UNIVERSITEIT GENT

FACULTY OF **BIOSCIENCE ENGINEERING**

Chlamydia suis, an emerging *Chlamydiaceae* species in pigs?

Katelijn Schautteet [(1)], Cora Miry [(2)], Frederick Vangroenweghe [(2)], Patrick Delava [(3)], Evelien De Clercq [(1)], Yannick Jönsson [(1)], Delphine S.A. Beeckman [(1)] and Daisy Vanrompay [(1)]

(1) Department of Molecular Biotechnology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
(2) Animal Health Care Flanders, Unit Services, Industrielaan 29, 8820 Torhout, Belgium.
(3) Vitamex N.V., Booiebos 5, 9031Drongen, Belgium

INTRODUCTION

Chlamydiaceae are Gram-negative obligate intracellular bacteria that can infect a broad range of animals and humans. To date, the chlamydial species *Chlamydophila* (*Cp.*) *pecorum*, *Cp. abortus*, *Cp. psittaci* and *Chlamydia* (*C.*) *suis* have been isolated from pigs (Everett *et al.*, 1999). *Chlamydia suis* can cause conjunctivitis, pneumonia, enteritis, reproductive problems and apparently asymptomatic infections. The type strain of this species, S45, is tetracycline sensitive (Tc^S) but tetracyline resistant (Tc^R) strains have been isolated in Italy and the USA (Di Francesco *et al.*, 2008; Andersen and Rogers, 1998). *Chlamydophila abortus* has been associated with abortion in pigs. This species is an zoonotic agent since it can induce abortion in pregnant women. *Chlamydophila pecorum* has been associated with abortion, polyarthitis, pneumonia, pleuritis and pericarditis in pigs. *Chlamydophila psittaci* primarily infects birds but has caused sporadic zoonotic infections in humans. Furthermore, it has been isolated from fattening sows.

OBJECTIVES

The purpose of this study was to examine the current serological status of the Belgian pig herd and to identify the chlamydial species involved in infecting Belgian pigs.

METHODS

To evaluate the *Chlamydiaceae* seroprevalence in Belgian pigs, serum samples were taken from 200 Belgian closed fattening farms. From each farm blood was drawn from 10 randomly chosen sows. Anti-MOMP antibodies in pig sera were determined by a direct ELISA as described previously (Verminnen *et al.* 2006) using *C. trachomatis* MOMP as antigen. Farms were divided into serologically negative (OD450 < 0.05), weakly positive (OD450 = 0.06-0.1), moderately positive (OD450 = 0.1–1.0) and strongly positive (OD450 = 1.0–2.0) farms.
Furthermore, 100 sows from 10 different pig fattening farms were selected in a pig slaughterhouse. From each sow a vaginal, lung and jejenal/rectal swab was taken. Farms were examined individually. DNA of five samples was pooled per tissue and per farm. Each pool was analyzed by microarray. Additionally, Animal Health Care Flanders send us 11 conjunctival and 16 lung swabs of a total of 10 sows and six weaned piglets to perform nucleic acid amplification tests. *Chlamydiaceae* species-specific array tube (AT) microarray was performed as previously described by Sachse *et al.* (2005). A sample was considered “chlamydia negative” when all signal intensities except for the internal staining control (biotin control) were below a normalized intensity of 0.075.

RESULTS

Chlamydiaceae seroprevalence in Belgian pigs

Pooled sera of each farm were tested for the presence of anti MOMP-specific antibodies. Only six of 200 farms (3%) were *Chlamydiaceae* seronegative (OD450 < 0.05). Twelve of 200 (6%) tested weakly positive (OD450 = 0.06-0.1), 155 farms (77.5%) were moderately positive (OD450 = 0.1–1.0) and 27 (13.5%) were strongly positive (OD450 = 1.0–2.0).

Molecular diagnostic study on slaughterhouse samples and on autopsy samples

Table 1: results of the AT DNA microarray for the lung samples from the pig slaughterhouse

Farm	Sample*	Biotinylation PCR	Hybridization result (species)
Farm 1	Pool 1/Pool 2	+/-	*C. suis*/NA[a]
Farm 2	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 3	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 4	Pool 1/Pool 2	+/+	*Cp. psittaci/ Cp. psittaci*
Farm 5	Pool 1/Pool 2	+/+	*C. suis*/ NI[b]
Farm 6	Pool 1/Pool 2	-/+	NA/ NI
Farm 7	Pool 1/Pool 2	+/+	NI/NI
Farm 8	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 9	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 10	Pool 1/Pool 2	+/+	NI/NI

Table 2: results of the AT DNA microarray for the vaginal samples from the pig slaughterhouse

Farm	Sample*	Biotinylation PCR	Hybridization result (species)
Farm 1	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 2	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 3	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 4	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 5	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 6	Pool 1/Pool 2	+/+	*C. suis*/ NI[a]
Farm 7	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 8	Pool 1/Pool 2	+/+	NI/ *C. suis*
Farm 9	Pool 1/Pool 2	+/+	*C. suis*/ NI
Farm 10	Pool 1/Pool 2	+/+	*C. suis/C. suis*

Table 3: results of the AT DNA microarray for the jejenal/rectal samples from the pig slaughterhouse

