Population samples and sampling for diagnostics, monitoring and surveillance

Marcelo Almeida, DVM, MS, PhD

Iowa State University Veterinary Diagnostic Laboratory
Department of Veterinary Diagnostic and Production Animal Medicine
College of Veterinary Medicine

Leman 2024, Chengdu, China

IOWA STATE UNIVERSITY

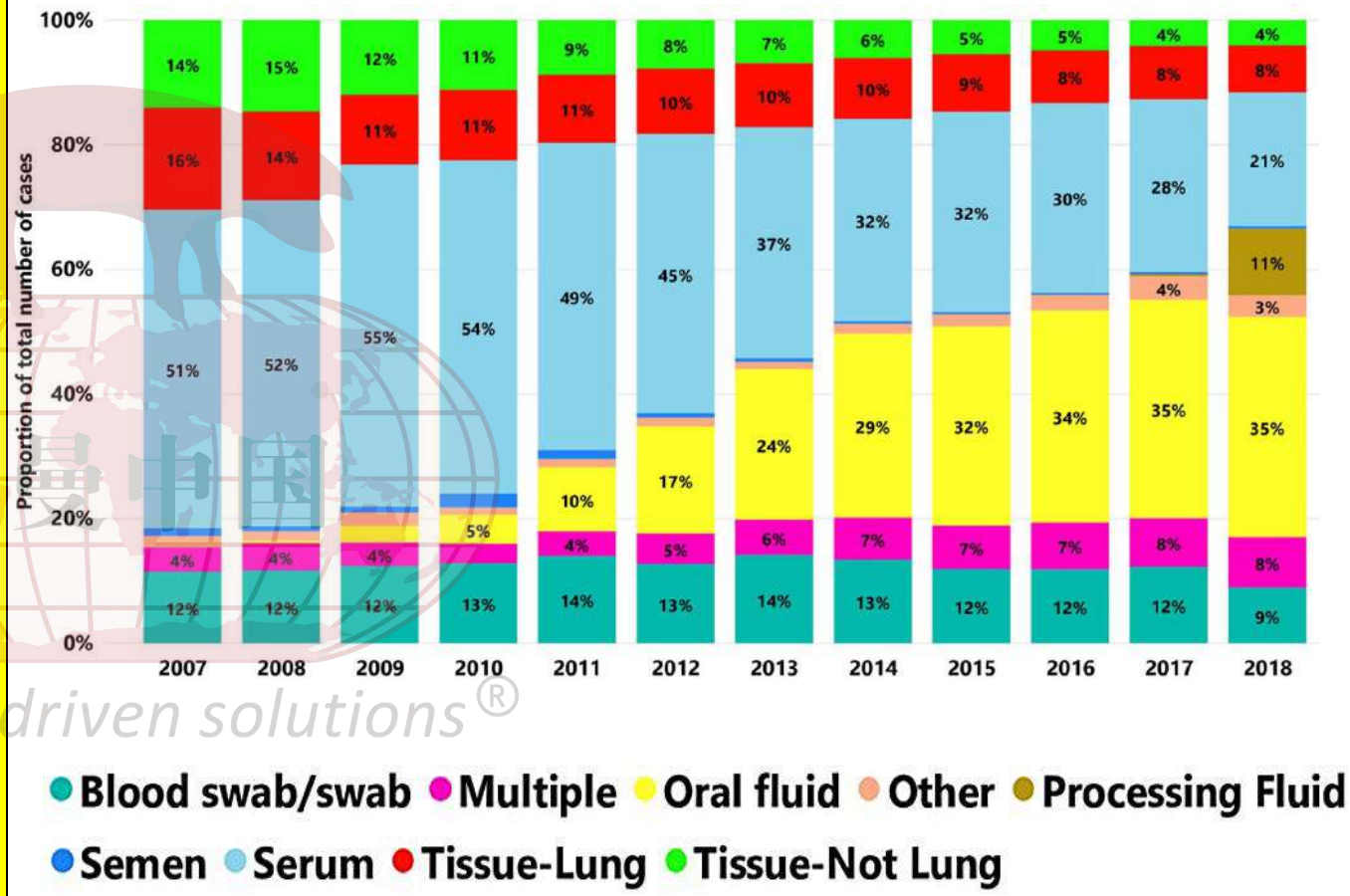
Agenda

- Trends in swine diagnostics in the
- The diagnostic process
- Population samples and diagnoses
- Population samples, sampling, and surveillance
  - Post weaning
  - Sow farms
- Take homes

Trends in swine diagnostics (Trevisan et al., 2019).

- PRRSV RT-PCR testing 2007 - 2018 at 3 U.S. VDLs (547,873 PRRS cases)

Specimen	Year	cases(%)
Tissues	2007	30%
	2018	12% ↓
Serum	2007	51%
	2018	21% ↓
Oral fluid	2007	0%
	2018	35% ↑
Proc fluid	2007	0%
	2018	11% ↑



Detection vs diagnosis

- Health challenges in pigs often present significant diagnostic dilemmas:
  - Many common pathogens are endemic on affected farms
    - Detection may or may not = disease
  - Available diagnostic tests may not readily differentiate pathogens from non-pathogens and/or vaccines
  - Disease expression is variable within and among farms
    - On-farm management factors impact disease expression
  - Mixed infections are common (if not the norm)

The Diagnostic Process

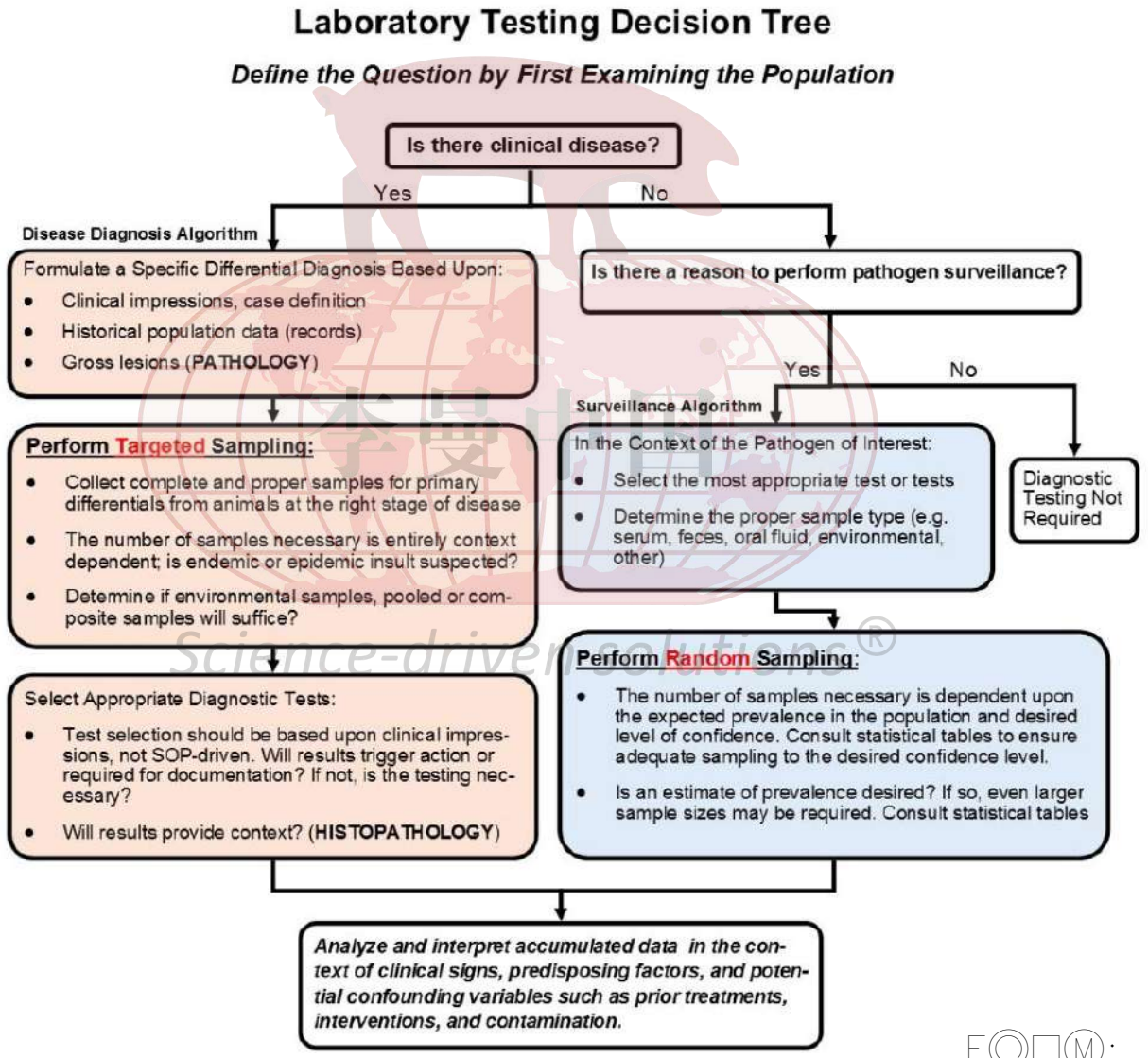
- For diagnostic investigations, finding the “right” answer begins with two fundamental concepts:

