



Chlamydia suis survival in dust: First insights

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ABSTRACT

Chlamydia (C.) suis, a zoonotic intracellular bacterium, is described as a causative agent for conjunctivitis, particularly in nursery and fattening pigs. *Chlamydiaceae* are claimed to survive drying and to persist in dust. The objective of this study was to evaluate the viability of *Chlamydia* in dust sampled in a fattening pig farm with a high appearance of chlamydial-induced conjunctivitis. Dust was collected and stored at room temperature. To evaluate bacterial load and survival over time, quantitative PCR (*Chlamydiaceae*, *C. suis*) and isolation in cell culture were performed every week for up to 16 weeks. While qPCR results remained highly positive with consistent bacterial loads between 10^3 and 10^4 copy numbers/100 μ L eluate over a period of 16 weeks and even after 40 weeks, it was not possible to isolate *Chlamydia* except for the initial sample. These results show only short-term viability of *C. suis* in dust. This is an important information regarding reduction of chlamydial loads in pig farms and risk for pigs and people to get infected via dust.

Chlamydial infections are assumed to be widespread in commercial pig production (Schautteet and Vanrompay, 2011). The most prevalent chlamydial species in pigs, *Chlamydia (C.) suis*, has zoonotic potential (Puyseleir et al., 2014) and is involved in the aetiology of a broad range of diseases in pigs including conjunctivitis (Unterweger et al., 2021) and reproductive disorders (Kauffold et al., 2006; Rypula et al., 2018). Within a pig herd, transmission mostly occurs via the faecal-oral route, but shedding via conjunctival, nasal and vaginal secretions is also known (Eggemann et al., 2000; Unterweger et al., 2021). The role of the barn environment, air and dust in the transmission of *Chlamydia* between pigs, but also between pigs and humans has not yet been clarified. Dust exposure is unavoidable, but has been measured to be higher on fattening pig farms than on sow farms (Louhelainen et al., 1987a) with concentrations in the breathing zone of 8.6 ± 2.7 mg dust/m³, resulting in high exposure not only of pigs, but also of pig farmers (Louhelainen et al., 1987b) and therefore impacts human health (Iversen et al., 2000; Normand et al., 2009). Transmission via dust contaminated from bird feathers is also described for *C. psittaci* (Andersen and Vanrompay, 2000; Schautteet and Vanrompay, 2011).

Current literature states that *Chlamydiaceae* in general can survive in

dust between 30 days to months (Becker et al., 2007; Broes et al., 2019) and dust might therefore be involved in the aetiology of chlamydial conjunctivitis (Hoffmann et al., 2015), but without citing any literature sources. In order to optimise cleaning and disinfection strategies and to avoid chlamydial (re)infections, knowledge on survival time of pathogens in the environment is crucial. This study was intended to contribute to the understanding of the survival of shed *C. suis* in dust of pig farms.

Fifty gram of dust from an Austrian fattening pig farm with confirmed *C. suis*-induced conjunctivitis and faecal *C. suis* shedding in all age groups and a high content of bedding material as well as a high burden of dust were collected from various locations (surfaces in the fattening barn and corridors) by wiping dust into a sterile plastic cup with small holes using sterile gloves. Last cleaning of the barn occurred two weeks earlier prior to the arrival of new animals, so the exact age of the dust samples was not definable. Starting on the collection day (week 0), every week over a period of 16 weeks and once more after 40 weeks, one gram of the homogenised original dust sample, stored at room temperature (19 °C - 20 °C), was transferred into a 1.5 mL Eppendorf tube with 1 mL sucrose phosphate glutamate (SP-medium) as described by Hoffmann et al. (2015). Each sample was promptly stored at -80 °C

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until further examination as described by Marti et al. (2018). In order to have an initial value for comparison of the results after freezing, week 0 sample was additionally examined before freezing.

DNA was extracted using a QIAmp DNA Mini Kit according to the manufacturer's instructions with an elution volume of 100 µL. The extract was examined on a Stratagene Mx3005P real-time PCR System using a 23S rRNA gene-based *Chlamydiaceae* family-specific PCR with an internal amplification control as described previously by Blumer et al. (2011). A sixfold dilution series of *C. abortus* DNA constituted the standard curve. A positively tested reference material served as positive control and DNA free water was used as negative control. The threshold line was set at 0.1 in each run for all samples. A cycle threshold (Ct-value) of <35 was considered positive. The corresponding *Chlamydiaceae* copy number per µL was calculated for each tested sample as described by Rohner et al. (2021). In case of a PCR positive result, the extract was further examined by a 23S rRNA based *C. suis* specific real-time PCR, as described by Pantchev et al. (2010).

In case of a positive PCR result, samples were examined for viable *Chlamydia* in cycloheximide-treated LLC-MK2 cells (Rhesus monkey kidney cell line, kindly provided by IZSLER, Brescia, Italy) using standard techniques (Donati et al., 2009) modified by Wanninger et al. (2016). Five dust samples plus a positive faecal swab control sample were cultivated in parallel and in duplicates. Simultaneously, cells were grown on a cover slip and stained via in-house immunofluorescence (IF) using anti-*Chlamydia* IgG3 antibody (Progen, ACI—P500, 1:400 in 1xPBS) and Cy3 conjugated goat anti-mouse IgG antibody (Invitrogen, M30010, 1:400 in 1xPBS). Stained coverslips were archived in aqueous mounting medium and scanned by a TissueFaxs (TissueGnostics GmbH, Axio Imager.Z1, Serial-Nr.: 3512001446, Carl Zeiss Microimaging GmbH, monochrome camera: PCO, PixelFlyUSB) at 20× magnification (Zeiss, Serial-Nr.: 421350–9970, LD Plan-Neofluar, 20×/0.4 Air). Cultures were considered negative for viable *Chlamydia* if no inclusions were detected by IF after three passages and *C. suis* specific real-time PCR of the harvested native cell suspension had a negative result.

