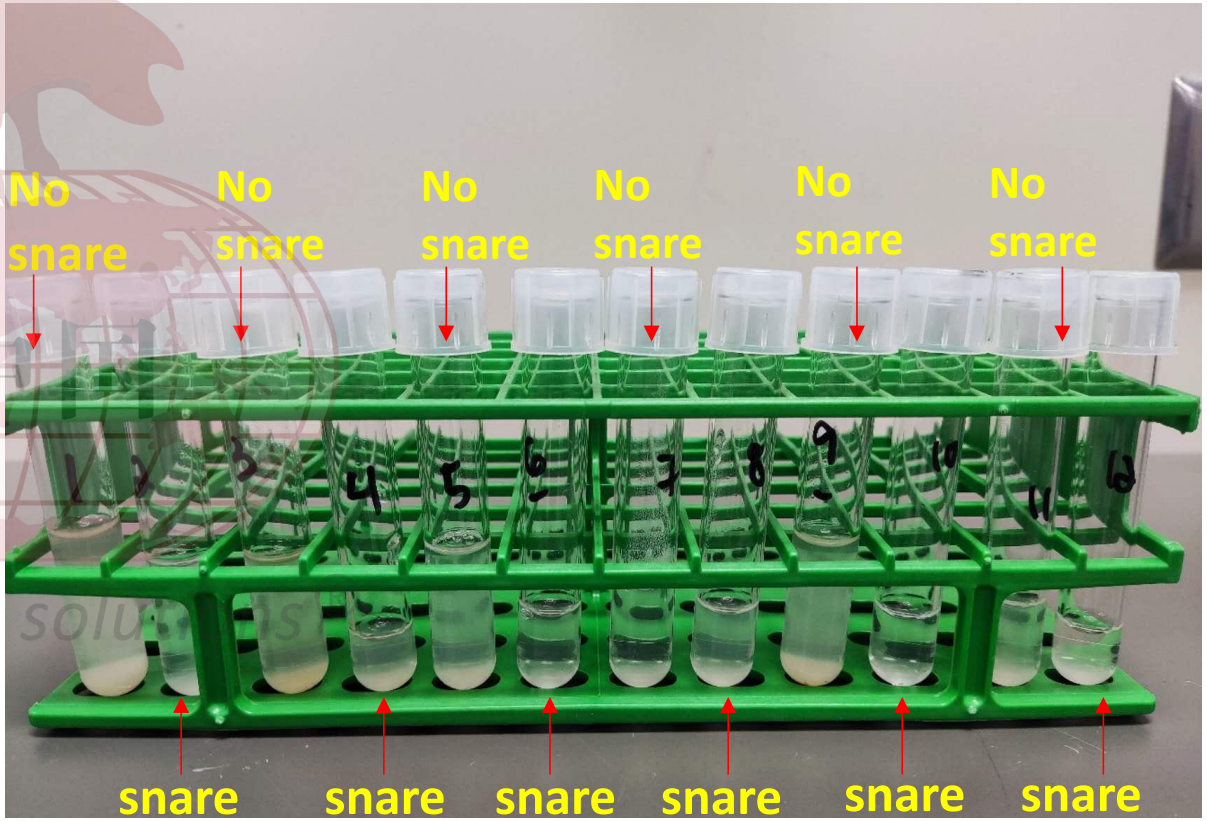
B


Hard palate
Soft palate
Tonsils
B
Soft palate
Tonsils
Pharynx
C
Tonsils
Tongu
D
Squamous epithelial cell
Cluster of immune cells
*
E
*
Squamous epithelial cells
Cluster of immune cells

Major differences of TOSc with other sample types
TOSc与其他样本的区别

- Without Snaring 不用绑定
  - Easy to collect 采集简单
  - more fluid and cell deposit 更多的液体与细胞沉淀物

- Scrubbing: 刮取
  - increase contact between tongue, tonsil and oropharyngeal areas by activating swallow reflex and adding an abrasive thimble;
  - 通过“凸点”和利用吞咽反射增加刮取的效果



