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Abstract

Parachlamydia acanthamoebae has been implicated as an emerging agent of pneumonia in humans. Recently, it has been linked to miscarriage and neonatal infections. In contrast, its role in atherosclerosis remains controversial. In animals, there is strong evidence for Parachlamydia as a new abortigenic in cattle, and further data on this agent in other animal species is accumulating. New diagnostic methods, such as specific real-time PCR and immunohistochemistry with specific anti-Parachlamydia antibodies to detect the organism within lesions, are now available to facilitate the diagnosis of parachlamydial infections in humans and animals. This review discusses parachlamydial infections in clinical settings in humans and animals, as well as their zoonotic potential and possible modes of transmission. Current diagnostic tools are presented, and finally, the antibiotic susceptibility of Parachlamydia is described for potential future preventive and therapeutic options. Nevertheless, more data on Parachlamydia and Chlamydia-like organisms are needed to elucidate their host range and pathogenic potential in humans and animals.
Parachlamydia acanthamoebae and its zoonotic risk

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Abstract

*Parachlamydia acanthamoebae* has been implicated as an emerging agent of pneumonia in humans. Recently, it has been linked to miscarriage and neonatal infections. In contrast, its role in atherosclerosis remains controversial. In animals, there is strong evidence for *Parachlamydia* as a new abortigenic in cattle and further data on this agent in other animal species is accumulating. New diagnostic methods such as specific real-time PCR and immunohistochemistry with specific anti-*Parachlamydia* antibodies to detect the organism within lesions are now available to facilitate the diagnosis of parachlamydial infections in humans and animals. This review discusses parachlamydial infections in clinical settings in humans and animals as well as their zoonotic potential and possible modes of transmission. Current diagnostic tools are presented and finally, the antibiotic susceptibility of *Parachlamydia* is described for potential future preventive and therapeutic options. Nevertheless, more data on *Parachlamydia* and *Chlamydia*-like organisms are needed to elucidate their host range and pathogenic potential in humans and animals.

Introduction

*Parachlamydia acanthamoebae* is a gram-negative obligate intracellular bacterium belonging to the order *Chlamydiales*, which currently include six family-level lineage, i.e. the *Chlamydiaceae, Parachlamydiaceae, Criblamydiaceae, Simkaniaceae, Rhabdoclamydiaceae* and *Waddliaceae* (1-5). Bacteria of the *Chlamydiaceae* family include well established animal and human pathogens of worldwide distribution and of high clinical importance. *Chlamydiaceae* are characterized by their particular
developmental cycle including elementary, intermediate and reticulate bodies taking place in eukaryotic hosts. *Parachlamydiaceae* developmental cycle is characterized by elementary and reticulate bodies comparable to the one of *Chlamydiaceae* and a third stage, the crescent body (Figure 1D) (6). *P. acanthamoebae* is naturally infecting free-living amoebae (Figure 1A) and may also enter and marginally replicate within pneumocytes and lung fibroblasts (7). The *Parachlamydiaceae* family includes other genus, species and species-level lineages (Table 1). However, this review will mainly focus on *P. acanthamoebae*, which has been more studied and for which there are some evidence for a pathogenic role towards animals and humans and for a zoonotic risk.

