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New application possibilities in the diagnostic methods
Multiserology via microarray

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Fachbereich Veterinärmedizin
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Intermediate Goal
4th: multiserology via ArrayStrip
3rd: multiserology via ArrayTube
2nd: meaningfulness of seroprofiles
1st: comparability meat juice vs. serum
The need

The need

Still high number of zoonotic diseases in humans

- zoonotic pathogens of inapparent infections like *Salmonella*, *Toxoplasma* or Hepatitis E stay undetected and uncontrolled during traditional and visual meat inspection

Societal demand for animal health and animal welfare

- evaluation of pathological lesions at slaughter is often **not** standardized, which can lead to vets and farmers **not** taking post mortem findings into account

Need for (cost-effective) diagnostics regarding zoonoses and production diseases

European & German legislation

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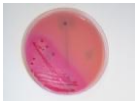
- 3 equal aims of the European food safety strategy (Reg. (EC) No 178/2002)


food safety

+


animal health

+

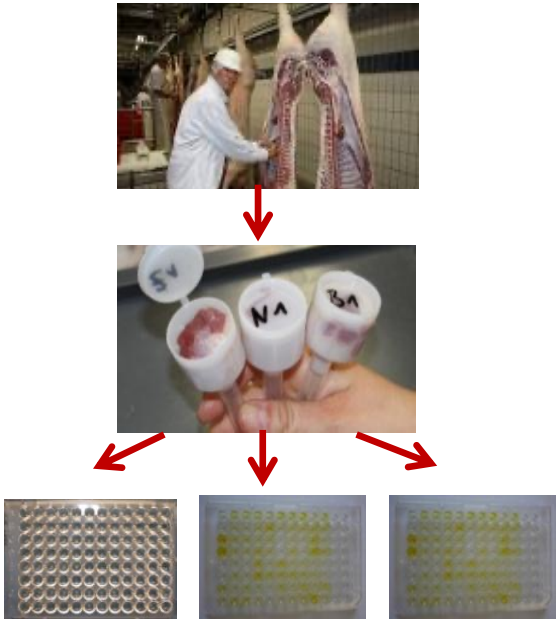

animal welfare

- demand for monitoring programmes to identify zoonotic pathogens in pig herds
  [Reg. (EU) No. 853/2004]
- ranking of zoonotic hazards which should be monitored in pigs:
  1. *Salmonella*, 2. *Trichinella*, 3. *Yersinia*, 4. *Toxoplasma*
  [EFSA´s scientific opinion (2011)]
- demand for evaluating animal health and human health risks
  [Reg. (EU) No. 219/2014]
- „serological *Salmonella* Monitoring Programme“ for pig herds in Germany
  [„German Regulation for *Salmonella* in pigs“ (2007)]

Principle of the „Meat Juice Multi-Serology“

(Meemken and Blaha, J Food Safety and Food Quality, 2011)

Testing meat juice samples (n ≥ 60/ herd) for antibodies against various pathogens for creating serological herd profiles



Taking the meat samples from diaphragm pillar at the slaughter line

↓

Producing meat juice by freezing and thawing the meat samples

↓ ↓ ↓

Testing the meat juice samples via various ELISA tests or other diagnostic methods
zoonoses & production diseases

Serum & meat juice as sample for multiserology

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Collection of blood samples in the herds

→ stress, pain, risk of infection

→ bleeding is veterinarian work only

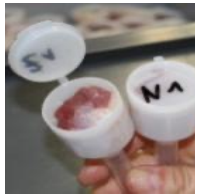
→ additional manpower needed

Collection of blood samples or diaphragm pillar muscle in the abattoirs

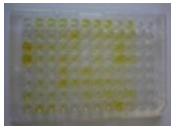
→ no risks for animal health, lower costs, no stress or pain for the animals

→ well-established working process due of the implemented *Salmonella* programme

→ no additional manpower needed



1st step: comparability of serological results meat juice vs. serum



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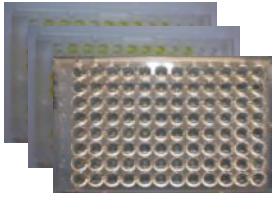
M&M: - comparative ELISA analysis of 291 pairs of samples (serum & meat juice)
- commercial ELISA tests for zoonoses and production diseases with (zoonoses) and without (production diseases) registrations for meat juice

	meat juice vs. serum (n=291)		
	Kappa	sensitivity	specificity
Salmonella spp.	0.87	87%	99%
Yersinia enterocolitica	0.93	100%	91%
Toxoplasma gondii	n.c.	100%	100%
Trichinella spp.	n.c.	100%	100%
Mycoplasma hyopneumoniae	0.86	91%	96%
Influenza A (H1N1)	0.66	61%	99%
Influenza A (H3N2)	0.65	55%	99%

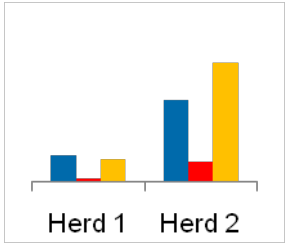
- tenfold lower dilution of meat juice than of blood serum ✓
- meat juice and serum are comparable specimens for ELISA test ✓

2nd step: meaningfulness of multiserological results → meat juice multiserology (Meemken et al., Prev. Vet. Med, 2014)

sampling
- taking muscle samples from 47 herds in the Northwest of Germany
- 47 herds x 60 samples= 2.820 meat juice samples

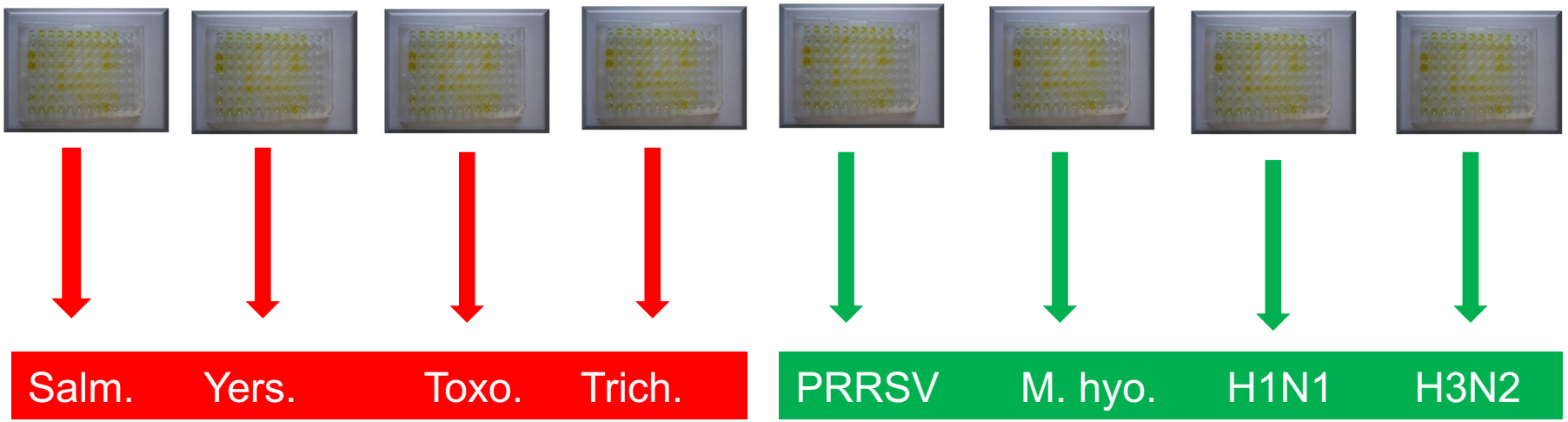


antibody testing
- 4 zoonotic diseases: *Salmonella*, *Yersinia*, *Toxoplasma*, *Trichinella*
- 4 production diseases: PRRSV, M. hyo., H1N1, H3N2



