

## RESEARCH ARTICLE

# Prevalence and diversity of *Chlamydiales* in Swiss ruminant farms

Margaux Dreyer<sup>1,2</sup>, Sébastien Aeby<sup>3</sup>, Anna Oevermann<sup>1</sup> and Gilbert Greub<sup>3,\*</sup>

<sup>1</sup>NeuroCenter, Division of Neurological Sciences, DCR-VPH, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland, <sup>2</sup>Graduate School for Cellular and Biomedical Sciences, University of Bern, Bremgartenstrasse 109a, CH-3001 Bern, Switzerland and <sup>3</sup>Institute of Microbiology, University Hospital Center and University of Lausanne, Bugnon 48, CH-1011 Lausanne, Switzerland

\*Corresponding author. Prof Gilbert Greub, Institute of Microbiology, Bugnon 48, CH-1011 Lausanne, Switzerland; Tel: +(41)-21-314-49-79;

Fax: +(41)-21-314-40-60; E-mail: [gilbert.greub@chuv.ch](mailto:gilbert.greub@chuv.ch)

**One-sentence summary:** Using a pan-*Chlamydiales* PCR followed by sequencing, *Chlamydiales* from four different family-level lineages were detected in Swiss ruminant farms indicating a high prevalence and biodiversity of *Chlamydiales* in this setting and the value of this broad-range pan-*Chlamydiales* PCR for epidemiological survey.

Editor: Viviane Bremer

## ABSTRACT

*Chlamydia* and *Chlamydia*-related bacteria are known to infect various organisms and may cause a wide range of diseases, especially in ruminants. To gain insight into the prevalence of these bacteria in the ruminant environment, we applied a pan-*Chlamydiales* PCR followed by sequencing to 72 ruminant environmental samples from water, feed bunks and floors. *Chlamydiales* from four family-level lineages were detected indicating a high biodiversity of *Chlamydiales* in ruminant farms. *Parachlamydiaceae* were detected in all three types of environmental samples and was the most abundant family-level taxon (60%). In contrast, only one bacterium from each of the following family-level lineages was identified: *Chlamydiaceae*, *Criblamydiaceae* and *Simkaniaceae*. The observed high prevalence of *Parachlamydiaceae* in water samples may suggest water as the main source of contamination for ruminants as well as their environment due to spoilage. The absence of reported infections in the investigated ruminant farms might indicate that either detected *Chlamydiales* are of reduced pathogenicity or infective doses have not been reached.

**Key words:** bovines; ovines; caprines; pan-*Chlamydiales* PCR; sequencing; environment

## INTRODUCTION

Bacteria belonging to the *Chlamydiales* order are known as obligate intracellular Gram-negative pathogens, able to infect invertebrates, vertebrates and free-living amoebae. These bacteria avoid lysosomal degradation by undergoing cell inclusion for replication. They multiply within the host cell from elementary bodies to reticulate bodies and back to elementary bodies for further cell infections, targeting mainly mucosal membranes. *Chlamydiales* were seen to replicate in human macrophages as well as lung fibroblasts and pneumocytes

(Corsaro and Greub 2006). With the recent discovery of new species, eight different family-level lineages have been established: *Chlamydiaceae*, *Parachlamydiaceae*, *Simkaniaceae*, *Waddliaceae* (Bavoil, Kaltenboeck and Greub 2013; Everett, Bush and Andersen 1999; Greub 2010), *Criblamydiaceae* (Thomas, Casson and Greub 2006), *Piscichlamydiaceae* (Draghi et al., 2004), *Rhabdochlamydiaceae* (Corsaro et al., 2007) and *Candidatus Parilichlamydiaceae* (Stride et al., 2013).

