LANDESUNTERSUCHUNGS-
ANSTALT FÜR DAS GESUNDHEITS-
UND VETERINÄRWESEN

Freistaat
SACHSEN

Paratuberculosis diagnostics in Saxony, Germany

Field study for the validation of a Real-Time PCR

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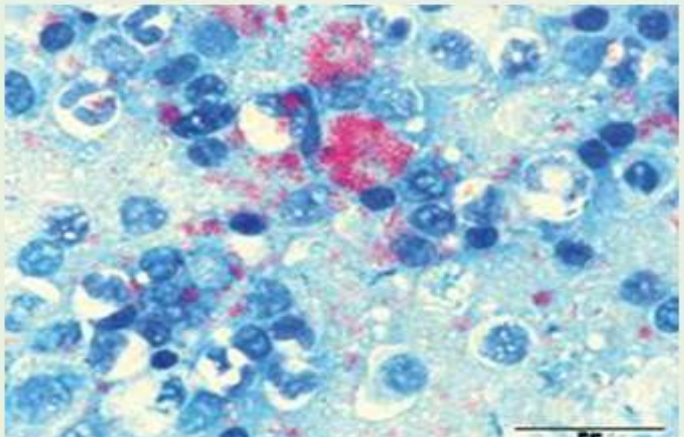
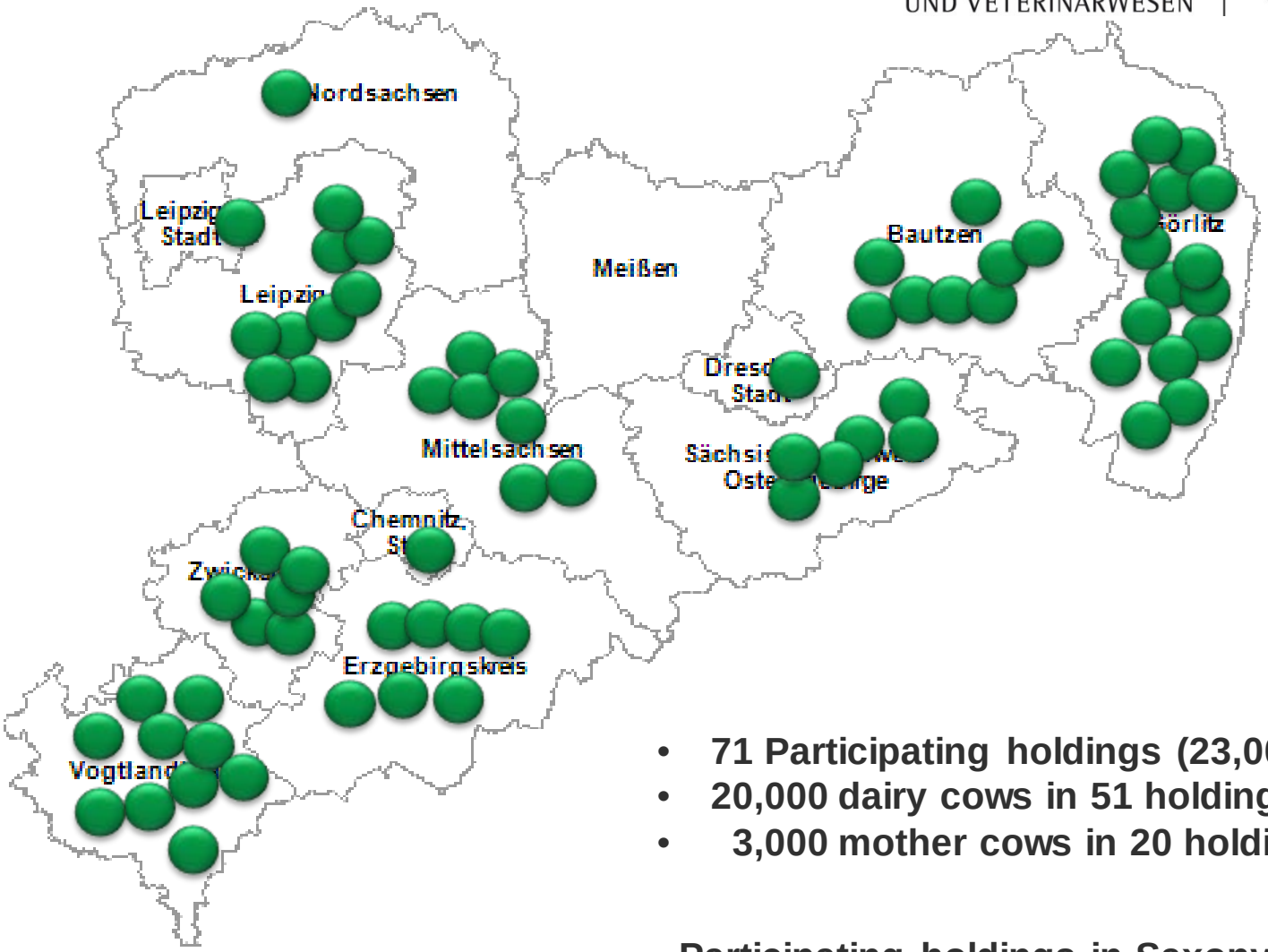