**Parachlamydia in ruminants**

*Chlamydia* causes abortion in large and small ruminants leading to significant economic losses worldwide (8). The typical chlamydial abortion in ruminants is caused by *Chlamydophila abortus* and around 20% of the sheep population in Switzerland is infected with *C. abortus* (9). Chlamydial abortion in cattle seems to be much less frequent compared to the situation in sheep and goat indicating species-specific differences (10). Despite comprehensive laboratory investigations, up to 70% of cattle abortion cases remain without an etiological diagnosis (11) suggesting the possible presence of new yet undescribed abortigenic agents. In 1986, *Waddlia chondrophila* type strain WSU 86-1044 was implicated as a new abortigenic agent in cattle, when it was isolated in lung, liver and other tissues of an aborted fetus in the USA (12,13). A second report described the same agent in a bovine abortion co-infected with *Neospora*
caninum (14), but could not clarify if abortion was caused by the former or the latter agent. Further reports of *W. chondrophila* associated to ruminant abortion are still lacking. Studies ongoing in the authors’s laboratory focusing on ruminant abortion did not show evidence of *Waddlia* by specific real-time PCR or immunohistochemistry. A previous study focusing on bovine chlamydial abortion (due to *C. abortus*) in the eastern part of Switzerland where chlamydial abortion in small ruminants is endemic (9), found *C. abortus* in only 4% of late-term abortion (10). In contrast, 18.3% of cases were positive for *Chlamydia*-like organisms by broad-range 16S rRNA PCR. Sequencing of PCR products surprisingly revealed no similarities to *W. chondrophila* but to *P. acanthamoebae* resulting in the first PCR-based detection of this organism in a bovine abortion. To confirm these preliminary findings, a follow-up study was initiated and demonstrated the presence of *Parachlamydia* in bovine placenta by immunhistochemical protocols using a specific anti-*Parachlamydia* antibody and by transmission electron microscopy (15). In 70% of abortion cases with amplification of *Chlamydia*-like organisms DNA, the presence of *Parachlamydia* could be further confirmed by positive immunohistochemistry demonstrating the infectious agent within the placental lesions (15). The development of more specific new molecular methods such as a *Parachlamydia*-specific real-time PCR (16) and further improvement of the *Parachlamydia*-specific immunohistochemistry (15) using mouse polyclonal antibodies (17) enabled a comprehensive investigation on retrospectively collected bovine abortion cases (18). Placenta samples from late-term abortion cases in cattle were investigated by real-time PCR diagnostic for *Chlamydiaceae* and *Parachlamydia* spp., respectively and histological sections of cases positive by real-time PCR were further examined by
immunohistochemistry to demonstrate the agent within the placental lesion.

Parachlamydial DNA was detected in 18.3% of cases and of these 81.4% was confirmed by immunohistochemistry. By immunohistochemistry, parachlamydial antigen was localized mainly intracytoplasmic within trophoblastic epithelium of the placenta (Figure 2D). The main histopathological feature in these parachlamydial abortion cases was purulent to necrotizing placentitis (Figure 1B). Severe placentitis will result in placental insufficiency and consecutive abortion. Presence of parachlamydial antigen within necrotic and inflamed placental tissue as demonstrated by immunohistochemistry could link placental damage to the bacteria. Although final proof is lacking by experimental infection in pregnant animals, there is strong evidence that Parachlamydia might represent a new abortigenic agent in cattle.

Similar data were obtained in small ruminant abortion when placenta and fetal tissue from 211 abortion cases of goat and sheep were investigated (19). Thus, 26.1% of abortion cases were positive for C. abortus whereas only 2 cases (0.9%) were positive for Parachlamydia by real-time PCR immunohistochemistry. In both cases, necrotizing placentitis was present and interestingly, in one case fetal organs were available showing interstitial pneumonia and mixed cellular periportal hepatitis. Parachlamydia could be further detected in the fetal lung by PCR and immunohistochemistry. Mixed infection with C. abortus and Parachlamydia was present in this case and simultaneous presence of both agents could be demonstrated in the necrotic trophoblastic epithelium neutrophilic exsudate of the placenta (19).

By 16S rRNA PCR, DNA of Chlamydia-like organisms was detected in semen samples of bulls and rams (20). The presence of these organisms suggests the possibility of
venereal transmission of *Parachlamydia* and of other members of this highly diverse
group of emerging pathogens.

*Parachlamydia* in other animals

**Reptiles**

A retrospective study on reptile tissues with granulomatous inflammation focused on the
presence of mycobacteria and chlamydiae. By 16S rRNA PCR, in 54.4% of cases DNA
of *Chlamydia*-like organisms with variable sequence similarity (88-97%) to *P.*
*acanthamoebae* and *Simkania negevensis* was detected in chelonians, lizards and
snakes from zoo, shops and privat owners (21). At that time, specific antibodies for
*Parachlamydia* were not available and samples are no more available. Thus,
demonstration of the infectious agent within the granulomatous lesions was not possible
and it remains unknown if *Chlamydia*-like organisms are involved in granuloma
formation in reptiles.