*Chlamydiaceae* infect a wide range of mammalian hosts, in particular humans and ruminants. In humans, these include major established human pathogens such as *Chlamydia*

Received: 15 September 2014; Accepted: 4 November 2014

© FEMS 2014. All rights reserved. For permissions, please e-mail: [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

*trachomatis* (urogenital infections and trachoma), *C. pneumoniae* (pneumonia) and *C. psittaci* (psittacosis). Moreover, the association between *Chlamydiaceae* and worldwide economic losses due to various infections in livestock is a major animal welfare issue. *Chlamydia abortus* is commonly linked to abortions, particularly in small ruminants (Chanton-Greutmann et al., 2002). *Chlamydia pecorum* is associated with abortions, enteric infections, mastitis, encephalomyelitis (Buss disease/sporadic bovine encephalomyelitis), arthritis and conjunctivitis in ruminants (Fukushi and Hirai 1992; Jelocnik et al., 2014; Shewen 1980). Additionally to the *Chlamydiaceae*, other members of the *Chlamydiales* order recently emerged as human and/or animal pathogens. For example, *Parachlamydia acanthamoebae* is associated with bovine abortions and pneumonia in humans (Borel et al., 2007; Greub 2009), but has also been identified in deer, which may act as wildlife reservoir (Regenscheit et al., 2012). *Simkania negevensis* has been detected in respiratory infections mostly in children (Friedman, Dvoskin and Kahane 2003; Greenberg et al., 2003), whereas *Waddlia chondrophila* are found in fetal tissues of humans as well as in bovine abortions (Henning et al., 2002; Dilbeck-Robertson et al., 2003; Wheelhouse et al., 2010; Barkallah et al., 2014).

Various members of the *Chlamydiales* order, especially *Parachlamydiaceae*, have been located in different aquatic habitats, likely due to the fact that they act as intracellular parasites of free-living amoebae, which are known to be ubiquitous in different environmental niches including fresh and marine waters (Kebbi-Beghdadi and Greub 2014). The role of amoebae as hosts for *Chlamydia*-related bacteria is supported by the recovery of some chlamydial strains using the *Acanthamoeba* co-culture (Thomas, Casson and Greub 2006; Corsaro et al., 2009; Lienard et al., 2011a).

Despite the impact of *Chlamydiales* on livestock health, little is known about their occurrence in the farm environment. Therefore, in this study, we investigated the prevalence of *Chlamydiales* in water, feed bunks and floor samples of bovine, ovine and caprine farms located in Switzerland.

## MATERIALS AND METHODS

We investigated eight farms (six bovine farms, one ovine farm and one caprine farm), chosen randomly throughout Switzerland. In each farm, three swabs (e-swabs from Copan, Brescia, Italy) were collected from water, feed bunks and floor resulting in a total of 72 samples. These e-swabs are devoid of chlamydial DNA, as shown previously by testing 60 non-inoculated e-swabs (Lienard et al., 2011a). To prevent contamination during DNA extractions, all extractions were performed automatically using the MagnaPure LC extraction system (Roche, Basel, Switzerland) coupled to a Hamilton robot. We then performed the specific *Chlamydiales* real-time TaqMan PCR (pan-*Chlamydiales* PCR) as described by Lienard et al. (2011a). For this pan-*Chlamydiales* PCR, the StepOne Plus system (Applied Biosystems, Zug, Switzerland) was used with the forward primer pan 16F2, the reverse primer pan16R2 and the probe panS (Eurogentec, Seraing, Belgium). The cycling conditions were 3 min at 95°C followed by 50 cycles of three steps: 15 s at 95°C, 15 s at 67°C and 15 s at 72°C. DNA-free water was used for all negative controls. All samples presenting a PCR Ct value of  $\leq 40$  were sequenced, as in our experience sequencing is successful when PCR products have a Ct value of  $< 40$ . The purification and sequencing of these samples was performed as in Lienard et al. (2011a), using the PanF seq and PanR seq primers. Positive sequences were compared, with

the BLAST server, to existing sequences on GenBank which classifies them based on the percentage identity of the best known species.

