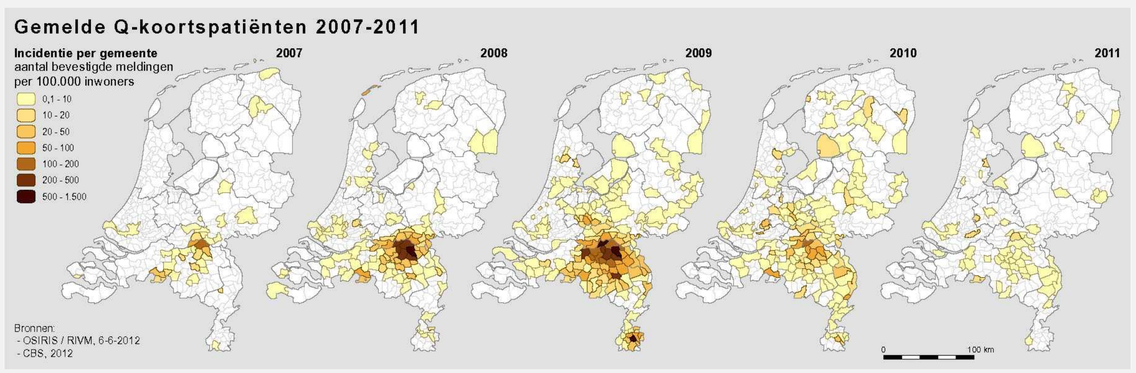
Q-detect™

Using cell mediated immunity to monitor *C. burnetii* infections

A. Garritsen[1], M. de Vries[1], D. van Houwelingen[1], P. Schneeberger[2], H-J. Roest[3], M. Kouwijzer[1]
[1]Innatoss Laboratories (Oss), [2]Jeroen Bosch Hospital ('s-Hertogenbosch), [3]WUR - Central Veterinary Institute (Lelystad)
THE NETHERLANDS

I . Introduction: Q fever

Q fever
Zoonosis - ruminants and cattle - abortion waves - transmission by air - human epidemic in the Netherlands between 2007-2012 (see figure)



Disease
Acute Q fever : flu-like, pneumonia, hepatitis
Q fever-associated **chronic fatigue syndrome**
Chronic Q fever: endocarditis – potentially lethal

Pathogen
Coxiella burnetii: intracellular bacteria - growth within the phagolysosome

Host defense against *C. burnetii*
=
Cell-Mediated Immunity

II. Q-Herpen-II (poster #624 by Morroy et al.)

Aim: identify latent cases of chronic Q fever and determine prevalence of *C. burnetii* infection based on the humoral and cellular immune response in the adult population of Herpen (N=2161).

Design
2 Laboratory tests
- Immune Fluorescence Assay IgG phase I and II (Focus Dx)
- C.b. IFNγ release assay (Q-detect™, Innatoss); Nine Mile RSA493 and a Dutch strain were used as antigen.

Questionnaire on health status

Results
1511 subjects were tested in the IFA as well as the IGRA. The distribution of positives and negatives is listed in the table below.

IGRA IFA >	Pos	Neg	Total
Pos	**462**	**400**	**862**
Neg	36	582	618
Inconclusive	15	16	31
Total	**513**	998	1511

Conclusions
- Q-detect™ gives 68% (862/513) more positives than IFA
- Sensitivity of the optimized Q-detect™ test is 94% (862/(513+400), a significant improvement over the prototype test (QHORT, sensitivity 78%)
- Specificity is not yet clear

III. Understanding Q-detect

Aim
- Additional study to determine specificity of Q-detect™
- Develop methods to detect low levels of antibodies and cellular responses that support Q fever infection in IFA-negative patients

Design
- Subjects: 109 random subjects in Enschede and 16 known Q fever patients
- Laboratory tests: Q-detect™. For QD-positives and patients a *Coxiella* immunoblot, IFA and cytokine profiling using Meso Scale Discovery technology was performed.