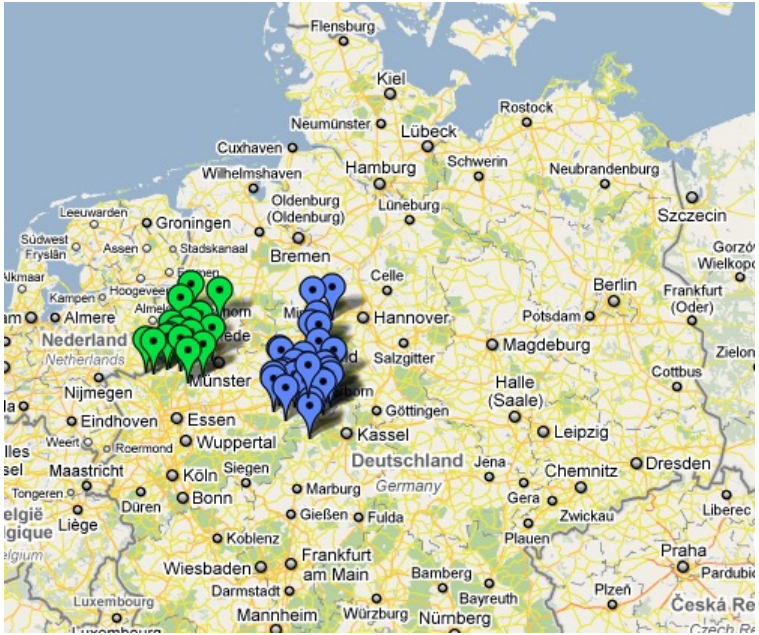
creating herd seroprofiles
- used by farmers and vets for continuous improvements in the herds
- used by official vets and food business operators for risk assessments

Serological herd profiles via sequential single-ELISA tests



2.820 samples x 8 single-ELISA tests = 22.560 results

Geographic position of the selected 47 herds in Nothwest Germany



- herd size >1000 slaughter pigs/year
- sampling of all herds in early spring 2011 from 3 slaughter batches each

Results: production diseases

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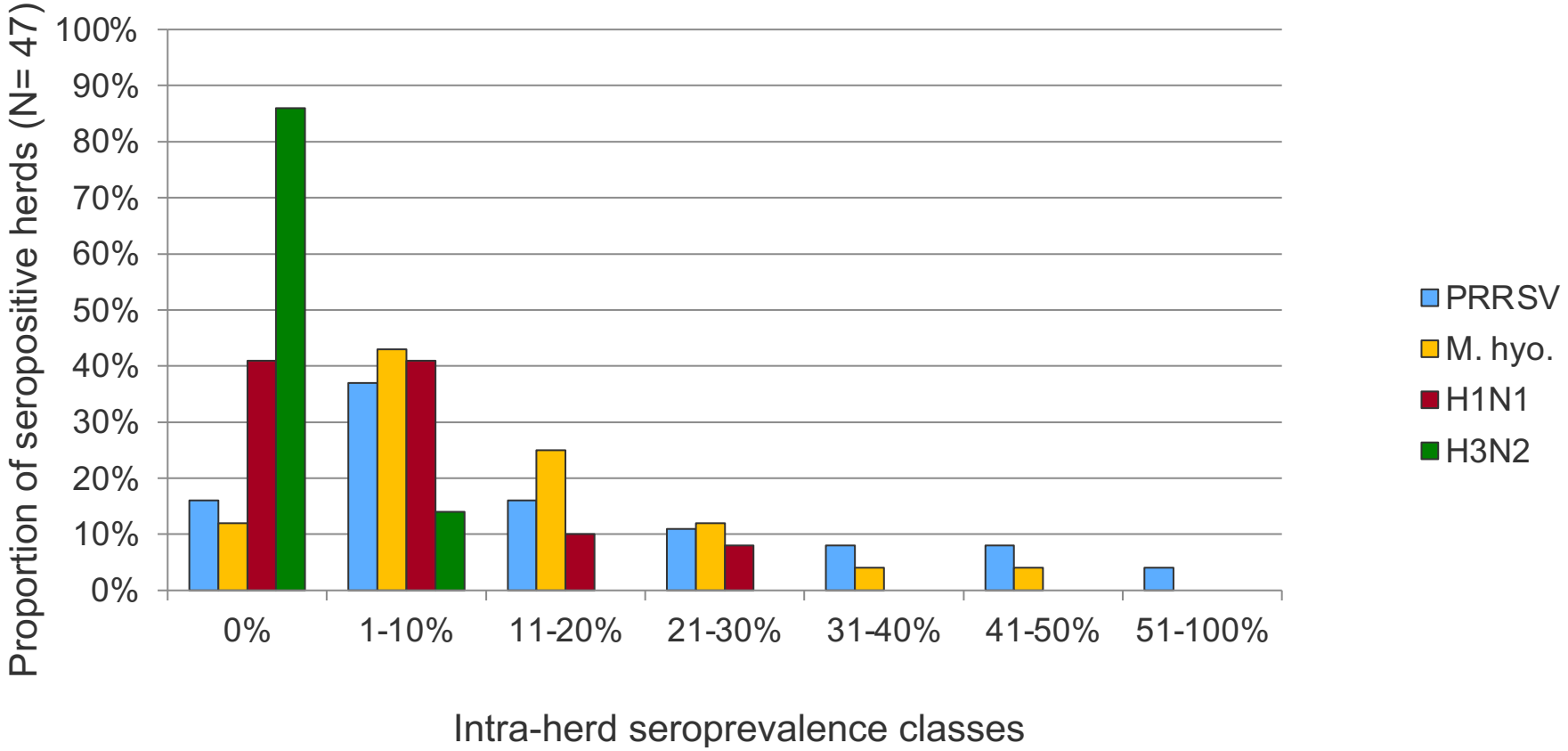
Seroprevalence per herd per seroprevalence class