Paratuberculosis control in Saxony

- 2005 — Program of the saxonian Animal Disease Fund (TSK) to monitor and control paratuberculosis
  - Serology testing of the whole herd, including specification of hygiene measures
- 2010: — Extension of the program
  - Implementation of feces culture as herd-level diagnostics
- Goals:
  - Knowledge of MAP prevalence in the herd
  - implement targeted action for MAP control
  - perform targeted action to establish and maintain negative status for paratuberculosis
- Protocol:
  - serology testing of all cattle older than 24 months in the herd (determination of status)
  - serological, bacteriological or pathological confirmation of clinical suspect cases
  - operational programs (hygiene management, diagnostics, stipulations for animal movement)
  - bacteriology on fecal samples to establish or maintain paratuberculosis negative status



- **71 Participating holdings (23,000 cows)**
- **20,000 dairy cows in 51 holdings**
- **3,000 mother cows in 20 holdings**

Participating holdings in Saxony

TSK
SÄCHSISCHE
TIERSEUCHENKASSE
ANSTALT
DES ÖFFENTLICHEN
RECHTS

Paratuberculosis program

- Culture: Standard method according to the German official method-collection
  - 3 g faeces – decontamination of sample with HPC
  - Inoculation of 3 tubes containing Herrold‘s Egg Yolk medium with mycobactin
  - First readout after 6 weeks, end result after 12 weeks
  - Wash-off of all cultures – PCR (F57, Herthnek et al., 2006)
    - negative result → test in pools of 10 samples, some as single samples
    - positive result → single sample testing

Fecal culture – tested samples

Cattle

Year	No. of samples	positive	Percentage positive
2009	641	212	33,0%
2010	7.772	447	5,8%
2011	12.945	370	2,9%
2012	13.573	339	2,5%
2013	14.042	485	3,5%
2014	17.882	677	3,8%

… takes a lot of time and space in the lab

⇨ Alternative: Real-Time PCR

Field study 2013

- Selection of RT-PCR

Goal: - registered PCR test
- automated sample preparation
- sensitivity and specificity comparable to fecal culture

⇨ VetMAX MAP Real-Time PCR Screening Kit,
Thermo Fisher Scientific

⇨ Nucleic acid isolation using Magnetic Beads in King Fisher 96 instrument with MagVet Mycobacterium Paratuberculosis Isolation Kit,
Thermo Fisher Scientific

⇨ High analytical sensitivity, 100% specificity
diagnostic sensitivity/specificity?