Assessment of predictive values of TOSc for liveborn PRRSV status

2. 评估母猪TOSc阴阳性能否预测出生仔猪蓝耳状态

Hypothesis & Objectives 假设与目的

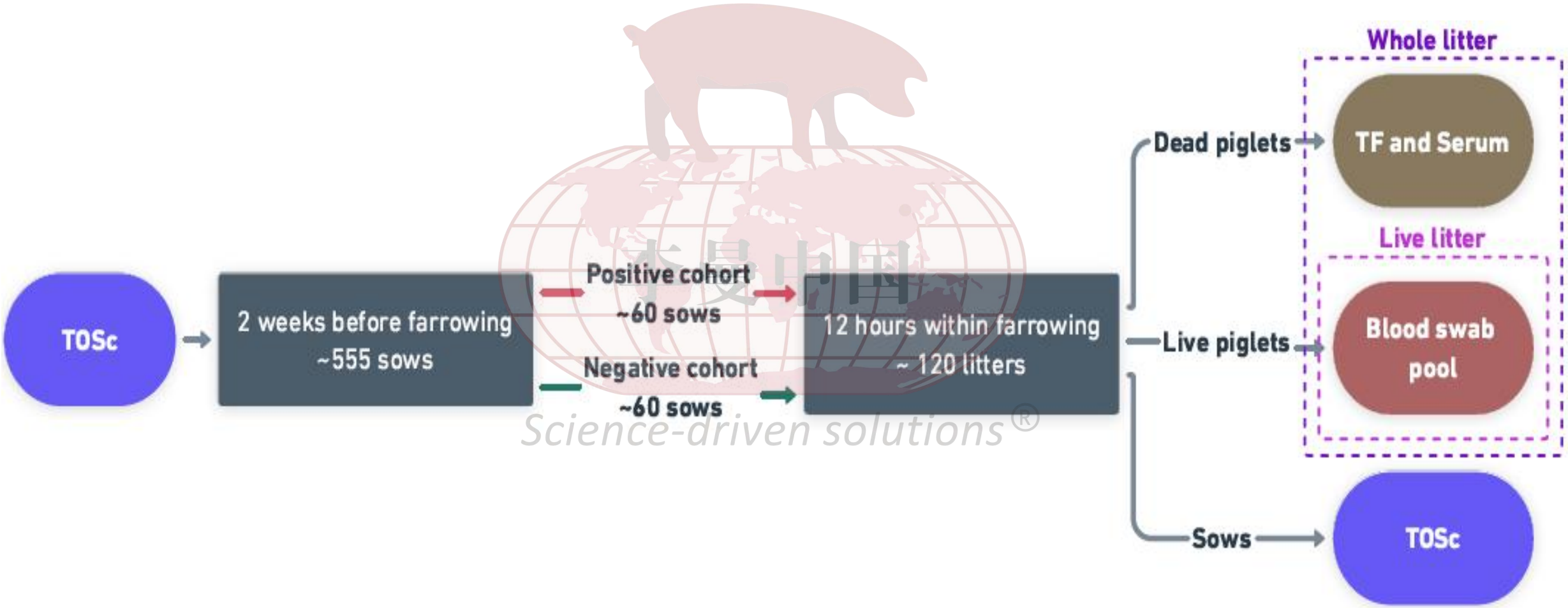
- **Hypothesis 假设**
- We hypothesize that TOSc from gestating sows reflect the status of subsequent liveborn pigs. 怀孕母猪蓝耳状态能够反映仔猪状态

- **Objectives 目的**

This was a field study monitoring individual sows before and after farrowing, and their offspring for PRRSV RNA by qPCR in different sample types. The objectives of this study are twofold: 两层目的

- Determine the positive and negative predictive values (PPV and NPV) of piglets based on qPCR results from sow TOSc. 母猪TOSc蓝耳状态对仔猪的阳性/阴性预测值（PPV/NPV）
- Describe the PRRSV qPCR results (positivity and Ct values) for each set of sows and piglet specimens; 母猪仔猪样本蓝耳状态分布

Study design 实验设计



PRRSV positivity and Ct values of TOScs, blood swab pools and TTF and dead serum 结果

