University of Zurich[UZH]

UNIVERSITÄT BERN

Institute of Veterinary Pathology

Occurrence of *Chlamydiaceae* in Swiss stray cats

Masterthesis
Bressan Michelle

Carried out under supervision of
Prof. Dr. med. vet. Borel Nicole

Zurich, 18 Juli 2019

Table of Contents

1 Zusammenfassung

Chlamydia felis (*C. felis*) ist ein obligat intrazelluläres Bakterium, welches in konjunktivalen Epithelzellen von Katzen zu finden ist und als wichtiger Erreger von Konjunktivitis gilt. Es wird angenommen, dass die natürliche Übertragung über Aerosole erfolgt, jedoch vermuten einige Wissenschaftler, dass das Bakterium auch den Verdauungstrakt von Katzen besiedelt und von dort in die Augen verschleppt oder fäko-oral auf andere Katzen übertragen werden kann. Eine im Jahr 2003 durchgeführte Studie untersuchte das Vorkommen von *C. felis* bei Schweizer Hauskatzen mittels Analyse von Konjunktivaltupfern. Es konnte gezeigt werden, dass 3.3 % bis 11.5 % der beprobten Tiere positiv für *C. felis* waren, jedoch lagen keine Daten zu streunenden Katzen vor. Studien aus anderen Ländern deuten aber darauf hin, dass Infektionen mit Chlamydien bei herrenlosen Katzen häufiger sind als bei Hauskatzen. Demzufolge ist davon auszugehen, dass möglicherweise eine beträchtliche Zahl an streunenden Katzen in der Schweiz mit *C. felis* infiziert ist.

Das Ziel dieser Studie war es, das Vorkommen von *Chlamydiaceae* bei streunenden Katzen in der Schweiz zu untersuchen, indem der Anteil infizierter Tiere bestimmt und die involvierten *Chlamydia* Spezies identifiziert wurden. Des Weiteren wurde untersucht, ob die Katzen Chlamydien über den Verdauungstrakt ausscheiden. Dazu wurden während mehreren Kastrationsaktionen zwischen 2017 und 2018 insgesamt 342 Augen- und Rektaltupfer von 154 streunenden Katzen für molekulardiagnostische Untersuchungen entnommen. Mittels *Chlamydiaceae* real-time PCR wurde eine erste Klassierung der Proben durchgeführt. Anschliessend unterzog man positive Proben einem DNA-Microarray Test oder einer Sanger Sequenzierung, um die beteiligten Chlamydienspezies zu identifizieren.

Von den 154 beprobten Katzen wurden 29 (18.8 %) positiv auf *Chlamydiaceae* getestet. Die meisten dieser Katzen (93.1 %) waren mit *C. felis* infiziert, bei einem Tier wurde jedoch überraschenderweise *C. abortus* gefunden. *C. felis* wurde signifikant häufiger bei Katzen mit Konjunktivitis detektiert verglichen mit Tieren, die keine Anzeichen für eine Augeninfektion aufwiesen. Während 53.8 % der symptomatischen Katzen positiv auf *C. felis* getestet wurden, konnte der Infektionserreger hingegen nur bei 3.6 % der asymptomatischen Tiere nachgewiesen werden. Zwar wiesen fünf Katzen (3.3 %) positive Rektaltupfer auf, dennoch deuten die gewonnen Daten nicht darauf hin, dass der Verdauungstrakt der Katze das Hauptreservoir für *C. felis* darstellt.

Diese Studie liefert erste Daten zur epidemiologischen Situation von Chlamydieninfektionen bei streunenden Katzen in der Schweiz. Aufgrund eines zu geringen Stichprobenumfangs sind die Resultate jedoch nicht repräsentativ für die gesamte Population. Ausserdem bleibt weiterhin ungeklärt, welche Rolle der Verdauungstrakt als Reservoir für *C. felis* spielt. Um das Wissen auf diesem Gebiet zu erweitern, sollten daher zusätzliche Studien durchgeführt werden.

2 Summary

Chlamydia felis (*C. felis*) is an obligate intracellular bacterium reckoned to be an important cause for conjunctivitis in cats that can be found in conjunctival epithelial cells. It is believed that natural transmission occurs most likely by aerosols, however, some authors hypothesise that the bacterium might colonise the digestive tract of the cat from where it could be translocated to the eyes or be transmitted to other cats by the faecal-oral route. In 2003, a study investigated the occurrence of *C. felis* in Swiss pet cats by analysing conjunctival swab samples. It revealed that 3.3 % to 11.5 % of the sampled cats were positive for *C. felis,* however, data on stray cats was not available. Studies carried out in other countries suggest though that chlamydial infections are even more common in stray cats compared to pet cats. Consequently, it must be assumed that a considerable number of Swiss stray cats might be infected with chlamydiae.

The aim of the present study was to investigate the occurrence of *Chlamydiaceae* in Swiss stray cats by determining the rate of infected animals, identifying the involved *Chlamydia* species as well as examining whether the cats shed chlamydiae from the digestive tract.

Therefore, a total of 342 ocular and rectal swab samples from overall 154 Swiss stray cats were collected for molecular diagnostics during several trap-neuter-return programs between 2017 and 2018. The investigation of these samples included a real-time PCR for *Chlamydiaceae* as a first screening method. Subsequently, positive samples were subjected to a DNA microarray assay or Sanger sequencing in order to identify the involved *Chlamydia* species.

Out of 154 sampled cats, 29 (18.8 %) were tested positive for *Chlamydiaceae*. While most of these cats (93.1 %) were infected with *C. felis*, *Chlamydia abortus* was found in one cat which was an unexpected finding. *C. felis* was significantly more often detected in cats with conjunctivitis compared to cats that did not show any signs of an ocular infection. While 53.8 % of symptomatic animals were tested positive for *C. felis*, the infectious agent was only found in 3.6 % of asymptomatic cats. Although five cats (3.3 %) had positive rectal swab samples, the collected data does not indicate that the digestive tract of the cat is the predominant reservoir for *C. felis*.

This study elucidates the epidemiological situation of chlamydial infections among Swiss stray cats however, the data is not representative for the entire population due to small sample size. Moreover, the role of the digestive tract in feline chlamydial infections still raises many questions. In order to expand the knowledge on this topic further studies are needed.

3 Introduction

3.1 *Chlamydiales*

The order *Chlamydiales* consists of several families and species, all of which are Gram-negative, obligate intracellular bacteria. They are widespread among many different hosts including humans, companion animals, livestock as well as wild animals. *Chlamydiales* infections are not only limited to mammals as some species also occur in birds, reptiles, amphibians and fish (Borel et al., 2018; Stride et al., 2014). The order currently consists of nine families including the *Chlamydiaceae* family with its single genus *Chlamydia (C.)* that, to date, comprises the following 13 species: *C. abortus*, *C. avium*, *C. caviae*, *C. felis*, *C. gallinacea*, *C. muridarum*, *C. pecorum*, *C. pneumoniae*, *C. poikilothermis, C. psittaci*, *C. serpentis, C. suis* and *C. trachomatis* as well as three *Candidatus (Ca.)* species: *Ca. C. corallus, Ca. C. ibidis* and *Ca. C. sanzinia* (Borel et al., 2018). All *Chlamydiales* share a biphasic developmental cycle that begins with an infectious, extracellular form referred to as elementary bodies (EBs) that enter a host cell by endocytosis. There, they form membrane-bound inclusions within the cytoplasm and differentiate into metabolically active reticulate bodies (RBs) that undergo multiple rounds of division before reforming EBs. They leave the host cell through cell lysis or extrusion in order to spread and infect new cells (Borel et al., 2018; Hybiske, Stephens, 2007; Moulder et al., 1991).
After resolution of clinical signs, the bacteria often remain associated with the host for a long period of time, hence the infection can get asymptomatic and clinical signs might recur later. The underlying mechanism of this phenomenon is still controversially debated as chlamydiae might behave differently *in vitro* and *in vivo* (Bavoil, 2014). In cultured cells, it has been observed that *Chlamydiales* can deviate from their usual developmental cycle under adverse conditions, i.e. exposure to β-lactam antibiotics or interferon-γ. They undergo a stress response that leads to the formation of aberrant bodies (ABs), which are viable yet non-infectious. These ABs have slowed down their metabolism, interrupted the division of RBs and do no longer differentiate into EBs. They can withstand harsh conditions and re-enter their normal developmental cycle as soon as the stressor is removed (Schoborg, 2011). Since it is not justified to simply extrapolate from cell culture models to chlamydial infections in a human or animal host, the AB form or so-called chlamydial persistence or the chlamydial stress response has not been fully proven to occur *in vivo*. Recently, another mechanism has been suggested to explain recurrent chlamydial infections in animals and humans. It was hypothesised that many chlamydial species are transmitted by the faecal-oral route and colonise the gastrointestinal tract where they live unnoticed as commensals and serve as a reservoir. Continually shed EBs might cause clinically relevant infections when they are translocated to different body sites representing a source for re-infection or infection of other hosts (Bavoil, 2014; Rank, Yeruva, 2014).

3.2 Historical background of *C. felis*

C. felis was first isolated from a cat with respiratory tract disease by Baker in 1942. Since all attempts of culturing the infectious agent on agar plates remained unsuccessful, it was assumed that the disease was caused by a virus (Baker, 1942). When scientists microscopically analysed infected cells they discovered elementary bodies resembling those in patients with trachoma, psittacosis and lymphogranuloma venereum that were already described in 1907, 1930 and 1935, respectively (Baker, 1942; Thygeson, 1951). After 1935, other chlamydial species were isolated from different hosts, including *C. felis*. They were initially called psittacosis-like viruses and were referred to the trachoma-psittacosis-lymphogranuloma venereum group of viruses before Page proposed the assignment of all group members to a single genus named *Chlamydia* (Thygeson, 1951; Moulder, 1966). With time,

scientists questioned the viral status of these microorganisms and Moulder reasoned in 1966 that they were rather intracellular bacteria (Moulder, 1966). Only two years later, the split of the genus *Chlamydia* into the species *Chlamydia trachomatis* and *Chlamydia psittaci* was suggested and it was believed that the agent of feline pneumonitis, which Baker isolated for the first time in 1942, was a *Chlamydia psittaci* strain (Page, 1968). Later, analyses of the ribosomal RNA gene sequences led to the controversial division of the genus *Chlamydia* into the two genera *Chlamydia* and *Chlamydophila* (Everett et al., 1999a). At that time, *C. psittaci* was split into four *Chlamydophila* species, and the *Chlamydia psittaci* feline pneumonitis agent became its own species named *Chlamydophila felis* (Everett et al., 1999a). More recently, it received its present name *Chlamydia felis* when the two separate genera were finally reunited into a single genus *Chlamydia* (Sachse et al., 2015).