Results: Zoonoses

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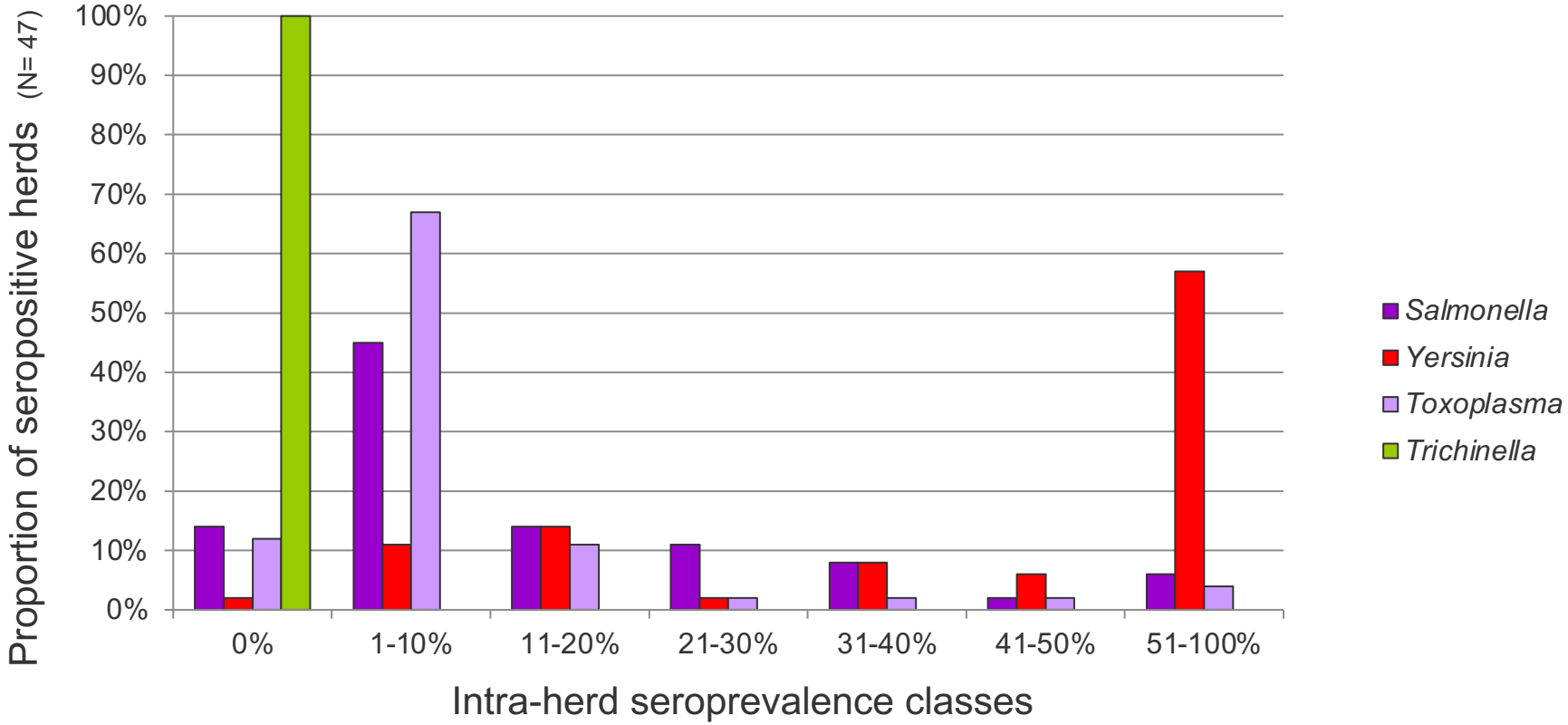
Seroprevalence per herd per seroprevalence class



Results: zoonoses

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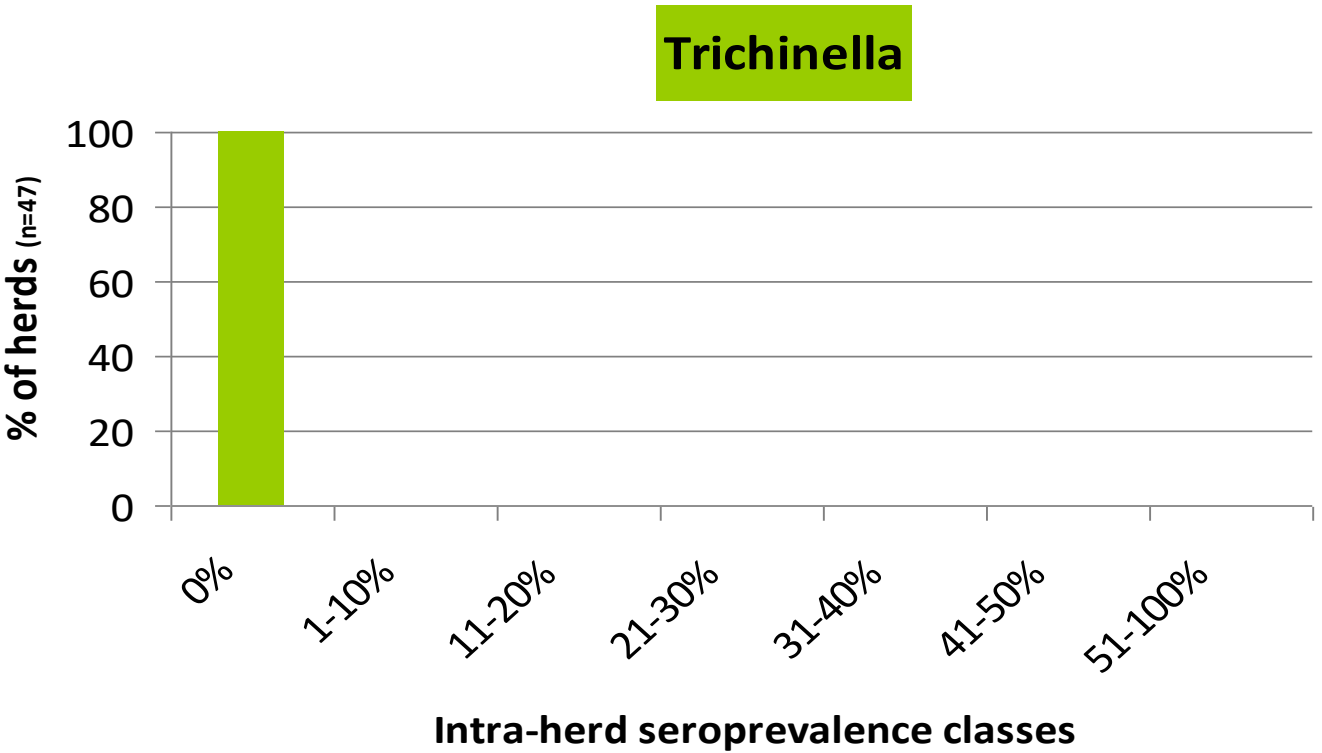
Classifying herds into seroprevalence classes



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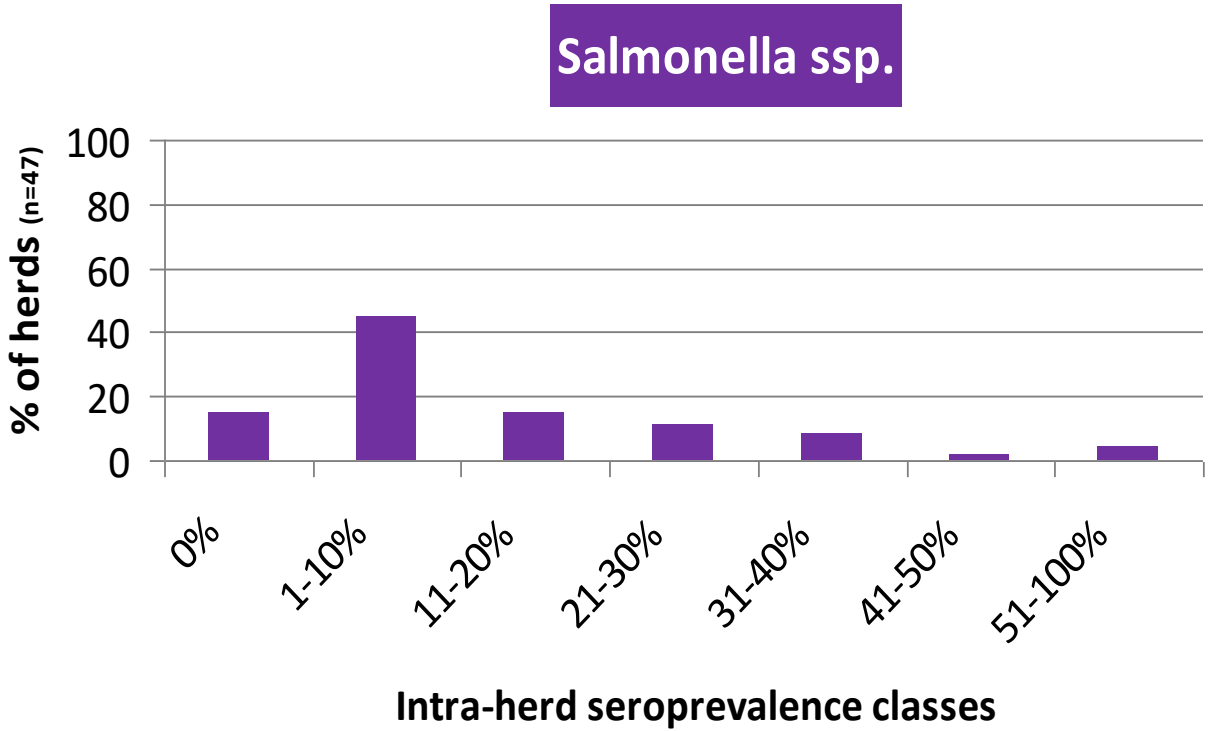
Results: zoonoses