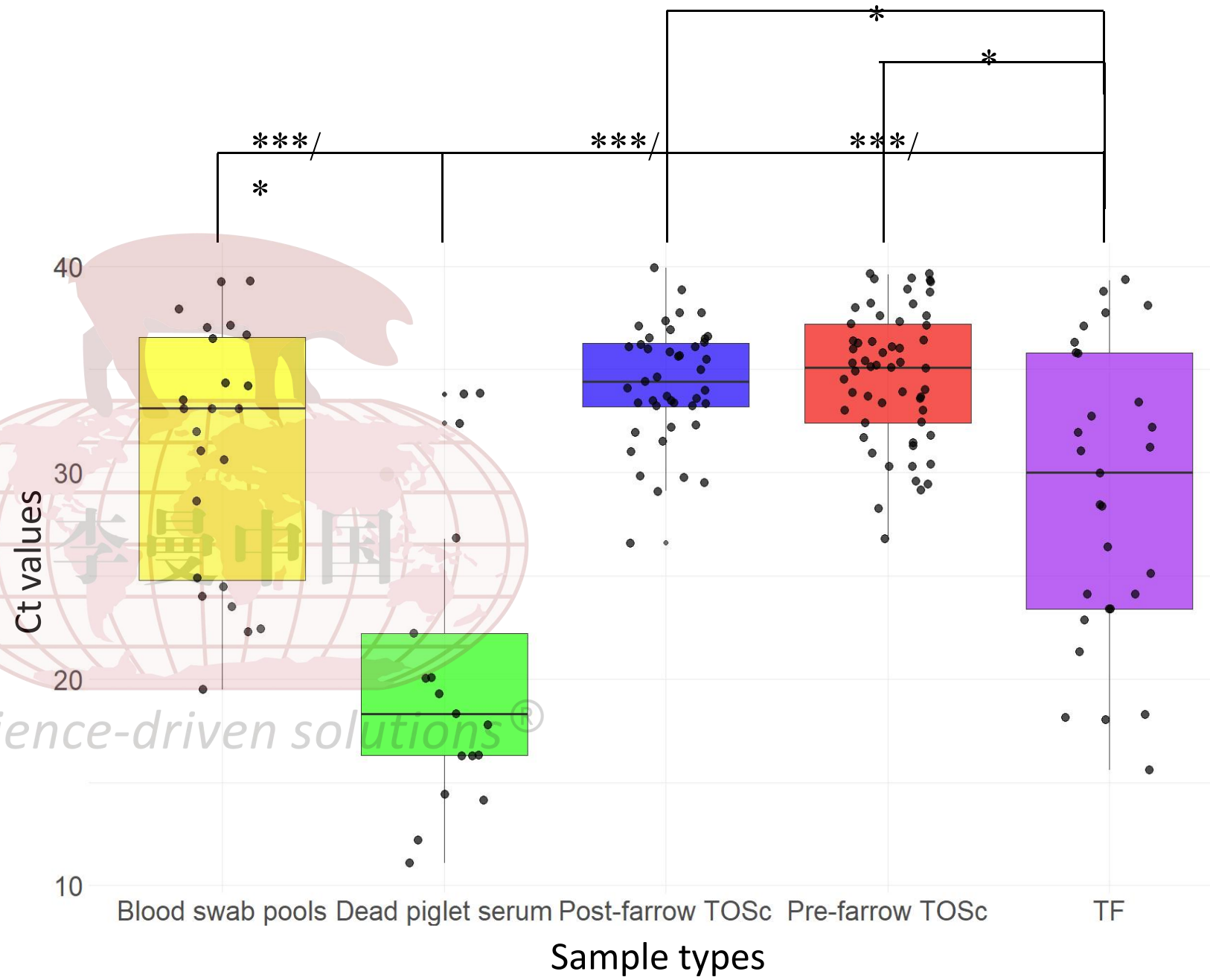
	Gestation TOSc 配怀 TOSc	Farrowing TOSc 产房TOSc	Blood swab pools 血拭子混样	TTF 舌尖液	Dead Serum 死猪血清
POD 蓝耳检出率	34.3% (20.6-51.1%) c	21.0% (11.2-35.9%) bc	8.0% (3.5-17.5%) a	10.9% (5.0-22.0%) ab	4.7%(1.8-11.8%a)
Positive Ct range Ct 值	34.7 (26-39.6)	34.2 (26.6-39.9)	31.2(19.5-39.3)	28.9 (15.6-39.3)	20.3(11.1-33.8)

a/b/c GLMixed, LSMean

- Higher detection rate in sow TOSc samples than litter samples: Blood swab pools, TTF and dead serum; 母猪阳性率更高
- Lower Ct values in litter samples than sow TOSc samples; 死亡仔猪病毒载量低