3.3 Host species and zoonotic potential

C. felis is most commonly found in cats but has also been detected in dogs (Pantchev et al., 2010). Very recently, *C. felis* was reported for the first time in a free-ranging Eurasian Lynx. The animal was suffering from unilateral conjunctivitis when it was captured in the Swiss Jura Mountains during a translocation program (Marti et al., 2018).
In 1969, *C. felis* was isolated from conjunctival scrapings of a man with follicular keratoconjunctivitis. Apparently, he got infected by close contact to his cat that had previously suffered from rhinitis and conjunctivitis. Hence, a zoonotic transmission of *C. felis* was documented for the first time (Schachter et al., 1969). Although since then only few such cases have been reported, cat owners and professionals working with cats seem to be at considerable risk due to their exposure to potentially infected animals (Wons et al., 2017; Di Francesco et al., 2006).

3.4 Clinical signs

In cats, *C. felis* seems to be primarily a pathogen of the conjunctiva and possibly the nasal epithelium (Sykes, 2005; Bart et al., 2000). It causes acute to chronic conjunctivitis with blepharospasm, chemosis and ocular discharge. Additionally, some animals might show inappetence, sneezing as well as nasal discharge (Sykes, 2005). Usually, the symptoms appear after an incubation period of three to five days and last for a few weeks to months (Sykes, 2005). Although *C. felis* was first isolated from the lung of a cat and was therefore named feline pneumonitis agent, it does not seem to predominantly affect the lower respiratory tract (Baker, 1942; Sykes, 2005; Bart et al., 2000).

3.5 Transmission routes and experimental infection

Natural transmission of *C. felis* occurs most likely by aerosols when either symptomatically or asymptomatically infected cats are in close contact to healthy cats (Sykes, 2005). Experimental infections were successful using the ocular or intranasal application route (Baker, 1943; TerWee et al., 1998; Shewen et al., 1978; Sykes et al., 1999b). Interestingly, experimental ocular infection of cats not only resulted in conjunctivitis and sometimes mild respiratory symptoms as expected but also led to vaginal and rectal excretion of the bacteria in 50 % and 40 % of the infected animals, respectively (Wills et al., 1987). Therefore, Wills et al. hypothesised that primary ocular Chlamydiosis may result in a persistent infection of the genital tract (Wills et al.,1987). Moreover, they suggest the gastrointestinal tract as another site of persistent infection, as do Rank and Yeruva (Wills et al., 1987; Rank, Yeruva, 2014). Therefore, the venereal as well as the faecal-oral route should be considered as potential transmission pathways as well. *C. felis* has also been isolated from internal organs of cats such as the

lung, peritoneum, liver and spleen, but it remains unclear so far whether those findings are clinically relevant (Sykes, 2005).

3.6 Occurrence of chlamydiae in cats

In 2003, Von Bomhard et al. investigated the occurrence of chlamydial infections in Swiss cats. When he analysed conjunctival swabs, he found that 11.5 % of the cats suffering from conjunctivitis and 3.3 % of healthy cats were positive for *C. felis*. Moreover, he detected other *Chlamydiales* in 38.9 % and 20 % of cats with and without conjunctivitis, respectively (Von Bomhard et al., 2003). However, no further studies have been carried out to pursue these findings and specific data on the occurrence of chlamydiae in Swiss stray cats is lacking. Studies from other countries confirm that *C. felis* is more frequently detected in cats suffering from conjunctivitis than in healthy animals (Low et al., 2007; Rampazzo et al., 2003; McDonald et al., 1998). Some studies also demonstrated that chlamydial infections were more common in stray cats compared to pet cats (Halánová et al., 2011; Yan et al., 2000). Table 1 displays the occurrence of chlamydiae in different cat populations in selected countries.

Table 1: Occurrence of chlamydiae in cats from different countries

Country	Material	Test method	Tested cats	Positive / total number of cats (%)		Reference
USA	Conjunctival swabs	Real-time PCR for *C. felis*	Stray cats from TNR[1] program with URI[2]	14 / 60	23.3 %	McManus et al., 2014
			Stray cats from TNR program without URI	3 / 64	4.7 %	
USA	Conjunctival swabs	Conventional PCR for *C. felis*	Pet cats that never suffered from conjunctivitis	0 / 37	0 %	Low et al., 2007
			Pet cats with history of conjunctivitis (resolved)	0 / 32	0 %	
			Pet cats with active conjunctivitis	4 / 55	7.3 %	
IT	Conjunctival swabs	Conventional PCR for *C. felis*	Cats with conjunctivitis	14 / 70	20 %	Rampazzo et al., 2003
			Cats without conjunctivitis	0 / 35	0 %	
CH	Conjunctival swab	Conventional PCR for *C. felis*	Cats with keratitis or conjunctivitis	26 / 226	11.5 %	Von Bomhard et al., 2003
			Healthy cats	1 / 30	3.3 %	
JP	Conjunctival swabs	Conventional PCR for *C. felis*	Pet cats with URI	7 / 26	26.9 %	Mochizuki et al., 2000
UK	Conjunctival swabs	Conventional PCR for *C. felis*	Pet cats with conjunctivitis and / or ocular discharge	20 / 113	17.7 %	McDonald et al., 1998
			Pet cats without history of conjunctivitis or URI	0 / 6	0 %	
AU	Conjunctival swabs	Multiplex PCR	Pet cats with URI	66 / 462	14.3 %	Sykes et al., 1999a
			Pet cats without URI	1 / 95	1.1 %	
ES	Oral or nasal swabs	Conventional PCR for chlamydial DNA	Cats in animal shelters with conjunctivitis	2 / 6	33.3 %	Ravicini et al., 2016
			Cats in animal shelters without conjunctivitis	1 / 110	0.9 %	
SK	Conjunctival swabs	Immuno-fluorescence	Domestic cats with conjunctivitis	17 / 55	30.9 %	Halánová et al., 2011
			Stray cats with conjunctivitis	25 / 38	65.8 %	
JP	Serum	Microimmuno-fluorescence	Stray cats	40 / 88	45.5 %	Yan et al., 2000
			Pet cats	22 / 127	17.3 %	
NZ	Conjunctival swabs	ELISA antigen kit	Cats with conjunctivitis	40 / 217	18.4 %	Gruffydd-Jones et al., 1995
			Healthy cats exposed to cats with conjunctivitis	7 / 116	6.0 %	
UK	Conjunctival swabs	Cell culture	Household cats with conjunctivitis	226 / 753	30.0 %	Wills et al., 1987
			Household cats without conjunctivitis	0 / 40	0.0 %	
			Feral cats	10 / 41	24.4 %	
			Cats on sheep farms	3 / 43	7.0 %	
	Rectal swabs	Cell culture	Cats on sheep farms	2 / 49	4.1 %	

[1] Trap-neuter-return: stray cats that are captured for neutering and afterwards returned to their place of origin.

[2] Upper respiratory infection

The present study is the first to investigate the occurrence of chlamydiae in Swiss stray cats. The aim was to collect data on the frequency and distribution of *Chlamydiales* infections in stray cats, identifying the involved chlamydial species and examine whether the gastrointestinal tract might serve as a reservoir and potential source for infection. In order to do so, ocular as well as rectal swab samples were collected from 154 Swiss stray cats that were captured and anaesthetised for neutering. The obtained samples were analysed with different molecular diagnostic methods at the Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland.

4 Material and Methods

4.1 Material

For the present study, a total of 342 ocular and rectal swab samples from 154 Swiss stray cats were collected between 2017 and 2018. The animals either lived in the canton Fribourg (FR) in western Switzerland or in the canton Nidwalden (NW), which is located in the centre of the country (Table 2). Local animal welfare organisations had captured the cats for neutering and samples were taken under general anaesthesia before the animals underwent surgery. In cats without ocular signs, a pooled swab sample was retrieved, whereas both eyes were sampled individually if an animal showed signs of conjunctivitis as for example ocular discharge, conjunctival redness or swelling. At the same time, a rectal sample was taken from each cat to detect potential intestinal shedding of the bacteria. The swabs were labelled with the animals' identification number, sex, estimated age (young or adult), sampling site (eye: pooled / left / right or rectum) and the sampling date. On the same day the samples were stored at -20°C until further processing.

Table 2: Number of tested cats and obtained samples in FR and NW

	2017	2018	2017 + 2018
Cats in total	63	91	154
Cats FR	28	29	57
Cats NW	35	62	97
Swabs in total (eye/rectum/NA*)	144 (80 / 62 / 2)	198 (111 /87 / 0)	342 (191 / 149 / 2)
Swabs FR (eye/rectum/NA*)	59 (30 / 29 / 0)	60 (32 / 28 / 0)	119 (62 / 57 / 0)
Swabs NW (eye/rectum/NA*)	85 (50 / 33 / 2)	138 (79 / 59 / 0)	223 (129 / 92 / 2)

* Information on sampling site unavailable

4.1.1 Fribourg

A total of 57 cats were sampled in FR between 2017 and 2018. From one animal, only a rectal sample was available. Since it was not documented whether this cat showed signs of conjunctivitis at the time of sampling and could therefore not be classified as symptomatic or asymptomatic, it was not included in the frequency calculations.
Out of the 56 cats, five animals (= 7.7 %) showed ocular symptoms. Thereof, rectal and bilateral ocular swabs were available for all five cats.
Out of the 51 asymptomatic cats, 50 were sampled entirely with rectal and pooled ocular swab whereas the rectal swab was missing in one animal.

4.1.2 Nidwalden

Between 2017 and 2018, 97 cats were sampled in NW. Of those, two cats were excluded from the frequency calculations because only one sample of unknown sampling site was taken, and it was not known if the cats showed clinical signs.
Out of the remaining 95 animals, 34 cats (= 35.8 %) showed signs of conjunctivitis. Of those, all samples were available from 31 animals whereas the rectal swab was missing in one cat and the second eye swab in two other cats.
From the 61 asymptomatic cats, all samples were available.