## RESULTS

Among the 72 collected samples, 25 had Ct values of  $\leq 40$  in the pan-*Chlamydiales* PCR (34.7%). Sequencing of the PCR products was successful in 15 samples (20.8%). The presence of more than one chlamydial 16S rRNA sequence in a sample prevented sequencing in 10 samples. Six out of eight farms were positive by PCR (75%), but for one farm the presence of *Chlamydiales* was not confirmed, since the PCR product could not be sequenced. The five remaining farms were positive for at least one family-level lineage. The prevalence of *Chlamydiales* was of 53.3% ( $n = 8$ ) for water swabs, 40% ( $n = 6$ ) for feed bunk swabs and 6.7% ( $n = 1$ ) for floor swabs. Twelve of the fifteen 16S rRNA sequences could be assigned to four families by BLAST GenBank analysis, whereas the three remaining sequences could not be affiliated to any family-level lineage. Nine sequences with hits in the BLAST analysis belonged to the *Parachlamydiaceae* and one each to the *Chlamydiaceae*, *Criblamydiaceae* and *Simkaniaceae*. Based on the cut-off designed by Everett, Bush and Andersen (1999), none of the sequences could be affiliated to a known species ( $\geq 98\%$  16S rRNA, sequence similarity). Six sequences were from a new species belonging to a known genus ( $< 98\%$  and  $\geq 95\%$  16S rRNA, sequence similarity). In addition, six sequences were from a new genus belonging to a known family ( $< 95\%$  and  $> 90\%$  16S rRNA sequence similarity). Details of BLAST analysis are given in Table 1.

## DISCUSSION

In this study, we identified different *Chlamydiales* in water, feed bunks and on floors of bovine, ovine and caprine farms. Interestingly, only one *Chlamydia* species has been detected using our broad-range pan-*Chlamydiales* PCR despite the frequent association of *C. abortus* with ruminant abortions and the established role of *C. pecorum* in ovine infections (Fukushi and Hirai 1992; Chanton-Greutmann et al., 2002).

Conversely, various new *Chlamydia*-related bacteria were documented, underlying the biodiversity and prevalence of *Chlamydia*-related bacteria in environmental samples. The most commonly detected family-level lineage is *Parachlamydiaceae*. This family is often found in water, generally associated with free-living amoebae (reviewed in Corsaro and Greub 2006; Lamoth and Greub 2010). Thus, it is not surprising that five of the eight positive PCRs performed on water samples were positive for this family. Furthermore, three feed bunk swabs and one floor swab were also positive for *Parachlamydiaceae*. These samples may have been spoiled with water by the ruminants. The high prevalence of positivity (60%) for *Parachlamydiaceae* is important to survey in our case as *Parachlamydia acanthamoebae*, a species of this family-level lineage, is known to cause abortions and lung infections, especially in bovines (Borel et al., 2007; Lohr et al., 2014). The absence of infections in the investigated farms may be either due to the apathogenicity of the detected *Parachlamydiaceae* or due to bacterial loads below the infective dose required to cause disease. In our work, only one sample from a feed bunk of a caprine farm was positive for *Parachlamydia* sp. However, animals from that farm appeared healthy. *Parachlamydia* might present a reduced pathogenicity in caprines as compared to its pathogenicity towards bovines.