- **Dead serum samples were of significantly lower Ct values than all other sample types;**

- **TTF were of significantly lower Ct values than TOScs**



Summary table for Predictive values
TOSc的阴阳性预测值

	Gestation TOSc testing for Live litter 配怀TOSc对新生活仔	Farrowing TOSc testing for Live litter 产房TOSc对新生活仔	TOSc Parallel testing for Live litter 串联TOSc检测对对新生活仔	TTF testing for Live litter with dead piglets
NPV	87.2% (80.2-94.3%)	89.0%(82.9-95.1%)	91.7%(85.5-98.1%)	95.4% (88.8-100%)
PPV	22.8% (11.9-33.7%)	30.2%(16.5-44.0%)	25.7%(15.5-36.0%)	58.3% (38.6-78.1%)

- Similar PPV for all sow groups; 阳性预测值基本一致25%
- NPV: TTF > Parallel TOScs > Farrowing TOSc > Gestation TOSc 阴性预测值：串联TOSc > 产房TOSc > 配怀TOSc

Conclusion & indications 1-virus distribution
结论 & 启示1

At farrowing Interface

- We can detect **more positive sows** than litters 3 months after LVI and closure; 血清接种3个月后，母猪阳性率要高于仔猪阳性率 （检测剔除？？）

- But serum and TTF from dead piglets are of significantly lower Ct values than TOSc from sows 仔猪血清，舌尖液Ct值显著低于其他样本 （McRebel，产房死猪处理模式变化）

Conclusion & indications 3-Predictive values

Repeatable in 2 farms:

- **Similar PPV for all sow groups; 阳性预测值基本一致25%**
- **NPV: TTF > Parallel TOScs > Farrowing TOSc > Gestation TOSc 阴性预测值：串联TOSc > 产房TOSc > 配怀TOSc**

- Potential use for **sow segregation/test removal of positive sows from negative sows; 检测剔除（隔离）的潜在应用**
- **Potential indicator of TOSc and TT as a classification criteria for negative herds TOSc在评估干预方案以及蓝耳分类的潜在应用**

下一步问题 Next question

- 何时检测剔除生产经济效益最高When to detect and eliminate the highest economic benefits of production
  - 蓝耳病在母猪群体感染动态如何 How is the infection dynamics of blue ear disease in sow populations
    - 长期携带者？Long term carrier?
    - 同一毒株再次“爆发”？Is the same strain outbreak again?
  - 低流行率：何时达到10%；5%？Low prevalence: When did it reach 10%; 5%?
- 对于哪一群体进行监测更加有效
- For which group is monitoring more effective
  - 全群监测？Whole group monitoring?
  - 产房批次性监测？Batch monitoring in the delivery room?

Characterizing PRRSV dynamics in the sow population using TOSc after whole-herd exposure by live virus

在繁殖群暴露后用TOSc调研母猪蓝耳感染动态

Background 背景

- Some questions on the dynamics of PRRSV infection in the sow population after LVI remains unanswered.LVI后母猪群体中PRRSV感染动态的一些问题仍未得到解答。

- For example, how long will it take to reach low prevalence in sows? 何时母猪群达到第流行率
- How many long-term carrier sows are there, and when do they turn negative? 多少“长期感染”母猪
- What are the risk factors affecting the sows PRRSV dynamics? 哪些因素导致长期感染

Hypothesis & objective 假说与目标

- **Hypothesis 假设**
- We hypothesized that TOSc can be used to characterize the dynamics PRRSV infection in the sow population over time. TOSc可以用于繁殖群蓝耳的监测