**Swine**

Cervical swabs and genital tracts of sows were examined in a recent study on the
diagnostic investigation into the role of chlamydiae in cases of increased rates of return
to estrus in pigs (22). By broad-range 16S rRNA PCR amplifying short-length
fragments, DNA of *Chlamydia*-like organisms were detected in 28.2% on the swabs
from sows with and 22.0% of sows without reproductive problems, respectively
(p>0.05). Although there was no significant difference between the two groups, the high
prevalence of *Chlamydia*-like organisms in cervical swabs, uteri, oviducts and tissues of aborted fetuses was unexpected. By the same method, DNA of *Chlamydia*-like organisms was detected in semen of boars (23). However, the significance of this finding related to reproductive disorders in boars and sows remains to be elucidated.

**Other mammals**

An association of ocular diseases in cats, koalas, guinea pigs, pigs and sheep with *Chlamydia*-like organisms was reported as DNA of these bacteria was amplified by PCR (24-29). However, the pathogenicity of these organisms remains unclear and need further investigation.

**Parachlamydia in humans**

**Respiratory Infections**

*P. acanthamoebae* is considered as an emerging agent of pneumonia in humans (recently reviewed by Greub, 2009) (5). The first association was provided by recovery of *P. acanthamoebae* strain Hall’s coccus from the water of a humidifier associated with an outbreak of fever (30). Then, serological and/or molecular evidence further supported the role of *Parachlamydia* in bronchitis (31), community-acquired pneumonia (32), aspiration pneumonia (33) and bronchiolitis (16). The source of infection (i.e aerosolized water, animals, or interhuman transmission) remained unidentified in all these subsequent reports. Noteworthy, some cases were identified among two
immunocompromised patients, i.e. an HIV-infected individual with low CD4 count (34) and a grafted patient (32).

The possible role of *Parachlamydia* was further supported by its ability to remain viable for more than 2 weeks in pneumocytes (7) and by its ability to resist to destruction by human macrophages (35,36), which are in the lower respiratory tract the first line of defense of innate immunity against invading pathogens. Finally, a murine model of pneumonia caused by *P. acanthamoebae* was developed: intratracheal inoculation of *P. acanthamoebae* induced a pneumonia in 100% of infected mice and led to a pneumonia-related mortality in 50% of them 5 days post-inoculation (37).

Histopathology of the lungs was characterized by purulent pneumonia in the acute stage (2-4 das post-infection) and interstitial pneumonia in the subacute phase (7-10 days post-infection) (Figure 1C). *Parachlamydia* could be demonstrated within the lung lesions by PCR, immunohistochemistry and electron microscopy (Figure 1D, 2C).

Moreover, living *Parachlamydia* could be recovered by amoebal co-culture fulfilling the Koch’s postulates.

**Miscarriage and neonatal infections**

*P. acanthamoebae* has been linked to human miscarriage and has been detected in cervical smears (38). Direct maternal-fetal transmission was documented by demonstrating the presence of parachlamydial DNA in an amniotic fluid taken from a female patient with cough and flu-like symptoms (39). A very recent study investigated the presence of *Parachlamydia* in premature neonates by specific real-time PCR (40). DNA of *Parachlamydia* was detected in 31% of respiratory samples from 29 neonates.
Most of these patients had severe respiratory distress syndrome and the authors hypothesized that superinfection with *Chlamydia*-related bacteria during mechanical ventilation could have led to more severe respiratory disease. The mode of transmission of *Parachlamydia* could have been either (i) through a systemic infection during pregnancy, (ii) a chorio-amnionitis, or (iii) an infection at delivery by *Parachlamydia* colonizing the vaginal mucosa. Alternatively, infection of the newborns with *Parachlamydia* could have taken place during mechanical ventilation, free-living amoebae being used as carrier by these strict intracellular bacteria (41).

*Atherosclerosis*

Some reports identified a possible role of *Parachlamydia* in atherosclerosis. Thus, *Parachlamydia* DNA was amplified from abdominal aortic aneurysms and from 18.5% of aortic and carotid atherosclerotic lesions (42). A very recent study did not confirm the former results as there was neither parachlamydial DNA amplification from peripheral blood mononuclear cells nor serological evidence of anti-*Parachlamydia* IgG in 354 patients with past history of atherosclerosis (43). Although it is difficult to definitively conclude based on these few studies, an association between atherosclerosis and *Parachlamydia* infection seems unlikely (43). In the past, atherosclerosis has been linked to various infectious agents but significant amount of data is mainly available for *Chlamydia pneumoniae* (44,45,46), which role in this setting remains nevertheless controversial.