4.2 Methods

4.2.1 DNA extraction

DNA extraction was performed using the Maxwell® 16 Buccal Swab LEV DNA Purification Kit #AS1295 (Promega, Madison, WI, USA) following the manufacturer's protocol.
For each sample, a 1.5 ml microtube and a clearing column were assembled. The swabs were cut off with clippers and added to the clearing columns. The equipment was cleaned with 70 % ethanol between handling different samples. Per sample, 300 µl of lysis buffer as well as 30 µl of proteinase K were prepared in a separate tube and a total of 330 µl was added to each clearing column. The tubes were vortexed for 10 seconds (s) followed by an incubation period of 20 minutes (min) at 56°C. Afterwards, the samples were centrifuged for 2 min at maximum speed. The flow-through was added to well #1 of a Maxwell® 16 LEV Cartridge while the clearing columns and swabs were discarded. Plungers were placed into well #8 of the cartridges. For further processing, the cartridges and corresponding elution tubes containing 50 µl of elution buffer were placed in the Maxwell® 16 LEV Cartridge Rack. After the extraction procedure was completed, the DNA concentration per µl eluate was determined using the Nanodrop-1000 version 3.8.1 (Witec AG, Lucerne, Switzerland). Afterwards the tubes were stored at 4 °C until further processing.

4.2.2 Real-time PCR for *Chlamydiaceae*

The selected real-time PCR for *Chlamydiaceae* targets a highly conserved, 111 bp long fragment of the 23S gene among all 13 members of the *Chlamydiaceae* family (Everett et al., 1999b; Sachse et al., 2014). It was therefore used as a first screening method in order to detect any *Chlamydia* species present in the obtained samples. The real-time PCR was performed with the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) following the protocol of Ehricht et al. (Ehricht et al., 2006) which consists of the primers and probe (Microsynth, Balgach, Switzerland) listed in Table 3.

Table 3: Primers and probe for detection of *Chlamydiaceae*

Primer/probe	Name	Sequence
Forward primer	Ch23S-F	5'-CTGAAACCAGTAGCTTATAAGCGGT-3'
Reverse primer	Ch23S-R	5'-ACCTCGCCGTTTAACTTAACTCC-3'
Probe	Ch23S-p	FAM-5'-CTCATCATGCAAAAGGCACGCCG-3'-TAMRA

As an internal control for successful DNA amplification, eGFP DNA was added to each reaction along with its corresponding primers and probe (Microsynth) (Table 4). A pre-made mixture called "IC2-Mix" composed of the two primers and probe in a 1:1:2 ratio was used for this PCR.

Table 4: Primers and probe for detection of eGFP DNA

Primer/probe	Name	Sequence
Forward primer	eGFP-1-F	5'-GACCACTACCAGCAGAACAC-3'
Reverse primer	eGFP-10-R	5'-CTTGTACAGCTCGTCCATGC-3'
Probe	eGFP-Hex	VIC-5'-AGCACCCAGTCCGCCCTGAGCA-3'-none

Each well of the PCR plate #4346906 (Applied Biosystems) consisted of the following reaction mixture: 1.75 µl Molecular Biology Grade water (Thermo Fisher Scientific, Waltham, MA, USA), 12.5 µl

TaqMan™ Fast Universal PCR Master Mix (2x) #4352042 (Applied Biosystems), 2.5 µl Ch23S-F (5 µM), 2.5 µl Ch23S-R (5 µM), 1 µl Ch23S-p (5 µM), 2 µl "IC2-Mix", 0.25 µl eGFP DNA template and 2.5 µl sample DNA template. This resulted in a total volume of 25 µl per well.
Samples containing more than 150 ng DNA per µl might cause DNA polymerase inhibition due to DNA overload. Therefore, a 1:10 dilution composed of 9 µl Molecular Biology Grade water and 1 µl sample DNA template was prepared, if the threshold value of 150 ng per µl was exceeded. All samples and their corresponding dilutions were tested in duplicate.
To exclude potential contamination with chlamydial DNA, each plate had at least two negative controls containing 2.5 µl of Molecular Biology Grade water instead of sample DNA template. Furthermore, 10-fold dilutions of *C. abortus* DNA ranging from 10^7 to 10 copies per µl were used as positive controls, as well as to calculate a standard curve for quantitation of the samples.
The amplification started with an initial step of activation/denaturation at 95°C for 20 s, followed by 45 cycles of denaturation and annealing/elongation at 95°C for 3 s and at 60°C for 30 s, respectively.
The Ct-value states the cycle number at which a certain amplification threshold is exceeded and hence was used for sample classification as presented in Table 5. The threshold for chlamydial DNA amplification was manually adjusted to 0.1 and the threshold for eGFP amplification had to be altered to 0.01.

Table 5: Sample classification according to Ct-values

Ct-values	Sample classification
2 x < 38	Positive
2 x > 38	Questionable positive
1 x < 38, 1 x undetermined	Questionable positive
1 x > 38, 1 x undetermined	Questionable negative
2 x undetermined	Negative

Normal Ct-values for eGFP ranged from 27 to 30. Protein and other contaminants might inhibit DNA amplification (Tichopad et al. 2004), resulting in higher or even undetermined Ct-values. *Chlamydia*-negative samples with either one or two high/undetermined eGFP Ct-values per duplicate were retested in the original concentration and additionally in a 1:10 dilution, as were questionable samples according to Table 5.

4.2.3 Species identification based on DNA microarray assay

Samples that tested positive for *Chlamydiaceae* in the 23S real-time PCR were further analysed using a DNA microarray assay in order to determine which chlamydiae the cats were carrying.
All *Chlamydia* species as well as other members of the order *Chamydiales* can be identified with a single DNA microarray assay that detects species-specific nucleotide sequences in a highly variable DNA region of the chlamydial 23S gene (Sachse et al., 2005). In this study, the Chlam Type-23S AS-4 Kit #246500096 (Alere Technologies GmbH, Jena, Germany) was used including 8-well strips based on the ArrayTube microarray technology (Alere Technologies GmbH) called ArrayStrips (Alere Technologies GmbH), which contain a DNA microarray at the bottom of each well. The microarray consists of several different probes of DNA oligonucleotides that are attached to a substrate. Each probe is characterised by an individual DNA sequence that is designed to hybridise with a certain chlamydial strain and therefore allows simultaneous testing for multiple *Chlamydia* species and other *Chlamydiales* (Table 6) including mixed infections (Sachse et al., 2005).

The sample DNA had to be 5'-biotin-labelled through biotinylation PCR before adding the PCR product to the microarray in order to visualise hybridisation by an enzymatic reaction involving the attached biotin molecule (Borel et al., 2008). Based on their hybridisation patterns, the samples were assigned to the according chlamydial species by the ArrayMate Reader #200501000 (Alere Technologies GmbH). Samples that either tested negative or could not be further classified by the ArrayMate were subjected to a subsequent analysis with a 16S PCR followed by DNA sequencing.

Table 6: Detectable *Chlamydiales* with the Chlam Type-23S AS-4 Kit

Chlamydia species	Other *Chlamydiales*
C. abortus	*Simkania* sp.
C. caviae	*Waddlia* sp.
C. felis	*Protochlamydia amoebophila*
C. psittaci	*Protochlamydia naegleriophila*
C. pecorum	*Neochlamydia hartmannellae*
C. pneumoniae	*Parachlamydia acanthamoebae*
C. suis	*Criblamydia sequanensis*
C. muridarum	*Chlamydiales xenoturbella*
C. trachomatis	*Estrella lausannensis*
C. avium	
C. gallinacea	

4.2.3.1 Biotinylation PCR

The amplification and biotinylation was performed with the Biometra TRIO thermal cycler (Analytik Jena AG, Jena, Germany) following a slightly modified protocol of Borel et al. (Borel et al., 2008). It comprises forward primers as well as 5'-biotinylated reverse primers (Microsynth) for both, chlamydial DNA (Table 7) and eGFP DNA (Table 8). EGFP served as an internal control to confirm successful DNA amplification.

Table 7: Primers for biotinylation PCR of chlamydial DNA

Primer	Name	Sequence
Forward primer	23-F	5'-ATTGAMAGGCGAWGAAGGA-3'
Reverse primer	23-R-Bio	Biotin-5'-GCYTACTAAGATGTTTCAGTTC-3'

Table 8: Primers for biotinylation PCR of eGFP DNA

Primer	Name	Sequence
Forward primer	eGFP-F	5'-CAGCCACAACGTCTATATCATG-3'
Reverse primer	eGFP-R-Bio	Biotin-5'-CTTGTACAGCTCGTCCATGC-3'

The following reaction mixture was added to a 0.2 ml PCR tube (Nolato Treff AG, Degersheim, Switzerland): 13.2 µl Molecular Biology Grade water (Thermo Fisher Scientific), 2 µl PCR buffer 10-fold concentrated including $MgCl_2$ (Roche Diagnostics International AG, Rotkreuz, Switzerland), 2 µl $MgCl_2$ (25mM) (Roche Diagnostics International AG), 0.2 µl deoxynucleotide triphosphate (dNTP) mix (10 nM) (PCR Nucleotide Mix, Roche Diagnostics International AG), 0.1 µl 23-F (100 µM), 0.1 µl 23-R-Bio (100 µM), 0.1 µl eGFP-F (10 µM), 0.1 µl eGFP-R-Bio (10 µM), 0.2 µl FastStart™ Taq DNA Polymerase (5 U/µl) (Roche Diagnostics International AG), 1 µl eGFP DNA template and 1 µl sample DNA template, resulting in a total volume of 20 µl per tube.

The amplification started at an initial temperature of 96°C that was held for 10 min, followed by 40 cycles of 94°C/30 s, 50°C/30 s and 72°C/30 s each and was completed after a final holding stage of 72°C for 4 min. The tubes were stored at 4°C until further use.

4.2.3.2 Hybridisation

The DNA hybridisation was performed with the Hybridization Kit #2452001000 (Alere Technologies GmbH) according to the manufacturer's manual including the components listed in Table 9. The amount of the biotinylated PCR product applied to the microarray assay was adjusted according to the Ct-values measured during the 23S real-time PCR in order to prevent hybridisation inhibition due to DNA overload. The biotinylated sample DNA was mixed with C1 buffer in separate tubes according to Table 10. First, the ArrayStrips™ (AS) (Alere Technologies GmbH) were activated by washing them with 200 µl Molecular Biology Grade water (Thermo Fisher Scientific). Then, the PCR product/C1 buffer mixture was added, and the AS were incubated at 58°C/60 min/550 rpm on a thermoshaker with ArrayStrip adapter (BioShake iQ, Quantifoil Instruments GmbH, Jena, Germany). The AS were washed and incubated twice with 200 µl C2 buffer at 43°C/10 min/550 rpm. To each well, 1.5 µl C3 conjugate and 150 µl C4 conjugate buffer were added followed by an incubation period of 30°C/10 min/550 rpm. The AS were washed with 200 µl C5 buffer before adding 100 µl D1 substrate at room temperature and the strips were incubated at 25°C/5 min/0 rpm. Lastly, D1 was removed and the microarrays were evaluated on the ArrayMate Reader (Alere Technologies GmbH).