- **Objective 目标**
- The objective of this proposal is to characterize the dynamics of PRRSV in breeding herds by TOSc over a complete reproduction cycle (21 weeks). Two specific aims are: 双重目标
- Aim 1 – Reveal the dynamics of PRRSV RNA detection in individual sows over time following whole-herd exposure to LVI. This aim will address the following questions: What is the variability between sows of test results from 1 to 21 weeks post-exposure? When will the herd achieve low prevalence (10%, 5% and 1%)? 群体何时能够到达低流行率
- Aim 2 – Characterize risk factors associated with long-term carriers. Key factors include: parity, gestation stage, PRRSV status before LVI**. 到达流行率的影响因素**

Study design 实验设计

LVI 血清接种

		week-1	week1	week5	week9	week13	week17	week21
妊娠（1-4周）	1st stage(1-4wks)							
妊娠（5-8周）	2nd stage (5-8wks)							
妊娠（9-12周）	3rd stage (9-12wks)							
妊娠（13-16周）	4th stage (13-16+wks)							
分娩断奶	5th stage (lactation + weaned)							

Each Gestation stage group of 60 animals contains 3 subgroups of different parities: 血清驯化前，每个生产阶段的母猪各60头；

(1) P0=20,

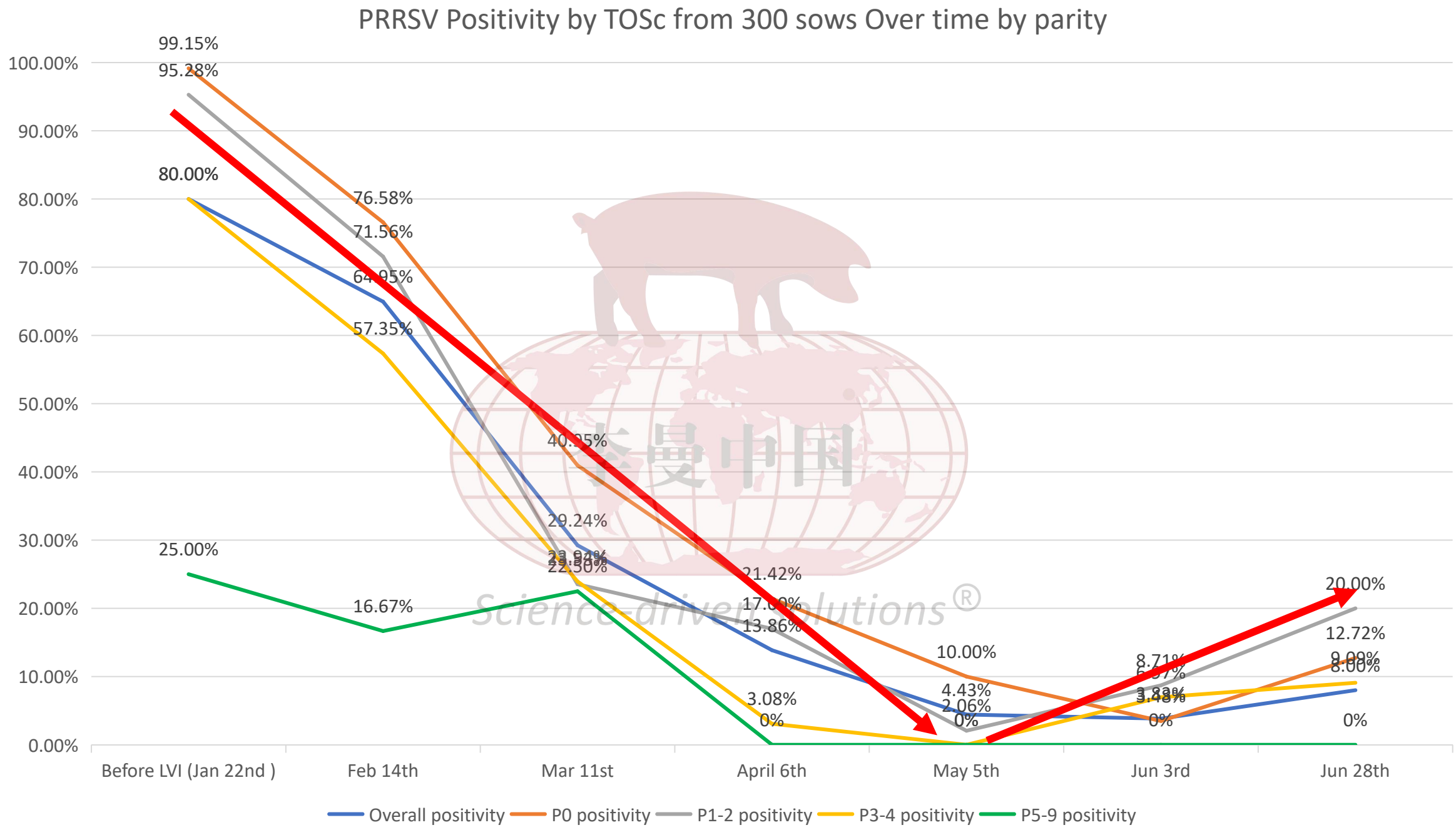
(2) P1,P2=20,

(3) P3+=20

Results-TOSc Positivity over time by parity
胎次对于TOSc阳性率的影响

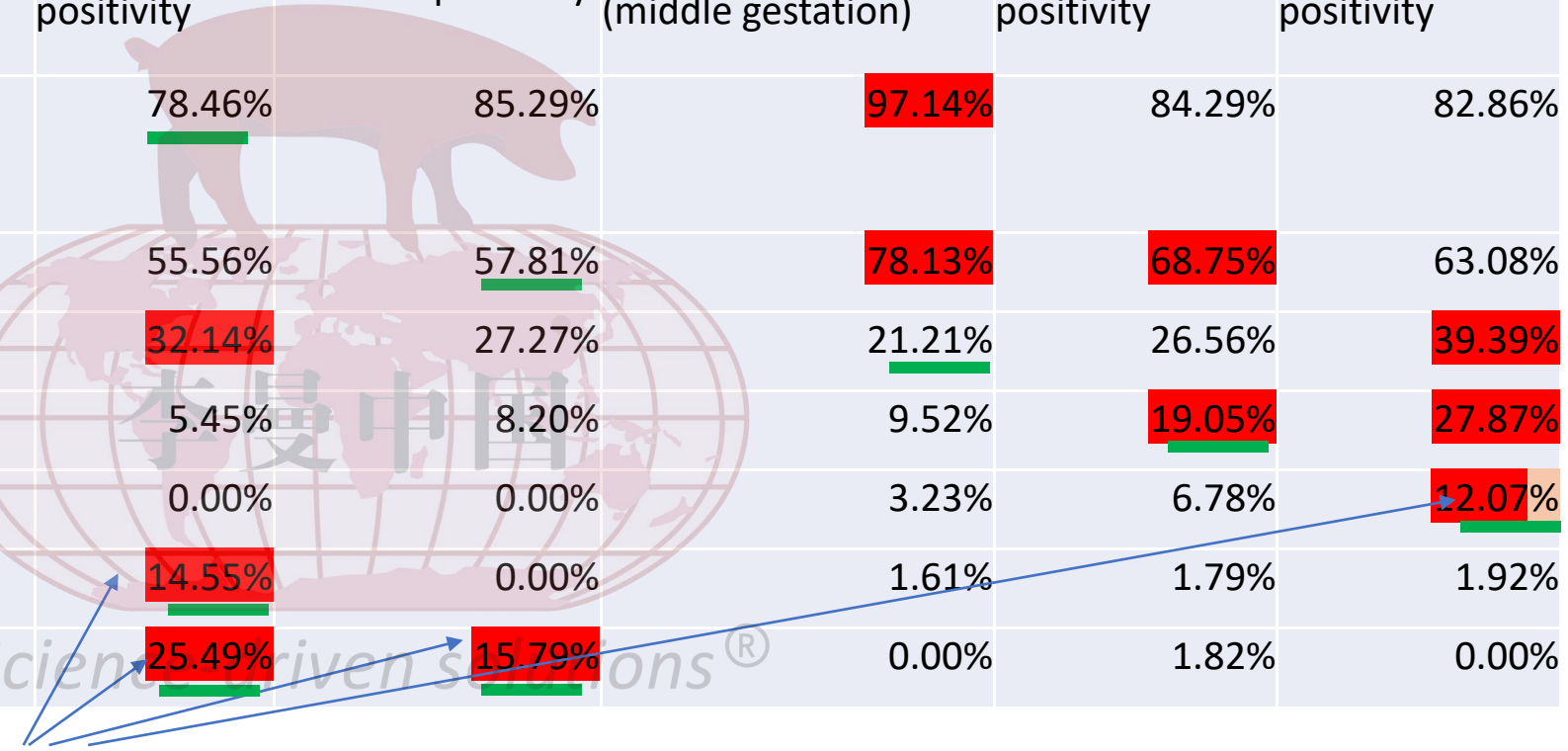
PRRSV positivity	Overall positivity	P0 positivity	P1-2 positivity	P3-4 positivity	P5-9 positivity
Before LVI (Jan 22nd)	80.00%	99.15%	95.28%	80.00%	25.00%
Feb 14th	64.95%	76.58%	71.56%	57.35%	16.67%
Mar 11st	29.24%	40.95%	23.53%	23.94%	22.50%
April 6th	13.86%	21.42%	17.00%	3.08%	0%
May 4th	4.43%	10.00%	2.06%	0%	0%
Jun 3rd	3.83%	3.48%	8.71%	6.97%	0%
Jun 28th	8.00%	12.72%	20.00%	9.09%	0%