The *Chlamydiaceae* PCR result of the dust sample taken from the dust pool directly on the sampling day at the farm was positive, regardless of whether the sample had been frozen or not (Table 1). This also applied for the *C. suis* specific PCR (Ct-value 28.22; quantity: 6.986e+003 before freezing compared to Ct-value 27.96; quantity 7.038e+003 after freezing) and isolation in cell culture proving that the original dust sample contained viable *C. suis*. By achieving the same results before and after freezing, it can be assumed that storage at –80 °C has no influence on the samples and their viability.

All dust samples ($n = 17$) taken between week 1 and week 16 and after 40 weeks, respectively, were positive by *Chlamydiaceae* PCR as well as by *C. suis* PCR. *C. suis* Ct-values, shown in Table 1, were stable over the entire 16-week period.

In comparison to the original sample, isolation was not possible in any sample collected at later time points, while isolation of positive controls was possible.

In this experimental setting, we could demonstrate that *C. suis* was viable in the collected dust, but lost its viability within one week after collection while Ct-values of *Chlamydiaceae* and *C. suis* PCR remained at the same level within a 16-week period and even after 40 weeks. This proves that viability of *Chlamydia* should never be concluded from PCR results, regardless of the level of Ct-values. However, this is done in diverse studies: Hulin et al. (2015), for instance, considered the environmental contamination, such as high chlamydial loads of soil, dust and water samples, to be the most probable source of exposure and transmission to the animals, although no isolation attempt was done. *Chlamydia* require living (epithelial) cells for reproduction, which are not part of dust, though. Consequently, detection of viable *Chlamydia* in dust is only possible in the environment of shedding pigs or other hosts. Two causes for the loss of viability might be realistic: First, the lack of reinfections after removal of a dust sample from a barn filled with animals, second, the storage of dust in a plastic cup, although with air supply, but nevertheless in completely different environmental conditions compared to the pig barn regarding temperature, harmful gases or humidity. Whilst this change of environment makes no difference to the growth of many other bacteria, this could be different for chlamydiae. Nevertheless, dust, a conglomeration of dead cells, plant pollen, hair, feed components and minerals from soil, is not a good medium for chlamydiae which need intact epithelial cells for survival.

Our results provide an indication of a low chlamydial infection risk for pigs and people via dust. Even, if cell culture is only a model, it is reasonable to conclude from a certain PCR chlamydial quantity, at least concerning the species *C. suis*, to viability and thus infectivity (Hoffmann et al., 2015; Wanninger et al., 2016).

This information might be useful for husbandry systems with a high level of dust exposure. Nevertheless, cleaning and disinfection measurements are important in order to reduce the chlamydial load in faeces and other bacterial, viral and fungal loads. Normand et al. (2009) compared the microbial (not chlamydial) diversity of dust and air in stables over time and could demonstrate for both materials, air and dust, a high microbial and fungal diversity which was lowered significantly in dust after a 3-month storage period at room temperature. Furthermore,

Table 1

Ct-values by *C. suis* specific real-time PCR, corresponding quantities per elution volume (100 µL SP medium) and results for *C. suis* isolation from dust samples.

Dust sample	Ct-value <i>C. suis</i> PCR*	<i>C.suis</i> copy numbers/elution volume	Isolation in cell culture
Week 0 before freezing	28.22	6.986e +003	Positive
Week 0 after freezing	27.96	7.038e +003	Positive
Week 1	26.59	2.552e+004	Negative
Week 2	27.14	1.642e+004	Negative
Week 3	27.18	1.190e+004	Negative
Week 4	28.19	7.001e+003	Negative
Week 5	27.06	1.658e+004	Negative
Week 6	28.36	6.278e+003	Negative
Week 7	27.05	1.612e+004	Negative
Week 8	28.90	4.099e+003	Negative
Week 9	26.72	1.854e+004	Negative
Week 10	29.16	3.338e+003	Negative
Week 11	26.30	3.214e+004	Negative
Week 12	27.10	1.697e+004	Negative
Week 13	27.01	1.825e+004	Negative
Week 14	26.95	1.921e+004	Negative
Week 15	26.15	3.198e+004	Negative
Week 16	26.30	3.214e+004	Negative
Week 40	28.63	5.059e+003	Negative

* Standards: 1.00e+002: Ct 34.44; 1.00e+003: Ct 29.42; 1.00e+004: Ct 27.25; 1.00e+005: Ct 25.41.

regular exposure to fine dust has been proven to affect the respiratory tract and to promote the colonisation by other pathogens, not only for pigs, but also for people.

We define our data as preliminary, but worth using them as a basis for further scientific studies, such as titration experiments to gain knowledge about the number of infectious particles within the sample, further survival studies in a variety of pig farms, investigations on specific influences inside the barn, comparisons of dust derived chlamydia and animal derived chlamydia as well as resistancy testing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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