Table 9: Components of Hybridization Kit #2452001000

Code	Reagent	Reference number
C1	Hybridisation buffer 1	245105000
C2	Washing buffer 1	245106000
C3	HRP conjugate	245107000
C4	Conjugate buffer	245108000
C5	Washing buffer 2	245109000
D1	HRP substrate	245110000

Table 10: Mixing proportions of PCR product and C1 buffer depending on Ct-values

Ct-value	PCR product	C1 buffer
< 20	4 µl	96 µl
20 – 30	8 µl	92 µl
> 30	10 µl	90 µl

4.2.4 Species identification based on Sanger sequencing

Samples that either tested negative in the DNA microarray assay or could not be assigned to a certain chlamydial species were subjected to Sanger sequencing by Microsynth. Prior to sequencing, a Pan-*Chlamydiales* PCR had to be performed on the samples in question.

4.2.4.1 Pan-*Chlamydiales* PCR

The conventional Pan-*Chlamydiales* PCR used in this study, herein referred to as short 16S PCR, amplifies a 298 bp long signature sequence of the chlamydial 16S rRNA gene (Everett et al., 1999a). The PCR was performed on a Biometra TRIO thermal cycler (Analytik Jena AG) using the primers

(Microsynth) (Table 11) published by Pospischil et al. (Pospischil et al., 2012), that match to all members of the *Chlamydiales* order according to Everett et al. (Everett et al., 1999a).

Table 11: Primers used for the short 16S PCR

Primer	Name	Sequence
Forward primer	16S-IGF (short)	5'-GATGAGGCATGCAAGTCGAACG-3'
Reverse primer	16S-IGR (short)	5'-CCAGTGTTGGCGGTCAATCTCTC-3'

The following reagents were added to a 0.2 ml PCR tube (Nolato Treff AG): 30.5 µl Molecular Biology Grade water (Thermo Fisher Scientific), 5 µl PCR buffer 10-fold concentrated including $MgCl_2$ (Roche Diagnostics International AG), 1 µl $MgCl_2$ (25 mM) (Roche Diagnostics International AG), 1 µl dNTP mix (10 mM) (Roche Diagnostics International AG), 5 µl 16S-IGF (10 µM), 5 µl 16S-IGR (10 µM), 0.5 µl FastStart™ Taq DNA Polymerase (5U/µl) (Roche Diagnostics International AG) and 2 µl sample DNA, adding up to 50 µl per tube. A positive and a negative control were carried out as well. For the positive control, 2 µl stock *Chlamydia suis* DNA was added instead of the sample DNA whereas no DNA was applied at all for the negative control.
The amplification was initialised at 95°C for 5 min, followed by 35 cycles of denaturation, annealing and elongation at 95°C/30 s, 65°C/30 s and 72°C/60 s, respectively. The final elongation temperature was kept for ten additional minutes before the PCR was completed.
The amplification of the target sequence was verified by gel electrophoresis using a 1.5 % agarose gel consisting of 0.6 g agarose diluted in 400 ml TAE buffer (Tris base, acetic acid and EDTA) and 4 µl of the nucleic acid stain GelRed® (Biotium inc., Fremont, CA, USA). For each sample and the corresponding controls, 5 µl of the PCR product were mixed with approximately 1 µl loading dye (Promega) and applied to the gel. Additionally, a 1 kb GeneRuler™ DNA ladder (Thermo Fisher Scientific) was added as a reference. The gel was run at 100 V voltage and 400 mA conduction for 45 min. Afterwards, the DNA fragments were visualised with the UVP BioDoc-It™ Imaging System (Analytik Jena AG).
It was assumed that the amplification of the desired DNA sequence was successful if a band appeared at a length of 300 bp. Such samples were purified with the Gene JET PCR Purification Kit #K0702 (Thermo Fisher Scientific) to remove residual dNTPs and primers, following the manufacturer's instructions.

4.2.4.2 Sanger sequencing

For each sample, two sequencing tubes were prepared containing 3 µl of either the forward or the reverse primer (10 µM) (Table 11), a certain amount of the PCR product depending on its DNA concentration measured beforehand with NanoDrop (Witec AG) diluted in water. This resulted in a total volume of 15 µl per tube with a DNA concentration of 4.5 ng/µl. The tubes were sent to Microsynth for Sanger sequencing. The obtained forward and reverse sequences were analysed by Geneious (http://www.geneious.com) and assembled to consensus sequences which were entered to BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

4.2.5 Statistics

Several statistical tests were applied to the present data in order to investigate whether certain risk factors affect the occurrence of chlamydial infections in Swiss stray cats. Animals that could not be

clearly classified as positive or negative due to missing samples were excluded from the calculations. The statistical analysis was carried out with the open source program R version 3.5.1.

In order to analyse whether *C. felis* occurs more often in cats with conjunctivitis compared to cats without ocular signs, a 2 x 2 contingency table was created based on the number of symptomatic and asymptomatic cats that were either positive or negative for *C. felis*. Since one cell had an expected value below five, the Fisher's exact test was chosen as statistical test. The confidence interval was set at 0.95.

It was investigated whether the age affects the occurrence of infections with *C. felis*. Therefore, the cats were grouped into young animals (sexually premature) and adults (sexually mature) according to their estimated age at the time of sampling. A 2 x 2 contingency table was created based on the number of young and adult cats that were positive or negative for *C. felis*, respectively. Fischer's exact test with a 0.95 confidence interval was chosen as statistical test.

In order to analyse whether the gender has an influence on the occurrence of infections with *C. felis,* a 2 x 2 contingency table was created consisting of the number of female or male cats that were positive or negative for *C. felis*. The statistical significance was investigated with a Pearson's Chi^2 test with a confidence interval of 0.95.

It was investigated whether *Chlamydiaceae* were significantly more often excreted from the eyes compared to the rectum. Therefore, the McNemar's test, which is suitable for paired samples, was used with a confidence interval of 0.95.

All 2 x 2 contingency tables as well as a list of the used statistical tests with according p-values can be found in the appendix.

5 Results

5.1 DNA extraction

A total of 342 swab samples was obtained from 154 cats and DNA was extracted with the Maxwell® DNA Purification Kit by Promega. The DNA concentration per eluate ranged from 3.46 ng/µl to 1696.81 ng/µl (mean 41.65 ng/µl, median 18.62 ng/µl) with a 260/280 purity between 0.07 and 33.94 (mean 1.53, median 1.53) and a 260/230 purity between -58.6 and 18.18 (mean 0.23, median 0.98).

5.2 Real-time PCR for *Chlamydiaceae*

The real-time PCR targeting the chlamydial 23S gene was performed on all 342 samples. It resulted in 296 (86.5 %) negative and 46 (13.5 %) positive samples, of which 39 (84.8 %) were of ocular, five (10.9 %) of rectal and two (0.4 %) of unknown origin. Thus, 29 (18.8 %) out of 154 cats were classified as positive, meaning that *Chlamydiaceae* DNA was found in at least one swab. A summary of the results is displayed in Table 12a and Table 12b.

Table 12a: Cats per year and region:

	2017			2018			2017 + 2018		
	All	Positive		All	Positive		All	Positive	
Cats in total	63	15	(23.8 %)	91	14	(15.4 %)	154	29	(18.8 %)
Symptomatic	20	11	(55.0 %)	19	11	(57.9 %)	39	22	(56.4 %)
Asymptomatic	40	2	(5.0 %)	72	3	(4.2 %)	112	5	(4.5 %)
Unknown	3	2	(66.7 %)	-	-	-	3	2	(66.7 %)
Cats FR	28	4	(14.3 %)	29	1	(3.4 %)	57	5	(8.8 %)
Symptomatic	3	3	(100 %)	2	1	(50 %)	5	4	(80 %)
Asymptomatic	24	1	(4.2 %)	27	0	(0 %)	51	1	(2.0 %)
Unknown	1	0	(0 %)	-	-	-	1	0	(0 %)
Cats NW	35	11	(31.4 %)	62	13	(21 %)	97	24	(24.7 %)
Symptomatic	17	8	(47.1 %)	17	10	(58.8 %)	34	18	(52.9 %)
Asymptomatic	16	1	(6.3 %)	45	3	(6.7 %)	61	4	(6.6 %)
Unknown	2	2	(100 %)	-	-	-	2	2	(100 %)

Table 12b: Samples per year and region:

	2017			2018			2017 + 2018		
	All	Positive		All	Positive		All	Positive	
Swabs in total	144	23	(16.0 %)	198	23	(11.6 %)	342	46	(13.5 %)
Ocular swabs	80	18	(22.5 %)	111	21	(18.9 %)	191	39	(20.4 %)
Rectal swabs	62	3	(4.8 %)	87	2	(2.3 %)	149	5	(3.4 %)
NK* swabs	2	2	(100 %)	-	-	-	2	2	(100 %)
Swabs FR	59	5	(8.5 %)	60	1	(1.7 %)	119	6	(5.0 %)
Ocular swabs FR	30	4	(13-3 %)	32	1	(3.1 %)	62	5	(8.1 %)
Rectal swabs FR	29	1	(3.4 %)	28	-	(0 %)	57	1	(1.8 %)
Swabs NW	85	18	(21.2 %)	138	22	(15.9 %)	223	40	(17.9 %)
Ocular swabs NW	50	14	(28.0 %)	79	20	(25.3 %)	129	34	(26.4 %)
Rectal swabs NW	33	2	(6.1 %)	59	2	(3.4 %)	92	4	(4.3 %)
NK* swabs NW	2	2	(100 %)	-	-	-	2	2	(100 %)

* Information on sampling site unavailable

5.2.1 Fribourg

During a collecting period of two years, 57 cats were sampled in Fribourg. Thereof, five (8.8 %) animals were tested positive according to the real-time PCR (Table 13). Every sample was tested in duplicate resulting in two Ct-values of which the mean Ct-value was calculated. The mean Ct-values ranged from 24.01 to 36.50 (mean 30.39, median 30.41). While the ocular sample was missing in one cat, the remaining 56 cats could be categorised as symptomatic or asymptomatic depending on the presence or absence of ocular signs. *Chlamydiaceae* were detected in four (80 %) out of five symptomatic and in one (2 %) out of 51 asymptomatic cats, respectively.

