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Extended semen for artificial insemination in swine as a potential transmission mechanism for infectious *Chlamydia suis*

G. Hamonic [a], J.A. Pasternak [a], T. Käser [a], F. Meurens [b,c,1], H.L. Wilson [a,*,1]

[a] *Vaccine and Infectious Disease Organization (VIDO)-International Vaccine Centre (InterVac), University of Saskatchewan, Saskatoon, Saskatchewan, Canada*
[b] *LUNAM Université, Oniris, Nantes-Atlantic College of Veterinary Medicine and Food Sciences and Engineering, UMR BioEpAR, Nantes, France*
[c] *INRA, UMR1300 Biology, Epidemiology and Risk Analysis in Animal Health, Nantes, France*

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ABSTRACT

Although typically unnoticed, Chlamydia infections in swine have been shown to be both widespread and may impact production characteristics and reproductive performance in swine. Serum titers suggest Chlamydia infection within boar studs is common, and infected boars are known to shed chlamydia in their ejaculates. Although the transmission of viruses in chilled extended semen (ES) is well established, the inclusion of antibiotics in commercially available extender is generally believed to limit or preclude the transmission of infectious bacteria. The objective of this study was to evaluate the potential of ES used in artificial insemination to support transmission of the obligate intracellular bacteria *Chlamydia suis* (*C suis*) under standard industry conditions. First, the effect of *C suis* on sperm quality during storage was assessed by flow cytometry. Only concentrations above 5×10^5 viable *C suis*/mL caused significant spermicidal effects which only became evident after 7 days of storage at 17 °C. No significant effect on acrosome reaction was observed using any chlamydial concentration. Next, an *in vitro* infection model of swine testicular fibroblast cells was established and used to evaluate the effect of chilled storage on *C suis* viability under variable conditions. Storage in Androhep ES reduced viability by 34.4% at a multiplicity of infection of 1.25, an effect which increased to 53.3% when the multiplicity of infection decreased to 0.1. Interestingly, storage in semen extender alone (SE) or ES with additional antibiotics had no effect on bacterial viability. To rule out a secondary effect on extender resulting from metabolically active sperm, *C suis* was stored in fresh and expended SE and again no significant effect on bacterial viability was observed. Fluorescent microscopy of *C suis* in ES shows an association between bacteria and the remaining gel fraction after storage suggesting that the apparent reduction of bacterial viability in the presence of semen is due to adherence to gel fraction. Taken together, the results of this study suggest that *C suis* remains viable and infectious during chilled storage and is globally unaffected by antibiotics in extender. Thus, ES used in artificial insemination may act as a viable transmission mechanism for *C suis* in swine.

1. Introduction

Chlamydia suis, *Chlamydophila abortus*, *Chlamydophila pecorum*, and *Chlamydophila psittaci* are obligate intracellular bacteria that infect swine [1,2] and may reduce

* Corresponding author. Tel.: 1-306-966-1537; fax: 1-306-966-7478.
E-mail address: heather.wilson@usask.ca (H.L. Wilson).
[1] These authors contributed equally to this work.

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performance of pigs in all stages of the production cycle including reproductive performance [2,3]. Recently *C suis* infection of slaughterhouse workers was confirmed [4] suggesting that it has crossed the species barrier and may represent a one-health concern. As with human chlamydia infections, no vaccines for pigs are available but reduced sow-to-piglet transmission rates have been achieved experimentally when sows were treated with probiotics before farrowing [1]. Although antibiotics effectively treat chlamydia infections, their use in animal agriculture continues to draw scrutiny and does not represent an effective long-term solution to prevent infections in a herd. Maintenance of a chlamydia-free herd may best be achieved through stringent biosecurity that restricts movement of animals and production materials. The high number of chlamydia-positive farms in regions where surveillance is performed suggests that transmission is still being achieved despite high biosecurity already in place [5]. One potential avenue for transmission may be through semen which is generally exempt from biosecurity [6]. Instead, semen for artificial insemination (AI) is typically obtained from high health, specific pathogen–free herds, and they are frequently tested for porcine reproductive and respiratory virus (PRRSV) and on occasion other porcine viruses (which do not regularly include *C suis* or any other bacteria) via polymerase chain reaction to avoid transmission. A number of important swine pathogens have been identified in the semen of infected boars including chlamydia [7], PRRSV [8], circovirus [9,10], and foot and mouth disease [11]. The following study was conducted to determine if chlamydia negatively impacted sperm viability and if commercial semen stored using industry-standard conditions was a potential mode of transmission for chlamydia [12].

