Diagnostic assay development & applications to infectious disease surveillance in swine

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Part 1. We are responsible for animal health

Part 2. Practical surveillance

Our animal health responsibilities:

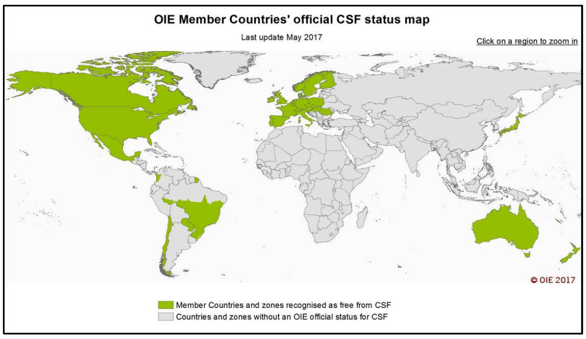
- Assure pig health and welfare.
- Help farmers keep farms profitable.
- Help produce safe, wholesome food.

We are responsible for animal health

- **How well are we doing?**
- Complicating factors ...
  - Massive transport of pigs, by-products, feedstuffs, etc.
  - Fewer herds / Bigger herds
  - Impact on disease ecology/disease control?

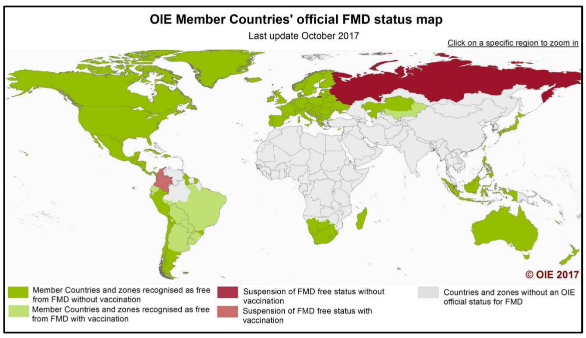
How well are we doing?

- Classical swine fever virus identified in 1903.
- CSFV eradication pays off - 1:13 cost:benefit ratio (USDA, 1981).
- 2017 - 32 of 181 (17.7%) OIE-member countries free of CSFV.



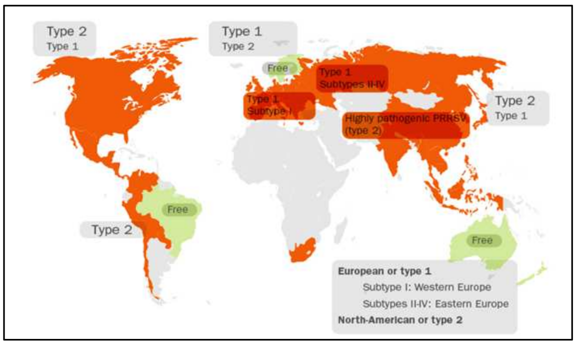
How well are we doing?

- FMDV identified in 1897.
- Annual losses $6.5 to $21 billion dollars annually (Knight-Jones, Rushton, 2013; Longjam et al, 2011; OIE, 2017a).
- 2013, 66 of 181 (36.5%) OIE countries "*FMD free where vaccination is not practiced*".



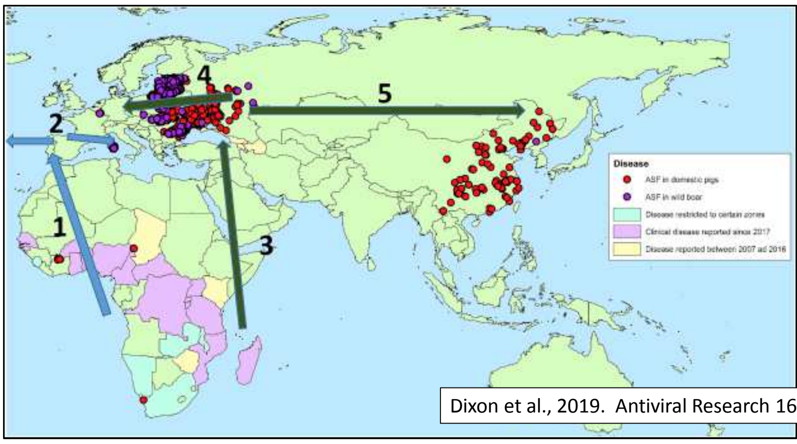
How well are we doing?

- PRRSV identified in 1991.
- U.S. producers' losses $664 million annually (Holtkamp et al., 2013).
- European producers' losses €126.79/sow/year and €3.77 per pig marketed in herds with "slight" PRRS (Nathues et al., 2017).



https://www.prrscontrol.com/portal/prrscontrol/prrs-knowledge/porcine-reproductive-and-respiratory-syndrome

African swine fever virus …



Dixon et al., 2019. Antiviral Research 165:34-41.

We are responsible for animal health

- How well are we doing?
- Complicating factors ...
  - **Massive transport** of pigs, by-products, feedstuffs, etc.
  - Fewer herds / Bigger herds
  - Impact on disease ecology/disease control?

Transportation

- In the UK, construction of railways in the 1800's → rinderpest outbreaks.

Response:

1. Movement restrictions
2. Formation of the British State Veterinary Service (1865)

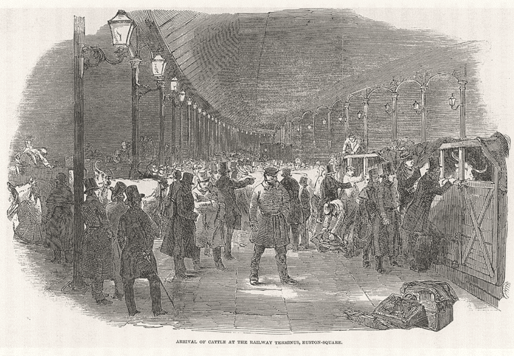
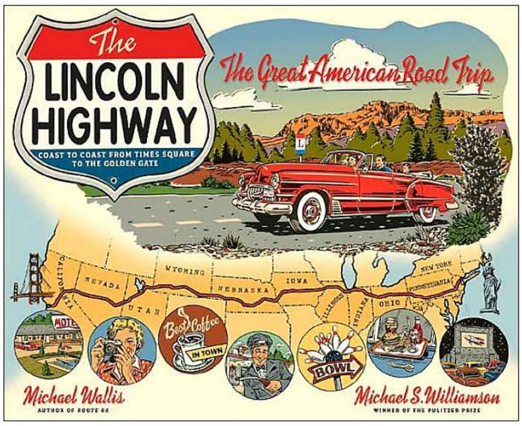


Fig. 16. Circumstances favourable to the spread of contagion: cattle transport arriving at Euston Square railway terminus, 1849, from *Illustrated London News* (courtesy Wellcome Institute Library, London).

Pig Transport

- Modern business model
- Moving pigs to feed is more efficient than moving feed to pigs.

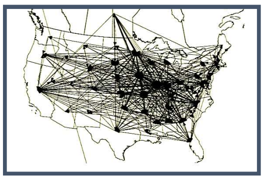


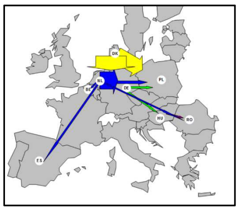
1915 - North America - transit ≥ 20 days.

http://hiddencityphila.org/wp-content/uploads/ 2013/09/1-www.restore-a-classic.com_.jpg

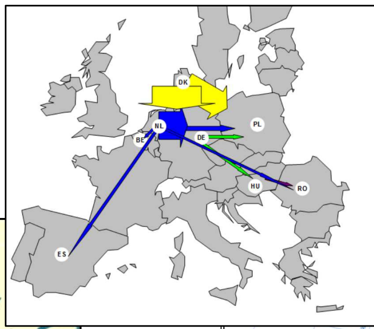
Pig transport ... connects distant farms

Year	Iowa	Indiana	Minnesota	Illinois	Total U.S.
1980	1,740,000	549,000	226,000	510,000	4,628,000
1990	1,400,000	240,000	262,000	359,000	4,317,000
2000	11,600,000	1,050,000	3,150,000	1,470,000	24,514,000
2010	21,200,000	2,632,000	7,089,000	1,898,000	39,571,000
2015	27,500,000	3,559,000	8,000,000	2,356,000	49,680,900
2017	30,400,000	4,100,000	8,850,000	1,684,000	55,238,400



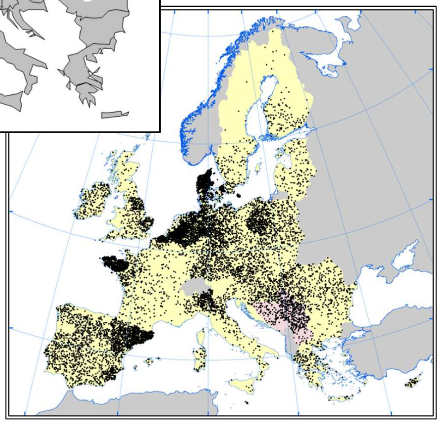


Continent-wide connections between "metapopulations"