1. Well-defined diagnostic question(s)
   - Formulated in context to the specific issue at hand

2. Proper sampling to address these specific question(s)
   - More is not always better, particularly if #1 is ill-defined

The diagnostic process

The diagnostic process



Relevant history and records

Primary complaint, historical issues, treatment process

Clinical observations and gross lesions

Subjective/objective/quantitative assessments

Assess risk factors: Environment, nutrition, commingling, etc

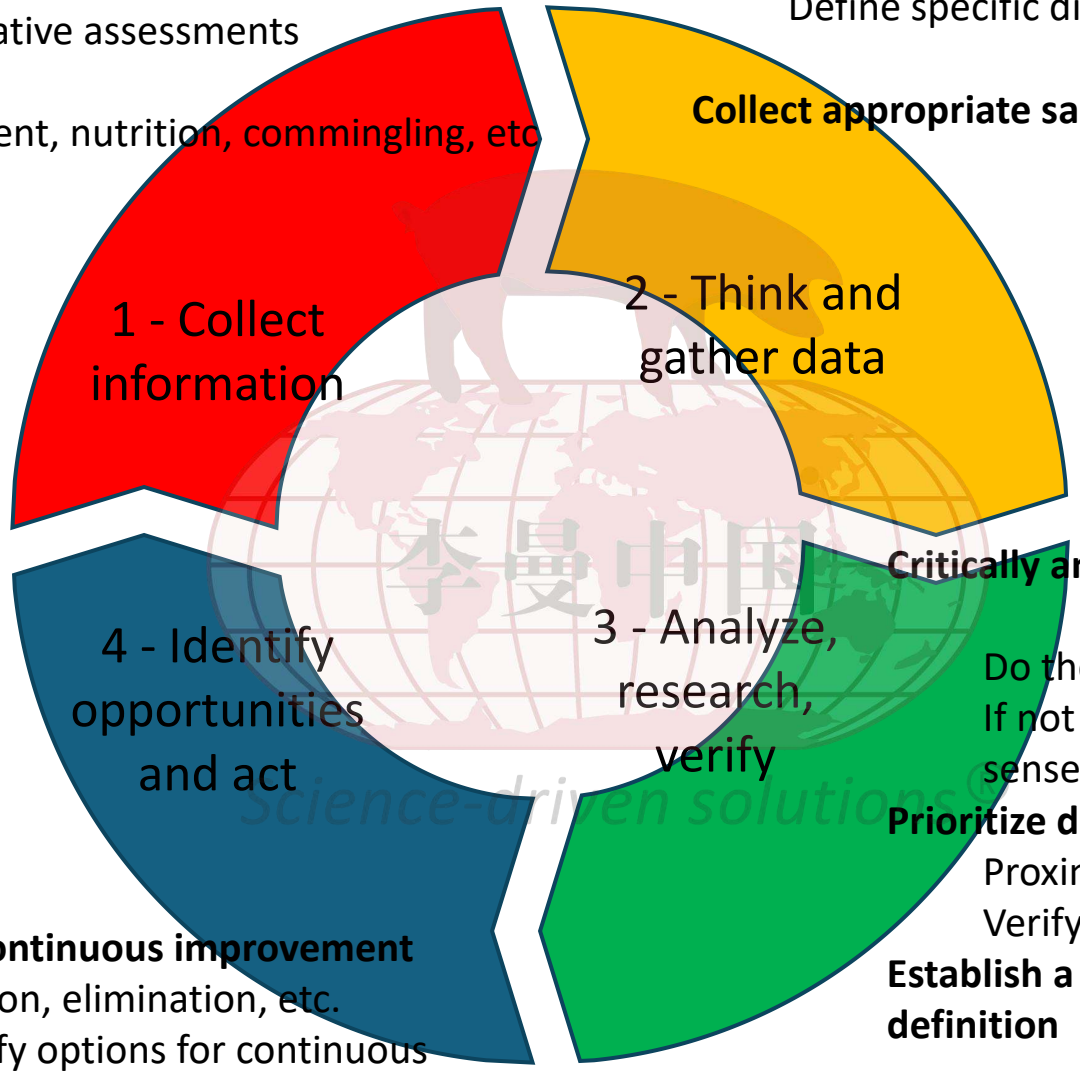
Create case definition/prioritize a realistic differential diagnosis

Formed with current clinical context

Consider laboratory testing

Define specific diagnostic questions that testing can answer

Collect appropriate samples from proper animals (critical step)



Critically analyze diagnostic data

Do the results align (“make sense”) with expectations?

If not alignment, reiterate process until results make sense

Prioritize detected agents and risk factors

Proximate cause(s) versus ultimate cause(s)

Verify with histopathology when possible

Establish a diagnosis and risk factors relevant to case definition

Interventions, monitoring, and continuous improvement

Treatment, control, prevention, elimination, etc.

Monitor and refine or identify options for continuous improvement

Too many specimens and assays

- Choose the specimen(s) to be collected and assay(s) to be used

Specimens

- Oral fluid
- Family oral fluid
- Placental umbilical cord serum
- Tongue fluids
- Processing fluids
- Wipes
  - Udder

Assays

- Antibody
  - ELISA, CF, HI, VN
- Nucleic acid
  - PCR
- Viable agent
  - Culture, VI

Considerations on

- Diagnostic sensitivity
- Diagnostic specificity
- Disease transition stages

The diagnostic process

Population samples for disease diagnosis

Sow farm

Udder wipes

2019 by Dr. Garrido-Mantilla

- Great for respiratory pathogens
  - PRRSV and IAV
- Can detect other pathogens
  - PEDV, PDCoV, SVA, rotavirus, etc.
- Tissues needed to confirm a diagnosis

Advantages

- Lower changes to miss detection
- Increase confidence in role of pathogens in disease occurrence

Family oral fluids

2021 by Dr. Almeida

Population samples, sampling, and surveillance in breeding herds

Serum x PF and FOF

Detecting PRRSV in nursing piglets

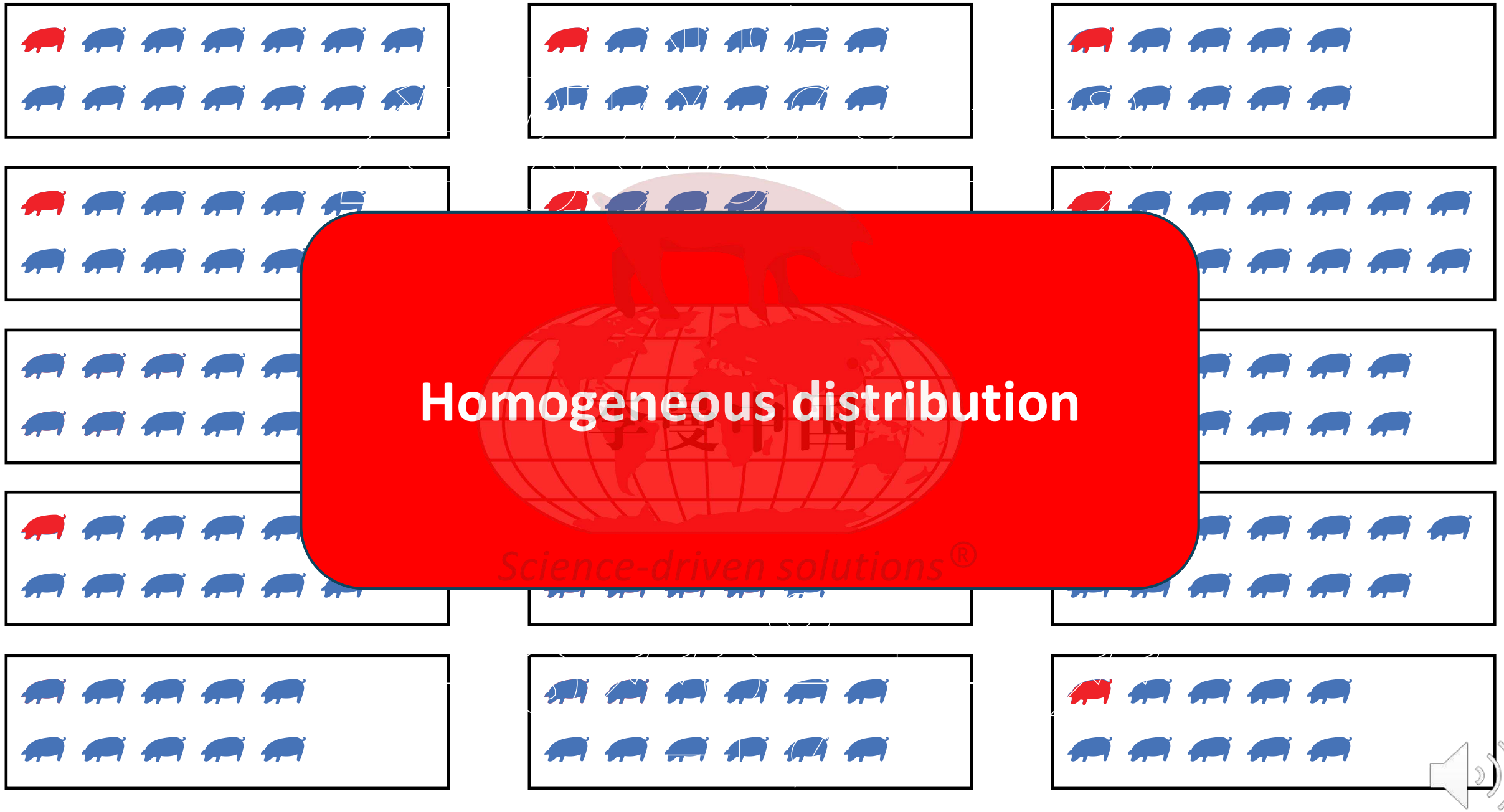
Population Size (Detecting One or More Positives)												
Prevalence Estimate % Positive	Confidence Level	100	200	400	600	800	1000	2000	4000	6000	8000	10000
>1%	70%	71	92	105	110	113	114	118	120	120	120	121
	80%	81	112	133	142	147	149	155	158	160	160	160
	90%	91	138	176	192	201	206	218	224	226	227	228
	95%	96	156	211	236	250	259	278	289	292	294	295
	99%	100	181	274	321	350	369	411	434	443	447	449
>2%	70%	46	53	57	58	59	59	60	61	61	61	61
	80%	56	67	74	76	77	78	80	80	81	81	81
	90%	69	88	101	105	108	109	112	114	114	115	115
	95%	78	106	125	133	137	139	144	147	148	148	149
	99%	91	137	175	191	200	205	217	223	225	226	227
>5%	70%	22	24	24	25	25	25	25	25	25	25	25
	80%	28	30	32	32	32	32	33	33	33	33	33
	90%	37	42	44	45	45	45	46	46	46	46	46
	95%	45	52	56	57	58	58	59	59	60	60	60
	99%	60	73	82	85	86	87	89	90	91	91	91
>10%	70%	12	13	13	13	13	13	13	13	13	13	13
	80%	16	16	16	17	17	17	17	17	17	17	17
	90%	21	22	23	23	23	23	23	23	23	23	23
	95%	26	28	29	29	29	30	30	30	30	30	30
	99%	37	41	43	44	44	44	45	45	45	45	45