Farm	Sample*	Biotinylation PCR	Hybridization result (species)
Farm 1	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 2	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 3	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 4	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 5	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 6	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 7	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 8	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 9	Pool 1/Pool 2	+/+	*C. suis/C. suis*
Farm 10	Pool 1/Pool 2	+/+	*C. suis/C. suis*

Table 4: results of the AT DNA microarray for the lung samples from the Belgian pig autopsy samples

Sample	Age	Biotinylation PCR	Hybridization result (Species)
10/051	Fattening pig (40 kg)	+	*C. suis*
10/052	Weaned piglet (10 kg)	-	NA[b]
10/053	Weaned piglet (10 kg)	-	NA
10/054	Fattening pig (45 kg)	+	*C. suis*
10/055	Fattening pig (45 kg)	+	*C. suis*
10/056	Fattening pig (45 kg)	+	*C. suis*
10/057	Weaned piglet (15 kg)	-	NA
10/058	Weaned piglet (15 kg)	-	NA
10/059	Weaned Piglet (8.5 kg)	+	*C. suis*
10/060	Fattening pig (35 kg)	-	NA
10/061	Fattening pig (45 kg)	+	*Cp. abortus*
10/062	Fattening pig	+	NI
10/063	Fattening pig	-	NA
10/064	Fattening pig (46 kg)	+	*C. suis*
10/065	Weaned piglet (14 kg)	+	*C. suis*
10/066	Fattening pig	+	NI

Table 5: results of the AT DNA microarray for the conjunctival samples from the Belgian pig authopsy samples

Sample	Age	Biotinylation PCR	Hybridization result (Species)
10/067	Fattening pig (40 kg)	+	*C. suis*
10/068	Weaned piglet (10 kg)	+	*C. suis*
10/069	Weaned piglet (10 kg)	+	*C. suis*
10/070	Fattening pig (45 kg)	+	*C. suis*
10/071	Fattening pig (45 kg)	+	*C. suis*
10/072	Fattening pig (45 kg)	+	*C. suis*
10/073	Weaned piglet (15 kg)	-	NA[d]
10/074	Weaned piglet (15 kg)	+	*C. suis*
10/075	Weaned Piglet (8.5 kg)	+	*C. suis*

* Each pool consisted of the DNA extract of 5 samples;
[a] NA = Not applicable as chlamydial DNA was not detected;
[b] NI = Not interpretable as hybridization signal was too low (< 0.075)

CONCLUSION

At present, 193 (97%) of 200 examined farms tested positive for *Chlamydiaceae*-specific antibodies. We tried to identify the *Chlamydiaceae* species using a diagnostic platform comprising of a *Chlamydiaceae*-species specific microarray. Our results were in accordance with the serological results, as *Chlamydiaceae*, and especially *C. suis* was highly prevalent in slaughtered pigs and in pigs ending up in the autopsy room of Animal Health Care Flanders. Furthermore, we could demonstrate *C. suis* in the eyes, the respiratory, the intestinal and the reproductive tract of sows. We found no other species, with the exception of one *Cp. psittaci* and one *Cp. abortus* strain. In conclusion, *C. suis*, for which Koch’s postulates have already been fulfilled in the past, are widespread in pigs in the Belgian pig population. Research towards the development of preventive measurements like probiotics or vaccines should be promoted.

REFERENCES:

Everett, K.D.E., Bush, R.M. & Andersen, A.A. (1999). Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. *International Journal of Systematic Bacteriology* **49:** 415-440.
Andersen, A.A. & Rogers, D.G. (1998). Resistance to Tetracycline and Sulfadiazine in Swine *C. trachomatis* Isolates. In *Chlamydial infections. Proceedings of the Ninth International Symposium on Human Chlamydial Infection*. Stephens (ed)., pp. 313-316.
Di Francesco, A., Donati, M., Rossi, M., Pignanelli, S., Shurdhi, A., Baldelli, R. & Cevenini, R. (2008). Tetracycline resistant *Chlamydia suis* isolates in Italy. *Veterinary Record* **163:** 253.
Suchland, R.J., Sandoz, K.M., Jeffrey, B.M., Stamm, W.E. & Rockey, D.D. (2009). Horizontal transfer of Tetracycline Resistance among *Chlamydia* spp. In Vitro. *Antimicrobial Agents and Chemotherapy* **47**; 636-642
Verminnen, K., Van Loock, M., Hafez, H.M., Ducatelle, R., Haesebrouck, F. & Vanrompay, D. (2006). Evaluation of a recombinant enzyme-linked immunosorbent assay for detecting *Chlamydophila psittaci* antibodies in turkey sera. *Veterinary Research* **37:** 623-632.
Sachse, K., Hotzel, H., Slickers, P., Ellinger, T. & Ehricht, R. (2005). DNA microarray-based detection and identification of *Chlamydia* and *Chlamydophila* spp. *Molecular and Cellular Probes* **19:** 41-50.
Dugan, J., Rockey, D.D., Jones, L. & Andersen, A.A. (2004). Tetracycline resistance in *Chlamydia suis* mediated by genomic islands inserted into the chlamydial inv-like gene. *Antimicrobial Agents and Chemotherapy* **48:** 3989-3995.

Contact: Katelijn.Schautteet@UGent.be