Classifying herds into seroprevalence classes



Results: zoonoses

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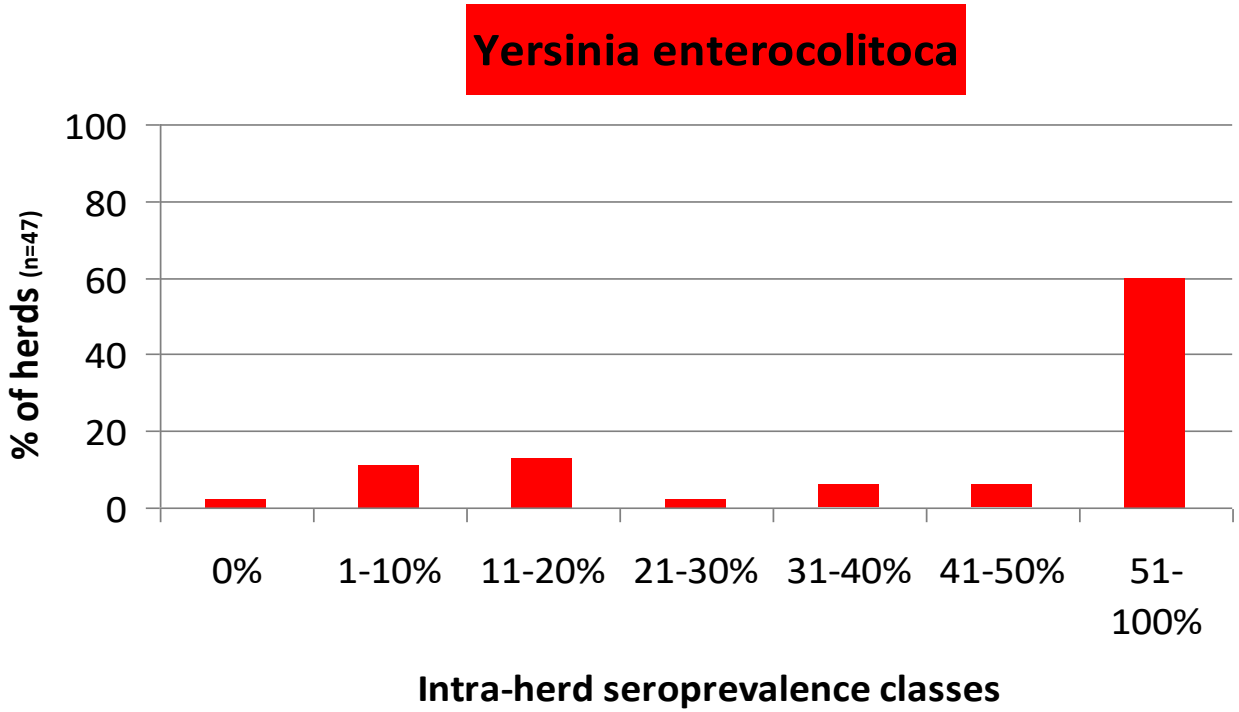
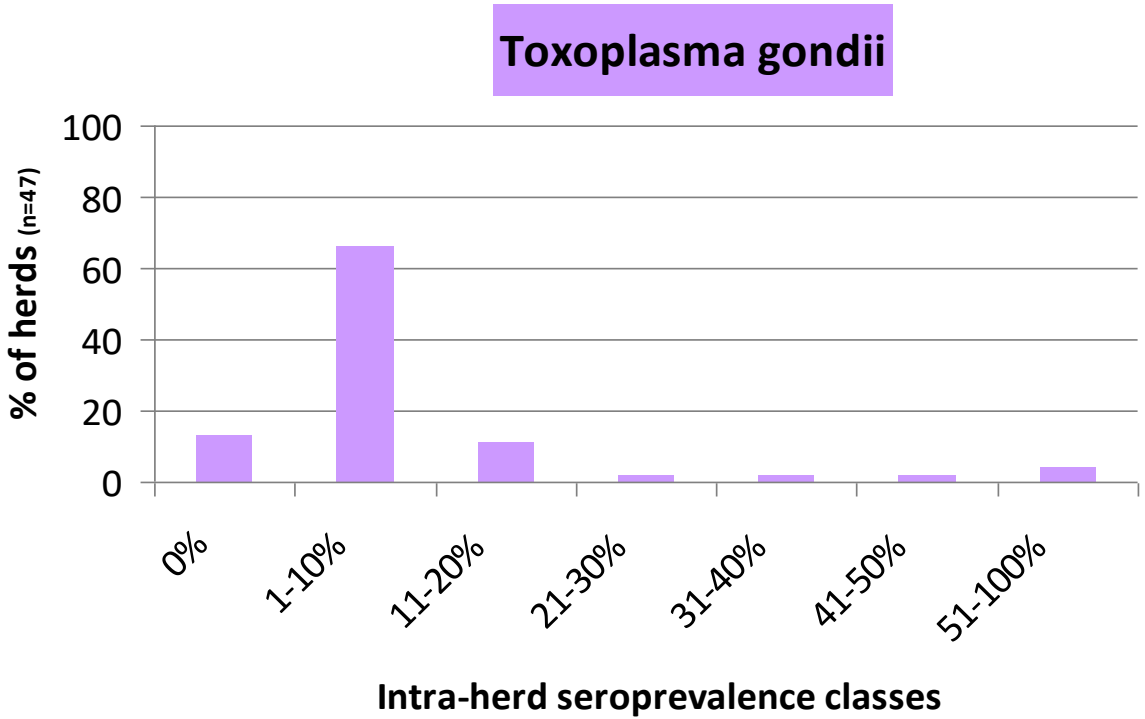


Results: zoonoses

Classifying herds into seroprevalence classes



Meaningfulness of serological herd profiles

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Salm.	Yersinia	Trichin.	Toxopl.	PRRSV	M. hyo	H1N1	H3N2
73%	0%	0%	20%	1%	63%	10%	92%

targeted herd investigation
- source of *Salmonella* infection
- optimising C&D

visual meat inspection/ logistic slaughter
- reducing cross contamination

use of meat
- no raw meat products

tageted herd investigation
- occurrence of cats in the barn

channeling of meat
- no raw meat products

- **targeted diagnostic measures**
  - necropsy, slaughter check, histology, PCR
- **reassessment of vaccination scheme**
  - time of vaccination

herd investigation: reasons for low seroprevalence
- implementation of these good measures in other herds

Conclusion: multiserology with sequential ELISA tests

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serological herd profiles:

- vary remarkably between herds even in the same region
- show infections with production diseases independently from the vaccination status (if vaccinated within first 2 months of life)
- help with vaccination decisions
- discriminates between low and high risk farms regarding meat safety hazards
- increase the informative value of the food chain information
- can be used for continuous improvement measures

next steps:

