Parachlamydia acanthamoebae in domestic cats with and without corneal disease

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*Parachlamydia acanthamoebae in domestic cats with and without corneal disease*

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ABSTRACT
Corneal samples of cats with and without corneal diseases were screened with a pan-
Chlamydiales PCR and specific PCRs for Parachlamydia, Protochlamydia,
Chlamydophila felis, Acanthamoeba and feline herpesviruses (FHV-1). Several corneal
samples tested positive for Parachlamydia and related Chlamydiales, indicating cat
exposure to these intracellular bacteria.
Introduction.

*Chlamydophila felis* (*Chlamydiaceae* family) is the only member of the *Chlamydiales* order, whose pathogenic role in cats has been established. Novel members of the *Chlamydiales* order have been recently detected in samples of humans and a number of animal species with respiratory, urogenital, granulomatous and ocular diseases (1). These new members of the *Chlamydiales* order have been assigned to different families such as the *Parachlamydiaceae*, the *Simkaniaceae*, and the *Waddliaceae* families (2). All these new bacterial species exhibit the same developmental stage than the members of the *Chlamydiaceae* family and share 80 to 90% 16S rRNA sequence similarity with the *Chlamydiaceae* (2). *Parachlamydiaceae*, such as *Parachlamydia acanthamoebae* and *Protochlamydia neagleriophila* have been associated with pneumonia in humans (2, 3). *Parachlamydia acanthamoebae* and other members of the *Parachlamydiaceae* family have also been detected in ocular samples from humans (4, 5) and animals (6-8) with conjunctivitis and/or keratitis. The presumed pathogenic potential of *Parachlamydia acanthamoebae* may be mediated by its ability to infect and multiply within human macrophages (9). *Acanthamoeba* are natural protozoan hosts for *Parachlamydiaceae* (10) and for other prokaryotes (11), and are causative organisms of granulomatous amoebic encephalitis and amoebic keratitis in humans (11). Although feline herpesvirus type 1 (FHV-1) is considered the main etiologic agent for corneal diseases in cats (12), an etiologic diagnosis is infrequently made in chronic corneal diseases (13), suggesting that other pathogens may be involved.

No data is currently available about the route of transmission, infectivity and pathogenicity of *Parachlamydiaceae* in humans and animals. The role of animals and especially of pets as source of infection for humans is still unknown. The aim of this study was thus to investigate the presence of *Parachlamydia acanthamoebae*, *Protochlamydia neagleriophila* and *Protochlamydia amoebophila* in eyes of domestic cats with and without corneal disease by using PCRs specific for the *Parachlamydia acanthamoebae* species and for the *Protochlamydia* genus, respectively. These samples have also been tested for the presence of *Chlamydophila felis* by using a species specific PCR and for the presence of any other *Chlamydiales* using a pan-**Chlamydiales** PCR, i.e a PCR amplifying any members of the *Chlamydiales* order, including *Chlamydiaceae*...
(Chlamydophila felis) and Parachlamydiaceae (Parachlamydia acanthamoebae, Protochlamydia neagleriophila). Moreover, these samples have been screened for FHV-1 and for the Acanthamoeba protist by using specific PCRs.

**Material and methods and results.**

Among 63 cats (63 eyes) included in this study, 44 cats had outdoor access, 14 cats were strictly kept indoors, and the habitat of 5 cats was not recorded. Ocular examination prior to sampling was performed for 40 eyes of 40 cats that presented to the Ophthalmology unit of our veterinary hospital with keratitis and/or other corneal lesions. As controls, we studied 23 eyes from 23 cats without any ocular disease and euthanized for unrelated reasons in our veterinary hospital.

Ocular signs were evaluated using a portable hand-held binocular biomicroscope (KOWA SL-14, Kowa, Tokyo, Japan). Fluorescein (Fluoreszein, Haag Streit, Koniz, Switzerland) staining was performed to visualize corneal erosions. Among the 40 eyes with keratitis and/or other corneal lesions, 7 eyes presented with active keratitis (vascularisation, cellular infiltrates) but intact epithelium, 9 eyes with corneal necrosis, 9 eyes with chronic corneal erosion, 11 eyes with mixed signs (erosion+necrosis, erosion+active keratitis, active keratitis+necrosis), and 4 eyes with eosinophilic keratitis.

To confirm eosinophilic keratitis, cytologic examination was done from corneal samples of cats with clinical evidence of eosinophilic keratitis, i.e. corneal vascularization and superficial multifocal whitish cellular infiltrates. Cytology smears were air-dried, stained with Wright's stain and evaluated by certified pathologists.

Corneal samples for PCRs were obtained by scraping of the corneal surface using a cytobrush (Cytobrush® plus, Medscand Medical®, Malmoe, Sweden) in 22/40 eyes with keratitis and in 22/23 normal eyes, by keratectomy in 8/40 eyes with keratitis and in 1/23 normal eyes or by both methods in 10/40 eyes with keratitis and/or other corneal lesions. Samples were put into a sterile tube and either processed immediately or stored at -20°C until DNA extraction was performed.

Nucleic acids were extracted from corneal swab samples or keratectomy specimens using the DNeasy™ Tissue Kit (Qiagen, Hombrechtikon, Switzerland). Each sample was mixed with 20μl proteinase and 200μl ATL-buffer and processed according
to manufacturer’s instructions. One negative extraction control was tested in each extraction run. Contamination of reactions by PCR products was avoided by strict separation of working areas and the use of filter-plugged pipette tips. Distilled water negative controls and DNA positive control samples were included in each PCR.