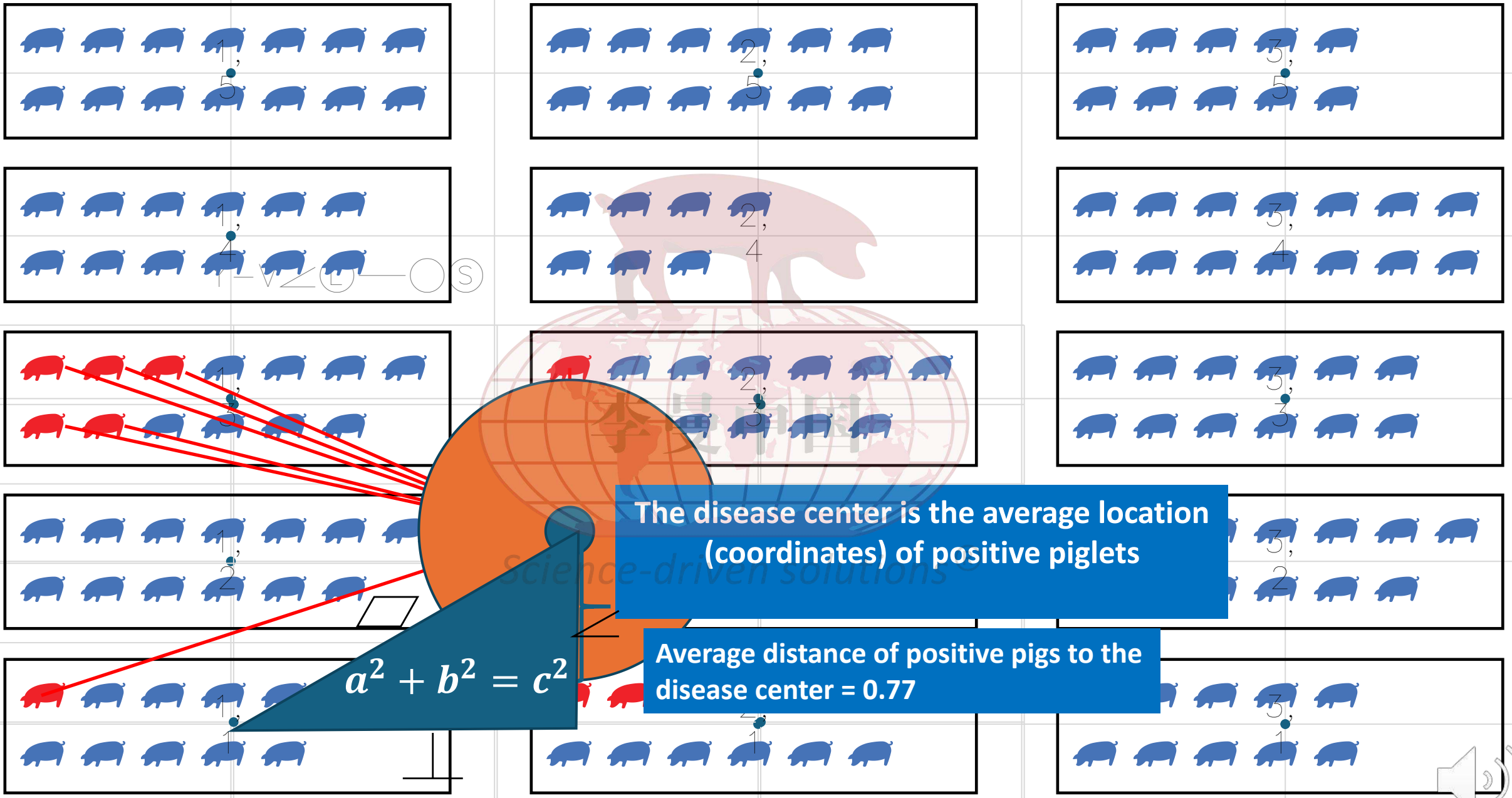
Assumptions:

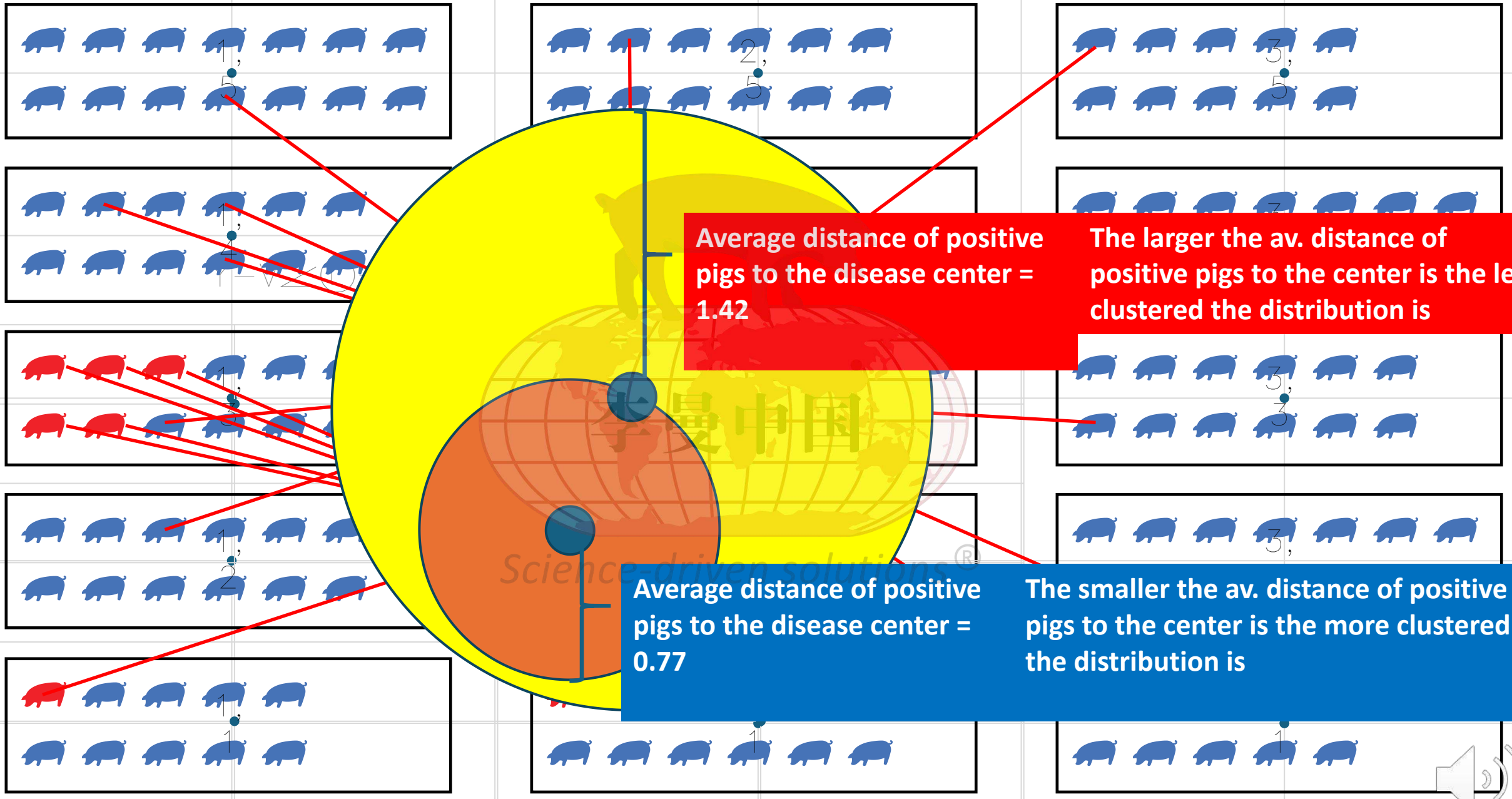
- Hypergeometric distribution
- Simple random sampling