**Zoonotic potential of *Parachlamydia* and mode of transmission**
Coxiella burnettii and C. abortus are well-established infectious causes of adverse pregnancy outcomes after contact with infected ruminants (48-50). The real case numbers are probably underestimated as these agents are obligate intracellular and need special diagnostic tools. Pregnant women should thus avoid contact to sheep and goats flocks during the lambing season (48). Parachlamydia has been related repeatedly to ruminant abortion (10,15,48,49), but is also an emerging respiratory pathogen in humans (5) and has been linked to human miscarriage (38). Thus, either aerosol (comparable to transmission of C. burnettii) or oral transmission (comparable to transmission of C. abortus) of Parachlamydia from ruminant abortion material is possible and should be considered as a potential zoonotic risk. This zoonotic risk is further supported by the fact that maternal-fetal transmission of P. acanthamoeba was documented in a young woman working as a butcher in a rural area known for cattle breeding near from a small village named Bercher (Figure 3) (39). Moreover, contact to animals as infection source was clearly observed in a serological study where 3 out of 482 healthy Swiss men were found positive for Parachlamydia sp., all coming from the same rural region near Lausanne (see figure 3) (51). Alternative routes of transmission are through contaminated water where free-living amoebae contain Parachlamydia or ingestion of uncooked meat or raw milk from infected ruminants.

Pet animals such as dogs, cats and guinea pigs are additional sources of potential zoonotic agents. Indeed, Chlamydia-like organisms have been reported in cats (24,29) and guinea pigs with conjunctivitis (26). In the latter study, Parachlamydia-DNA was found in six symptomatic and two asymptomatic guinea pigs. Interestingly, the owner
contact lenses also harbored DNA of *P. acanthamoebae* suggesting a zoonotic transmission.

**Diagnosis of parachlamydial infections**

Diagnosis of parachlamydial infections is hampered by its obligate intracellular growth, by its relatively recent discovery and the very recent availability of specific diagnostic tools. Nucleic acid amplification of parachlamydial DNA was initially performed by broad-range 16S rRNA PCR (1), and by a variation of it (3) followed by sequencing. Diagnosis of well-known chlamydial infections is mostly either based on species-specific PCR tests for detection of *Chlamydia trachomatis* in humans or *Chlamydiaceae*-specific PCR methods followed by sequencing or Microarray in animals. Such PCR assays are not able to detect new *Chlamydia*-like organisms such as *Parachlamydia*. Thus, specific real-time PCR have then been developed allowing the detection of as few as 10 DNA copies of *Parachlamydia* in clinical samples (16,34). One of these PCRs is targeting the ADP/ATP translocase, an enzyme only present in Chlamydiae, Rickettsiae and plant plastids and absent from all other bacterial species (including those colonizing the oral cavity and potentially contaminating the lower respiratory tract samples), thus explaining the excellent specificity of the test (34). The other PCR is targeting the 16S rRNA and this will likely amplified also some other yet undiscovered species within the *Parachlamydia* genus (16). These PCRs have been applied to various human and animal samples.

To define the possible pathogenic potential of these novel organisms, new diagnostic tool were needed to demonstrate the agent within tissue lesions. In clinical settings, samples could have been taken from a contaminated environment in the first place, i.e.
deep respiratory specimens contaminated by bacteria present in the upper airway. Thus, it is important to ensure that the infectious agent diagnosed is related to the lesion. Immunofluorescence and immunohistochemistry techniques are then the methods of choice and can be used as second confirmatory tests in addition to PCR. Mouse sera against various *Chlamydia*-like organisms were raised in the senior authors’ lab and the serological cross-reactivity between different *Chlamydia*-related organisms was determined in a preliminary study by immunofluorescence and Western Blotting (17). Subsequently, the specificity of these antisera was tested by Tissue Microarray (TMA) technology associated with immunohistochemistry on formalin-fixed and paraffin-embedded pellets (52) (Figure 2A & 2B). As expected, mice antisera against *P. acanthamoebae* strain Hall’s coccus exhibited strong cross-reactivity to *P. acanthamoebae* strain BN9, but not with distantly related species (52). Antisera against *P. acanthamoebae* strain Hall’s coccus were then already successfully applied to formalin-fixed and paraffin-embedded bovine placenta specimens and resulted in the first report of *Parachlamydia* in bovine abortion (15) and follow-up studies on ruminant abortion (18,19) (Figure 2D). Rabbit anti-*Parachlamydia* antibodies have been applied to lung samples from mice experimentally infected with *P. acanthamoebae* showing good correlation with histopathological lesions and real-time PCR (27) (Figure 2C).