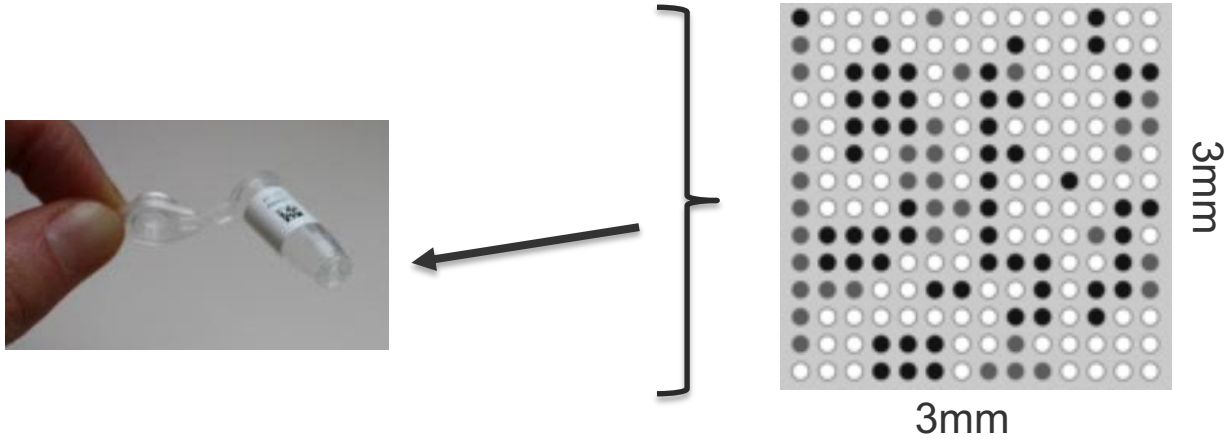
development and validation of (cost-effective) **simultaneous test systems** for production diseases and zoonoses (microarray, bead technology, lateral flow…)

3rd: development of multiserology via microarray

→ microrray in ArrayTube format (Hahne, 2014)

microarray:

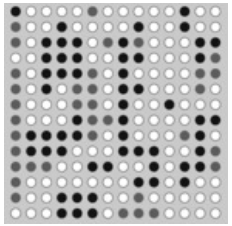
- a laboratory tool used to analyze large numbers of antibodies at one time
- antigens are placed in a pattern onto a glass slide (3x3mm) to detect antibodies



Test principle of protein microrrays

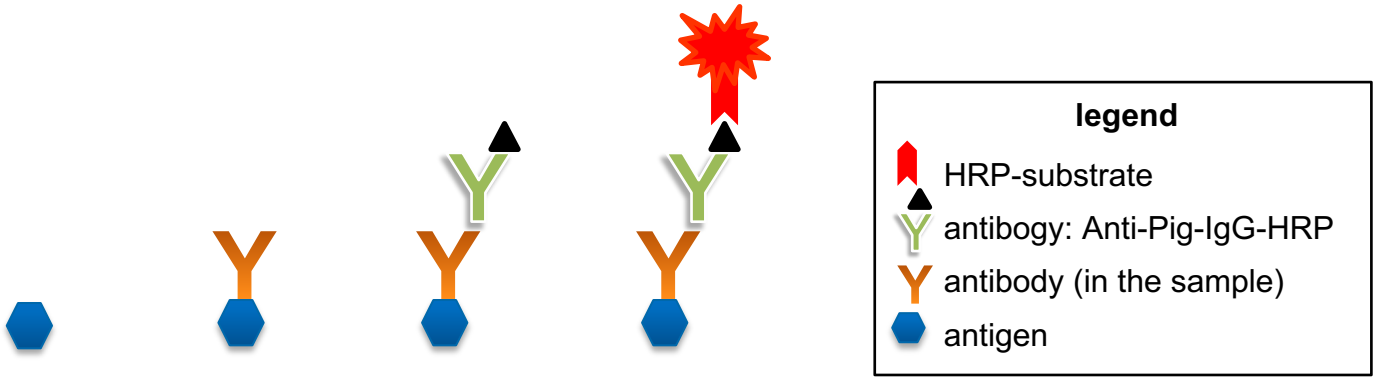
microarray in ArrayTube format:

- microarray chip (= glass slide) at the bottom of an Eppendorf cup (= reaction vessel)
- substances are added to detect the antibodies in the meat juice or serum
- manual processing neccessary



test principle:

- same principle and conjugats as for ELISA tests
- the darker the spot, the more antibodies in the sample



Development of a swine-specific protein microarray for simultaneaously testing production diseases and zoonoses

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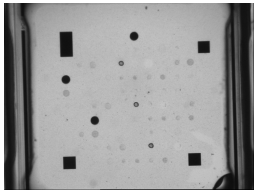
1st step

- acquiring antigens from ELISA test producers (QIAGEN, Alere)
- testing the feasibility of meat juice and serum as specimen
- developing a useful testing procedure

2nd step

- comparing the results of the microarray with single-ELISA results using the same samples



3rd step

- statistical analysis of the results
- finding the optimal sensitivity and specificity for the new test

The swine-specific protein microarray

designing a “swine-specific protein microarray” assisted by QIAGEN and Alere

Spotting recombinant or native antigens on the microarray chip:

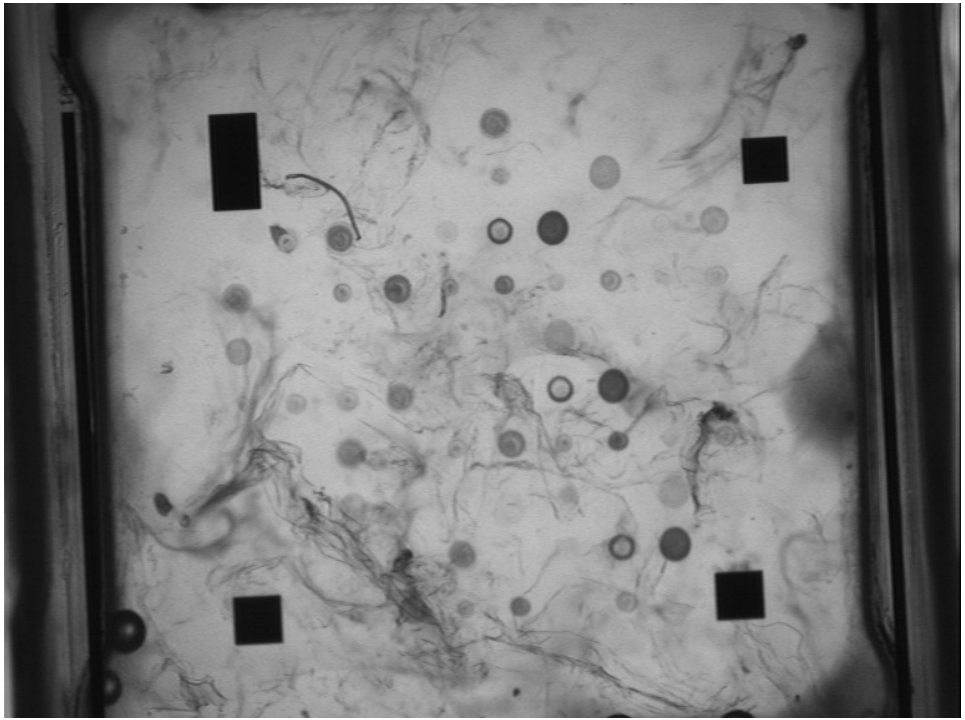
- *Salmonella spp.*
- *Toxoplasma gondii*
- *Trichinella spiralis*
- *Yersinia enterocolitica*
- *Hepatitis E virus*

} zoononic agents

- Swine Influenza Virus
- *Mycoplasma hyopeumoniae*
- PRRSV
- *Actinobacillus pleuropneumoniae*