Table 13: Positive cats in Fribourg

Cat	Year	Ocular signs	Positive samples	DNA (ng/µl)	Ct-values		Mean Ct-value
A6E	2017	Yes	Eye 1	51.26	24.88	25.07	25.00
			Eye 2	108.15	24.04	23.98	24.01
A7E	2017	Yes	Eye 1	236.62	36.59	35.60	36.10
A75A	2017	No	Rectum	9.87	24.95	24.82	24.89
AO47	2017	Yes	Eye 1	9.13	35.05	36.64	35.85
DD2	2018	Yes	Eye 1	16.75	36.90	36.10	36.50

Ocular swabs were available from 56 cats. *Chlamydiaceae* were detected in the eyes of four (7.1 %) cats which all showed ocular signs. While chlamydial DNA was found in both eyes of the first (25 %) cat, the other three (75 %) cats were positive in only one eye.
In total, 56 rectal swabs were available, of which one (1.8 %) was tested positive. It came from an asymptomatic cat with negative pooled eye swabs. Hence, none of the cats with ocular signs or *Chlamydiaceae* in the eyes had a positive rectal swab.

5.2.2 Nidwalden

Of the 97 cats that were sampled in Nidwalden, 24 (24.7 %) were positive for *Chlamydiaceae* (Table 14) with mean Ct-values ranging from 22.26 to 37.82 (mean 31.37, median 31.61). Of two positive cats (marked with * in Table 14), only one sample of unknown origin was available thus, they were excluded from further frequency calculations. Chlamydial DNA was found in 18 (52.9 %) out of 34 symptomatic and in four (6.6 %) out of 61 asymptomatic cats, respectively.

Table 14: Positive cats in Nidwalden

Cat	Year	Ocular signs	Positive samples	DNA (ng/µl)	Ct-values		Mean Ct-value
BC2	2017	Yes	Eye 1	14.70	35.17	34.97	35.07
			Eye 2	14.35	35.01	34.77	34.89
BF1	2017	Yes	Eye 1	78.25	31.56	31.33	31.44
			Eye 2	13.31	36.73	35.50	36.12
BT2	2017	No	Eye pooled	14.84	34.31	34.55	34.43
BX2	2017	Yes	Eye 1	11.61	37.94	36.53	37.23
			Eye 2	109.00	32.80	32.86	32.83
BY1	2017	Yes	Eye 1	60.25	34.24	33.55	33.90
BY2	2017	Yes	Eye 1	36.49	31.14	31.50	31.32
			Rectum	13.07	32.11	33.93	33.02
CS2	2017	Yes	Eye 1	20.41	26.13	25.88	26.01
			Eye 2	7.87	32.03	32.04	32.04
CS4*	2017	Yes	Unknown	6.39	29.72	29.80	29.76
CS5	2017	Yes	Eye 1	8.36	33.83	33.51	33.67
			Rectum	15.06	36.65	36.27	36.46
CS6*	2017	Yes	Unknown	11.84	31.21	31.49	31.35
CS8	2017	Yes	Eye 1	7.03	33.39	33.01	33.20

			Eye 2	11.93	29.74	29.80	29.77
ED7	2018	Yes	Eye 1	23.55	37.68	37.96	37.82
			Eye 2	20.66	35.86	36.82	36.34
ED8	2018	Yes	Eye 1	179.59	32.03	32.04	32.04
			Eye 2	135.15	32.48	33.06	32.77
EI1	2018	Yes	Eye 1	18.85	28.50	28.41	28.45
			Eye 2	15.30	29.69	29.84	29.77
			Rectum missing	-	-	-	-
EI2	2018	No	Eye pooled	14.65	30.64	30.88	30.76
EI3	2018	Yes	Eye 1	87.11	27.87	27.83	27.85
			Eye 2	191.33	26.05	25.89	25.97
			Rectum	32.74	28.37	28.36	28.36
EI4	2018	Yes	Eye 1	84.97	25.28	24.98	25.13
			Eye 2	294.84	22.16	22.36	22.26
			Rectum	32.93	32.92	32.74	32.83
EK1	2018	Yes	Eye 1	30.60	34.62	33.79	34.21
			Eye 2	226.23	29.97	31.02	30.49
EM1	2018	No	Eye pooled	28.01	28.80	28.76	28.78
EM3	2018	Yes	Eye 1	84.53	25.61	25.79	25.70
			Eye 2 missing	-	-	-	-
EM4	2018	No	Eye pooled	355.93	28.48	28.64	28.56
EM12	2018	Yes	Eye 1	16.64	31.02	31.36	31.19
			Eye 2	15.06	34.21	33.84	34.02
EM13	2018	Yes	Eye 2	306.49	27.22	27.20	27.21
ES2	2018	Yes	Eye 1	46.59	32.18	31.39	31.78

Ocular swabs were available from 95 cats, thereof 22 (23.2 %) were positive in at least one eye. Of these 22 cats, four (18.2 %) were asymptomatic and therefore had a pooled eye sample taken whereas 18 (81.8 %) had their eyes individually tested resulting in 12 (54.5 %) bilaterally, five (22.7 %) unilaterally affected cats and one (4.5 %) cat remaining unknown whether both eyes were positive since the second ocular swab was missing.
Out of the 94 rectal swabs, *Chlamydiaceae* were found in four (4.3 %) samples. Rectal excretion was only detected in symptomatic cats with one or two positive eye swabs.

5.3 DNA microarray assay

All samples that were tested positive by the *Chlamydiaceae* real-time PCR (n = 46) were subjected to a DNA microarray assay (Alere Technologies GmbH) in order to identify the involved chlamydial species. Out of 46 positive samples, 38 (82.6 %) were clearly identified as *C. felis*, five (10.7 %) were assigned to the family *Chlamydiaceae* but could not be further classified and four (8.7 %) were negative according to the DNA microarray assay. Moreover, *C. abortus* was found in one (2.2 %) ocular swab sample of a symptomatic cat that was sampled in Fribourg in 2018. Neither the other eye nor the rectal swab was positive in the *Chlamydiaceae* PCR. The four negative samples as well as the five samples that could not be further classified were subjected to a Pan-*Chlamydiales* PCR followed by Sanger sequencing by Microsynth.

5.4 Sanger sequencing

The short 16S PCR was performed successfully on all nine samples. The sequencing was achieved in seven samples, all of which were identified as *C. felis*. They had a 99 – 100 % nucleotide identity with the partial sequence of the 16S ribosomal RNA gene (JN606073.1) of the strain NS9 and 99 % identity to all other *C. felis* sequences available in GenBank. The sequencing of two rectal samples (A75Ar and

CS5r) was unsuccessful. The first sample came from an asymptomatic cat with negative eye swab whereas the second was obtained from a symptomatic cat that was positive for *C. felis* in one eye.
Combining the information from the *Chlamydiaceae* PCR, the DNA microarray assay and the DNA sequencing led to the following results: out of 46 positive samples, 43 (93.5 %) were identified as *C. felis*, one (2.2 %) as *C. abortus* and two samples (4.3 %) belonged to the family *Chlamydiaceae* but could not be further classified. Hence of 154 cats, 26 (16.9 %) were positive for *C. felis*, one (0.6 %) for *Chlamydiaceae* (rectum), one (0.6 %) for *C. felis* (eye) as well as *Chlamydiaceae* (rectum) and one (0.6 %) for *C. abortus* (eye).

6 Discussion

This study investigated the occurrence of *Chlamydiaceae* in two populations of Swiss stray cats that were sampled during trap-neuter-return programs in Fribourg and Nidwalden. Positive samples were further analysed to identify the involved chlamydial species. Moreover, it was tested how often rectal shedding occurred in order to determine the role of the intestinal tract as a possible reservoir for *Chlamydiaceae* in cats.

6.1 Occurrence of *Chlamydiaceae* and species identification

Out of 154 sampled cats, 29 (18.8 %) were tested positive for *Chlamydiaceae* while 122 (79.2 %) were negative and three (1.9 %) could not be definitely categorised due to missing samples. Diagnostic tests for species identification recognised *C. felis* in 27 (17.5 %) cats and *C. abortus* in one (0.6 %) cat. Two positive rectal samples could not be further classified since DNA microarray assay as well as DNA sequencing were unsuccessful. Both samples had been stored at -20 °C for almost a year before DNA was extracted which might have decreased the DNA quality to a point where species identification based on the 16S rRNA gene was no longer possible, although the quantity and quality of the DNA determined with NanoDrop® did not markedly differ from other samples.

6.1.1 *C. felis*

As expected, most *Chlamydiaceae* positive cats (93.1 %) were infected with *C. felis* which was the only *Chlamydia* species known to affect cats (Von Bomhard et al., 2003). In this study, *C. felis* was detected in 17.5 % of stray cats which exceeds the occurrence of 3.3 % to 11.5 % that Von Bomhard et al. assessed in Swiss pet cats in 2003 (Von Bomhard et al., 2003). The observation that stray cats are more often infected with *C. felis* compared to pet cats is concordant with previous studies (Halánová et al., 2011; Yan et al., 2000). Von Bomhard et al. also discovered non-*C. felis Chlamydiales* in 38.9 % and 20 % of cats with and without conjunctivitis, respectively, while analysing conjunctival swabs with a broad range pan-*Chlamydiales* PCR assay targeting a sequence of the 16S rRNA gene (Von Bomhard et al., 2003). DNA sequencing identified the non-*C. felis Chlamydiales* as *Neochlamydia (N.) hartmannellae*, which belongs to the order *Parachlamydiaceae* and inhabits a free-living amoeba named *Hartmannella vermiformis*, that can be found in soil, water as well as on plants or animals (Von Bomhard et al., 2003; Horn et al., 2000). Since the present study was based on a screening method for *Chlamydiaceae* rather than *Chlamydiales*, it is possible that *N. hartmannellae* or other *Chlamydiales* were present in the samples but remained undetected.