2. Materials and methods

2.1. Cell culture and chlamydia propagation

Both swine testicular fibroblast (ST; CRL-1746) and McCoy (CRL-1696) cells were obtained from American Type Culture Collection (Cedarlane, Burlington, Ontario, Canada). ST cells were cultured with minimum essential media (MEM; Sigma–Aldrich, St. Louis, MO, USA) containing 5% fetal bovine serum (FBS; Gibco, Life Technologies, Carlsbad, CA, USA), and 1X Antibiotic-Antimycotic (Gibco) and McCoy cells were cultured with MEM containing 10% FBS, 1 μg/mL cycloheximide (Sigma–Aldrich) and 10 μg/mL gentamicin (Bio Basic, Markham, Canada). Cells were grown in sterile T-150 cm^2 flasks at 37 °C at 5% CO_2. *C suis* (VR-1474) was obtained from American Type Culture Collection and propagated in McCoy cells. Purification of the infectious elementary bodies (EB) of *C suis* was performed using standard methodologies as previously described [13]. Infectious inclusion forming units per milliliter (IFU/mL) were determined by a titrated infection of ST cells followed by analysis via flow cytometry.

2.2. Semen analysis

Commercially extended semen (ES) was obtained from Total Swine Genetics (Tillsonburg, Canada) in weekly batches and stored at 17 °C over the course of the experiment. The ES was mixed with chlamydia at ratios ranging from 5×10^4 to 6.25×10^5 *C suis*/mL and stored at 17 °C until sperm analysis. Evaluation of sperm viability and acrosome reaction was conducted on Days 0, 2, and 7 (Fig. 1) via flow cytometry after sample incubation at 37 °C for 30 minutes. Sperm were stained with propidium iodide (PI; BioVision, Milpitas, CA, USA) at a concentration of 5 μg/mL and peanut agglutinin (PNA) conjugated to Alexa-648

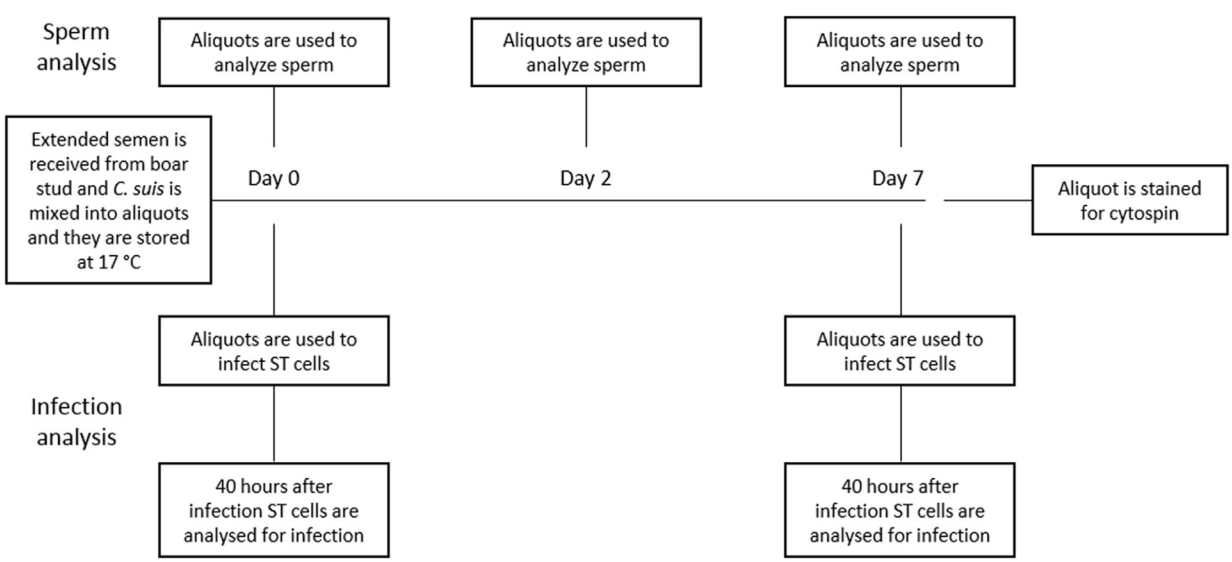

Fig. 1. Flowchart showing experimental design and timing of analyses.