**Parachlamydia and antimicrobial susceptibility**

When tested in an amoebal co-culture system, *Parachlamydia acanthamoebae* appeared to be susceptible to macrolides and doxycycline and to be resistant to quinolones (53), exhibiting a similar susceptibility pattern than that of *Waddlia*...
chondrophila (54). This resistance to quinolones is likely due to mutations in the quinolone-resistance determining region of the *gyrA* and *parC* encoding genes of *Parachlamydia acanthamoebae* (55). Although, no animal models have confirmed these in vitro data, we thus currently recommend to use macrolides and/or doxycycline to treat infections due to any *Parachlamydiaceae*.

**Conclusion**

The role of *P. acanthamoebae* as an emerging agent of pneumonia in humans and as an abortigenic agent in ruminants has led to further study the pathogenesis of this strict intracellular bacteria and its biology, and has triggered the development of *Parachlamydia*-specific diagnostic tools to diagnose parachlamydial infections in humans and animals. The parallel detection of this new bacterial agent suggested a possible zoonotic risk. However, zoonotic transmission of *Parachlamydia* between animals and humans remains to be proven.

Persons in contact with aborted material from ruminants such as owner, animal caretakers, veterinarians should be aware of the potential zoonotic risk of well-known abortigenic agents such as *C. burnettii* and *C. abortus*, and of more recently described agents with yet poorly defined impact on human health such as *P. acanthamoebae*.

**Acknowledgements**

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References


**Figure legends**

Figure 1. Histopathology (Hematoxylin and eosin staining, H & E) and transmission electron microscopy in infections with *Parachlamydia acanthamoebae*. A. Histopathology of *Acanthamoeba castellanii* infected with *P. acanthamoebae* strain Hall's coccus (52). Parachlamydial infection within amoebae is visible as finely basophilic stippling. H & E staining. B. Histopathology of a bovine placenta. Purulent to necrotizing placentitis in a case of parachlamydial abortion positive by real-time PCR and immunohistochemistry for *P. acanthamoebae* (18). H & E staining. C. Lung histopathology of a mouse experimentally infected with living *P. acanthamoebae* at day 7 post-inoculation (37). Interstitial pneumonia is present. H & E staining. D. Transmission electron microscopy of the lung of a mouse experimentally infected with living *P. acanthamoebae* and sacrificed 4 days post-infection (37). Presence of several *Parachlamydia* in an apoptotic cell including the crescent stage.
Figure 2. Immunohistochemistry (IHC) with mouse antiserum against *Parachlamydia acanthamoebae* strain Hall’s coccus. A. Uninfected *Acanthamoeba castellanii*. IHC with mouse antiserum against *P. acanthamoebae* strain Hall’s coccus (negative control). B. *A. castellanii* infected with *P. acanthamoebae* strain Hall’s coccus. IHC with mouse antiserum against *Parachlamydia acanthamoebae* strain Hall’s coccus (52). C. Positive IHC of the lung of a mouse infected with living *Parachlamydia* (37). D. IHC of a bovine placenta with the anti-*Parachlamydia* antibody. Presence of positive granular reaction within trophoblastic epithelium (18). All sections were stained with the AEC/peroxidase method, hematoxylin counterstain.

Figure 3. Map of Switzerland showing the location where the major outbreak of bovine abortion due to *Parachlamydia acanthamoebae* occurred (15) and the rural area near Bercher where a cluster of human infection due to *Parachlamydia* has been observed during a seroepidemiological study in young Swiss men (n=3) and during an investigation of amniotic fluids (n=1) (39,51).
Table 1. Pathogenicity of *Parachlamydiaceae* in humans and animals

<table>
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<th>Species</th>
<th>Host</th>
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GENEVA
LAUSANNE
NEUCHATEL
BERN
LUZERN
LOCARNO
BASEL
ZURICH

RURAL AREA NEAR BERCHER
MATERNO-FETAL TRANSMISSION & 3 SEROPOSITIVE PATIENTS

DOCUMENTED CASES OF ABORTION IN BOVINES

GRAUBUNDEN