6.1.2 *C. abortus*

Surprisingly, *C. abortus* was found unilaterally in an eye swab of a cat with conjunctivitis. Although the pathogen is usually associated with abortion in sheep and goats and to a lesser extent cattle, pigs and horses, a case of *C. abortus*-induced unilateral follicular keratoconjunctivitis in a dog has been described by Hoelzle et al. (reviewed in Longbottom, Coulter, 2003; Hoelzle et al., 2005). In 2011, Sostaric-Zuckermann et al. detected *C. abortus* in the heart tissue of a cat with arteriosclerotic lesions, however, conjunctivitis caused by *C. abortus* has not been reported in this species so far (Sostaric-Zuckermann et al., 2011). *C. abortus* was responsible for approximately 39 % and 23 % of abortions in Swiss sheep and goats, respectively, between 1996 and 1998 (Chanton-Greutmann et al., 2002). Therefore, it is possible that cats which live or hunt in close proximity to farm animals could encounter

C. abortus in Switzerland. The *Chlamydiaceae* real-time PCR resulted in a mean Ct-value of 36.5 which is at the higher end of the scale, implying that the number of chlamydial DNA copies in the sample was low. In fact, many other infectious agents are more likely to have caused conjunctivitis in this case, as for example feline herpes virus (FHV), feline calicivirus (FCV), *Bordetella bronchiseptica* or *Mycoplasma felis* (Mitchell, 2006), however, diagnostic tests for the presence of other pathogens were not carried out. Since the conjunctivitis could have been caused by an undetected pathogen or mechanical irritation, it remains unclear whether the cat's eye was infected or just contaminated with *C. abortus*.

6.2 Correlation between *C. felis* infection and conjunctivitis

C. felis was significantly ($p = 1.261e^{-11}$) more often detected in cats with conjunctivitis. While 55.3 % of symptomatic animals were positive, *C. felis* was only found in 3.6 % of asymptomatic cats. This result is consistent with previous studies of McManus et al. and Ravicini et al. who had already reported that chlamydial infections were more frequent in cats with upper respiratory infection and conjunctivitis, respectively (McManus et al., 2014; Ravicini et al., 2016). Although the trend was the same, the percentage of positive cats varied amongst the three studies. Whereas 55.3 % of Swiss stray cats with conjunctivitis were positive for *C. felis*, McManus et al. identified *C. felis* in only 23.3 % of trap-neuter-return cats with upper respiratory disease and Ravicini et al. reported chlamydial infections in 33.3 % of shelter cats with conjunctivitis. In contrast to Ravicini et al. who did not find any chlamydiae in cats without conjunctivitis, the present study revealed positive ocular swabs in asymptomatic cats, as did McManus et al. (McManus et al., 2014; Ravicini et al., 2016). Since the medical history of the cats is unknown, it is not possible to tell whether they were still in the pre-patent period, had almost overcome the disease or never developed clinical signs. It cannot be excluded that some samples were positive due to contamination, however, the Ct-values were similar to those of positive cats with conjunctivitis.
By comparing the two sampling locations, it was striking that Nidwalden had a much higher percentage of *C. felis* positive cats (25.0 %) compared to Fribourg (5.6 %). This might be explained by the positive correlation between cats with conjunctivitis and *C. felis* infection: 35.8 % of cats in Nidwalden had conjunctivitis whereas only 8.9 % of cats in Fribourg showed ocular signs. According to Hwang et al., the prevalence of some pathogens that are transmitted through direct contact, i.e. the feline leukaemia virus, is higher in areas where stray cats are being fed and therefore gather in large groups where infections are easily spread (Hwang et al., 2018). If stray cats were fed more often or if the feline population density was higher in Nidwalden, the number of direct contacts between cats might have been more frequent promoting the transmission of *C. felis* and therefore contributing to higher positivity rates compared to Fribourg. However, further investigations are necessary to verify this hypothesis.
By analysing other potential risk factors, it was observed that sexually premature cats were significantly ($p = 0.005$) less affected by chlamydiae which is contradictive to the studies by Sykes et al. and Wills et al. who reported that the prevalence was significantly higher in cats aged six weeks to nine months and five weeks to nine months, respectively (Sykes et al., 1999a; Wills et al., 1987). It is assumed that the findings of this study deviate from others because the categorisation into the two age groups was presumably inaccurate. Since the age of the cats was not documented it had to be estimated based on the opinion of the handling personnel which was difficult since many cats were supposedly aged closely around puberty.
Sex predisposition was not observed in this study and neither did Sykes et al. (Sykes et al., 1999a). However, Wills et al. reported that male cats had a significantly higher prevalence of chlamydial infections than females (Wills et al., 1987).

6.3 Chlamydial reservoir in the gastrointestinal tract

Chlamydiae can remain associated with their host for a long period of time without manifestation of clinical signs, however, the mechanism of this phenomenon has not been fully explained yet (Bavoil, 2014). Chlamydiae have been detected in faecal samples of cattle, sheep, pigs and other hosts while the intestines did not show any pathological response (York, Baker, 1951; Clarkson, Philips, 1997; Hoffmann et al., 2015; Rank, Yeruva, 2014). Therefore, it was assumed that the gastrointestinal tract might be the natural habitat for chlamydiae in most animals and that they are primarily transmitted by the faecal-oral route (Rank, Yeruva, 2014; Bavoil, 2014). Assuming this concept and also applying to *C. felis*, it was expected to observe rectal shedding more frequently compared to ocular shedding and to find a considerable number of asymptomatic cats with rectal excretion of *C. felis*, similar to the findings of Hoffmann et al. in Swiss fattening pigs (Hoffmann et al., 2015). The latter authors detected a mean herd prevalence of 38.7 % for conjunctival swabs and 93.0 % for faecal swabs, showing that almost all pigs carried *C. suis* in the intestinal tract. (Hoffmann et al., 2015). For the present study, the same approach was applied to Swiss stray cats. However, *Chlamydiaceae* were detected in the rectal swabs of only five (3.3 %) animals whereas ocular excretion occurred in 17.3 % of sampled cats and was therefore observed significantly ($p < 2.2e^{-16}$) more often. Moreover, only one (0.9 %) out of 111 asymptomatic cats showed rectal shedding. Therefore, the intestinal tract is unlikely to be the predominant habitat for *C. felis* in cats nor does the pathogen seem to be primarily transmitted by the faecal-oral route. However, chlamydiae were still rectally detected in four (16 %) out of 25 cats with positive conjunctival swabs. Since the rectal swabs of all four cats had higher Ct-values and therefore contained less chlamydial DNA compared to the conjunctival swabs, rectal shedding has probably occurred secondary to ocular chlamydiosis. *C. felis* might have been ingested via grooming or by ocular fluids draining through the nasolacrimal duct and transitioned the gastrointestinal tract. Eventually, *C. felis* is able to stay in the intestines for some time and still being shed after ocular symptoms have resolved which would explain the finding of an asymptomatic cat with a positive rectal swab. It would have been interesting to investigate how long the shedding of *C. felis* continued and whether it was shed intermittently from the rectum, however, the cats were set free on the same day of neutering, which made a follow-up impossible.

6.4 Conclusion

In summary, the occurrence of *Chlamydiaceae* in Swiss stray cats is comparable to prevalences reported in other countries though data on this topic is scarce. In almost all cases, the species could be identified as *C. felis*. While sex and age predisposition were not observed or remained unclear, the data clearly showed a correlation between conjunctivitis and ocular chlamydiosis. Although rectal shedding was observed in some cases, the data does not indicate that *C. felis* inhabits the intestinal tract of most stray cats nor that it is predominantly transmitted by the faecal-oral route. It has also been shown that the occurrence of *C. felis* can markedly vary between different sampling locations, therefore, future studies need to be extended and should include cats from other Swiss cantons as well. Although this study gave an insight into the occurrence of *Chlamydiaceae* in Swiss stray cats, many questions remain open and further investigations are needed to clarify if, for example, *C. felis* is able to replicate in the intestinal tract and how it remains associated with its host.

7 References

Baker, J. A. (1943). A virus causing pneumonia in cats and producing elementary bodies. Journal of Experimental Medicine 1944 Feb 1; 79(2), 159–172.

Bart, M., Guscetti, F., Zurbriggen, A., Pospischil, A., & Schiller, I. (2000). Feline infectious pneumonia: a short literature review and a retrospective immunohistological study on the involvement of *Chlamydia* spp. and Distemper Virus. Veterinary Journal 2000 May; 159(3), 220–230. DOI: 10.1053/tvjl.1999.0451

Bavoil, P. M. (2014). What' s in a word: the use, misuse, and abuse of the word "persistence" in *Chlamydia* biology. Frontiers in Cellular and Infection Microbiology 2014 Mar 4; 4, 27. DOI: 10.3389/fcimb.2014.00027

Borel, N., Kempf, E., Hotzel, H., Schubert, E., Torgerson, P., Slickers, P., Ehricht, R., Tasara, T., Pospischil, A., & Sachse, K. (2008). Direct identification of chlamydiae from clinical samples using a DNA microarray assay: a validation study. Molecular and Cellular Probes 2008 Feb; 22(1), 55–64. DOI: 10.1016/j.mcp.2007.06.003

Borel, N., Polkinghorne, A., & Pospischil, A. (2018). A review on chlamydial diseases in animals: still a challenge for pathologists? Veterinary Pathology 2018 May; 55(3), 374–390. DOI: 10.1177/0300985817751218

Chanton-Greutmann, H., Thoma, R., Corboz, L., Borel, N., & Pospischil, A. (2002). Aborte beim kleinen Wiederkäuer in der Schweiz: Untersuchungen während zwei Ablammperioden (1996-1998) unter besonderer Beachtung des Chlamydienabortes. Schweizer Archiv für Tierheilkunde 2002; 144(9), 483–492. DOI: 10.1024/0036-7281.144.9.483

Clarkson, M. J., & Philips, H. L. (1997). Isolation of faecal *Chlamydia* from sheep in Britain and their characterization by cultural properties. Veterinary Journal 1997 May; 153(3), 307–310. DOI: 10.1016/S1090-0233(97)80064-0

Di Francesco, A., Donati, M., Mazzeo, C., Battelli, G., Piva, S., Cevenini, R., & Baldelli, R. (2006). Feline chlamydiosis: a seroepidemiological investigation of human beings with and without contact with cats. The Veterinary Record 2006 Dec 2; 159(23), 778–779. DOI: 10.1136/vr.159.23.778

Ehricht, R., Slickers, P., Goellner, S., Hotzel, H., & Sachse, K. (2006). Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Molecular and Cellular Probes 2006 Feb; 20(1), 60–63. DOI: 10.1016/j.mcp.2005.09.003

Everett, K. D., Bush, R. M., & Andersen, A. A. (1999a). 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. International Journal of Systematic Bacteriology 1999 Apr; 49(2), 415–440. DOI: 10.1099/00207713-49-2-415

Everett, K. D., Hornung, L. J., & Andersen, A. A. (1999b). Rapid detection of the *Chlamydiaceae* and other families in the order *Chlamydiales*: three PCR tests. Journal of Clinical Microbiology 1999 Mar; 37(3), 575–580.