The homogeneous population assumption

- The disease is homogeneously distributed in the population
- Every piglet has an equal chance of being PRRSV-viremic
- Approaches for testing
  - Homogeneity of disease among litters
    - Fisher's exact test Fisher
  - Clustering of viremic piglets within the room
    - Permutation test and Euclidian distance


Homogeneous distribution


1, 5
2, 5
3, 5
1, 4
2, 4
3, 4
1, 3
2, 3
3, 3
1, 2
3, 2
1, 1
2, 1
3, 1
$a^2 + b^2 = c^2$
The disease center is the average location (coordinates) of positive piglets
Average distance of positive pigs to the disease center = 0.77


Average distance of positive pigs to the disease center = 1.42
The larger the av. distance of positive pigs to the center is the less clustered the distribution is
Average distance of positive pigs to the disease center = 0.77
The smaller the av. distance of positive pigs to the center is the more clustered the distribution is

Homogeneity and clustering analysis

Farm	Room	Positive piglets	Population homogeneity analysis				p-value	Clustering analysis	
			Expected positive litters[1]		Observed positive litters				
			n	Avg. No. of positive piglets per litter	n	Avg. No. of positive piglets in positive litters		Clustered (Y/N)	p (probability)
A	1	90	17	0.57	14	0.66	< 0.01	N	0.684
	2	13	20	0.06	4	0.36	< 0.01	Y	0
	3	29	17	0.19	5	0.58	< 0.01	Y	0
	4	2	5	0.04	1	0.20	> 0.05	N	0.185
C	1	8	7	0.13	1	1.00	< 0.01	Y	0
	3	4	10	0.03	1	0.33	< 0.01	Y	0.001
E		38	13	0.28	8	0.44	< 0.01	Y	0
G	2	3	22	0.01	2	0.14	< 0.05	N	0.219
H		30	19	0.17	8	0.38	< 0.01	Y	0.001
I		66	24	0.24	13	0.49	< 0.01	Y	0
J	1	117	20	0.55	17	0.65	< 0.01	Y	0
	2	58	21	0.36	16	0.46	< 0.01	Y	0.03
K	1	14	7	0.21	4	0.42	< 0.01	Y	0
	2	10	19	0.05	3	0.37	< 0.01	N	0.315
	3	7	4	0.18	2	0.64	< 0.01	Y	0.024
	4	36	17	0.21	13	0.28	< 0.01	N	0.329

Sample size to detect at least one positive using either SRS, 2SS, or RBS

Risk-based sampling

>

Two-stage sampling

>

Simple random sampling

Weekly PF results and follow-up with FOF

FARM A
PROCESSING FLUIDS

# Neg Wks	# positive / # total
	5
1	5
2	4
3	4
	5
1	4
2	5
3	4
	1 of 3
	1 of 5
1	5
2	5
3	5
4	1

FARM B
PROCESSING FLUIDS

# Neg Wks	# positive / # total
1	5
2	3
	0
1	5
2	4
3	5
4	5
5	4
6	4
7	6
8	6
9	3
10	4
11	5

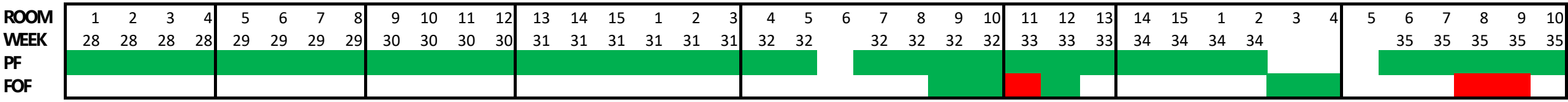
FARM C
PROCESSING FLUIDS

# Neg Wks	# positive / # total
	4
1	2
	5
	4
1	6
	4
1	5
2	4
3	4
4	2
5	4
6	2
7	4
8	4

FARM D
PROCESSING FLUIDS

# Neg Wks	# positive / # total
1	4
2	4
	5
1	5
	5
	5
	3
1	5
2	3
3	6
4	6
	2 of 4
1	4
	1 of 4

FARM B



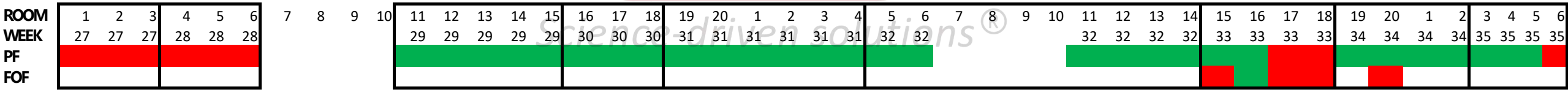
3 farrowing turns of negative PF (11 weeks)

Week 9 FOF positive (1 of 47) with a Ct of 29.2 (1-7-4)

Week 11 FOF positive with a Ct of 35.6 (1 of 43)

Negative PF ≠ Negative FOF

FARM D



Intermittent positive results by week and room and PF + = FOF +

Comparative sample size FOF x Serum when all samples test negative

Serum samples (n)	FOF samples (n)	95% credible interval
24	2	0.0 - 11.7
38	5	0.0 - 7.4
57	8	0.0 - 5.0
71	10	0.0 - 4.0
92	15	0.0 - 3.1
105	20	0.0 - 2.7
113	25	0.0 - 2.5
133	30	0.0 - 2.1
147	35	0.0 - 1.9
154	40	0.0 - 1.8

Population samples for disease diagnosis

Post weaning

Always pair population sampling with histopathology

- Detection does not equal causation
- Confirmation of role is essential for placement of adequate interventions

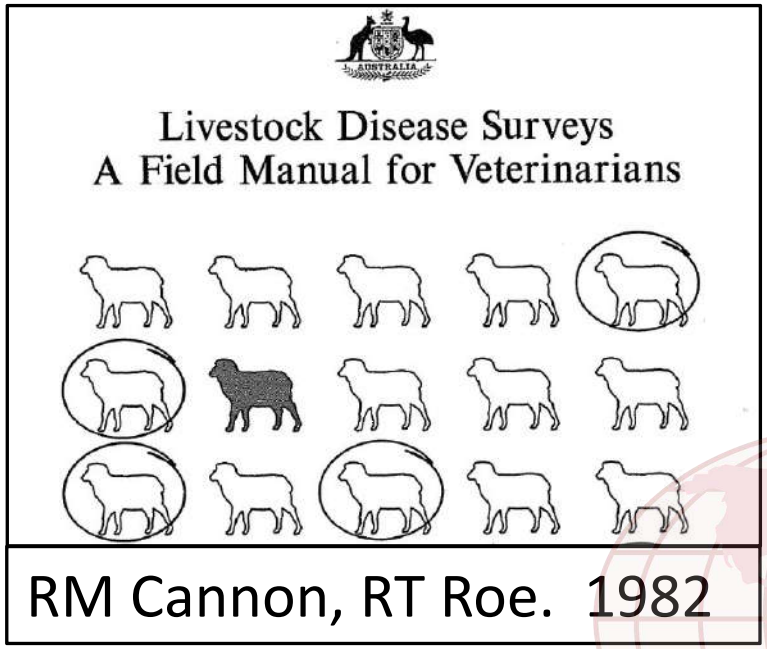
Ballellas et al., 2021
Machado et al., 2022, 2023

- PRRSV, IAV, enteric coronaviruses, *Lawsonia,* APP, ASV, CSF, etc.

Dr. Prickett et al., 2008

Representative sampling and sample size ...

