Results of two inter-laboratory comparisons:
1. *Toxoplasma gondii* nucleic acid detection in veterinary medicine
2. Results of an inter-laboratory comparison on serological detection of antibodies against *Toxoplasma gondii*… (…, *Neospora caninum* and *Besnoitia besnoiti*)

Schares G.*, Tuschy, M., Bärwald, A.

Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, National Reference Laboratory for Toxoplasmosis, Greifswald-Insel Riems, Germany

*email: gereon.schares@fli.de

Interlaboratory comparison on *Toxoplasma gondii* DNA detection

- Organized by the German NRL for Toxoplasmosis in 2020/2021
- Aim
  - Potential differences in the analytic sensitivity of various polymerase chain reaction methods applied by various laboratories
  - To confirm specificity
  - To unravel false positive reactions due to cross-contamination or carry-over
- Participants
  - State veterinary laboratories (n=12)
  - German university (n=1)
  - Foreign university (n=1)
  - Private, commercial laboratories (n=5)

Interlaboratory comparison on *Toxoplasma gondii* DNA detection

- Samples
  - 22 DNAs was provided consisting of spiked ruminant carrier DNA
    - carrier DNA extracted by phenol-chloroform-extraction from bovine liver
    - 16 DNAs varying but defined concentrations of *T. gondii* DNA
  - 4 negative control DNAs
  - 2 DNAs of related parasites
    - *Neospora caninum*
    - *Besnoitia besnoiti*
- Sample delivery
  - 50 µl of sample
  - Speed-vacuum-dryed and delivered including a molecular grade water to reconstitute
  - Send by postage, except samples for the foreign laboratory
  - 4 different coded panels, samples in random order and haphazadly distributed to labs to avoid exchange of results among laboratories

All laboratories which agreed in participation provided results

- Results of 30 PCRs from 19 laboratories
  - Results of 4 endpoint PCRs
  - Results of 26 real time PCRs
    - 15 commercial realtime PCRs (all Adiagene ToxoFast)
      - 5 different types of equipment (BioRad CFX 96, Qiagen Rotor-Gene Q 5plex, LightCycler480II, AgilentMx3005P)
    - 10 inhouse realtime PCRs
      - Published by Talabani et al., 2009 (529 bp); Reischl et al. 2003 (529bp), Costa et al. 2000 (B1)
      - Unpublished ITS1 realtime PCR
    - 1 High Resolution Melting (HRM) PCR
  - 25 PCRs employed an inhibition control.
    9 PCRs were used without inhibition control.

Good news: only two laboratories with indications of false positive reactions

- 1 laboratory reporting a false positive result
- 1 laboratory reporting Ct/Cq values for negative samples which were not regarded as positive reactions due to high Ct/Cq values
- No laboratory detected *Neospora caninum* or *Besnoitia besnoiti* DNA as *Toxoplasma gondii* positive