Field study 2013/14

Implementation of RT-PCR and comparison to culture

- Receipt of samples via the HIT-submission form – Registration of ear tags with a hand-held scanner
- Parallel investigation of approx. 2000 feces samples with culture and PCR
- Sampling from selected holdings with high prevalence, in order to detect all phases of bacterial shedding

Operations

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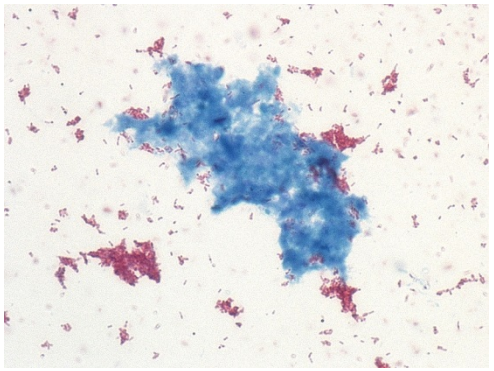
	# Cows	Serology since	Feces exam. since	Feces prevalence			Seroprevalence	
				2010	2011	2012	2011	2012
Operation A	150	2005	2010	45%*	14%	2.3%*	14.90%	
Operation B	400	2011	2011		50%*	50%*	3.60%	4.10%
Operation C	600	2002	2010	6.60%	8.50%	3.30%	3.30%	
Operation D	350	2009	2012			32.5%*	7.00%	6.10%
Operation E	50		2011		38.30%	26.70%		
Operation F	120	2009	2010	26.70%	7.90%	9.80%	4.20%	1.80%
Operation G	60	2012	2012			50%*		6.40%

… as well as additional operations that were clinically and/or serologically conspicious

Challenge with DNA-Isolation of Mycobacteria

- Distribution: uneven, lying in clusters
- Complex cell wall – Resistant to acids



→ Thorough homogenisation of the sample

→ Strong mechanical and chemical processing of samples

Sample preparation and DNA isolation

2g feces in 30 ml Water
→ Vortex with 3 beads (∅ 5mm), 30-60 sec

Let feces sediment (5-10 min)

Discard 1,8 ml supernatant,
centrifuge 10 min, 15.000 g

Discard supernatant,

Pellet + Lysisbuffer + Zirkonium beads (∅ 0,1mm)
→Homogenisation with bead beater (10 min., 30 Hz.)

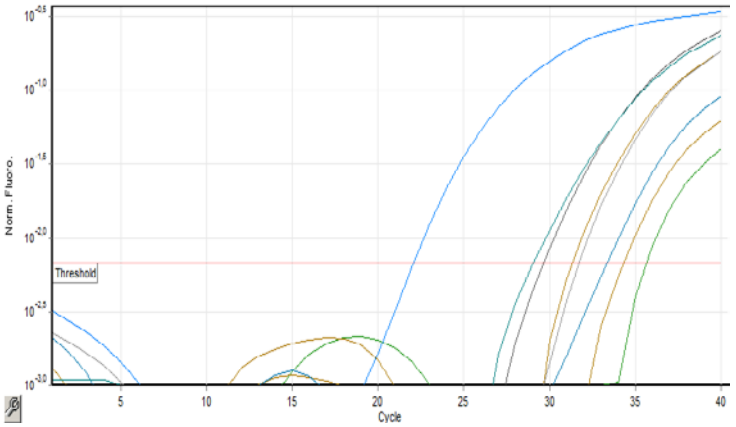
Centrifuge 3 min, 15.000 g

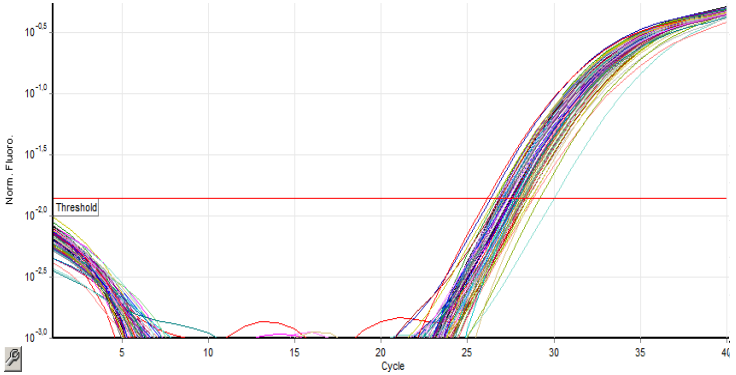
DNA isolation from supernatant with the KingFisher 96 (approx. 45 min) with the addition of internal control