Sample size - based on SRS



RM Cannon, RT Roe. 1982

PRV eradication guidelines

< 100 pigs - test 25
100-200 - test 27
201-999 - test 28
≥ 1,000 - test 29

Population N	Prevalence 30.0%	20.0%	10.0%
80	8	13	24
90	9	13	25
100	9	13	25
120	9	13	26
140	9	13	26
160	9	13	26
180	9	13	27
200	9	13	27
250	9	14	27
300	9	14	28
350	9	14	28
400	9	14	28
450	9	14	28
500	9	14	28
600	9	14	28
700	9	14	28
800	9	14	28
900	9	14	28
1000	9	14	29

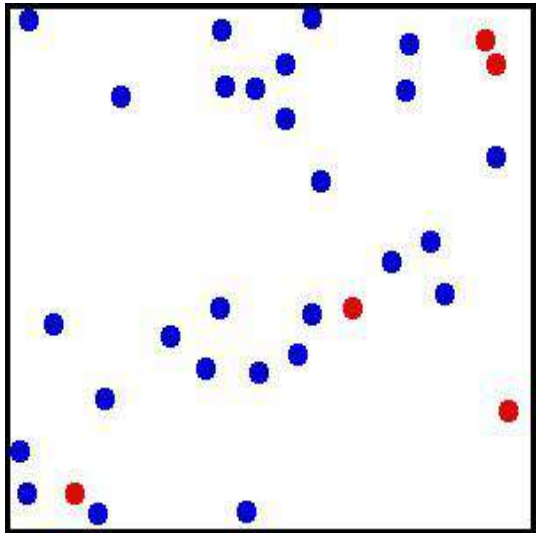
Sample size numbers come from the binomial distribution formula

$$P(x) = \frac{n!}{(n-x)!x!} p^x q^{n-x}$$

Assumptions of binomial distribution?

Assumptions of binomial distribution:

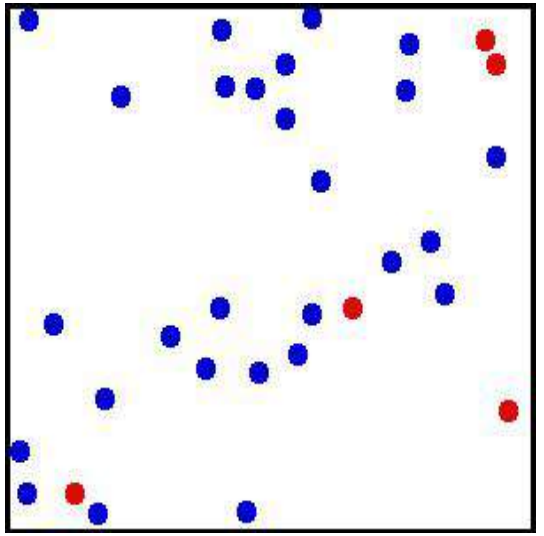
1. Finite population.
2. Binary outcome (pos/neg).
3. Subjects are independent.
   - One sample does not predict the next.
4. Population is homogenous.
   - Equal probability of being selected.



Assumptions of binomial distribution:

1. Finite population.
2. Binary outcome (pos/neg).
3. Subjects are independent.
   - One sample does not predict the next.
4. Population is homogenous.
   - Equal probability of being selected.

How many marbles to sample?
N = 100. Prevalence = 10%.
Sample size to include ≥ 1 red?

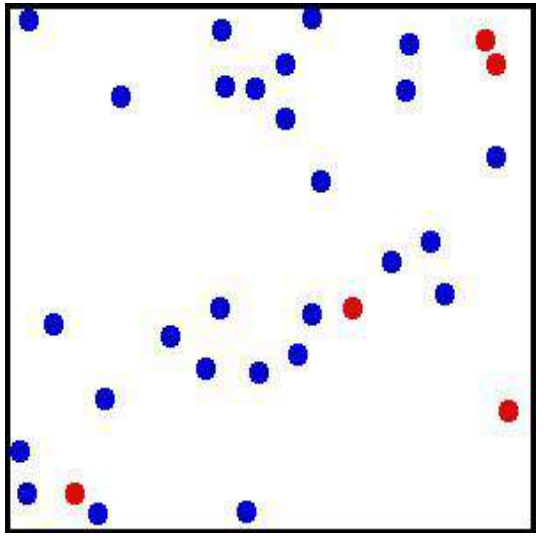


Look at sample size table for answer.

How many marbles to sample?

N = 100. Prevalence = 10%.

Sample size to include ≥ 1 red?

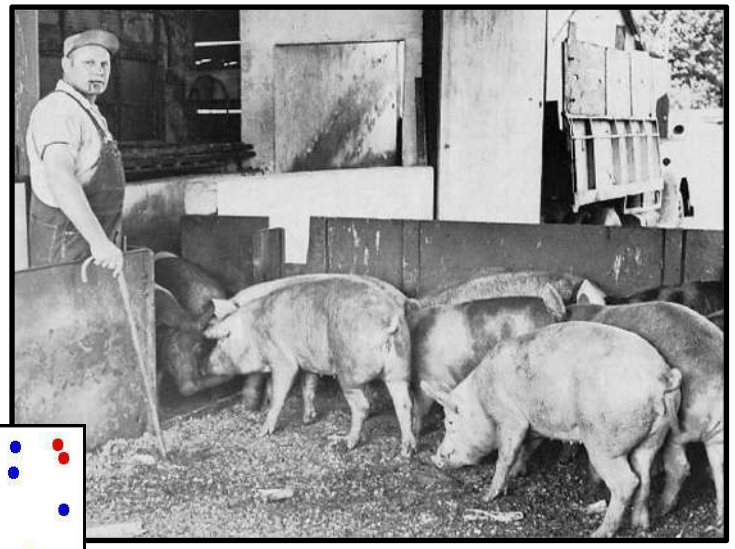


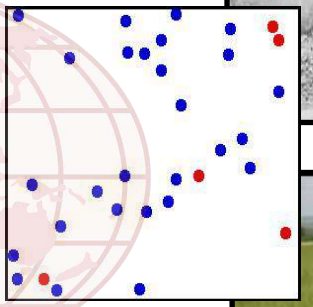
Population N	Prevalence 30.0%	20.0%	10.0%
80	8	13	24
90	9	13	25
100	9	13	25

Look at sample size table for answer.

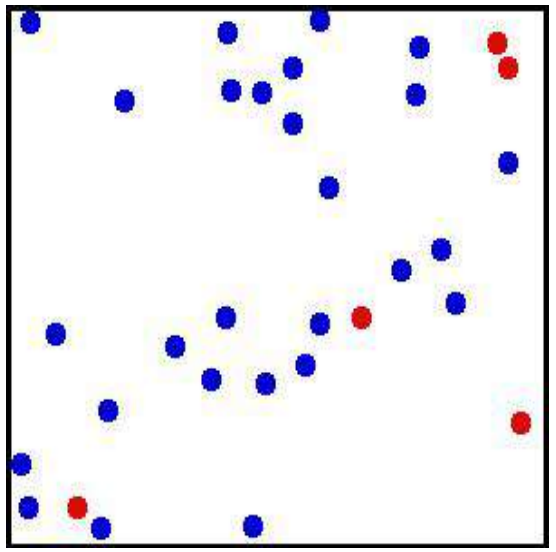
Assumptions of binomial distribution?

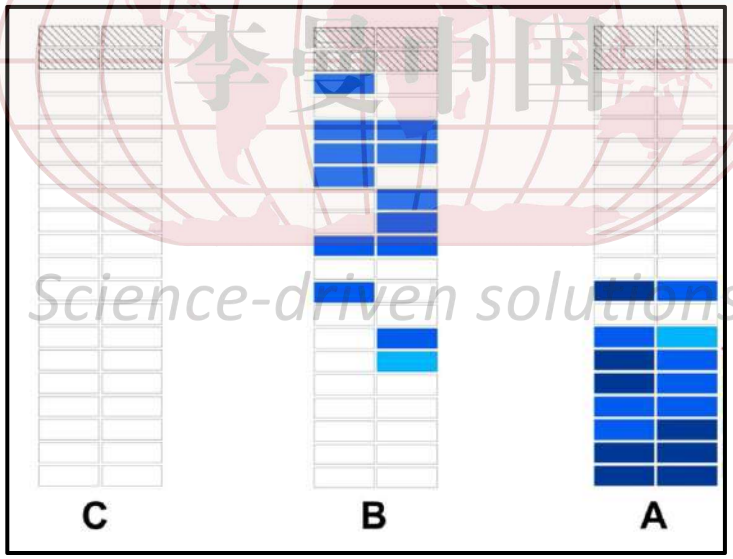
1. Finite population.
2. Binary outcome (pos/neg).
3. Subjects are independent.
4. Population is homogenous.

