

## Absence of *Chlamydia*-like organisms in pigs

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### Abstract

Porcine reproductive failure, especially abortion, causes significant economic loss in the pig industry. *Waddlia chondrophila* and *Parachlamydia acanthamoebae* are potential abortigenic agents for pigs. Therefore, we investigated the presence of these two *Chlamydia*-like organisms in abortion-related samples originating from Belgian pig farms. All investigated samples remained negative. New Microbes and New Infections © 2015 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

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Porcine abortion presents significant economic losses for pig breeders. However, for most abortions, a cause often cannot be determined. Chlamydiaceae infections are frequently identified in pigs, and *Chlamydia abortus* has been associated with abortion and neonate morbidity in pigs [1,2]. Waddliaceae and Parachlamydiaceae families are *Chlamydia*-like organisms belonging to the order Chlamydiales. These Gram-negative obligate intracellular bacteria exhibit reproductive cycles similar to that of Chlamydiaceae and have a high 16S rRNA sequence similarity with Chlamydiaceae (80–90%). *Waddlia*

*chondrophila* was originally isolated from aborted bovine fetuses in the United States [3] and Germany [4]. *Parachlamydia acanthamoebae* was first discovered in *Acanthamoeba amoebae* recovered from human nasal mucosa [5] and then suspected to cause pneumonia in humans (reviewed in [6]).

*W. chondrophila* and *P. acanthamoebae* have recently been implicated as putative abortigenic agents in both ruminants [7–9] and humans [10–12]. *Waddlia* and Parachlamydiaceae are regularly detected in aborted ruminant fetal tissues [13–15] or in vaginal swabs of animals with reproductive problems [16]. Ruhl et al. [17] analysed 235 aborted bovine samples, while Barkallah and colleagues [8] tested over 600 bovine samples from 150 cases and 64 control animals from 20 dairy herds. *P. acanthamoebae* was detected in 13–18% of all cases [7,8], strongly suggesting an association with bovine abortion. *W. chondrophila* was found less frequently, with approximately 0.9% of all samples being positive using *Waddlia* real-time PCR [7]. Furthermore, *in vitro* infection of ruminant AH-I trophoblasts with *W. chondrophila* elicit innate immunoresponses comparable to *C. abortus* infection [9].

Koschwanez et al. [18] were the first to investigate the occurrence of Waddliaceae and Parachlamydiaceae in pigs. They tested 286 fetuses from 113 sows with fertility disorders for Chlamydiales and other urogenital tract pathogens. The sows originated from 59 Swiss pig breeding farms. Apart from common causative agents of abortion (such as PCV-2 and PEV) and a single *C. abortus* infection, no *Waddlia* or Parachlamydiaceae infections could be identified. As far as we know, there is no information on the occurrence of Waddliaceae or Parachlamydiaceae infections on Belgian pig breeding farms.

We therefore tested 23 fetal lung and liver samples of 11 aborted piglets from 8 different Belgian pig breeding farms for the presence of Waddliaceae and Parachlamydiaceae. The farms were located in Limburg ( $n = 2$ ) and West Flanders ( $n = 6$ ). We also tested 25 vaginal swabs of 25 sows with reproductive disorder (early embryonic death after artificial insemination). The sows originated from three Belgian pig breeding farms. All samples had been previously tested for Chlamydiaceae [19] and were found to be negative.

DNA extraction was performed with the MagNA Pure 96 Instrument (Roche). All samples were analysed in duplicate for both *Chlamydia*-like organisms using specific quantitative PCRs (qPCR) for *P. acanthamoebae* [20] and *W. chondrophila* [21]. Samples were considered positive if duplicate samples had a Ct value of <40. Positive samples were confirmed by a follow-up qPCR detecting the *secY* gene [22], coding for a preprotein translocase membrane subunit. All 48 samples were negative for both *Waddlia* and Parachlamydiaceae.

To our knowledge, our study is the first to analyse Belgian samples for *Waddlia* and Parachlamydiaceae. Our study

investigated only a small number of samples. However, despite the small sample size, results of the current study are in agreement with the prior findings in Swiss pig breeding farms [18]. Nonetheless, further investigations into these organisms are required in order to determine or rule out their potential to cause abortions in pigs.

### Conflict of interest

None declared.

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