**Table 1:** Sequencing results of positive pan-*Chlamydiales* PCRs from Swiss barn samples

| Type of sample | Farm no. | Species grown | Ct values | Family-level lineage | Most closely related species                 |                       |            |
|----------------|----------|---------------|-----------|----------------------|----------------------------------------------|-----------------------|------------|
|                |          |               |           |                      | Species name                                 | GenBank accession no. | % Identity |
| Water          | 1        | Caprine       | 39.63     | Parachlamydiaceae    | <i>Candidatus metachlamydia lacustris</i>    | GQ221847              | 92.8       |
|                | 1        | Caprine       | 38.37     | Chlamydiaceae        | <i>Candidatus amphibiichlamydia ranarum</i>  | JN402380              | 91.3       |
|                | 1        | Caprine       | 38.12     | Criblamydiaceae      | <i>Estrella lausannensis</i>                 | EU074225              | 91.4       |
|                | 1        | Caprine       | 36.92     | ND                   | ND                                           |                       |            |
|                | 2        | Ovine         | 38.9      | Parachlamydiaceae    | <i>Protochlamydia naegleriophila</i>         | FJ976102              | 93.1       |
|                | 5        | Bovine        | 32.01     | Parachlamydiaceae    | <i>Neochlamydia hartmanellae</i>             | NR_025037             | 95.9       |
|                | 5        | Bovine        | 32.07     | Parachlamydiaceae    | <i>Neochlamydia hartmanellae</i>             | NR_025037             | 97.4       |
| Feed bunk      | 5        | Bovine        | 34.98     | Parachlamydiaceae    | <i>Neochlamydia hartmanellae</i>             | NR_025037             | 95.6       |
|                | 1        | Caprine       | 39.2      | Parachlamydiaceae    | <i>Parachlamydia acanthamoebae</i>           | NR_074972             | 97.3       |
|                | 2        | Ovine         | 37.04     | Parachlamydiaceae    | <i>Candidatus protochlamydia amoebophila</i> | NR_074271             | 92.5       |
|                | 2        | Ovine         | 37.78     | Parachlamydiaceae    | <i>Protochlamydia naegleriophila</i>         | DQ632609              | 91.3       |
|                | 6        | Bovine        | 34.48     | Simkaniaceae         | <i>Simkania negevensis</i>                   | NR_074932             | 97.1       |
|                | 8        | Bovine        | 34.28     | ND                   | ND                                           |                       |            |
|                | 8        | Bovine        | 31.19     | ND                   | ND                                           |                       |            |
| Floor          | 8        | Bovine        | 34.61     | Parachlamydiaceae    | <i>Protochlamydia naegleriophila</i>         | NR_115817             | 97.4       |

ND, no classification could be determined.

Criblamydiaceae were found only in one water sample. This is in agreement with the literature, since both species belonging to this lineage were isolated from water (Thomas et al., 2008; Lienard et al., 2011b). A single Simkaniaceae was detected in a feed bunk from a bovine farm. The source and significance of this bacterium in such a setting is unknown.

In summary, the results of this study showed the significant prevalence and diversity (12 new species-level lineages) of *Chlamydiales* in environmental samples being in direct contact with farm ruminants. Parachlamydiaceae is the most abundant family-level lineage detected using our pan-*Chlamydiales* PCR. Moreover, water was the likely contamination source, but did not result in animal infections.

## ACKNOWLEDGEMENTS

We thank farm operators for giving their consent for sampling their farms after obtaining previous information on the study aims.

## FUNDINGS

This work was supported by funds allocated to GG from the Naef foundation, a foundation involved in animal welfare.

**Conflict of interest statement.** None declared.

## REFERENCES

Barkallah M, Gharbi Y, Hassena AB, et al. Survey of infectious etiologies of bovine abortions during mid- to late gestation in dairy herds. *PLoS One* 2014;9:e91549.

Bavoil P, Kaltenboeck B, Greub G. In *Chlamydia veritas*. *Pathog Dis* 2013;67:89–90.

Borel N, Ruhl S, Casson N, et al. Parachlamydia spp. and related Chlamydia-like organisms and bovine abortion. *Emerg Infect Dis* 2007;13:1904–7.

Chanton-Greutmann H, Thoma R, Corboz L, et al. Abortion in small ruminants in Switzerland: investigations during two

lambling seasons (1996–1998) with special regard to chlamydial abortions. *Schweiz Arch Tierh* 2002;144:483–92.

Corsaro D, Feroldi V, Saucedo G, et al. Novel *Chlamydiales* strains isolated from a water treatment plant. *Environ Microbiol* 2009;11:188–200.

Corsaro D, Greub G. Pathogenic potential of novel *Chlamydiae* and diagnostic approaches to infections due to these obligate intracellular bacteria. *Clin Microbiol Rev* 2006;19:283–97.

Corsaro D, Thomas V, Goy G, et al. ‘*Candidatus Rhabdochlamydia crassificans*’, an intracellular bacterial pathogen of the cockroach *Blatta orientalis* (Insecta: Blattodea). *Syst Appl Microbiol* 2007;30:221–8.

Dilbeck-Robertson P, McAllister MM, Bradway D, et al. Results of a new serologic test suggests an association of *Waddlia chondrophila* with bovine abortions. *J Vet Diag Invest* 2003;15:568–9.

Draghi A, Popov VL, Kahl MM, et al. Characterization of “*Candidatus Piscichlamydia salmonis*” (order *Chlamydiales*), a *Chlamydia*-like bacterium associated with epitheliocystis in farmed Atlantic salmon (*Salmo salar*). *J Clin Microbiol* 2004;42:e5286–97.