Results
- Q-detect™ identified 19 (18%) positive subjects in the 'low incidence' region. Three were inconclusive.
- The number of subjects without any increase in IFN-γ (i.e. a relative *Coxiella* response < 0,2; figure 2 dark green) was remarkably higher than Herpen (50% vs 15%) (Figure 2).
- In 12 out of 19 positives, additional proof a past Q fever infection was found: antibodies (N=9) and/or increases in IL-2 (N=8). 5 were overlapping.

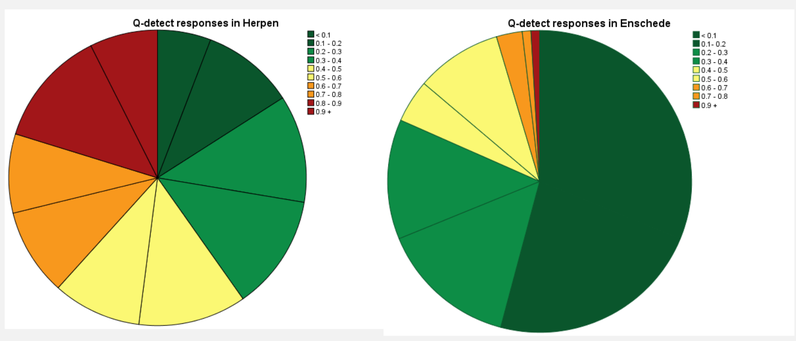


Figure 2. Coxiella-specific IFNγ production was measured. The relative increase vs PHA (positive control) was calculated and the number of subjects per group determined.

Green = CoxRR < 0,4 (negative)
Yellow = CoxRR 0,4-0,6 (pos; weak)
Orange = CoxRR 0,6-0,8 (pos; moderate)
Red = CoxRR > 0,8 (pos; strong)

IV. Methods

Antigen preparation: Coxiella antigen was prepared by the Central Veterinary Institute. Strain Cb2009-02629 was isolated from goat placenta and cultured under cell-free conditions (Omsland, Adv Exp Med Biol, 984, 215-29; 2012). Antigen characterization was performed by Innatoss.

Measuring IFNγ production: Blood samples were incubated for 24 h at 37 °C after which IFN-γ was determined by ELISA (Sanquin) (Schoffelen , Clin Infect Dis. 56:1742-51, 2013)

Data analysis: Log-transformed values were used for calculating means and the **relative *Coxiella* response**.

Coxiella immunoblot: heat-killed *Coxiella* was subjected to SDS-PAGE and Western blotting. The Western Breeze kit was used to detect goat or human IgG against *C. burnetii.*

Cytokine profiling: A Meso Scale Discovery V-Plex assay was used to determine IFN-γ, TNFα, IL-2, IL-1β and IL-10 concentrations.

Ethics: Humans studies were approved by METC Utrecht and Brabant. Donors gave written consent.

V. Conclusions

- Q-detect™ has been validated in > 1600 subjects.
- Sensitivity for detecting a previous *C. burnetii* exposure is 94%
- A *Coxiella* immunoblot and measuring IL-2 in addition to IFNγ, provided additional proof of a *Coxiella* infection.
- A false positive rate below 7% was determined, resulting in a specificity of at least 92% in a low incidence population.
- The percentage of positive results in the 'low' incidence population was higher than expected. This deserves follow-up.

VI. Acknowledgements

Financial support was provided by the Ministry of Health, Welfare and Sports (UIT-13016959). Statistical support was provided by Numerus Ltd (Tubingen, Germany) through an In2LifeSciences grant. Q-Herpen-II Core Team members G. Morroy, J. van der Velden and C. Wijkmans are acknowledged for their support. We also thank A. Olde Loohuis and Medlon BV for their contribution to the Enschede study. Last but not least, we thank all volunteers involved in these studies.