MagVet Mycobacterium Paratuberculosis Isolation Kit, of Thermo Fisher Scientific

VetMax™ MAP Real-Time PCR Screening Kit

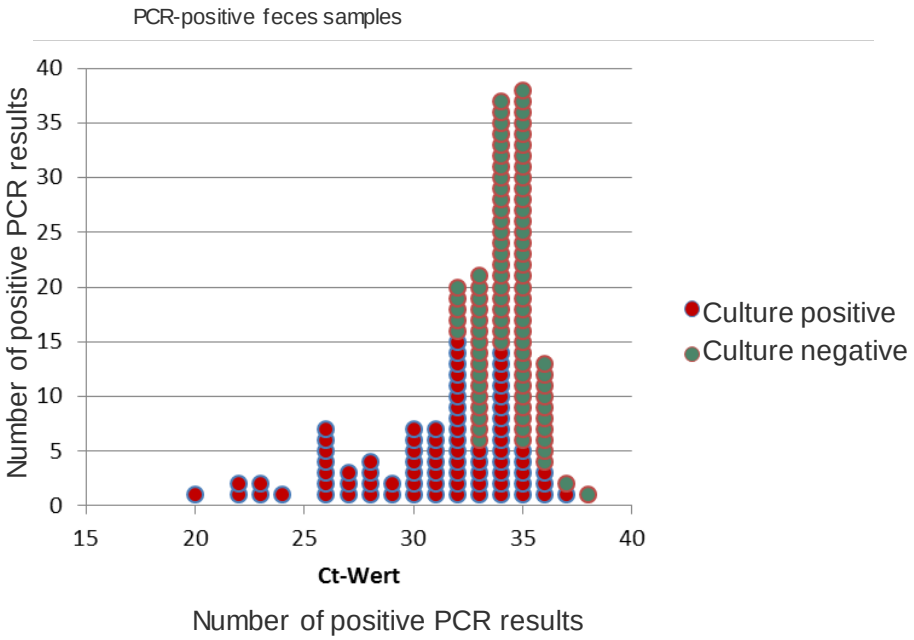
Target: non IS900
Duplex-PCR with internal control





Results

		PCR			
		positive	negative	Total	%
Culture	positive	97	99	196	9,36
	negative	97	1.802	1.899	90,64
Total		194	1.901	2.095	100
%		9,26	90,74	100	



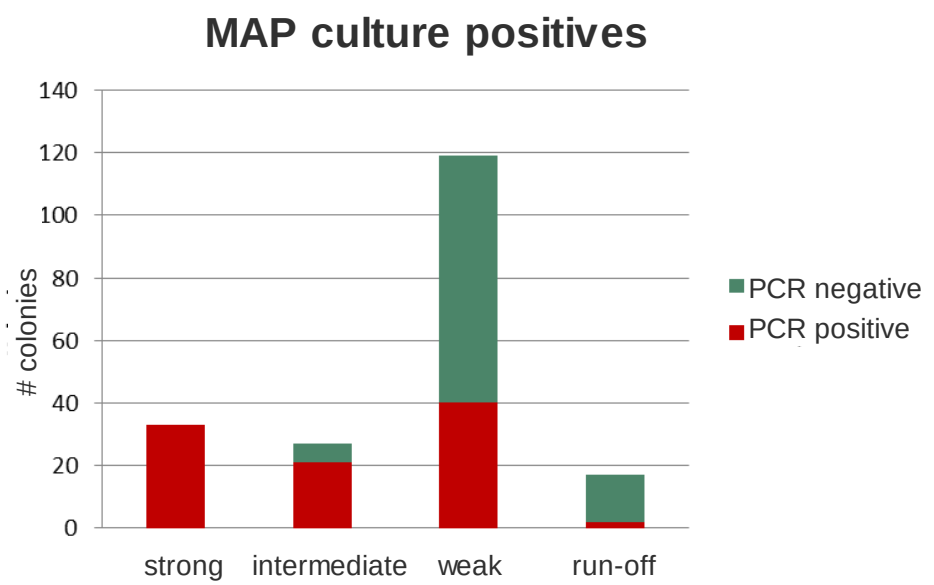
Ct ≤31: Confirmation with culture: 100%
Ct 32-34: Confirmation with culture: approx. 48%
Ct >34: Confirmation with culture: approx. 20%