Net exchange of weaned pigs (left)

Number of sows by region (below)



FAO 2016	LIVE PIG EXPORTS
Denmark	13,335,658
Netherlands	6,411,841
Canada	5,671,386
China	2,830,589
Germany	2,464,606
Spain	1,519,351
Belgium	1,033,794
Slovakia	552,672
France	545,597
Hungary	425,479
Finland	103,533
Poland	54,829
USA	48,227
Brazil	10,376
Norway	1,220
United Kingdom	589

FAO 2016	LIVE PIG IMPORTS
Germany	15,542,605
Poland	6,283,760
USA	5,656,011
China	1,815,173
Italy	1,603,232
Netherlands	1,330,748
Portugal	1,245,399
Romania	1,088,627
Hungary	871,643
Belgium	851,598
Spain	795,735
France	194,899
Mexico	19,961
Canada	2,565
Brazil	322
Denmark	278

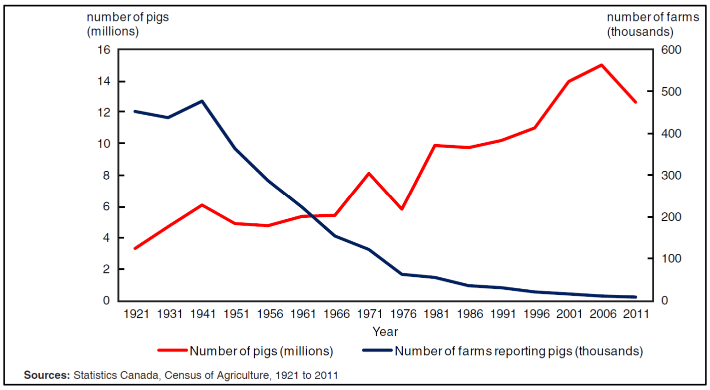
FAO 2013	PIG MEAT EXPORTED TONS
Germany	925,928
Denmark	722,909
Spain	627,808
Belgium	619,641
Netherlands	589,353
USA	431,299
France	388,066
Poland	305,545
Canada	162,988
United Kingdom	133,257
China	108,930
Austria	78,798
Hungary	73,206
Ireland	65,370
Italy	55,626
Brazil	52,076

We are responsible for animal health

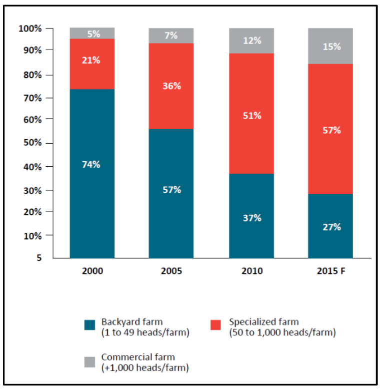
- How well are we doing?
- Complicating factors ...
  - Massive transport of pigs, by-products, feedstuffs, etc.
  - **Fewer herds / Bigger herds**
  - Impact on disease ecology/disease control?

Fewer herds / bigger herds

Canada

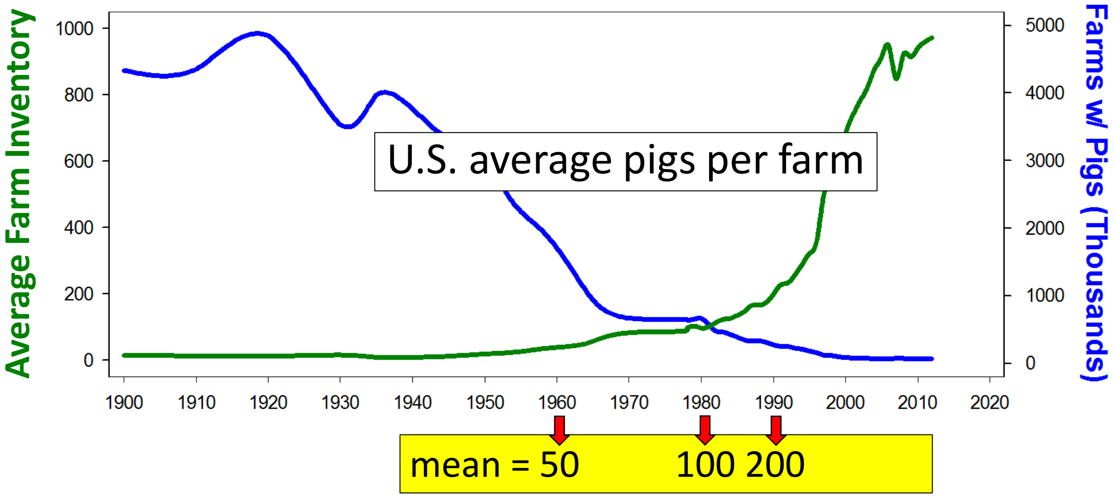


China



FAO. March 2018. Animal Health Risk Analysis. Assessment No. 05.

Fewer herds / bigger herds



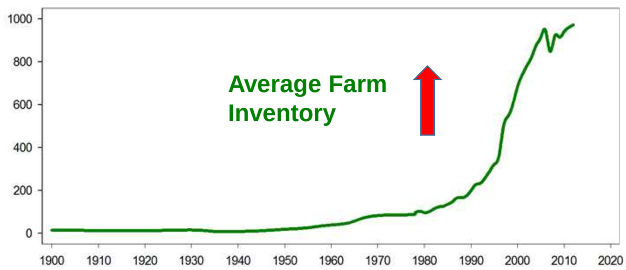
We are responsible for animal health

- How well are we doing?
- Complicating factors ...
  - Massive transport of pigs, by-products, feedstuffs, etc.
  - Fewer herds / Bigger herds
  - **Impact on disease ecology/disease control?**

Impact on disease and disease control?

Increasing herd size ...

- Increased probability of becoming infected
- Decreased ability to achieve herd immunity
- Increased frequency and magnitude of disease outbreaks



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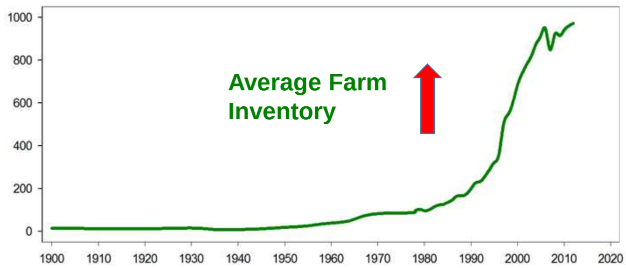
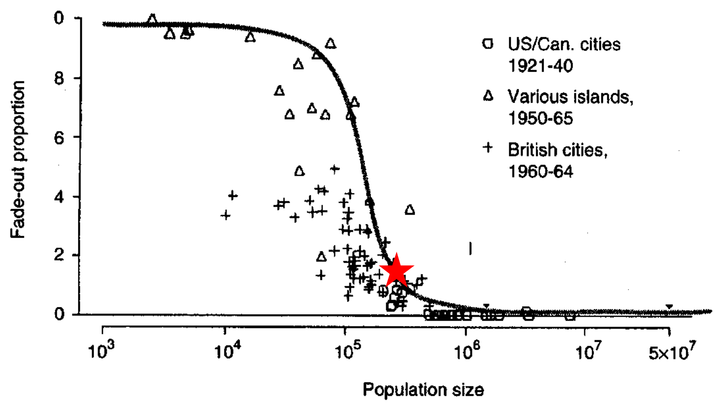


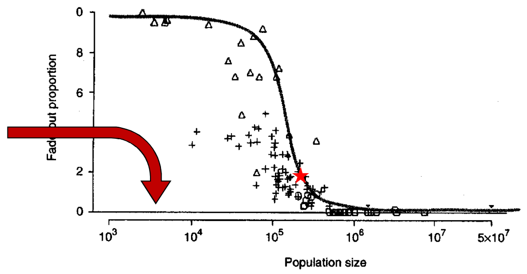


"Critical community size" = the population required to maintain an infection. CCS for measles = 250,000 - 500,000

Pitzer et al., 2016. High turnover drives prolonged persistence of influenza in managed pig herds. J R Soc Interface *13*(119), 20160138.