Gruffydd-Jones, T. J., Jones, B. R., Hodge, H., Rice, M., & Gething, M. A. (1995). *Chlamydia* infection in cats in New Zealand. New Zealand Veterinary Journal 1995 Oct; 43(5), 201–203. DOI: 10.1080/00480169.1995.35890

Halánová, M., Sulinová, Z., Čisláková, L., Trbolová, A., Páleník, L., Weissová, T., Halán, M., Kalinová, Z., & Holičková, M. (2011). *Chlamydophila felis* in cats - are the stray cats dangerous source of infection? Zoonoses and Public Health 2011 Nov; 58(7), 519–522. DOI: 10.1111/j.1863-2378.2011.01397.x

Hoelzle, K., Wittenbrink, M. M., Corboz, L., & Hoelzle, L. E. (2005). *Chlamydophila abortus*-induced keratoconjunctivitis in a dog. The Veterinary Record 2005 Nov 12; 157(20), 632–633.

Hoffmann, K., Schott, F., Donati, M., Di Francesco, A., Hässig, M., Wanninger, Sidler, X., & Borel, N. (2015). Prevalence of chlamydial infections in fattening pigs and their influencing factors. PLoS One 2015 Nov 30; 10(11), 1–19. DOI: 10.1371/journal.pone.0143576

Horn, M., Wagner, M., Müller, K. D., Schmid, E. N., Fritsche, T. R., Schleifer, K. H., & Michel, R. (2000). *Neochlamydia hartmannellae* gen. nov., sp. nov. (*Parachlamydiaceae*), an endoparasite of the amoeba *Hartmannella vermiformis*. Microbiology 2000 May; 146(5), 1231–1239. DOI: 10.1099/00221287-146-5-1231

Hwang, J., Gottdenker, N. L., Oh, D. H., Nam, H. W., Lee, H., & Chun, M. S. (2018). Disentangling the link between supplemental feeding, population density, and the prevalence of pathogens in urban stray cats. PeerJ 2018 Jun 25; 6:e4988. DOI: 10.7717/peerj.4988

Hybiske, K., & Stephens, R. S. (2007). Mechanisms of host cell exit by the intracellular bacterium *Chlamydia*. Proceedings of the National Academy of Science of the USA 2007 Jul 3; 104(27), 11430–11435. DOI: 10.1073/pnas.0703218104

Longbottom, D., & Coulter, L. J. (2003). Animal chlamydioses and zoonotic implications. Journal of Comparative Pathology 2003 May; 128(4), 217–244. DOI: 10.1053/jcpa.2002.0629

Low, H. C., Powell, C. C., Veir, J. K., Hawley, J. R., & Lappin, M. R. (2007). Prevalence of feline Herpesvirus 1, *Chlamydophila felis*, and *Mycoplasma* spp DNA in conjunctival cells collected from cats with and without conjunctivitis. American Journal of Veterinary Research 2007 Jun; 68(6), 643–648. DOI: 10.2460/ajvr.68.6.643

Marti, I., Pisano, S. R. R., Wehrle, M., Meli, M. L., Hofmann-Lehmann, R., & Ryser-Degiorgis, M. P. (2018). Severe conjunctivitis associated with *Chlamydia felis* infection in a free-ranging Eurasian lynx (Lynx lynx), Journal of Wildlife Diseases 2018 Oct 30; 55(2). DOI: 10.7589/2018-05-142

McDonald, M., Willett, B. J., & Addie, D. D. (1998). A comparison of DNA amplification, isolation and serology for the detection of *Chlamydia psittaci* infection in cats. The Veterinary Record 1998 Jul 25; 143(4), 97–101.

McManus, C. M., Levy, J. K., Andersen, L. A., McGorray, S. P., Leutenegger, C. M., Gray, L. K., Hilligas, J., & Tucker, S. J. (2014). Prevalence of upper respiratory pathogens in four management models for unowned cats in the Southeast United States. The Veterinary Journal 2014 Aug; 201(2), 196–201. DOI: 10.1016/j.tvjl.2014.05.015

Mitchell, N. (2006). Feline ophthalmology Part 2: clinical presentation and aetiology of common ocular conditions. Irish Veterinary Journal 2006 Apr; 59(4), 223–232.

Mochizuki, M., Kawakami, K., Hashimoto, M., & Ishida, T. (2000). Recent epidemiological status of feline upper respiratory infections in Japan. The Journal of Veterinary Medical Science 2000 Jul; 62(7), 801–803.

Moulder, J. W. (1991). Interaction of chlamydiae and host cells in vitro. Microbiological Reviews 1991 Mar; 55(1), 143–190.

Moulder, J. W. (1966). The relation of the psittacosis group (chlamydiae) to bacteria and viruses. Annual Review of Microbiology 1966; 20, 107–130. DOI: 10.1146/annurev.mi.20.100166.000543

Page, L. A. (1968) Proposal for the recognition of two species in the genus *Chlamydia* Jones, Rake, and Stearns, 1945. International Journal of Systematic Bacteriology 1968 Jan; 18(1), 51–66. DOI: 10.1099/00207713-18-1-51

Pantchev, A., Sting, R., Bauerfeind, R., Tyczka, J., & Sachse, K. (2010). Detection of all *Chlamydophila* and *Chlamydia* spp. of veterinary interest using species-specific real-time PCR assays. Comparative Immunology, Microbiology and Infectious Diseases 2010 Dec; 33(6), 473–484. DOI: 10.1016/j.cimid.2009.08.002

Pospischil, A., Kaiser, C., Hofmann-Lehmann, R., Lutz, H., Hilbe, M., Vaughan, L., & Borel, N. (2012). Evidence for *Chlamydia* in wild mammals of the Serengeti. Journal of Wildlife Diseases 2012 Oct; 48(4), 1074–1078. DOI: 10.7589/2011-10-298

Rampazzo, A., Appino, S., Pregel, P., Tarducci, A., Zini, E., & Biolatti, B. (2003). Prevalence of *Chlamydophila felis* and feline Herpesvirus 1 in cats with conjunctivitis in northern Italy. Journal of Veterinary Internal Medicine 2003 Nov-Dec; 17(6), 799–807.

Rank, R. G., & Yeruva, L. (2014). Hidden in plain sight: chlamydial gastrointestinal infection and its relevance to persistence in human genital infection. Infection and Immunity 2014 Apr; 82(4), 1362–1371. DOI: 10.1128/IAI.01244-13

Ravicini, S., Pastor, J., Hawley, J., Brewer, M., Castro-López, J., Beall, M., & Lappin, M. R. (2016). Prevalence of selected infectious disease agents in stray cats in Catalonia, Spain. Journal of Feline Medicine and Surgery Open Reports 2016 Feb 29; 2(1), 205511691663410. DOI: 10.1177/2055116916634109

Sachse, K., Bavoil, P. M., Kaltenboeck, B., Stephens, R. S., Kuo, C. C., Rosselló-Móra, R., & Horn, M. (2015). Emendation of the family *Chlamydiaceae*: proposal of a single genus, *Chlamydia*, to include all currently recognized species. Systematic and Applied Microbiology 2015 Mar; 38(2), 99–103. DOI: 10.1016/j.syapm.2014.12.004

Sachse, K., Hotzel, H., Slickers, P., Ellinger, T., & Ehricht, R. (2005). DNA microarray-based detection and identification of *Chlamydia* and *Chlamydophila* spp. Molecular and Cellular Probes 2005 Feb; 19(1), 41–50. DOI: 10.1016/j.mcp.2004.09.005

Sachse, K., Laroucau, K., Riege, K., Wehner, S., Dilcher, M., Creasy, H. H., Weidmann, M., Meyer, G., Vorimore, F., Vicari, N., Magnino, S., Liebler-Tenorio, E., Ruettger, A., Bavoil, P. M., Hufert, F. T., Rosselló-Móra, R., & Marz, M. (2014). Evidence for the existence of two new members of the family *Chlamydiaceae* and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov. Systematic and Applied Microbiology 2014 Mar; 37(2), 79–88. DOI: 10.1016/j.syapm.2013.12.004

Schachter, J., Ostler, H. B., & Meyer, K. F. (1969). Human infection with the agent of feline pneumonitis. The Lancet 1969 May 31; 293(7605), 1063–1065. DOI: 10.1016/S0140-6736(69)91703-6

Schoborg, R. V. (2011). *Chlamydia* persistence – a tool to dissect *Chlamydia*-host interactions. Microbes and Infection 2011 Jul; 13(7), 649–662. DOI: 10.1016/j.micinf.2011.03.004

Shewen, P. E., Povey, R. C., & Wilson, M. R. (1978). Case report. Feline chlamydial infection. The Canadian Veterinary Journal 1978 Oct; 19(10), 289–292.

Sostaric-Zuckermann, I. C., Borel, N., Kaiser, C., Grabarevic, Z., & Pospischil, A. (2011). *Chlamydia* in canine or feline coronary arteriosclerotic lesions. BMC Research Notes 2011 Sep 9; 4(1), 350. DOI: 10.1186/1756-0500-4-350

Stride, M. C., Polkinghorne, A., & Nowak, B. F. (2014). Chlamydial infections of fish: diverse pathogens and emerging causes of disease in aquaculture species. Veterinary Microbiology 2014 May 14; 170(1–2), 19–27. DOI: 10.1016/j.vetmic.2014.01.022

Sykes, J. E. (2005). Feline chlamydiosis. Clinical Techniques in Small Animal Practice 2005 May; 20(2), 129–134. DOI: 10.1053/j.ctsap.2004.12.018

Sykes, J. E., Anderson, G. A., Studdert, V. P., & Browning, G. F. (1999a). Prevalence of feline *Chlamydia psittaci* and feline Herpesvirus 1 in cats with upper respiratory tract disease. Journal of Veterinary Internal Medicine 1999 May-Jun; 13(3) 153–162.

Sykes, J. E., Studdert, V. P., & Browning, G. F. (1999b). Comparison of the polymerase chain reaction and culture for the detection of feline *Chlamydia psittaci* in untreated and doxycycline-treated experimentally infected cats. Journal of Veterinary Internal Medicine 1999 May-Jun; 13(3), 146–152.