A *Chlamydiales* specific PCR was performed on all samples to screen for the presence of any member of the *Chlamydiales* order. Practically, the complete 16SrRNA encoding gene (about 1500 bp) was amplified using primers 16SIGF and rP2Chlam as described (14). DNA of *Chlamydiales* was detected in 3/50 corneal samples from 3/40 cats with keratitis and/or other corneal lesions (40 eyes) (Table 1), and in 5/23 samples from 5/23 cats with normal eyes (23 eyes).

To screen for infections with FHV-1 and *Chlamydophila felis*, well known and common ocular pathogens in cats, specific real-time TaqMan® PCRs were performed according to Vogtlin et al. (15) and Helps et al. (16), respectively, using the ABI PRISM 7700 (Applied Biosystems, Rotkreuz, Switzerland). Feline herpesvirus DNA was detected in 13 of 50 samples from 12/40 cats with keratitis and/or other corneal lesions (Table 1), and in 11/20 samples from 11/23 cats with normal eyes. *Chlamydophila felis* DNA was detected in 2/50 samples from 2/40 cats with keratitis and/or other corneal lesions, and was not detected in any sample from 23 cats with normal eyes.

Detection of *P. acanthamoebae* and *Protochlamydia* spp. DNA were performed by real-time TaqMan® PCRs as described elsewhere (3, 17) using the ABI PRISM 7000 (Applied Biosystems, Rotkreuz, Switzerland). Each sample was amplified in duplicate. *Parachlamydia acanthamoebae* DNA was detected in 5/50 corneal samples from 5/40 cats with keratitis and/or other corneal lesions (Table 1), and in 6/23 samples from 6/23 cats with normal eyes. *Protochlamydia* DNA was not detected in any sample from any cat. Three samples were positive both with the broad-range *Chlamydiales* PCR and the *Parachlamydia* PCR, hence excluding false positivity by PCR contamination since both PCRs amplify different DNA segments.

Samples positive for 16S rDNA, but negative for *Chlamydophila felis*, *Parachlamydia acanthamoebae* and any member of the *Protochlamydia* genus were submitted for sequencing. Sequence analyses did not reveal any meaningful results,
perhaps due to low number of copies and/or due to use of primers amplifying large sequences (about 1400 base-pairs), at the expense of sensitivity.

Statistical analysis (Chi-Square test, Fisher’s exact test) revealed no significant difference in the rate of *Parachlamydia acanthamoebae* and *Chlamydiales* order specific PCRs positivity between cats with and without corneal disease. However, among 8 diseased eyes positive for *Parachlamydia acanthamoebae* DNA and *Chlamydiales* order specific 16S rDNA, 7 suffered from chronic corneal erosions and/or corneal necrosis with damaged epithelium, and the remaining eye exhibited an active keratitis with intact epithelium (Table 1). The habitat of the cat (indoors versus outdoors) did not correlate with presence of *Parachlamydia acanthamoebae* DNA and *Chlamydiales* order specific DNA.

All samples were also screened with an *Acanthamoeba* specific 18S rDNA gene PCR for the presence of *Acanthamoeba* species, which are natural hosts for *Parachlamydia acanthamoebae*. The *Acanthamoeba* specific primer pair JDP1 and JDP2 were used as described by Schroeder *et al.* (18), with slight modifications of the forward primer JDP1-mod (5′ GGCCCAGATCGTTTACCGTG-3′) and the reverse primer JDP2-mod (5′-CACAAGCTGCTAGGGGAGTC-3′) (6). *Acanthamoeba* DNA was not detected in any sample of any cat.

Discussion.
The detection of *Chlamydiales* order specific DNA and *Parachlamydia acanthamoebae* DNA in corneal samples from cats with and without corneal disease indicates exposure of cats, or strictly speaking, of the feline eye to these obligate intracellular bacteria. Eyes of cats living strictly indoors and eyes of cats having outdoor access were equally exposed to *Parachlamydia acanthamoebae* and other *Chlamydiales*. Interestingly, *Acanthamoeba* DNA was not detected in any cat. Therefore, transmission of *Parachlamydia acanthamoebae* and other *Chlamydiales* to the feline eye remains unknown. The absence of *Acanthamoeba* DNA in samples positive for *Parachlamydia acanthamoebae* is unlikely to be due to false negative *Acanthamoeba* PCR, but rather suggest that epithelial cells of cat’s eyes might allow persistence of *Parachlamydia acanthamoebae* in the absence of an amoebal host, as suggested by the persistence of this strict intracellular bacteria in different mammalian cell lines (19-21). Since cats
frequently live in close contact with humans, cats might be a possible source of infection for humans and the associated zoonotic risk merits further investigation.

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References


**Table 1:** PCR results of 50 samples taken from 40 cats (40 eyes) with keratitis, conjunctivitis and/or other corneal lesions. Please note that some cat’s eyes presented corneal necrosis and corneal erosion or active keratitis with or without corneal necrosis, explaining that the total of the denominators provided in a column is higher than 50 and that the total of the numerators is higher than the actual number of eyes (cats) positive for a given pathogen.

<table>
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<tr>
<th></th>
<th>Chlamydiales °</th>
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</table>

° Unidentified *Chlamydiales* other than *Parachlamydia acanthamoebae* and *Chlamydophila felis*