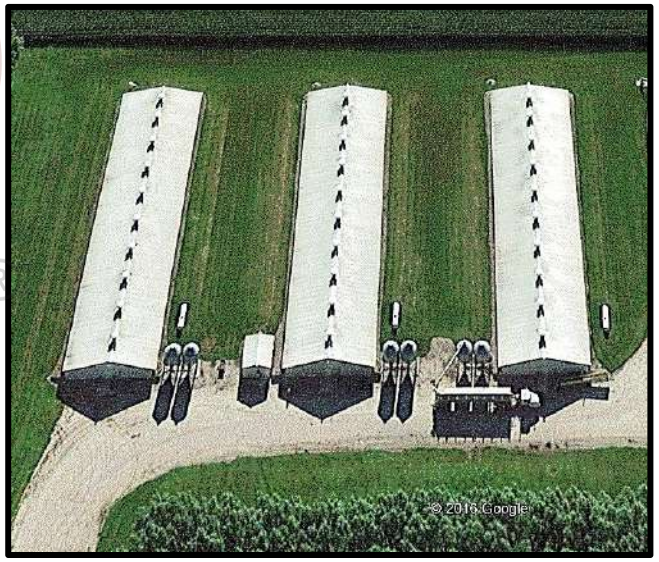


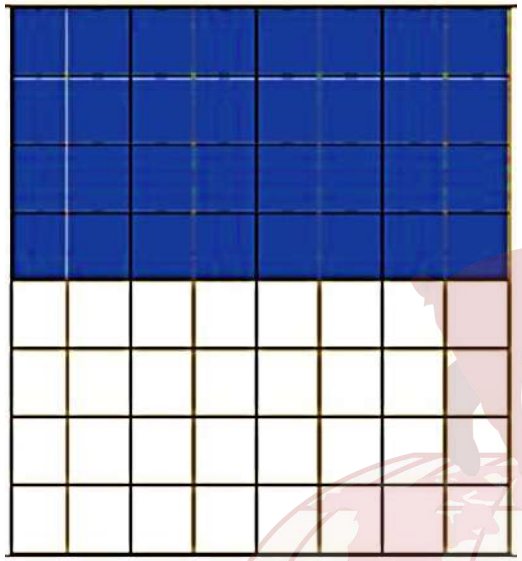


1. Finite population.
2. Binary outcome (pos/neg).
3. Subjects are independent.
4. Population is homogenous.

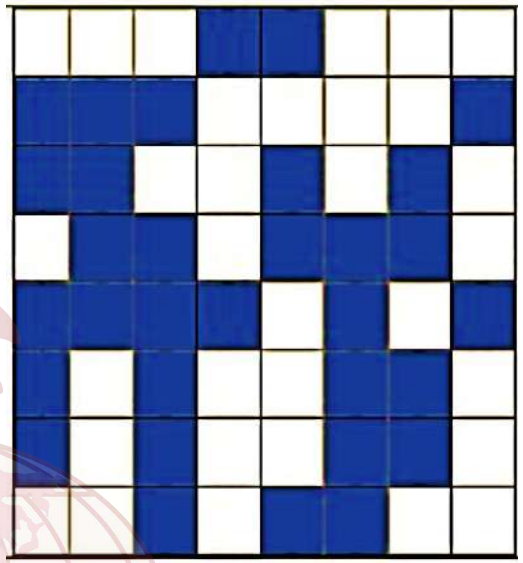




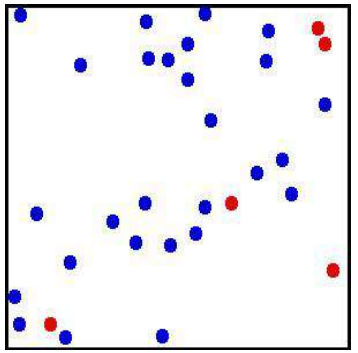


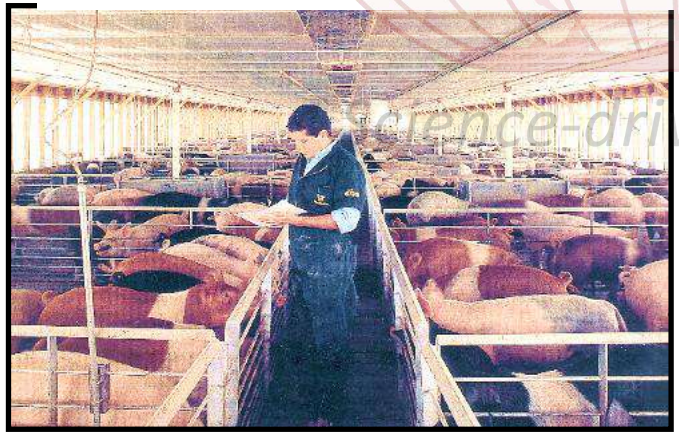


Spatial autocorrelation



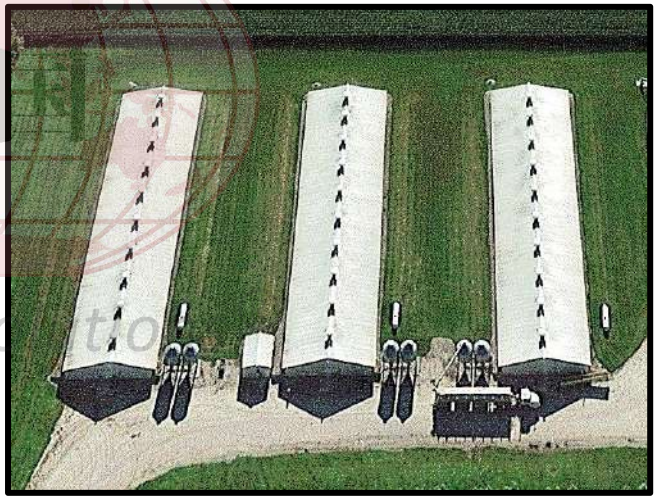
No spatial autocorrelation





Population N	Prevalence 30.0%	20.0%	10.0%
80	8	13	24
90	9	13	25
100	9	13	25
120	9	13	26
140	9	13	26
160	9	13	26
180	9	13	27
200	9	13	27
250	9	14	27
300	9	14	28
350	9	14	28
400	9	14	28
450	9	14	28
500	9	14	28
600	9	14	28
700	9	14	28
800	9	14	28
900	9	14	28
1000	9	14	29

This does not apply to *this*.

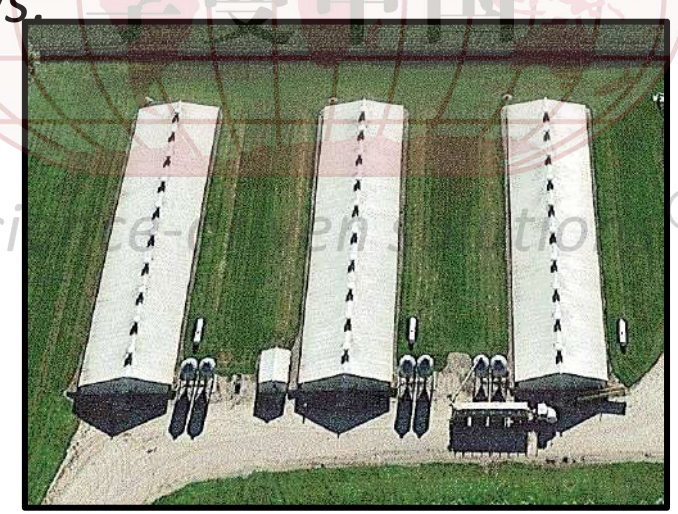


Finding a compromise …

Official PRV random-sample test (95/10). 95% probability of detecting PRV in a herd in which ≥ 10% are seropositive.

*Each segregated group of swine **on a premise** must be considered **a separate herd** and sampled as follows:*

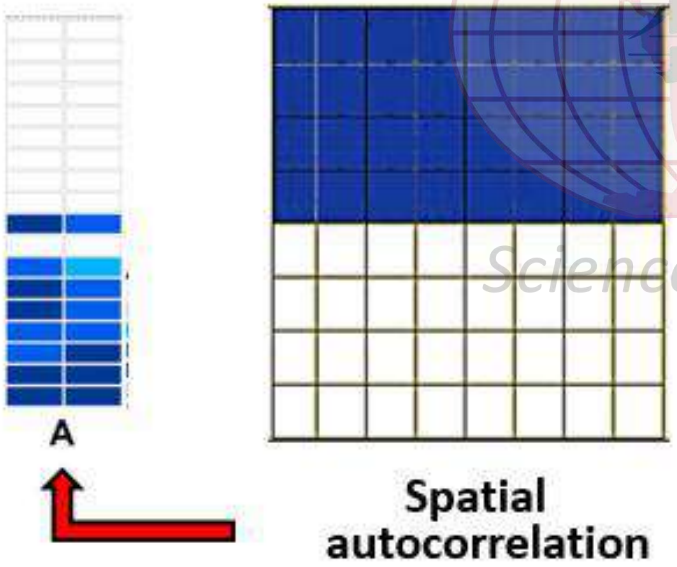
< 100 head	- test 25
100 - 200	- test 27
201 - 999	- test 28
≥ 1,000	- test 29