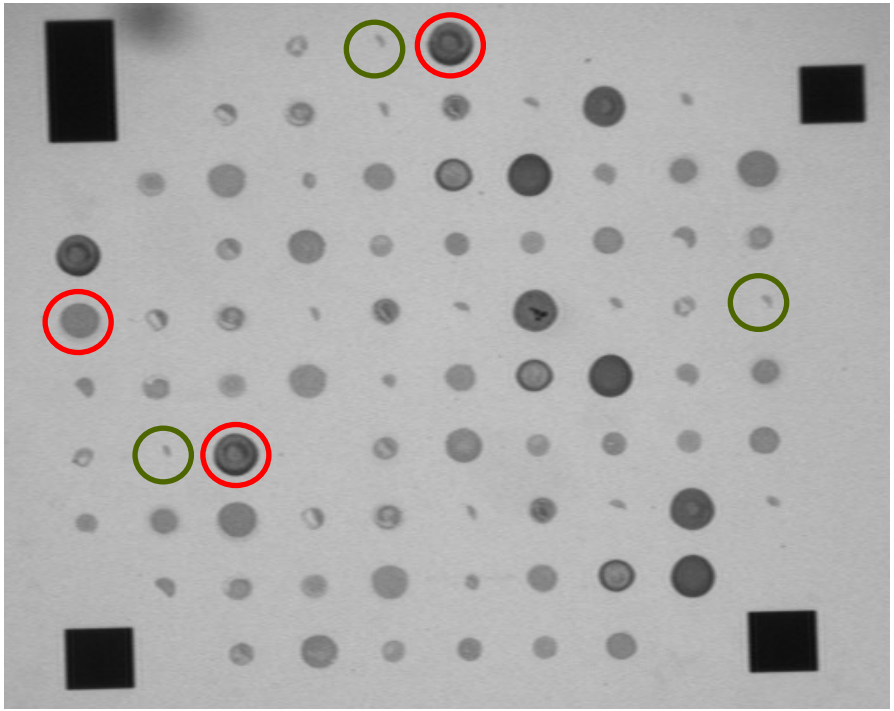
} production disease agents

Results



clouds caused by using wrong washing solution

Results

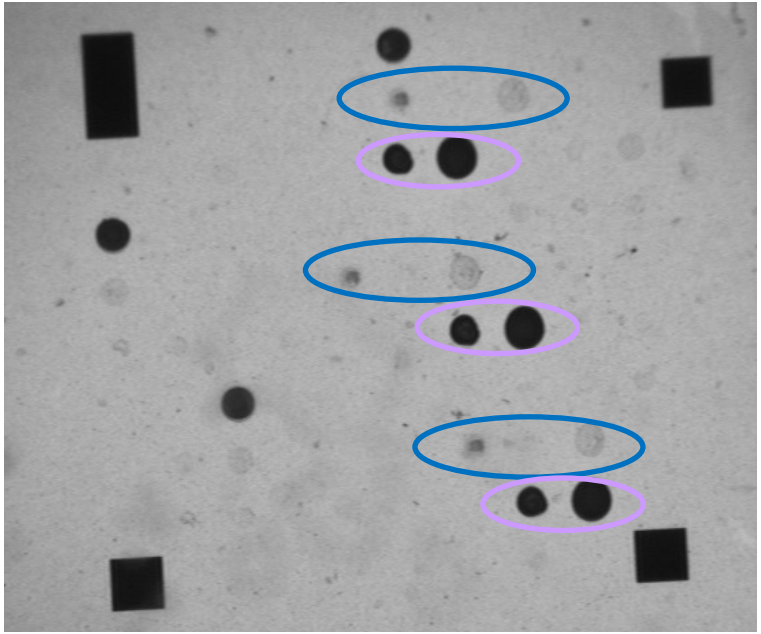


positive controls

negativ controls

unspecific bindings caused by using the wrong conjugate

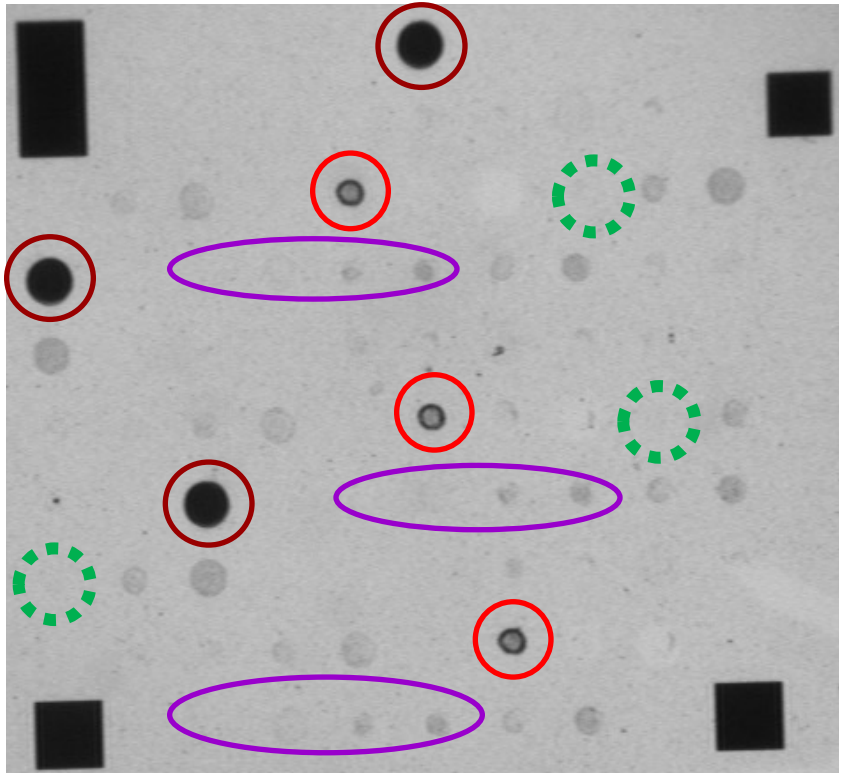
Seropositive meat juice for *Toxoplasma* and PRRSV



Toxoplasma gondii

PRRSV

Seropositive meat juice field sample for *Yersinia enterocolitica* & *S. Typhimurium*