- 250% annual turnover in growing pig populations
- 40-50% annual turnover in breeding herds

Pitzer et al. (2016) predicts IAV persistence in populations ≥ 3000



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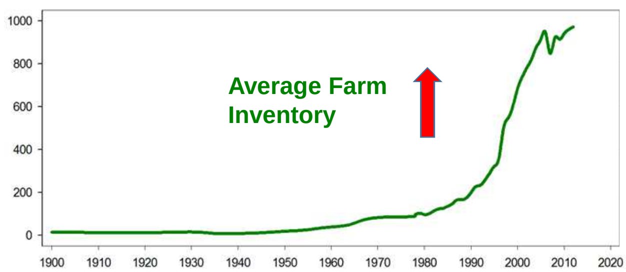
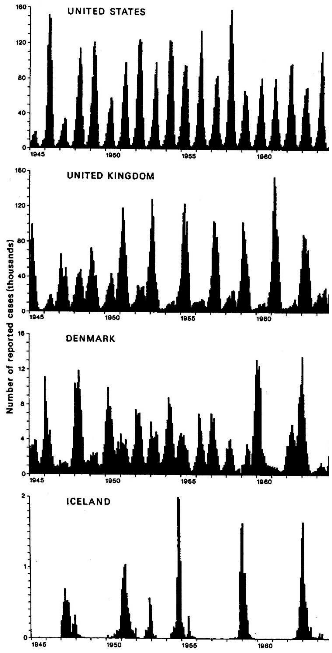




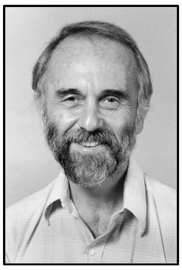
Measles cycles and population size.
Cases of measles per month for four countries by descending order of population size.

Larger populations have:
1. Continuous viral circulation
2. More frequent outbreaks
3. Bigger outbreaks

The virus is the same …

Haggett. 2000. The Geographical Structure of Epidemics.

- Calvin Schwabe – (Prev Vet Med 1982 1:5-15)
  - In the 1970's - "production diseases".
  - New patterns of disease.
- How can we do better? Schwabe's idea …
  - Use continuous on-farm surveillance to establish levels/patterns of disease.
  - Measure impact of intervention by comparing baselines.



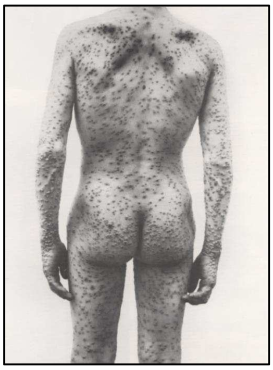
Dr. Calvin Schwabe
(1926 – 2006)

Part 1. We are responsible for animal health

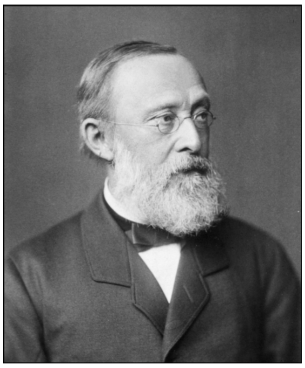
Part 2. Practical surveillance

- Two distinct objectives need to be met:
  - Regional/country level = detection/elimination
  - Herd level = improved productivity through disease control
- Which surveillance approach?
  - Risk-based surveillance
  - Syndromic surveillance
  - "Schwabe model" (continuous herd surveillance)

Syndromic surveillance?



Smallpox (1900)



Rudolf Virchow
"Father of Pathology"
1821 - 1902

"When CSFV entered … it took between one week and 2 months before the owner suspected the disease."

Morilla and Rosales, 2002. Trends in Emerging Viral Infections of Swine

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- Ultimately - regardless of the surveillance approach - we rely on sampling and testing.

1. Which specimen to collect for testing?
2. Which target? Nucleic acid vs antibody …
3. How to sample? Statistical validity ...

1. **Which specimen?**
2. Which target? Nucleic acid vs antibody.
3. How to sample in the field? Statistical validity.

Which specimen?

Garrido-Mantilla *et al. BMC Veterinary Research* (2019) 15:61
https://doi.org/10.1186/s12917-019-1805-0
BMC Veterinary Research

RESEARCH ARTICLE Open Access

Comparison of individual, group and environmental sampling strategies to conduct influenza surveillance in pigs

Jorge Garrido-Mantilla[1], Julio Alvarez[1,2,3], Marie Culhane[1], Jayaveeramuthu Nirmala[1], Jean Paul Cano[4] and Montserrat Torremorell[1*]

Which specimen?

Influenza A detection by rRT-PCR in WTF (5 herds)

Nasal Swabs	Nasal Wipes	Oral Fluids	Surface Wipes
15/50 (30%)	16/50 (32%)	30/48 (63%)	25/50 (50%)

Adapted from Garrido-Mantilla et al. BMC Vet Res 2019 15:61

Individual animal samples … good for diagnostics …

Figure 3-7.—Tonsil tissue, a prime location for early detection of hog cholera virus, could be obtained quickly in the field from a living animal. It could then be tested within hours after the sample reached the laboratory.

… but not practical for surveillance.

Which specimen?

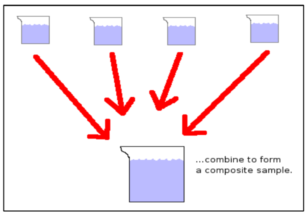
- Surveillance samples must be ...
  - Easy to collect by one person
  - Inexpensive to collect
  - Sensitive, efficient detection
- Trend to "aggregate" samples
  - Environmental samples
  - “Processing fluid”
  - Oral fluid samples

Good for surveillance.

Aggregate samples ≠ pooled samples

- Aggregate sample
  - ≥ 2 animals contribute to the sample.
  - Has defined source, location, time
  - Testing produces statistically valid data
- “Pooled sample”
  - ≥ 2 individual specimens combined into one for testing (Dorfman, 1943).
  - Depending on pooling strategy, statistical analysis may be problematic.

Environmental studies



Oral fluid testing in 3 US vet diagnostic labs

Pathogen	2010	2011	2012	2013	2014	2015	2016
PRRSV	14,603	46,239	77,756	109,868	126,165	144,773	148,526
Influenza A virus	4,785	16,495	34,297	46,940	48,688	48,895	47,454
Mycoplasma hyopneumoniae	760	4,514	7,079	10,286	11,203	11,741	13,178
PCV2	751	2,047	4,147	2,149	5,676	4,807	3,176
Actinobacillus pleuropneumoniae	-	37	4	93	14	287	3,306
TGEV	-	34	-	4,651	32,848	12,497	12,996
PEDV	-	-	-	14,361	75,965	76,063	73,494
Lawsonia intracellularis	-	-	-	454	1,519	3,290	2,443
PDCoV	-	-	-	-	21,393	46,366	58,513
Senecavirus A	-	-	-	-	-	1,597	3,598
Other*	64	1,630	1,919	1,804	2,010	2,595	2,755
Total	20,963	70,996	125,202	190,606	325,481	352,911	369,439

Source: Bjustrom-Kraft et al. 2018. J Swine Health Prod 26:262-269.

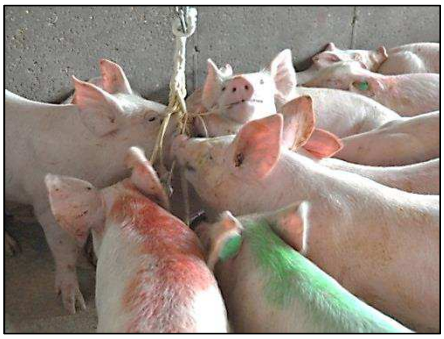
2010 - 2016 = ~1,500,000

Courtesy of Dr. Marcelo Almeida

Can aggregate samples provide sensitive, specific detection?

Pen level comparison of serum vs oral fluid samples ...

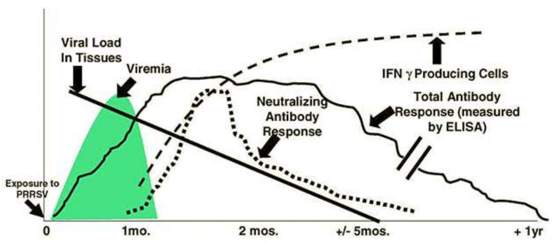
Olsen et al. 2013. Probability of detecting PRRSV infection using pen-based swine oral fluid specimens as a function of within-pen prevalence. J Vet Diagn Invest 25:328-335



Pens held 25 pigs each. Positives = MLV vaccinated exactly 14 days earlier.

Within-pen prevalence	One oral fluid sample (rate of detection) **PCR | ELISA**	
5%	31%	17%
10%	79%	59%
15%	94%	85%
20%	98%	94%
25%	99%	97%

Olsen et al. 2013. J Vet Diagn Invest 25:328-335.



Impact of timing on the results?