Overall, P0 and P1-2 had highest PRRSV positivity **0-2 胎阳性率最高**

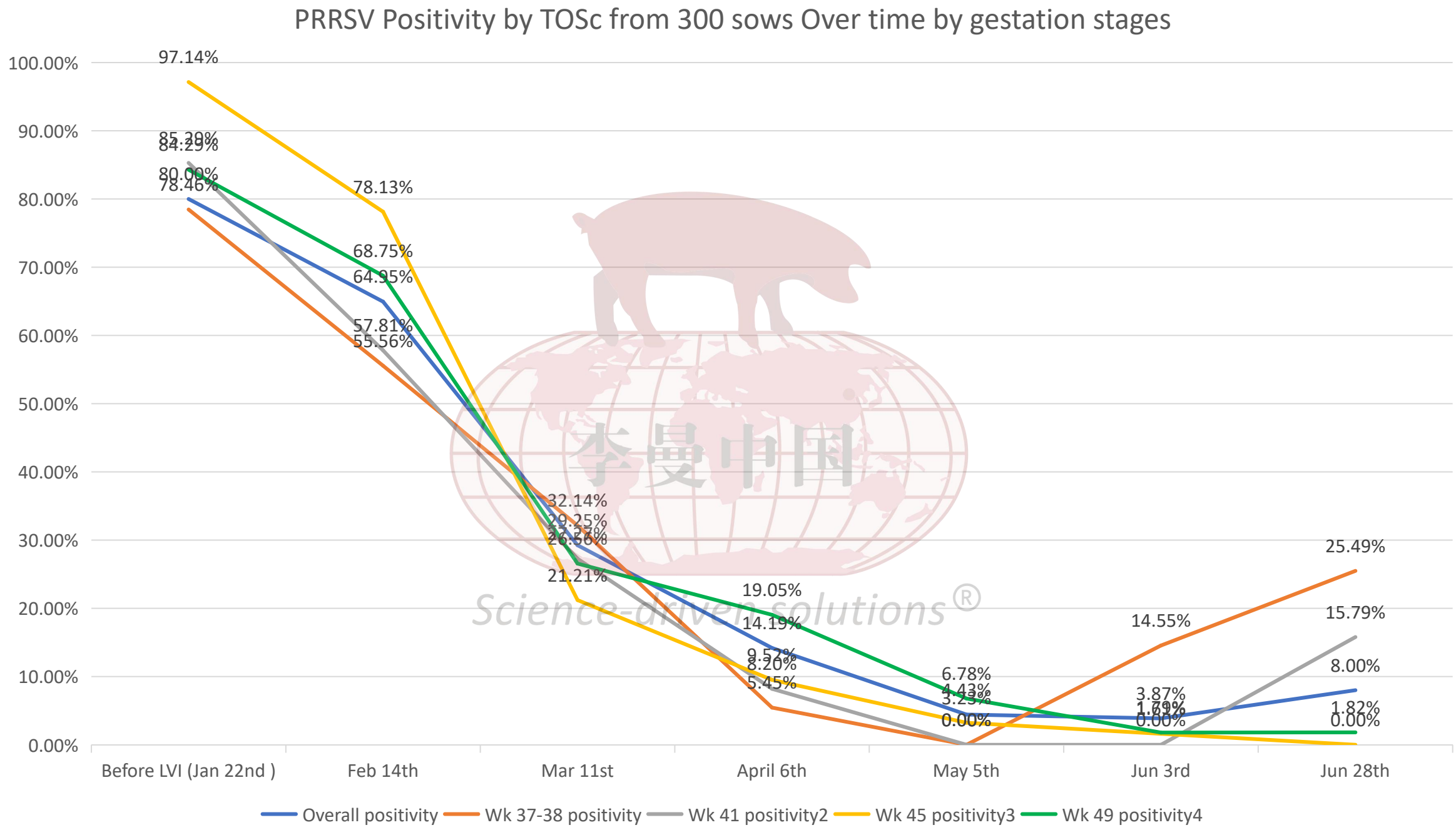


Results TOSc positivity over time by gestation stages
妊娠日龄对于TOSc阳性率的影响

PRRSV positivity	Number of samples	Overall positivity	Week 37-38 positivity	Week 41 positivity	Week 45 positivity (middle gestation)	Week 49 positivity	Week 1 positivity
Before LVI (Jan 22nd)	343	80.00%	78.46%	85.29%	97.14%	84.29%	82.86%
Feb 14th	309	64.95%	55.56%	57.81%	78.13%	68.75%	63.08%
Mar 11st	318	29.25%	32.14%	27.27%	21.21%	26.56%	39.39%
April 6th	303	14.19%	5.45%	8.20%	9.52%	19.05%	27.87%
May 4th	293	4.43%	0.00%	0.00%	3.23%	6.78%	12.07%
Jun 3rd	287	3.87%	14.55%	0.00%	1.61%	1.79%	1.92%
Jun 28th	275	8.00%	25.49%	15.79%	0.00%	1.82%	0.00%



Blue arrow: Farrow to wean batch appear to have highest positivity at low prevalence scenario, especially at Jun 28th, 17 out of 23 positive sows were at farrow-wean stage. 13 out from the 17 farrow-wean sows were recycled sows. (cells underlined with blue were at farrow-wean phase),
indicating that farrowing stress and recycling might be associated with increased PRRSV positivity.