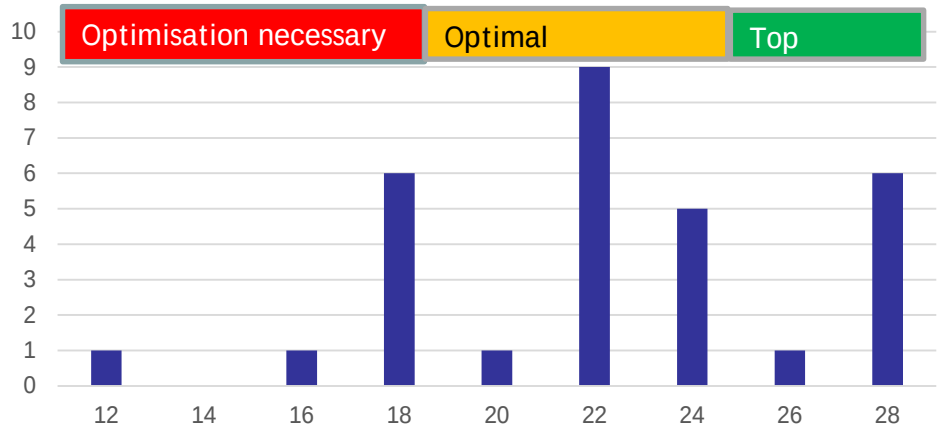
Analysis of results

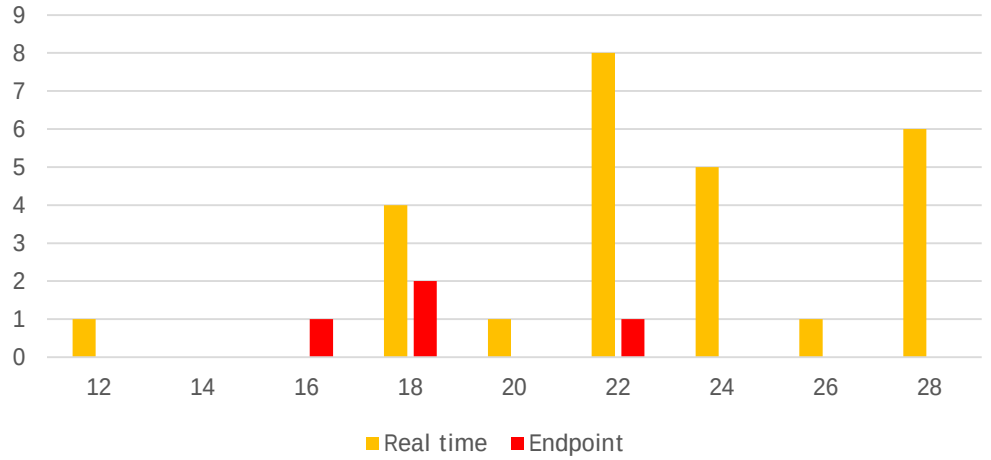
- Samples spiked with *T. gondii* DNA

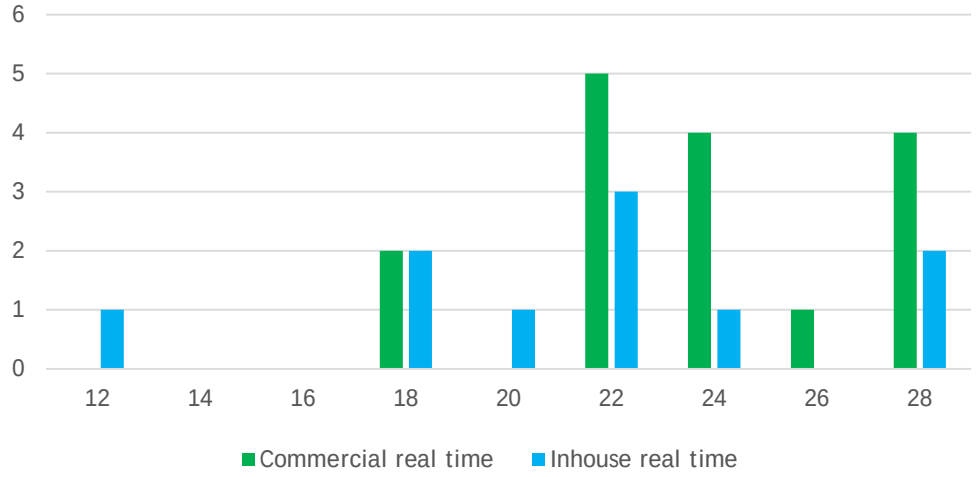
  - Scoring sensitivity
    - 4 samples resembling 500 parasites/10 µl
      -> 1 point per reporting positive (0.5 for inconclusive)
    - 4 samples resembling 50 parasites/10 µl
      -> 2 points per reporting positive (1 for inconclusive)
    - 4 samples resembling 5 parasites/10 µl
      -> 3 points per reporting positive (1.5 for inconclusive)
    - 4 samples resembling 0.5 parasite/10 µl
      -> 4 points per reporting positive (2 for inconclusive)

  - Based on the 33% and 66% percentils, results for each parameter were classified into three classes „Optimisation necessary“, „optimal“ and „top“.

Summary of scoring results on analytical senstivity







PCR problems – low analytic sensitivity

	Sensitivity problems			
	No	**Yes**		
Commercial test	14	2	**16**	
In-house test	7	7	**14**	
	20	**9**		
	The Fisher exact test statistic value is 0.0457.			

Inter-laboratory comparison on serological detection of antibodies against *Toxoplasma gondii* (, *Neospora caninum* and *Besnoitia besnoiti*)

Organized by the German NRL for Toxoplasmosis in 2021/2022

Aim: Assessment of sensitivity and specificity

Participation:

- In total, 19 laboratories participated (Toxoplasma, Neospora, Besnoitia) (17 from Germany, 2 from neighboring countries)
- Toxoplasma: only 8 laboratories participated (two detection tests)

Specificity – no problems at all
Sensitivity – some laboratories had problems with weak positive sera

	Sample	1	2	4	5	3	6	7	8	9	10	11	12
	Type of sample	Pig, strong *T. gondii* positive	Pig, weak *T. gondii* positive	Goat, strong *T. gondii* positive	Goat, weak *T. gondii* positive	Pig, negative	Goat, negative	Cattle, strong *N. caninum* positiv	Cattle, weak *N. caninum* positive	Cattle, weak *N. caninum* positive	Cattle, strong *B. besnoiti* positive	Cattle, weak *B. besnoiti* positive	Cattle, negative
ID Screen Toxoplasma	Positive	9	7	9	5	0	0	0	0	0	0	0	0
	In-conclusive	0	2[a]	0	1[b]	0	0	0	0	0	0	0	0
	Negative	0	0	0	3[c]	9	9	9	9	9	9	9	9
Immuno-blot	Positive	NA	NA	1	0	0	0	0	0	0	0	0	0
	Negativ	NA	NA	0	1[d]	1	1	1	1	1	1	1	1

a, Lab 3, 15 b, Lab 8 **c, Lab 3, 9, 15** **d, Lab 8**

Correct
False, inconconclusive
False negative

We thank all the laboratories for their participation.

Thank you for your attention.