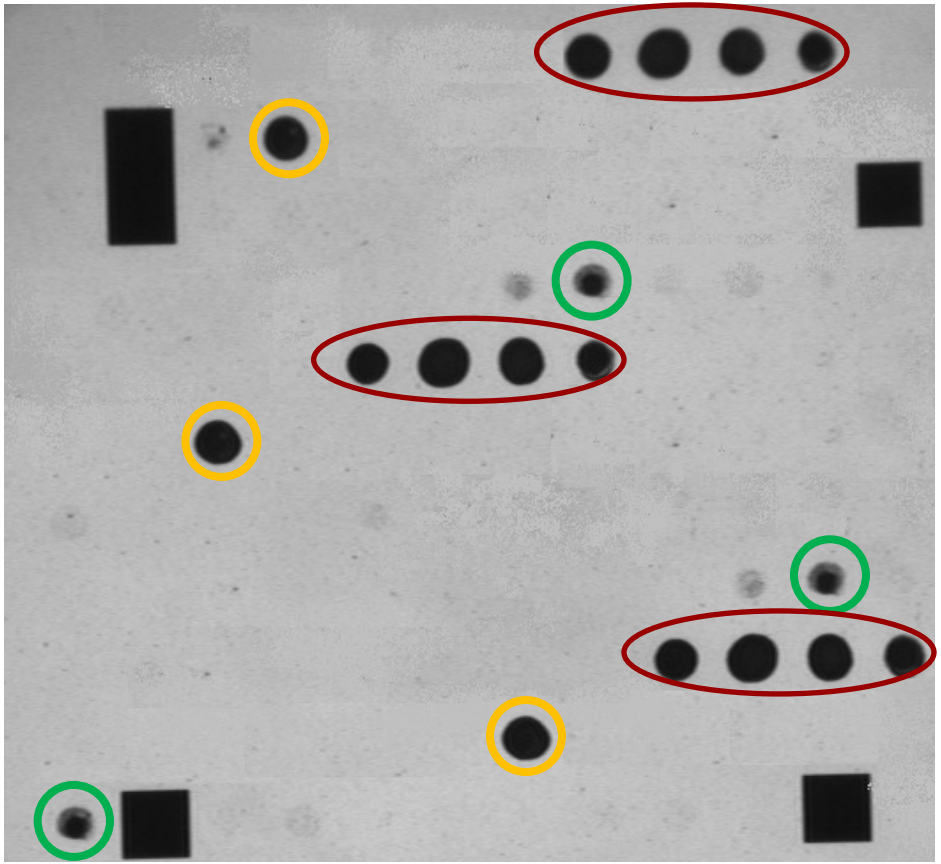
positivs controls

Yersinia enterocolitica

Salmonella Typhimurium

no antibodies against Trichinella

Seropositive meat juice from reference labor for *Trichinella spiralis* (Prof. Dr. Nöckler, BfR)



- Positiv controls
- *Trichinella*
- Hepatitis E Virus

Comparable results

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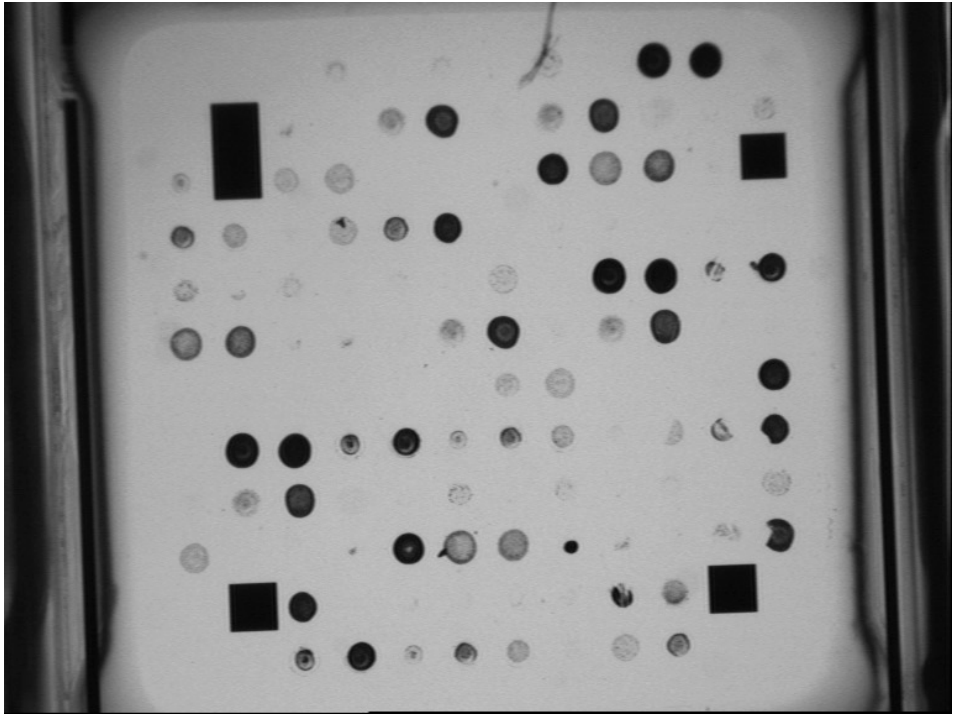
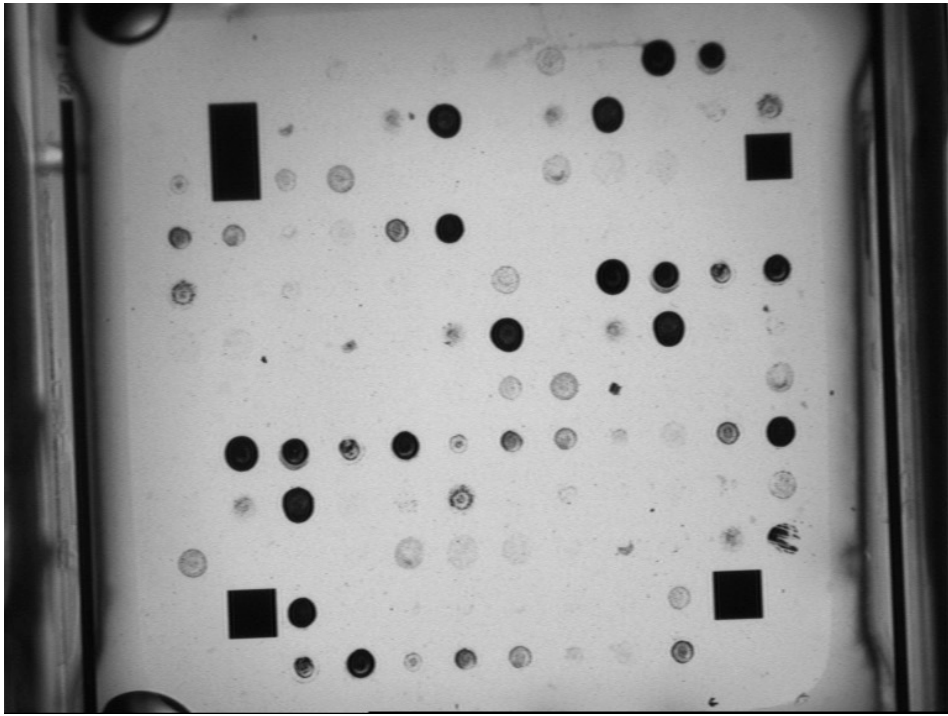


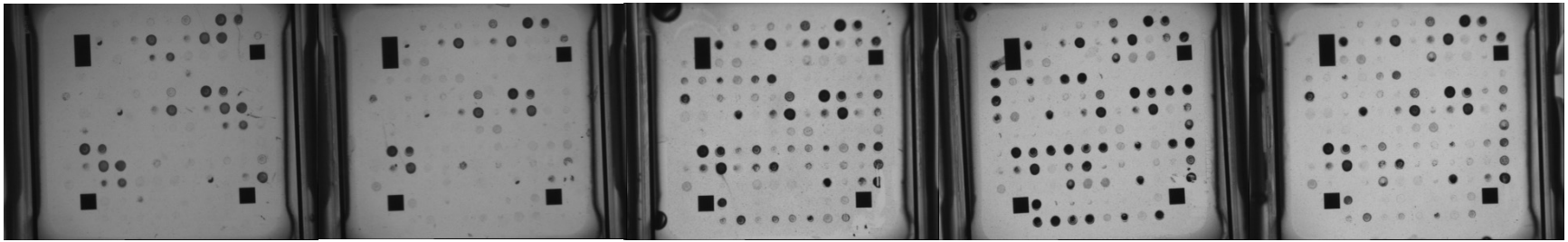
meat juice (dilution: 1:2)



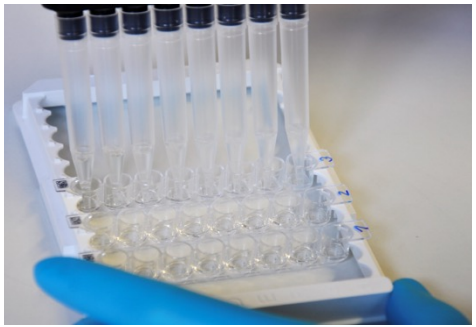
blood serum (dilution 1:20)