生产应激及返情母猪似乎会增加蓝耳的阳性率



Conclusion & indications 结论 & 启示

- Linear decline of PRRSV positivity based on TOSc after LVI and reached 10% 4 months after LVI;
- 血清接种后，母猪阳性率线性下降, 4个月后阳性率达到~10% （预测达低流行率时间；用于母猪监测的参考 3-4月后开始进行监测；4个月左右开始检测剔除）

- Low parity sows (p0-p2); farrowed sows and recyclers seems to be associated with higher TOSc positivity.
- 低胎龄群体（p0-p2）;分娩母猪和返情母猪与蓝耳高阳性率有关 (产后母猪的监测)

Conclusions & Discussions 结论与讨论

How to test-remove/ segregate如何检测剔除/隔离

- Start TOSc screening on due to farrow or/and farrowing population 3 month after LVI (Normally Ct value of PF starts to increase)
- 血清接种后3月后对待分娩或/及将要分娩母猪进行检测;

- Parallel testing increase the chance of testing TOSc positive
- 间歇性检出；多次采样能够增加TOSc 检出率

- Non-negotiables: Low parity sows (p0-p2); farrowed sows and recyclers
- 低胎龄群体，分娩母猪和返情母猪**必须检测**；

- 3000 sows:120-130 sows/week; 25-26 test/week
- 3000 母猪场：120头母猪/周；25-26 检测/周

- Until 9-13 consecutive week of PF/ TOSc negative
- 连续9-13周睾丸液/TOSc阴性！

- 谢谢
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