Technology offer

ELISA for diagnosis of infections with *Chlamydia suis*

Chlamydia suis-specific antibody ELISA (indirect) with high specificity and sensitivity

Target market and value

Chlamydia suis infections, and especially infections with Tc^R *Chlamydia suis* strains are emerging in the pig industry and the transmission of *Chlamydia suis* to humans has been demonstrated (Dean et al., 2013; De Puysseleyr et al., 2014; De Puysseleyr et al., 2017; Kieckens et al., 2017). Given the importance of *Chlamydia suis* as a swine pathogen and as a recently emerged tetracycline resistant zoonotic pathogen this sensitive *Chlamydia suis*-specific antibody ELISA is a valuable tool to monitor *Chlamydia suis* infections (in pig and human) and support its control.

Chlamydia suis in pigs has been associated with asymptomatic infections but also with a variety of clinical symptoms such as conjunctivitis, rhinitis, pneumonia, enteritis, reproductive disorders such as irregular return to oestrus, early embryonic dead in inseminated sows and inferior semen quality in boars (decrease of sperm cell motility and dead of sperm cells) (Rogers and Andersen, 1999; Rogers and Andersen, 2000; Schautteet et al., 2010; Englund et al., 2012; Schautteet et al., 2013; Rypula et al., 2014; Hoffmann et al., 2015).

Chlamydia suis infections could be successfully treated with tetracyclines until the appearance of a tetracycline resistant (Tc^R) phenotype, which was first isolated on pig farms in Iowa and Nebraska. Soon thereafter, tetracycline resistant *Chlamydia suis* strains appeared in other countries including Belgium, Cyprus, Germany, Israel, Italy, Switserland and The Netherlands. The emergence of Tc^R *C. suis* strains raises considerable concern because *Chlamydia suis* shares on average 80% nucleotide identity with the human pathogen *C. trachomatis*. As *C. trachomatis* is the leading cause of sexually transmitted diseases and preventable blindness (trachoma) worldwide the control of *Chlamydia suis* is also relevant for human health.

Nowadays, *Chlamydia suis* infections are often unnoticed because diagnosis is still not routinely performed in veterinary diagnostic laboratories as: i) tetracycline resistant *C. suis* strains relatively recently emerged, ii) *Chlamydia suis* strains are hard to culture and iii) *Chlamydia suis*-specific molecular diagnostic techniques, such as real-time PCR and DNA micro-array, only became available in recent years and are too expensive for the pig industry.

Our *Chlamydia suis*-specific ELISA has the following advantages:

- easily applicable for routine analysis
- acceptable cost for swine producers

- can be used to support the production of *Chlamydia suis* sero-negative pigs/breeding stock
- can be used to prevent venereal transmission by serological monitoring of boars on the farms and in artificial insemination centers
- can be used on human samples
- can detect persistent asymptomatic *Chlamydia suis* infections

Assay

We have developed an indirect ELISA described in De Pusseleyr et al., 2017. *Chlamydia suis* PmpC was chosen for the selection of the antigen for the ELISA as this protein is absent in *C. abortus*, *C. pecorum* and *C. psittaci* which also infect pigs and as the protein contains *Chlamydia suis*-specific amino acid regions that are absent in *C. trachomatis* PmpC. An immunodominant B cell epitope in *Chlamydia suis* PmpC was identified using experimental porcine sera and selected as the antigen for the ELISA.

Sensitivity, specificity and validation

Sensitivity and specificity of the PmpC ELISA

Experimental porcine sera were tested by the PmpC peptide ELISA and results were compared to those of our in house made recombinant MOMP (rMOMP) ELISA and of the complement fixation test (CFT) (Table 1). All positive control sera of group 1 (n= 40 ; see legend of table 1 for description of the groups) reacted positive in the rMOMP ELISA and in the PmpC ELISA. However, only 29 of 40 (72.5%) positive control sera reacted positive in the CFT. All positive control sera of group 2 (n= 35) reacted positive in the rMOMP ELISA and in the PmpC ELISA. However, only 30 of 35 (85.7%) sera reacted positive in the CFT. Pigs of group 4, which were simultaneously infected with two different *C. suis* strains reacted positive in all three tests.

Regarding the specificity, all negative control sera of group 3 reacted negative in the three tests. However, all 9 pigs of group 5, which were simultaneously infected with three species other than *C. suis*, reacted positive in the rMOMP ELISA as well as in the CFT. Only the PmpC ELISA gave a negative result for these sera.

Table 1. Evaluation of ELISA's and CFT with experimental sera[a]

C. suis serological test	Sensitivity (%) with sera of			*C. suis* specificity % with sera of	
	Group 1	Group 2	Group 4	Group 3	Group 5
rMOMP ELISA	100	100	100	100	0%
PmpC peptide ELISA	100	100	100	100	100
CFT	72.5	85.7	100	100	0%

[a] Group 1, 2 and 4, positive control sera of animals infected with *C. suis* strains S45, H7 or R19; group 3, negative control sera; group 5, sera of pigs experimentally infected with *C. abortus*, *C. pecorum* or *C. psittaci* (all lacking the PmpC coding gene).

Validation of the PmpC ELISA on field sera

82 Belgian field sera (Group 6) were collected at the slaughterhouse and originated from 21 different farms. Other sera originated from clinically affected sows (n=10 ; Group 7) or boars (n=10) from farms in Israel or Estonia, respectively. All field sera were tested at the same time by the PmpC ELISA and the rMOMP ELISA (Table 2).

All field sera (n = 102) appeared to be positive in the rMOMP ELISA, while 101 of 102 (99%) sera reacted positive in the PmpC ELISA. Sera of sows and boars with reproductive failure (Groups 7 and 8) due to a proven *C. suis* infection, all reacted positive in both ELISA's, although *Chlamydia suis* real-time PCR was not always positive in all animals (Group 7).

Table 2. Evaluation of ELISA's with field sera.

C. suis serological test	Group 6	Group 7	Group 8
rMOMP ELISA	102/102 (100)	10/10 (100)	10/10 (100)
PmpC peptide ELISA	101/102 (99)	10/10 (100)	10/10 (100)

Technology offer

IP-position

A patent application has been filed and is currently pending:

Patent Application in name of Universiteit Gent
Title: “A method and peptides for the detection of Chlamydia suis”
Priority date: 24.03.2015
Publication number: WO2016/150930

Partnering

We are looking for partners that are interested to take a license on the IP and market the ELISA.

We can offer

- scientific and technical support during the transfer of the assay to the industrial partner
- well defined samples for test validation
- know-how and research capacity on *Chlamydia suis* and other Chlamydia species

References

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