Within-pen prevalence	One oral fluid sample (rate of detection) PCR | ELISA	No. serum samples to equal the oral fluid detection rate PCR | ELISA
5%	31%	8
10%	79%	11
15%	94%	12
20%	98%	13
25%	99%	13

Within-pen prevalence	One oral fluid sample (rate of detection) PCR	ELISA	No. serum samples to equal the oral fluid detection rate PCR	ELISA
5%	31%	17%	8	5
10%	79%	59%	11	7
15%	94%	85%	12	9
20%	98%	94%	13	10
25%	99%	97%	13	11

Within-pen prevalence	One oral fluid sample (rate of detection) **PCR | ELISA**		No. serum samples for 95% probability of detection for designated prevalence*
5%	31%	17%	24
10%	79%	59%	16
15%	94%	85%	13
20%	98%	94%	11
25%	99%	97%	9

*assumes 100% dx se & sp

1. Which specimen? *Aggregate samples*.
2. **Which target?** Nucleic acid vs antibody.
3. How to sample in the field? Statistical validity.

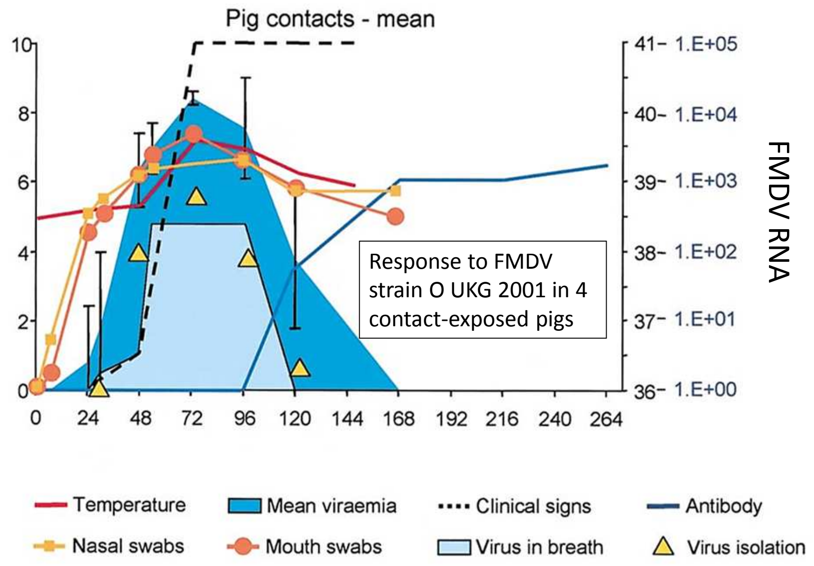
Detection of nucleic acids in oral fluid specimens

- African swine fever virus
- Classical swine fever virus
- Foot-and-mouth disease virus
- Influenza viruses
- PCV2, PCV3
- Aujeszky’s disease virus
- PEDV
- PRRSV
- *Erysipelothrix* spp.
- *M. hyorhinis*
- *M. hyosynoviae*
- *M. hyopneumoniae*

Detection of nucleic acids in oral fluid specimens

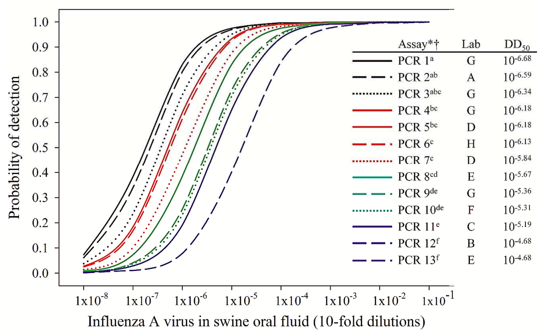
- Majority of work in PRRSV
- Detection of ASFV, CSFV, FMDV in oral fluids (Grau et al. 2015)
- Grau's comments:
  - Oral fluid sampling was uniformly successful.
  - Sampling infected pigs was successful
    - before the onset of disease
    - as long as they could move after onset of fever
    - after recovery from illness.

Alexandersen et al. 2003.
J Comp Path 129:268–282



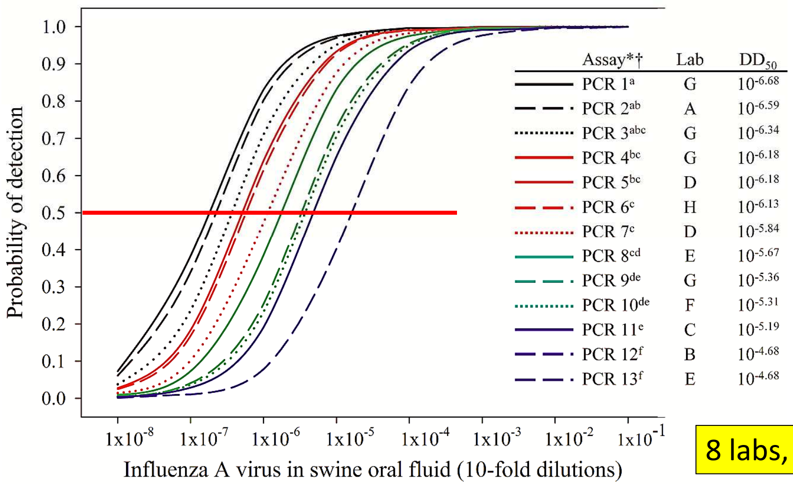
Cautions on PCRs ...

- PCRs must be optimized for aggregate matrices, e.g., oral fluids.
- Not all PCRs are created equal - evaluate and compare.
- Improvement is a continual process.



Comparison of influenza A virus RT-PCRs

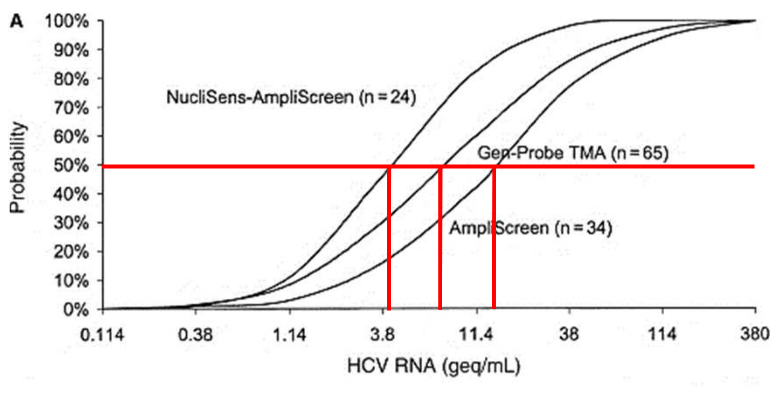
Goodell et al., 2016.
Can J Vet Res 80:12-20.



8 labs, 13 RT-PCRs

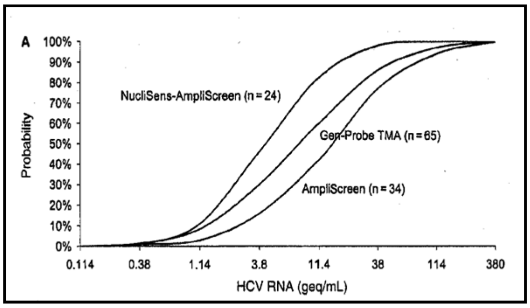
Evaluate and compare ...

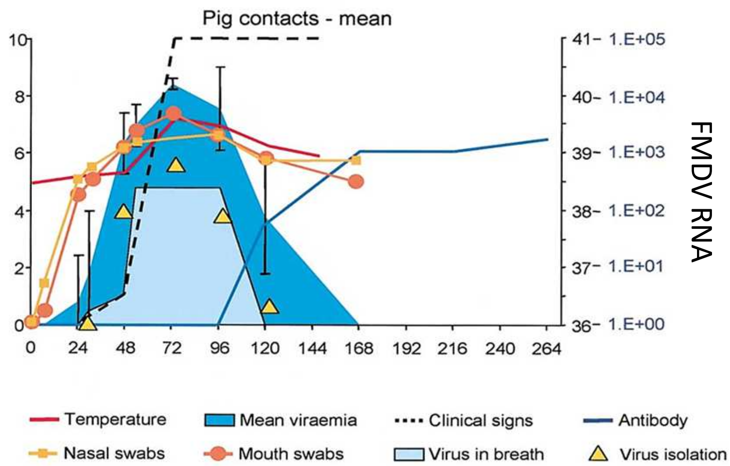
Lelie et al., 2002.
Transfusion 42:527-536



Which parameters can you calculate?