Results

		PCR			
		positive	negative	**Total**	%
Culture	positive	97	99	196	9,36
	negative	97	1.802	1.899	90,64
Total		194	1.901	2.095	100
%		9,26	90,74	100	

PCR on feces samples	Culture positive			
	Strong > 100 colonies	Intermediate 10-100 colonies	Weak <10 colonies	Culture run-off
negative	0	6	79	15
positive	33	21	40	2
total	33	27	119	17

94% of culture positive, PCR negative results are in the range of low shedding – over two thirds of these had only one colony/ 3 tubes or were only positive after run-off



Results - Summary

PCR evaluation

		PCR			
		positive	negative	**Total**	%
Culture	positive	97	99	196	9,36
	negative	97	1.802	1.899	90,64
Total		194	1.901	2.095	100
%		9,26	90,74	100	

Diagnostic sensitivity:
97/196*100 = 49%

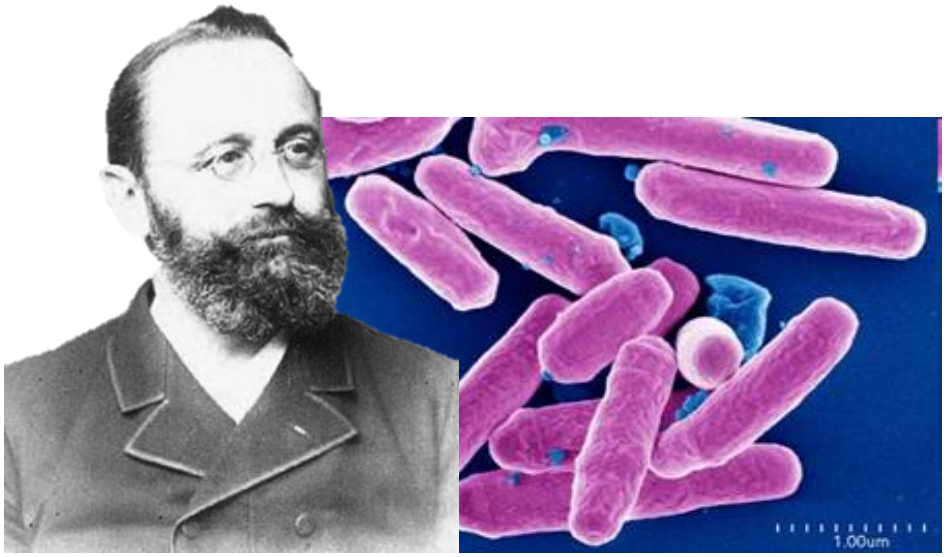
Diagnostic Specificity:
1.802/1.899*100 = 95%

Relative trueness:
(1.802+97)/2.095*100 = 90%

Summary

- PCR and culture give comparable detection rates
- Good agreement between bacterial load detected with the culture method and the Ct value obtained with the PCR test
- PCR is well suited for the detection of strong and intermediate shedders
- Automated DNA-isolation enables high throughput detection system

Thank you for your attention!



Albert Johne Paratuberculosis: 1895