Everett KD, Bush RM, Andersen AA. 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. *Int J Syst Bacteriol* 1999;4:415–40.

Friedman MG, Dvoskin B, Kahane S. Infections with the *Chlamydia*-like microorganism *Simkania negevensis*, a possible emerging pathogen. *Microbes Infect* 2003;5:1013–21.

Fukushi H, Hirai K. Proposal of *Chlamydia pecorum* sp. nov. for *Chlamydia* strains derived from ruminants. *Int J Syst Bacteriol* 1992;42:306–8.

Greenberg D, Banerji A, Friedman MG, et al. High rate of *Simkania negevensis* among Canadian Inuit infants hospitalized with lower respiratory tract infections. *Scand J Infect Dis* 2003;35:506–8.

Greub G. *Parachlamydia acanthamoebae*, an emerging agent of pneumonia. *Clin Microbiol Infect* 2009;15:18–28.

- Greub G. International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of the *Chlamydiae*: minutes of the inaugural closed meeting, 21 March 2009, Little Rock, AR, USA. *Int. J. Syst Evol Micr* 2010;**60**:2691–3.
- Henning K, Schares G, Granzow H, et al. *Neospora caninum* and *Waddlia chondrophila* strain 2032/99 in a septic stillborn calf. *Vet Microbiol* 2002;**85**:285–92.
- Jelocnik M, Forshaw D, Cotter J, et al. Molecular and pathological insights into *Chlamydia pecorum*-associated sporadic bovine encephalomyelitis (SBE) in Western Australia. *BMC Vet Res* 2014;**10**:121.
- Kebbi-Beghdadi C, Greub G. Importance of amoeba as a tool to isolate amoeba- resisting microorganisms and for their ecology and evolution: the *Chlamydia* paradigm. *Environ Microbiol Rev* 2014;**6**:309–24.
- Lamoth F, Greub G. Amoebal pathogens as emerging causal agents of pneumonia. *FEMS Microbiol Rev* 2010;**34**:260–80.
- Lienard J, Croxatto A, Aeby S, et al. Development of a new *Chlamydiales*-specific real-time PCR and its application to respiratory clinical samples. *J Clin Microbiol* 2011a;**49**:2637–42.
- Lienard J, Croxatto A, Prod'homme G, et al. *Estrella lausannensis*, a new star in the *Chlamydiales* order. *Microbes Infect* 2011b;**13**:1232–41.
- Lohr M, Prohl A, Ostermann C, et al. A bovine model of a respiratory *Parachlamydia acanthamoeba* infection. *Pathog Dis* 2014, DOI: 10.1111/2049-632X.12201.
- Regenscheit N, Holzwarth N, Greub G, et al. Deer as a potential wildlife reservoir for *Parachlamydia* species. *Vet J* 2012;**193**:589–92.
- Shewen PE. Chlamydial infection in animals: a review. *Can Vet J* 1980;**21**:2–11.
- Stride MC, Polkinghorne A, Miller TL, et al. Molecular characterization of “*Candidatus Parilichlamydia carangidicola*,” a novel *Chlamydia*- Like epitheliocystis agent in yellowtail kingfish, *Seriola lalandi* (Valenciennes), and the proposal of a new family, “*Candidatus Parilichlamydiaceae*” fam. nov. (order *Chlamydiales*). *Appl Environ Microb* 2013;**79**:1590–7.
- Thomas V, Casson N, Greub G. *Criblamydia sequanensis*, a new intracellular *Chlamydiales* isolated from Seine river water using amoebal coculture. *Environ Microbiol* 2006;**8**:2125–35.
- Thomas V, Loret JF, Jousset M, et al. Biodiversity of amoebae and amoebae-resisting bacteria in a drinking water treatment plant. *Environ Microbiol* 2008;**10**:2728–45.
- Wheelhouse N, Katzer F, Wright F, et al. Novel *Chlamydia*-like organisms as cause of bovine abortions, UK. *Emerg Infect Dis* 2010;**16**:1323–4.