- Diagnostic sensitivity = ______
- Diagnostic specificity = ______
- Predictive value (+) = ______
- Predictive value (-) = ______
- Accuracy = ______





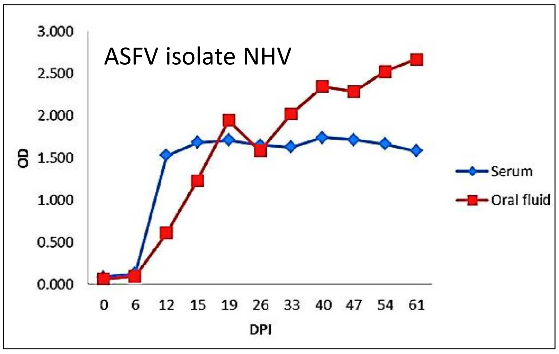
Develop test performance estimates in the context of the disease process

Detection of antibody in oral fluid specimens

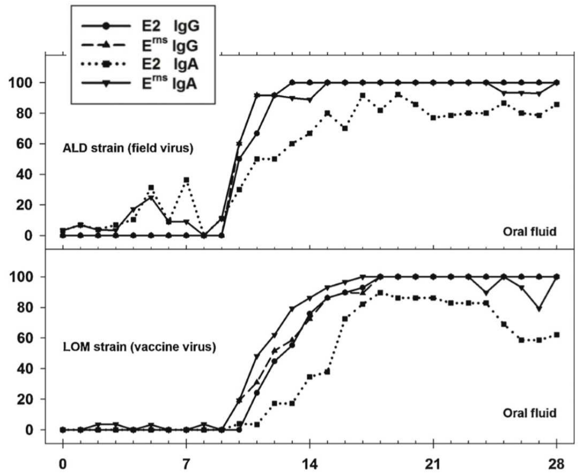
- African swine fever virus
- Aujeszky's disease virus
- Classical swine fever virus
- FMD virus *(DIVA)*
- Influenza viruses
- PCV2
- PEDV
- PRRSV

- *Actinobacillus pleuropneumoniae*
- *Erysipelothrix spp.*
- *Salmonella* spp.
- *Lawsonia intracellularis*

Any pathogen for which we can develop a good serum ELISA



ASFV p30 ELISA antibody
Giménez-Lirola et al., 2014

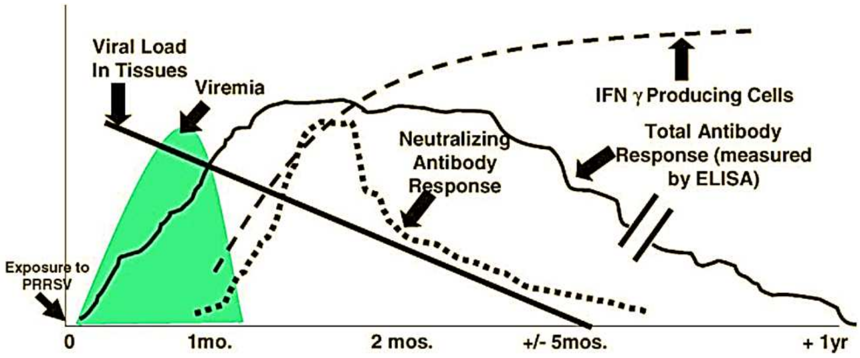


CSFV E2 and E^{rns} antibody
Panyasing et al., 2018

" Nice, but I see it more as a *gadget* than a real invention. "

Does antibody detection have a role in a "molecular world"?

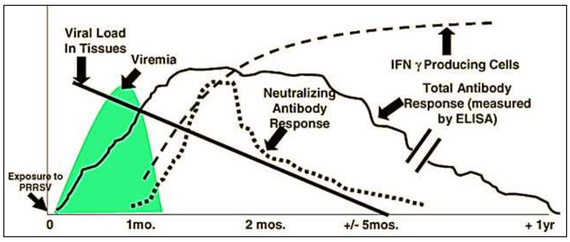
Does antibody detection have a role in a molecular world?



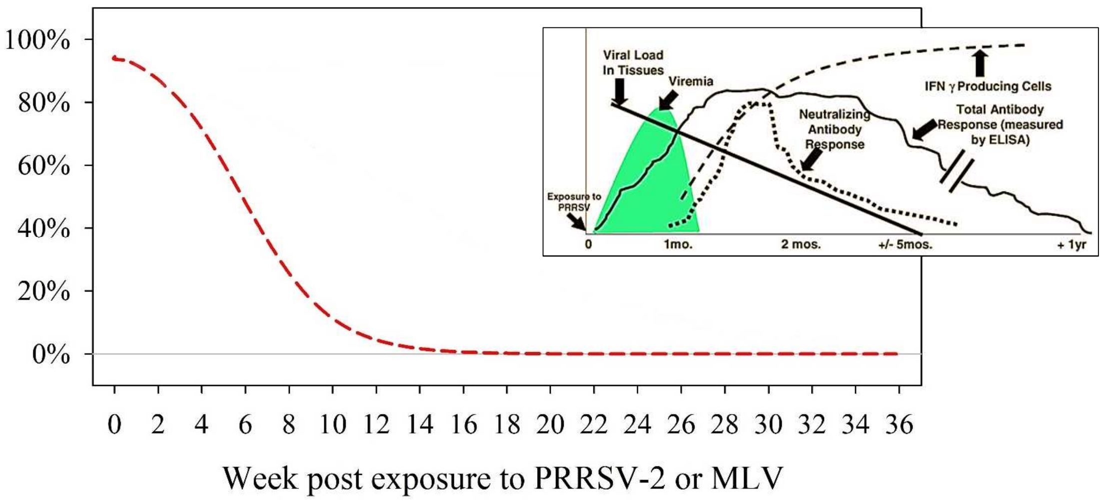
Lopez and Osorio. 2004. Vet Immunol Immunopathol 102:155-163

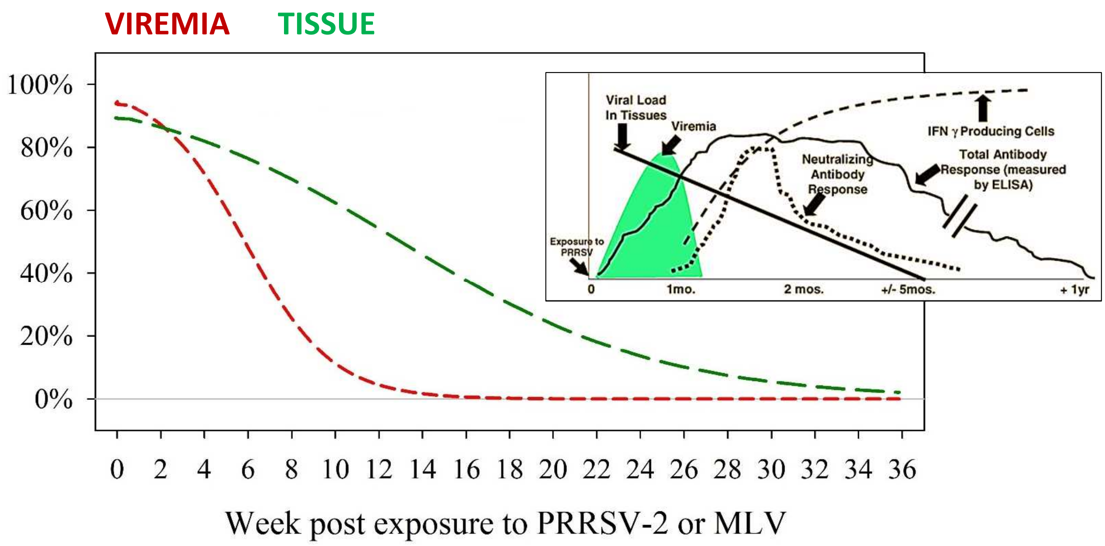
Henao-Diaz (2019): meta-analysis fitted with a logistic regression based on data (n = 3766) from 19 refereed publications (1995-2018).

- Viremia (RT-PCR-positive serum samples)
- Carrier animals (bioassay positive)
- Antibody positive (ELISA)

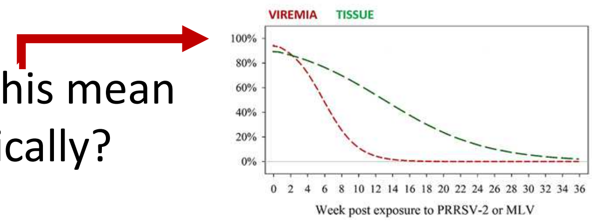


VIREMIA

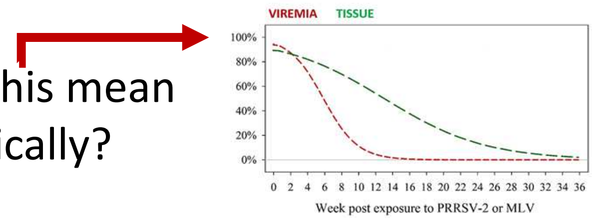




What does this mean diagnostically?