Conclusion

- meat juice and blood serum are equally suitable specimens
- only a small amount of antigen is needed (= lower cost)
- test duration is comparable to ELISA tests (1:45h)
- possibility to test up to 100 different pathogens via microarray
- approach is in line with the requirements of the EU Food Safety Strategy

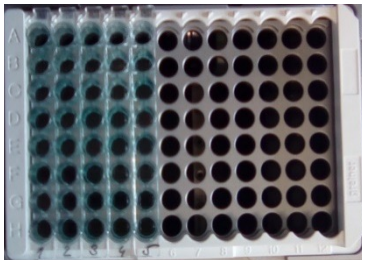


4th: development of automated multiserology
→ microarray strip format (Loreck et al. 2019, 2020)



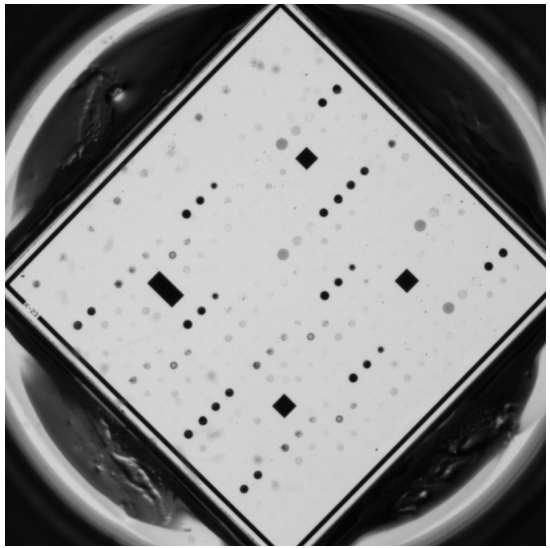
ArrayTube format

ArrayStrip Format

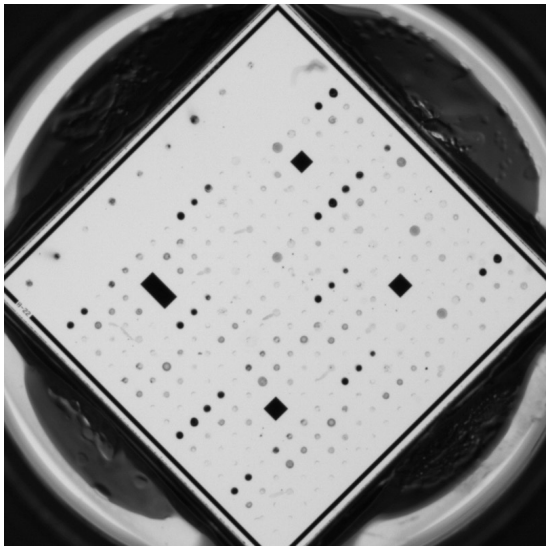


Multiserologie via Microarray

Comparing a paired sample (meat juice and serum from the same animal

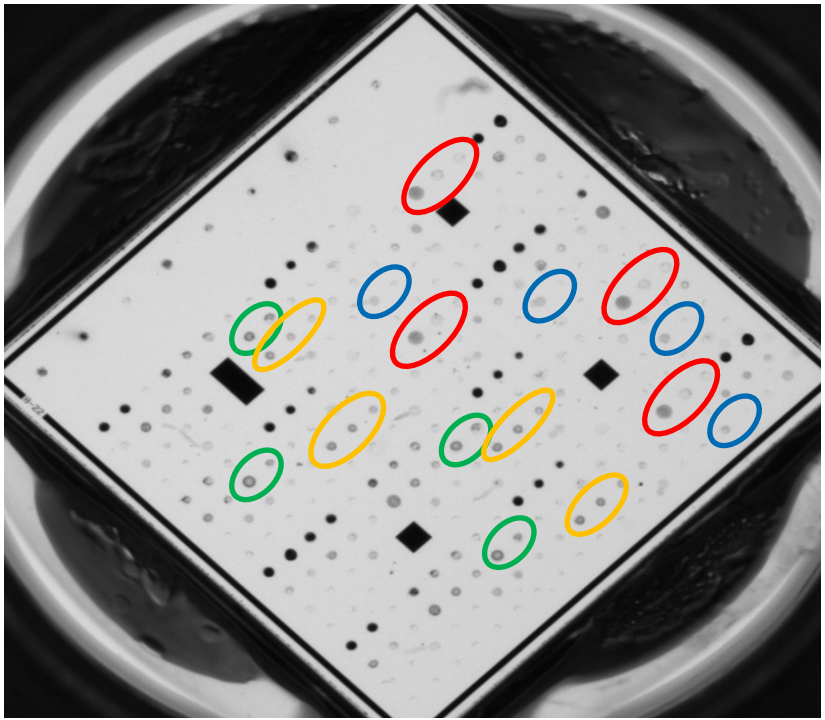


meat juice



blood serum

Multiserologie via Microarray



- *Mycoplasma hyopneumoniae*
- *Toxoplasma gondii*
- *Salmonella*
- PRRS-Virus

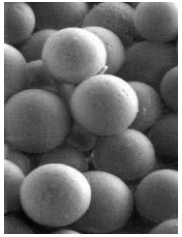
Intermediate goal

successful development of multiserology

- for production diseases & zoonoses
- with serum & meat juice as specimen
- via ArrayTube format & automated ArrayStrip format
- up to 10 serological results for approx. 5€ (order volume of 1 milion arrrays)

potential next steps:

- development of multiserology via lateral flow or bead technology“
  - more flexible, cheaper and currently available diagnostics



Thank you for your attention!

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Special thanks to our funders

2nd step **Ministry for Climate Protection, Environment, Agriculture, Nature Conservation and Consumer Protection of the State of North Rhine-Westphalia**

3nd step **„ Federal Ministry of Food, Agriculture and Consumer Protection on the basis of a Resolution of the German Bundestag“**

„ Federal Office for Agriculture and Food (BLE), Grant no. 28011HS013“

Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz

Bundesanstalt für Landwirtschaft und Ernährung

4th step

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Publications along the path of development

Meemken, D, Blaha T (2011): **"Meat Juice Multi-Serology" – A tool for the continuous improvement of herd health and food safety in the framework of the risk-based meat inspection of slaughter pigs, J Food Safety and Food Quality**

Nobmann JA, Blaha T, Beyerbach M, Kreienbrock L, Meemken D. (2011): **[Comparing the results of the serological detection of Salmonella antibodies in blood serum and meat juice from different muscles from slaughter pigs].** Berl Munch Tierarztl Wochenschr. 2011 Jul-Aug;124(7-8):313-9. German. PMID: 21848039.

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Meemken D, Tangemann AH, Meermeier D, Gundlach S, Mischok D, Greiner M, Klein G, Blaha T. (2014): **Establishment of serological herd profiles for zoonoses and production diseases in pigs by "meat juice multi-serology".** Prev Vet Med. 2014 Mar 1;113(4):589-98. doi: 10.1016/j.prevetmed.2013.12.006. Epub 2013 Dec 25. PMID: 24411983.

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Loreck, K.; Mitrenga, S.; Heinze, R.; Ehricht, R.; Engemann, C.; Lueken, C.; Ploetz, M.; Greiner, M.; Meemken, D. (2020): **Use of meat juice and blood serum with a miniaturised protein microarray assay to develop a multi-parameter IgG screening test with high sample throughput potential for slaughtering pigs. BMC veterinary research**; 16, S. Article number: 106

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