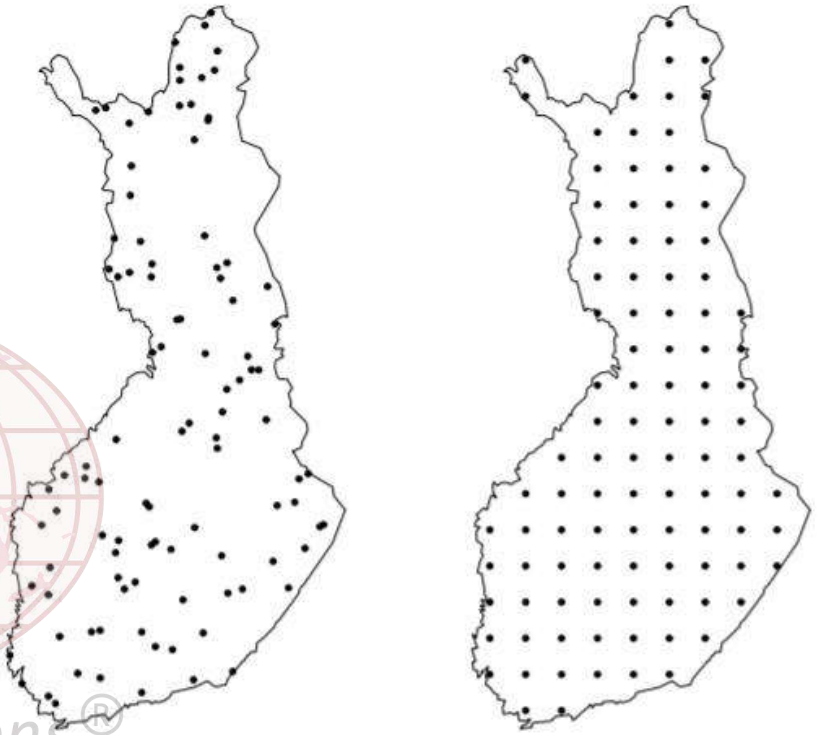


= test 87 pigs

Spatial sampling is better (than representative sampling) *when there is autocorrelation.*

Wang et al. 2012. *Spatial Statistics 2*:1-14.

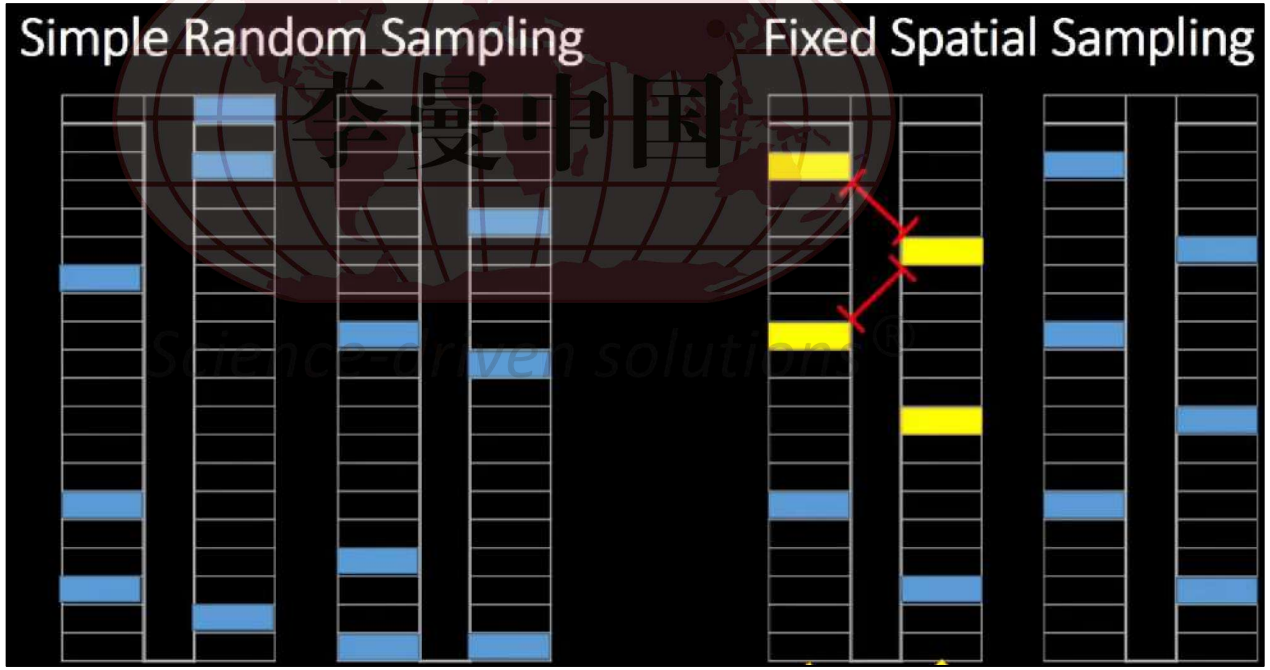




Heikkinen, J. (2006). Assessment of uncertainty in spatially systematic sampling. *Forest Inventory*, 155.

Fixed spatial sampling

- Select pens equidistant to each other and on alternate sides of the alleyway over the length of the barn.
  - Accounts for spatial autocorrelation.



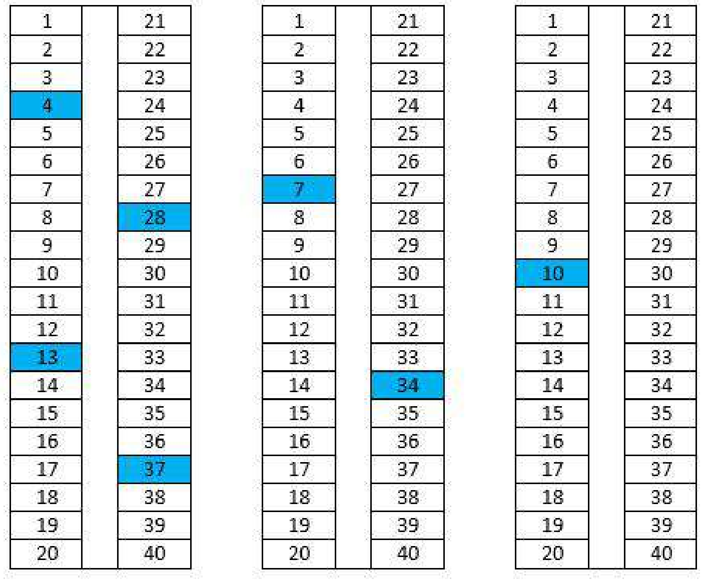
Sample size for fixed spatial sampling

1.Decide how many samples you can collect and test routinely – some is better than none.

2. Use “fixed spatial sampling”, i.e., same pens over time.

3. Use the best test (based on your objective, pathogen biology, and cost)

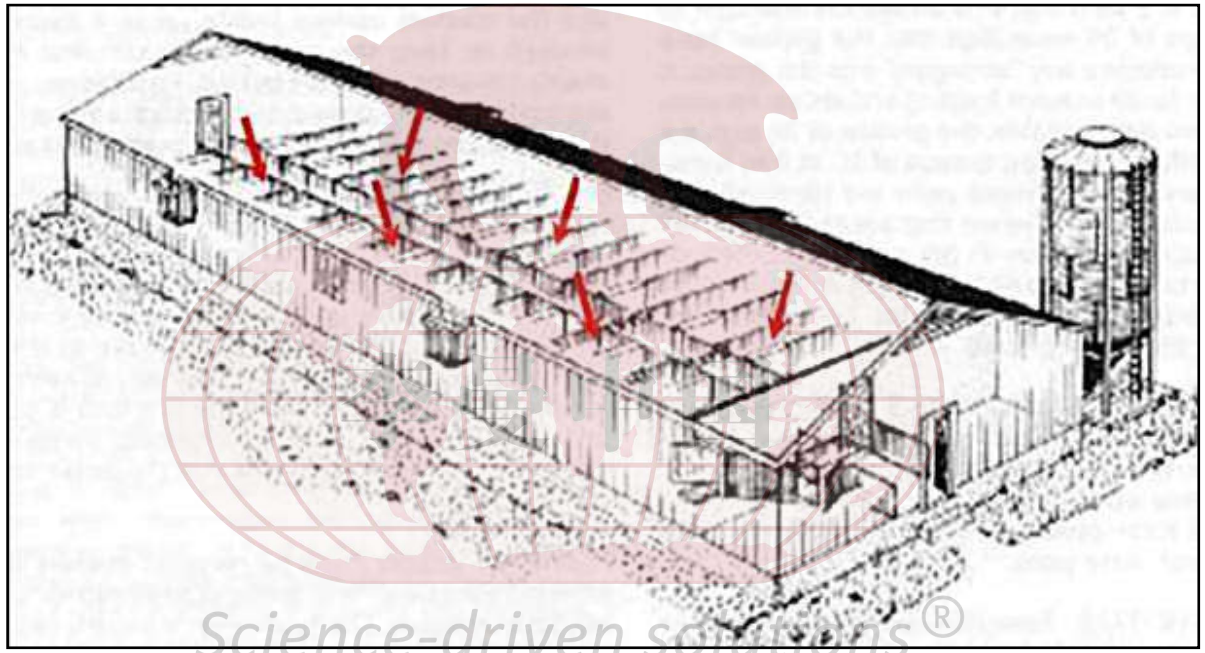
4. Time reveals all!