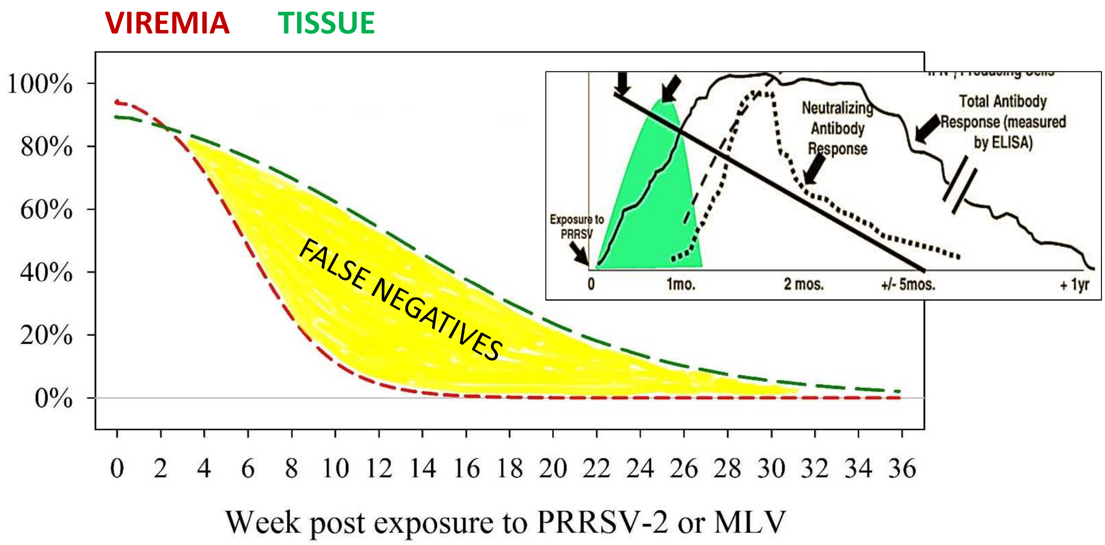


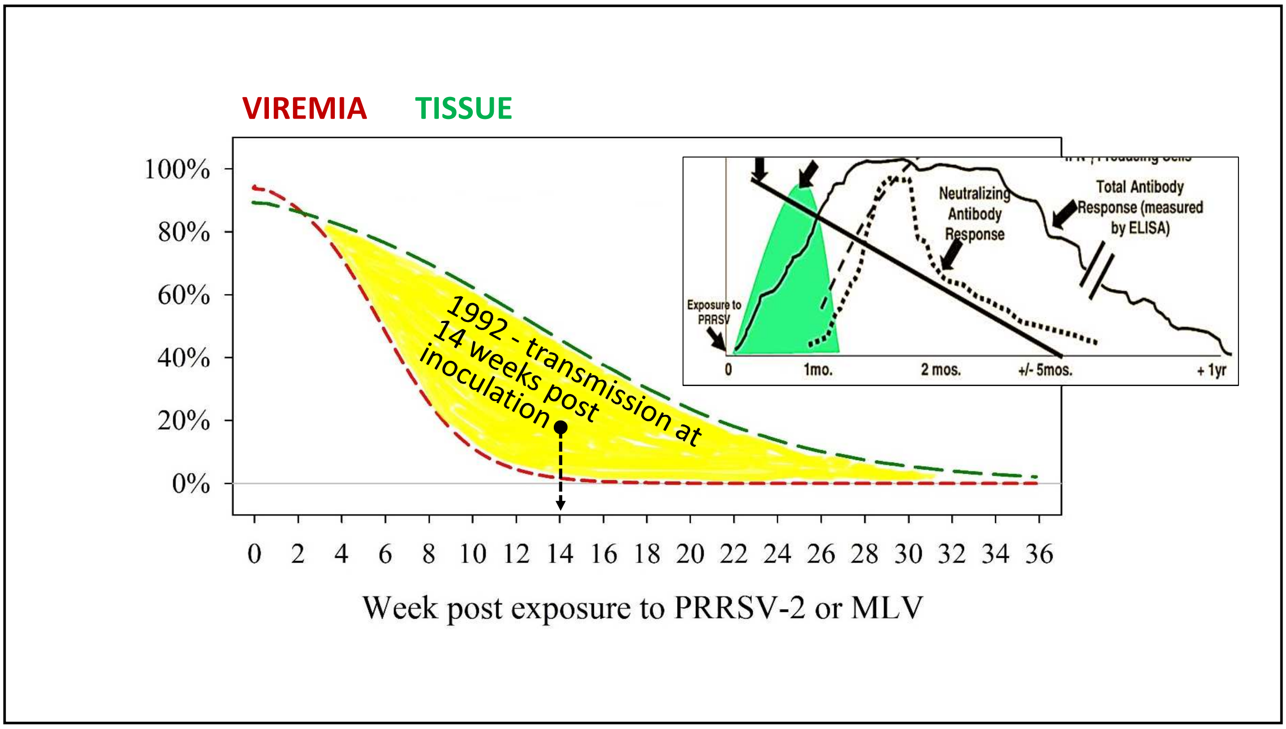
What does this mean diagnostically?

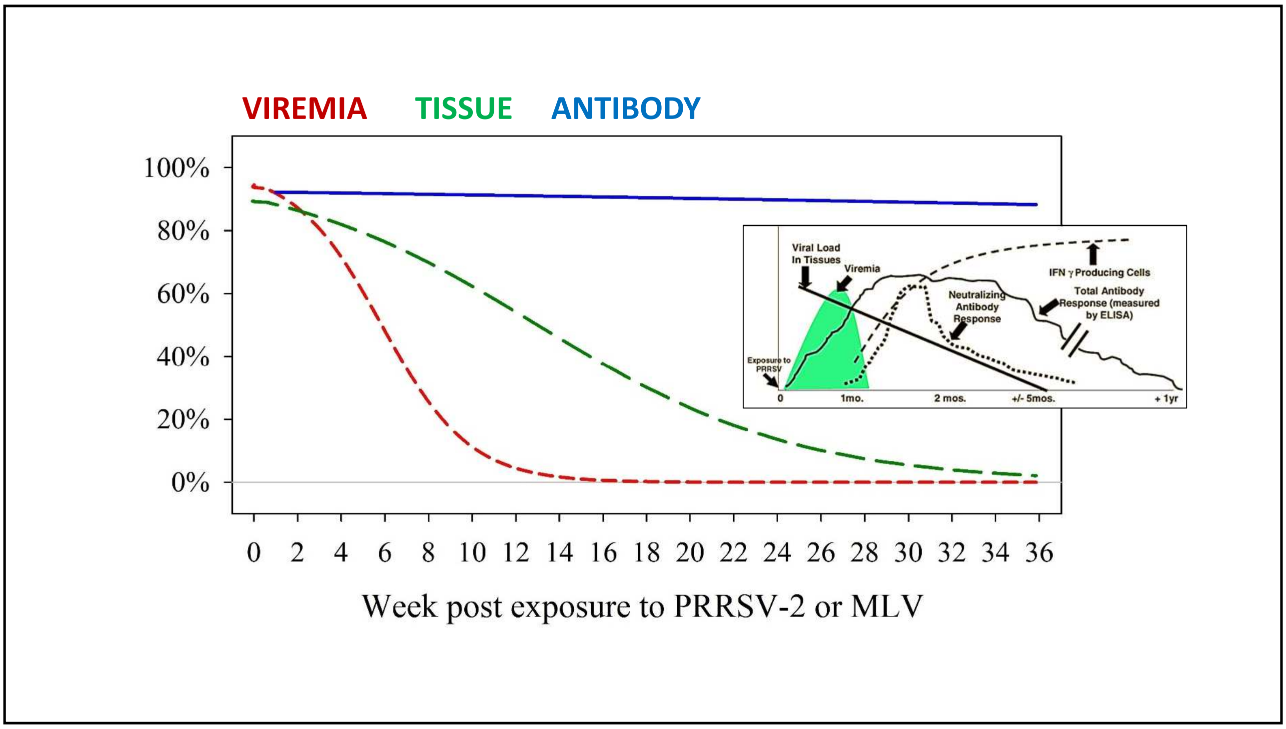


	ASSAY	DPI 28	DPI 56	DPI 84
SERUM	Virus isolation	2/28 (7%)	0/28	0/28
	RT-PCR	7/28 (25%)	1/28 (4%)	0/28
TONSIL	Virus isolation	9/28 (32%)	4/28 (14%)	0/28
	RT-PCR	27/28 (96%)	21/28 (75%)	20/28 (71%)

Wills et al., 2003. Duration of infection and proportion of pigs persistently infected with PRRSV. J Clin Microbiol 41:58-62




VIREMIA
TISSUE
100%
80%
60%
40%
20%
0%
1992 - transmission at 14 weeks post inoculation
Neutralizing Antibody Response
Total Antibody Response (measured by ELISA)
Exposure to PRRSV
0
1mo.
2 mos.
+/- 5mos.
+ 1yr
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Week post exposure to PRRSV-2 or MLV

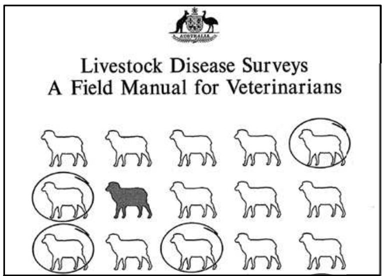

VIREMIA
TISSUE
ANTIBODY
100%
80%
60%
40%
20%
0%
Viral Load In Tissues
Viremia
Neutralizing Antibody Response
IFN γ Producing Cells
Total Antibody Response (measured by ELISA)
Exposure to PRRSV
0
1mo.
2 mos.
+/- 5mos.
+ 1yr
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Week post exposure to PRRSV-2 or MLV

Q: Which target?

