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RESEARCH ARTICLE

Prevalence and diversity of *Chlamydiales* in Swiss ruminant farms

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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.

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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*

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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 ≤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 ≤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 (≥98% 16S rRNA, sequence similarity). Six sequences were from a new species belonging to a known genus (<98% and ≥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 accesion no.	% Identity
Water	1	Caprine	39.63	*Parachlamydiaceae*	*Candidatus metachlamydia lacustris*	GQ221847	92.8
	1	Caprine	38.37	*Chlamydiaceae*	*Candidatus amphibiichlamydia ranarum*	JN402380	91.3
	1	Caprine	38.12	*Criblamydiaceae*	*Estrella lausannensis*	EU074225	91.4
	1	Caprine	36.92	ND	ND		
	2	Ovine	38.9	*Parachlamydiaceae*	*Protochlamydia naegleriophila*	FJ976102	93.1
	5	Bovine	32.01	*Parachlamydiaceae*	*Neochlamydia hartmanellae*	NR˙025037	95.9
	5	Bovine	32.07	*Parachlamydiaceae*	*Neochlamydia hartmanellae*	NR˙025037	97.4
	5	Bovine	34.98	*Parachlamydiaceae*	*Neochlamydia hartmanellae*	NR˙025037	95.6
Feed bunk	1	Caprine	39.2	*Parachlamydiaceae*	*Parachlamydia acanthamoebae*	NR˙074972	97.3
	2	Ovine	37.04	*Parachlamydiaceae*	*Candidatus protochlamydia amoebophila*	NR˙074271	92.5
	2	Ovine	37.78	*Parachlamydiaceae*	*Protochlamydia naegleriophila*	DQ632609	91.3
	6	Bovine	34.48	*Simkaniaceae*	*Simkania negevensis*	NR˙074932	97.1
	8	Bovine	34.28	ND	ND		
	8	Bovine	31.19	ND	ND		
Floor	8	Bovine	34.61	*Parachlamydiaceae*	*Protochlamydia naegleriophila*	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.

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Conflict of interest statement. None declared.

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