“Spatial sampling is better (than random sampling) *when there is autocorrelation."*

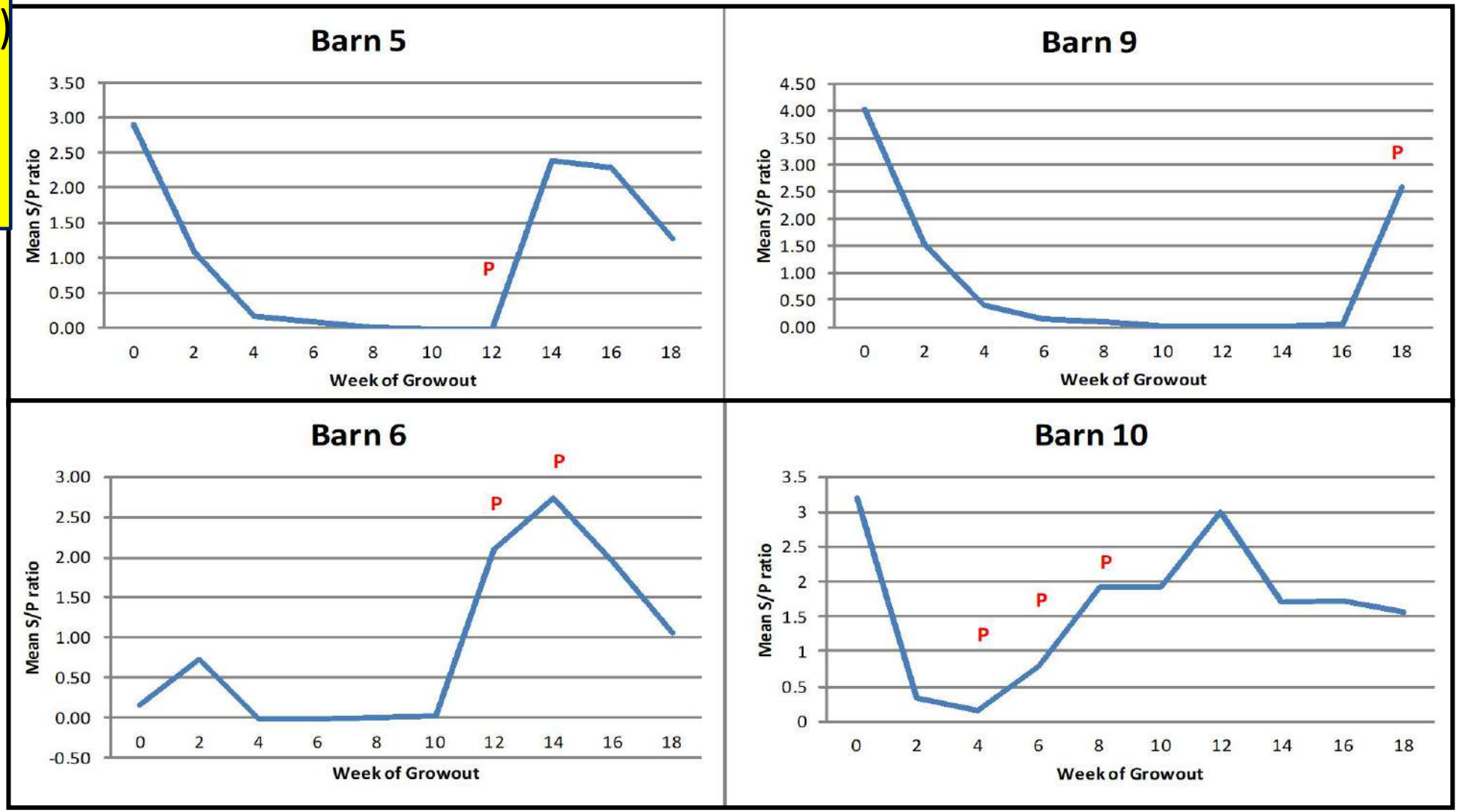
Wang et al. 2012. *Spatial Statistics 2*:1-14.

Sample the same pens every time

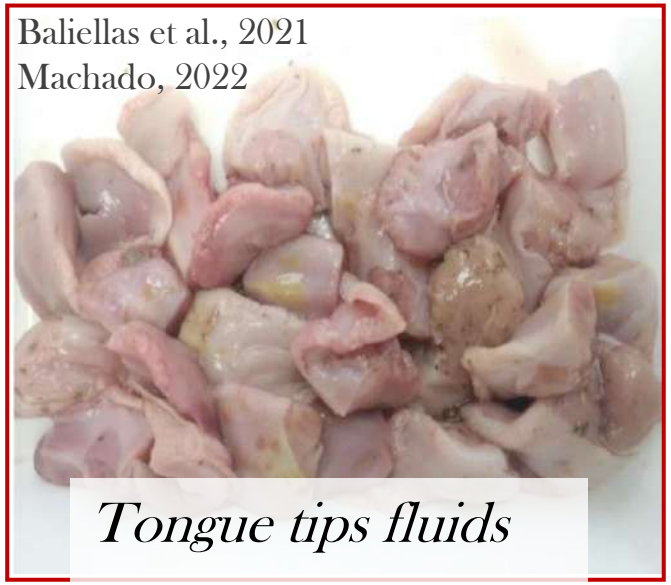


10 sites x 6 pens in each barn x sampling each 2 weeks for 18 weeks.

Results (averages)
ELISA S/P values
RT-PCR positives (**P**)



Baliellas et al., 2021
Machado, 2022



Tongue tips fluids

Lopez et al., 2017

Processing fluids

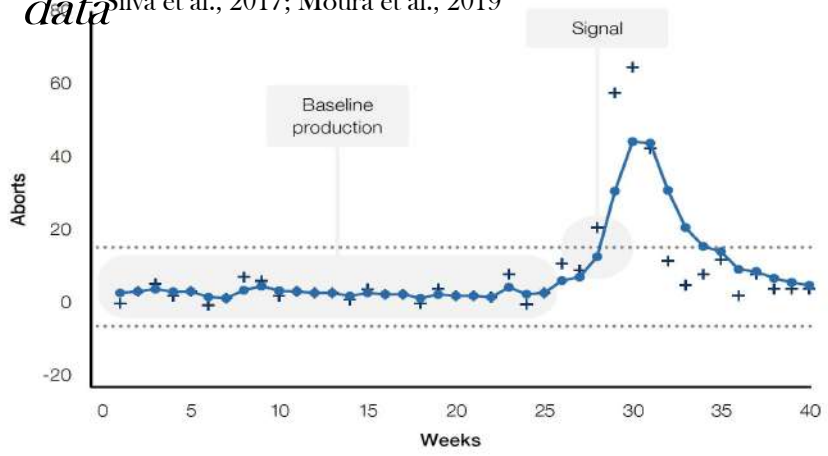
Almeida et al., 2018

Family oral fluids

Population-based monitoring and surveillance systems

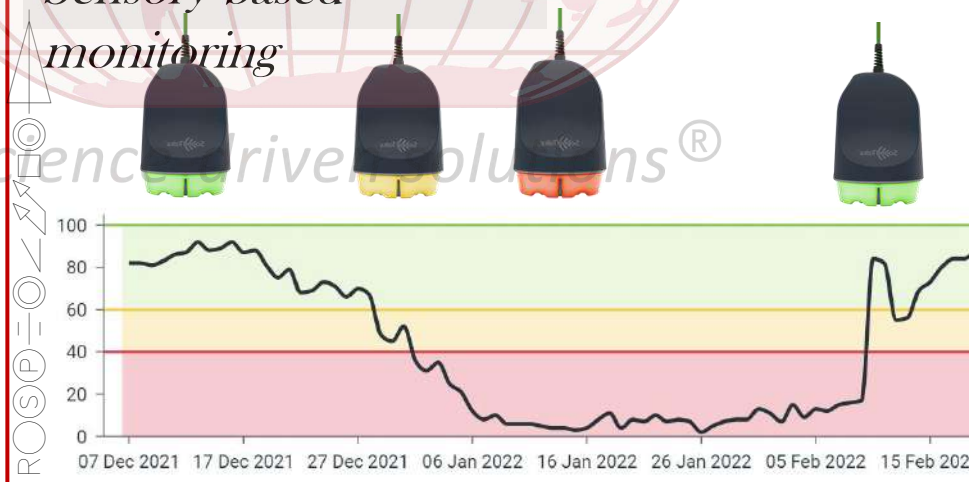
Ongoing monitoring of productivity data

Silva et al., 2017; Moura et al., 2019



Sensory-based monitoring

Courtesy Dr. Alonso, 2022



Oral fluids

Take homes

- Combination of strategies is the best strategy
- Frequency of testing matters
- Sample size and representativeness
  - 8 wks of negative processing fluid results (less pooling as time goes by)
  - + 6 wks of FOF or other due-to-wean piglet testing (adjust sample size to detect 1-2% prevalence)
  - OF post weaning → 6 pens per barn using fixed spatial sampling

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Marcelo Almeida, DVM, MS, PhD
Assistant Professor
malmeida@iastate.edu