A: We need both nucleic acid and antibody detection for surveillance

1. Which specimen? *Aggregate samples*.
2. Which target? *Both nucleic acid & antibody*.
3. **How to sample in the field?** Statistical validity.

First question is always “*how many samples*”?



RM Cannon, RT Roe. 1982

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

Sample size - PRV eradication

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

1. Where do these numbers come from?
2. Can we use these tables for oral fluids?

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

Sample size - PRV eradication

Population N	Prevalence 30.0%	20.0%	10.0%
80	8	13	24
90	9	13	25
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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
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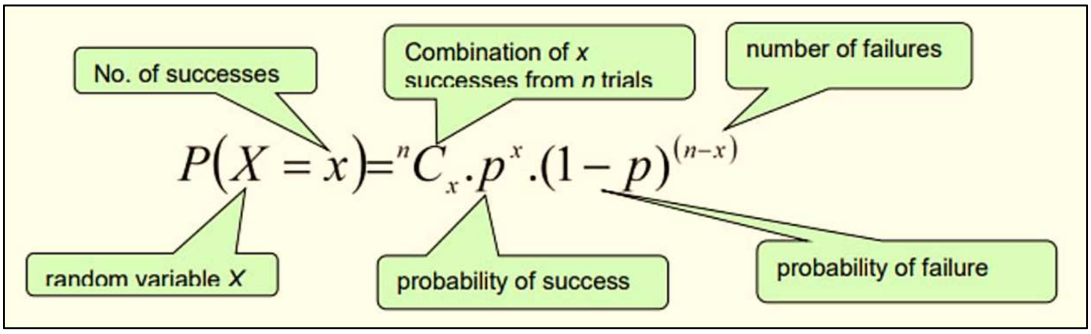
700	9	14	28
800	9	14	28
900	9	14	28
1000	9	14	29

Sample size numbers come from the binomial distribution formula

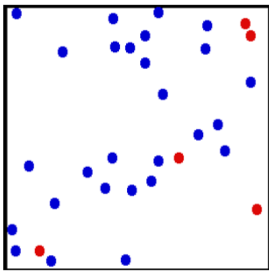


$$P(X = x) = {}^{n}C_{x} . p^{x} . (1-p)^{(n-x)}$$

Assumptions of binomal sampling?

Assumptions of
binomial distribution?

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



Assumptions of
binomial distribution?

1. Finite population.
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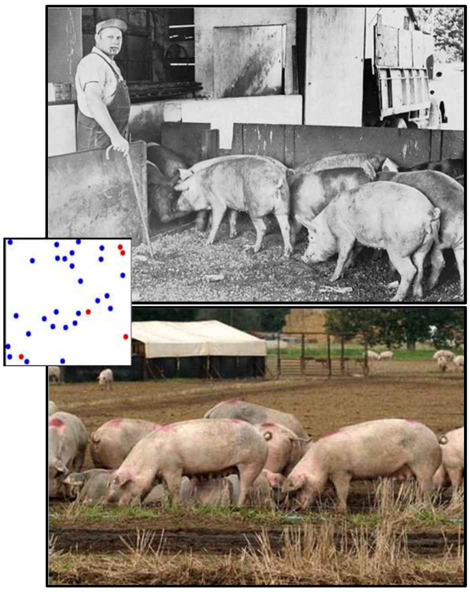
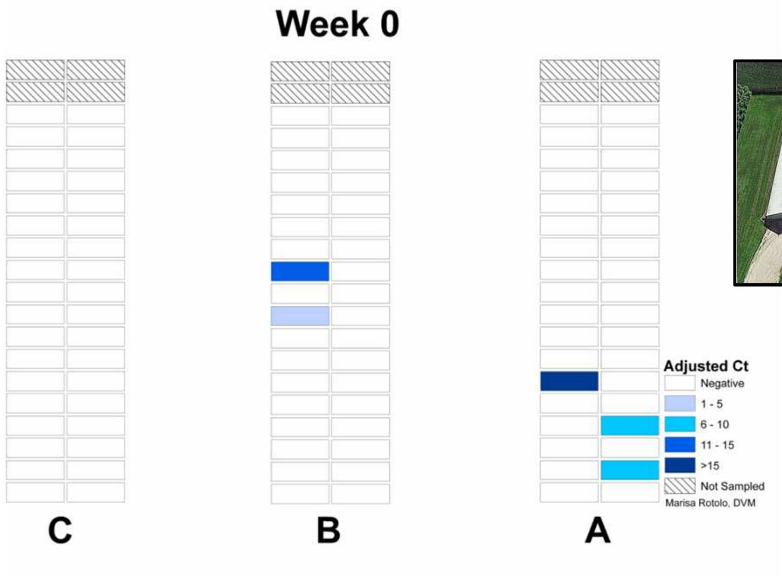
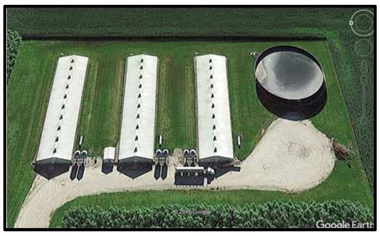


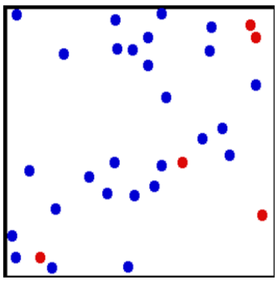


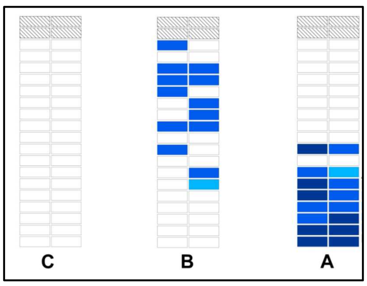


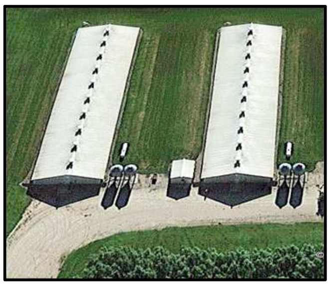
PRRSV oral fluid testing - field study.

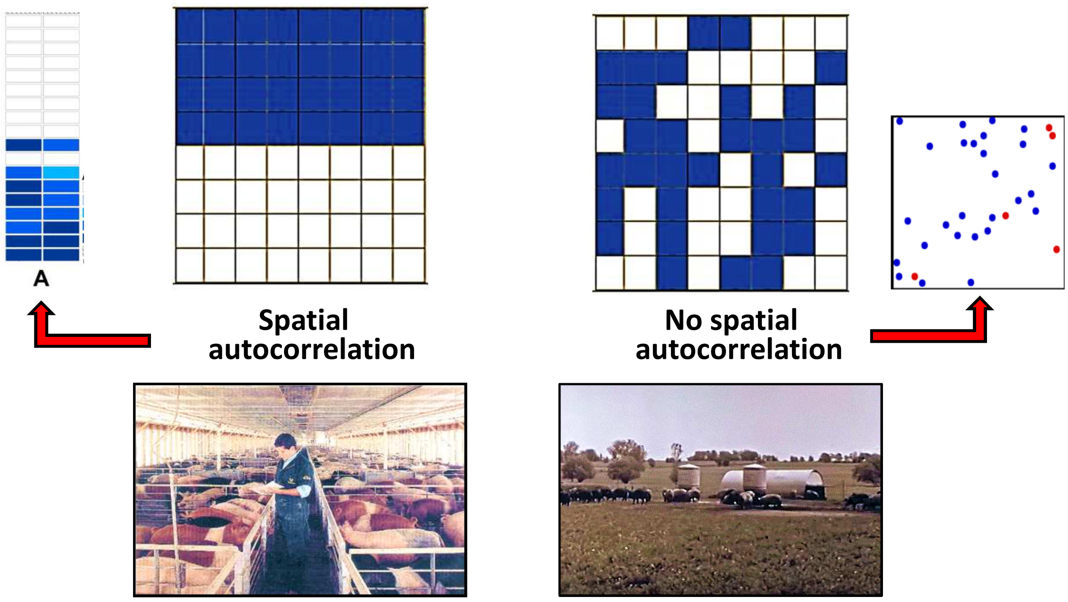
Rotolo et al. 2017. Vet Microbiol 209:20-29

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



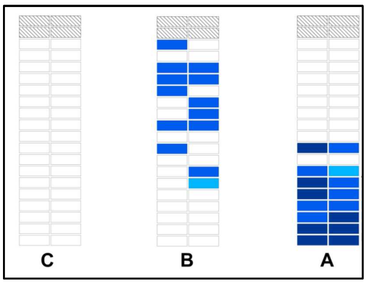






Spatial autocorrelation

- 1st Law of Geography (Tobler) "*everything is related to everything else, but near things are more related than distant things*.“



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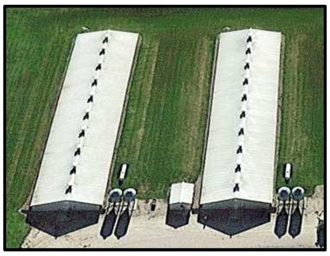
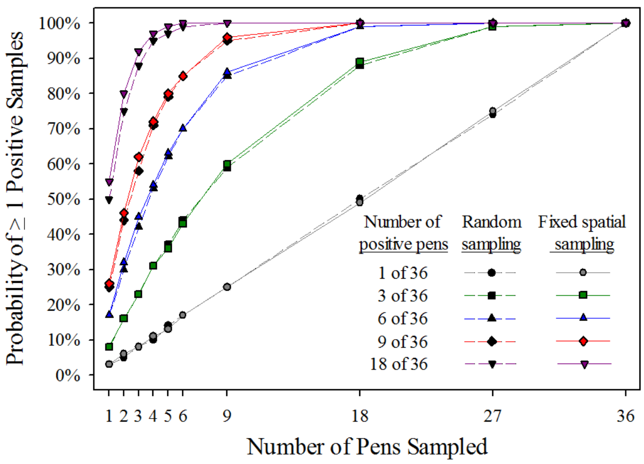
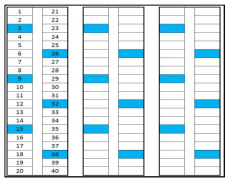
This does not apply to *this*.



Rotolo et al. 2017. Vet Microbiol 209:20-29





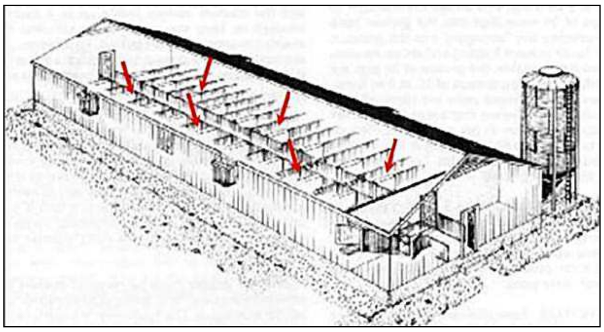
Statistical analysis: fixed spatial sampling was **EQUAL OR BETTER** than random sampling. Why?

"Spatial sampling is better (than random sampling) *when there is autocorrelation"* Wang et al. 2012. *Spatial Statistics 2*:1-14.

1. Decide how many samples you can collect routinely.
2. Sample the same pens every time.
3. Routine sampling - even with a few samples - is better than more samples collected infrequently.

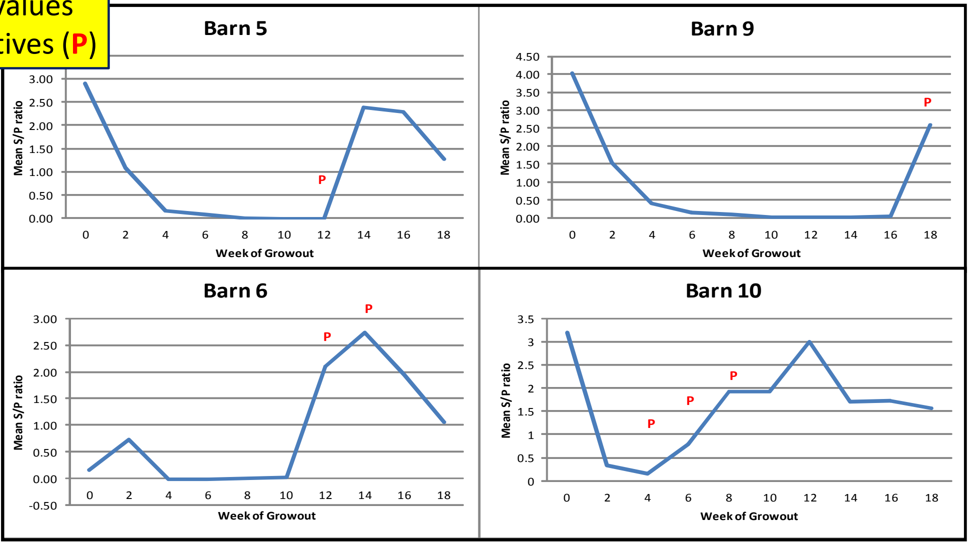
1	21	1	21	1	21	1	21
2	22	2	22	2	22	2	22
3	23	3	23	3	23	3	23
4	24	**4**	24	4	24	4	24
5	25	5	25	5	25	5	25
6	**26**	6	26	6	26	6	26
7	27	7	27	**7**	27	7	27
8	28	8	**28**	8	28	8	28
9	29	9	29	9	29	9	29
10	30	10	30	10	30	**10**	30
11	31	11	31	11	31	11	31
12	**32**	12	32	12	32	12	32
13	33	**13**	33	13	33	13	33
14	34	14	34	14	**34**	14	34
15	35	15	35	15	35	15	35
16	36	16	36	16	36	16	36
17	37	17	**37**	17	37	17	37
18	**38**	18	38	18	38	18	38
19	39	19	39	19	39	19	39
20	40	20	40	20	40	20	40

Sample the same pens every time at a fixed interval



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)



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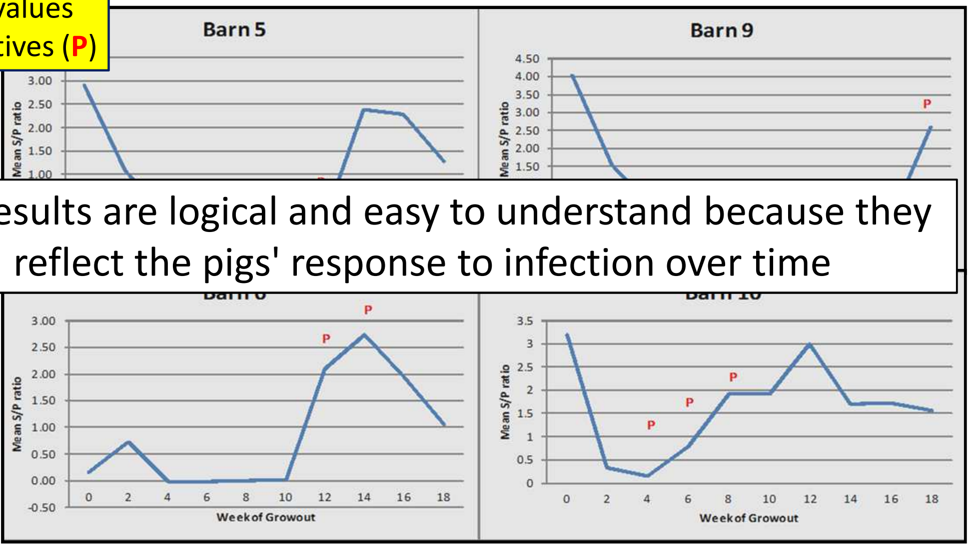
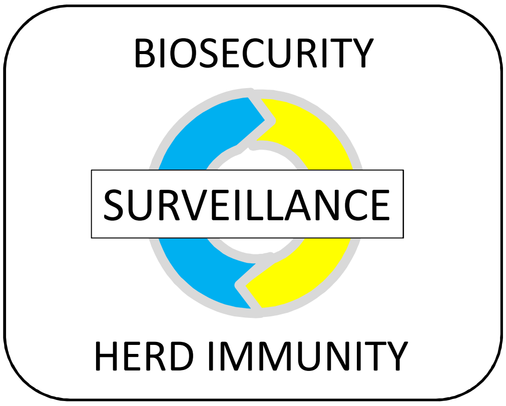


The results are logical and easy to understand because they reflect the pigs' response to infection over time

Conclusions

- Surveillance will play an increasingly important role in pig health.
- More efficient approaches are needed ... and are coming!
- Much accomplished/much remains to be done.



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- Christa Goodell
- Ramon Molina
- Marisa Rotolo

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Veterinary Diagnostic Laboratory

Thank you.

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