TerWee, J., Sabara, M., Kokjohn, K., Sandbulte, J., Frenchick, P., & Dreier, K. J. (1998). Characterization of the systemic disease and ocular signs induced by experimental infection with *Chlamydia psittaci* in cats. Veterinary Microbiology 1998 Jan 31; 59(4), 259–281.

Tichopad, A., Didier, A., & Pfaffl, M. W. (2004). Inhibition of real-time RT-PCR quantification due to tissue-specific contaminants. Molecular and Cellular Probes 2004 Feb; 18(1), 45–50. DOI: 10.1016/j.mcp.2003.09.001

Thygeson, P. (1951). The trachoma-psittacosis lymphogranuloma venereum group of viruses: the *Chlamydozoaceae*. American Journal of Ophthalmology 1951 May; 34(5 2), 7–34. DOI: 10.1016/0002-9394(51)90005-0

Von Bomhard, W., Polkinghorne, A., Lu, Z. H., Vaughan, L., Vögtlin, A., Zimmermann, D. R., Spiess, B., & Pospischil, A. (2003). Detection of novel chlamydiae in cats with ocular disease. American Journal of Veterinary Disease 2003 Nov; 64(11), 1421–1428.

Wills, J. M., Gruffydd-Jones, T. J., Richmond, S. J., Gaskell, R. M., & Bourne, F. J. (1987). Effect of vaccination on feline *Chlamydia psittaci* Infection. Infection and Immunity 1987 Nov; 55(11), 2653–2657.

Wons, J., Meiller, R., Bergua, A., Bogdan, C., & Geissdörfler, W. (2017). Follicular conjunctivitis due to *Chlamydia felis* – case report, review of the literature and improved molecular diagnostics. Frontiers in Medicine 2017 Jul 17; 4, 105. DOI: 10.3389/fmed.2017.00105

Yan, C., Fukushi, H., Matsudate, H., Ishihara, K., Yasuda, K., Kitagawa, H., Yamaguchi, T., & Hirai, K. (2000). Seroepidemiological investigation of feline chlamydiosis in cats and humans in Japan. Microbiology and Immunology 2000; 44(3), 155–160.

York, C. J., & Baker, J. A. (1951). A new member of the psittacosis-lymphogranuloma group of viruses that causes infection in calves. The Journal of Experimental Medicine 1951 Jun; 93(6), 587–604.

Alere Technologies https://alere-technologies.com/products/lab-solutions/chlamydia/chlamydia-identification-23s.html (11.10.18)

Alere Technologies https://alere-technologies.com/products/lab-solutions/platforms/arraystrip-as.html (11.10.2018)

8 Acknowledgements

First, I would like to thank my supervisor Prof. Dr. med. vet. Nicole Borel from the Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland for her dedicated support and valuable advice during the entire process of this Master thesis. I would also like to thank Theresa Pesch, Barbara Prähauser, Hanna Marti und Jasmin Kuratli from the Institute of Veterinary Pathology in Zurich for their excellent support regarding technical questions as well as laboratory work. Many thanks to Bettina Gächter, Nicole Winter, Karen Barmettler and Helin Bozu to whom I am very grateful for helping me collecting samples. Lastly, I would like to thank the animal welfare organisation NetAP (Network for Animal Protection) who kindly let us attend their neutering programs in order to collect samples and therefore enabled this study.

9 Annex

9.1 Statistical analysis

Correlation between ocular signs and the occurrence of *C. felis*

	Asymptomatic	Symptomatic
Negative	106	16
Positive	5	22

Correlation between age and infection with *C. felis*

	Adult	Young
Negative	51	48
Positive	20	4

Correlation between sex and infection with *C. felis*

	Female	Male
Negative	74	49
Positive	15	12

Difference between ocular and rectal excretion of *Chlamydiaceae*

	Eyes	Rectum
Negative	123	143
Positive	25	5

Summary of statistical analysis:

Risk factor	Test	Test-value	Critical value*	p-value	Conf. interval	significant
Ocular signs	Fishers's exact	-	-	1.261e-11	0.95	Yes
Age	Fisher's exact	-	-	0.005307	0.95	Yes
Sex	Pearson's Chi-squared	0.19475	3.84	0.659	0.95	No
Sampling site	McNemar's Chi-squared	82.881	3.84	$< 2.2e^{-16}$	0.95	Yes

* according to the statistical consulting of the University of Zurich

9.2 Data sets

Data cats

Year	Canton	Animal ID	Age	Sex	Conjunctivitis	Missing samples
2017	FR	A18I	Unknown	Female	No	-
2017	FR	A19AA	Young	Female	No	-
2017	FR	A19J	Unknown	Female	No	-
2017	FR	A25K	Adult	Female	No	-
2017	FR	A2F	Adult	Female	No	-
2017	FR	A30E	Unknown	Female	No	-
2017	FR	A3F	Young	Female	No	-
2017	FR	A4F	Adult	Female	Unknown	Ocular swab(s)
2017	FR	A4GN	Adult	Male	No	-
2017	FR	A52BB	Young	Female	No	-
2017	FR	A54CC	Adult	Female	No	-
2017	FR	A5F	Unknown	Male	No	-
2017	FR	A6E	Adult	Female	Yes	-
2017	FR	A75A	Young	Female	No	-
2017	FR	A76A	Young	Female	No	-

2017	FR	A79B	Unknown	Female	No	-
2017	FR	A7E	Unknown	Female	Yes	-
2017	FR	A80E	Young	Female	No	-
2017	FR	A8G	Young	Female	No	-
2017	FR	A90C	Young	Female	No	-
2017	FR	A9G	Adult	Female	No	-
2017	FR	AC81	Young	Male	No	-
2017	FR	AC83	Young	Female	No	-
2017	FR	AC92	Young	Female	No	-
2017	FR	ACC56	Adult	Female	No	-
2017	FR	AH24	Unknown	Female	No	-
2017	FR	AM44	Unknown	Male	No	-
2017	FR	AO47	Adult	Female	Yes	-
2017	NW	BC2	Adult	Male	Yes	-
2017	NW	BE1	Adult	Female	No	-
2017	NW	BF1	Adult	Female	Yes	-
2017	NW	BI1	Young	Male	No	-
2017	NW	BL4	Adult	Male	No	-
2017	NW	BO1	Young	Female	No	-
2017	NW	BO2	Adult	Male	No	-
2017	NW	BT2	Adult	Male	No	-
2017	NW	BX2	Adult	Male	Yes	-
2017	NW	BY1	Adult	Female	Yes	-
2017	NW	BY2	Adult	Female	Yes	-
2017	NW	BZ1	Adult	Male	No	-
2017	NW	BZ2	Young	Female	Yes	-
2017	NW	BZ3	Young	Female	No	-
2017	NW	CB1	Adult	Male	Yes	-
2017	NW	CB2	Unknown	Female	No	-
2017	NW	CD1	Unknown	Female	Yes	-
2017	NW	CD3	Unknown	Male	Yes	-
2017	NW	CE2	Unknown	Male	Yes	-
2017	NW	CE3	Unknown	Female	No	-
2017	NW	CE4	Unknown	Male	Yes	-
2017	NW	CE5	Unknown	Male	No	-
2017	NW	CE6	Unknown	Male	No	-
2017	NW	CS1	Unknown	Female	Yes	-
2017	NW	CS2	Unknown	Female	Yes	-
2017	NW	CS4	Unknown	Female	unknown	unknown
2017	NW	CS5	Adult	Female	Yes	-
2017	NW	CS6	Unknown	Female	unknown	unknown
2017	NW	CS8	Unknown	Female	Yes	-
2017	NW	CT1	Unknown	Female	Yes	-
2017	NW	CW1	Unknown	Female	No	-
2017	NW	CW2	Unknown	Female	No	-
2017	NW	CW4	Unknown	Male	Yes	-
2017	NW	CW5	Unknown	Female	No	-
2017	NW	CX1	Unknown	Female	No	-
2018	FR	DA1	Young	Female	No	-
2018	FR	DC1	Adult	Male	No	-
2018	FR	DC2	Young	Female	No	-
2018	FR	DD1	Unknown	Female	No	-
2018	FR	DD2	Young	Male	Yes	-
2018	FR	DD3	Adult	Male	No	-
2018	FR	DE1	Adult	Female	No	-
2018	FR	DE10	Adult	Female	No	-
2018	FR	DE11	Young	Male	No	-
2018	FR	DE2	Young	Female	No	-
2018	FR	DE3	Adult	Male	No	-
2018	FR	DE5	Young	Female	No	-
2018	FR	DE6	Young	Male	No	-
2018	FR	DE9	Adult	Female	No	-
2018	FR	DF1	Adult	Female	No	-
2018	FR	DF2	Adult	Female	No	-
2018	FR	DF3	Young	Female	No	-
2018	FR	DF4	Young	Female	No	-
2018	FR	DG1	Young	Male	No	-
2018	FR	DG2	Adult	Female	No	-
2018	FR	DG3	Adult	Female	No	-
2018	FR	DH1	Young	Female	No	-
2018	FR	DH4	Young	Female	No	-

2018	FR	DH5	Adult	Male	No	-
2018	FR	DH6	Adult	Female	No	Rectal swab
2018	FR	DI1	Young	Female	No	-
2018	FR	DJ1	Young	Female	No	-
2018	FR	DK1	Adult	Female	Yes	-
2018	FR	DL3	Adult	Male	No	-
2018	NW	EA1	Adult	Male	No	-
2018	NW	EA2	Young	Female	No	-
2018	NW	EA3	Adult	Female	No	-
2018	NW	EB1	Young	Male	No	-
2018	NW	EB2	Young	Female	No	-
2018	NW	EB3	Young	Female	No	-
2018	NW	EB4	Young	Male	No	-
2018	NW	EB5	Adult	Male	Yes	-
2018	NW	EC1	Young	Male	No	-
2018	NW	EC6	Adult	Male	No	-
2018	NW	ED5	Young	Male	No	-
2018	NW	ED6	Adult	Male	No	-
2018	NW	ED7	Adult	Male	Yes	-
2018	NW	ED8	Adult	Male	Yes	-
2018	NW	EE1	Adult	Male	No	-
2018	NW	EF1	Young	Male	No	-
2018	NW	EF2	Young	Female	No	-
2018	NW	EF3	Young	Female	No	-
2018	NW	EG2	Adult	Male	No	-
2018	NW	EG3	Adult	Female	No	-
2018	NW	EG4	Young	Male	No	-
2018	NW	EH1	Adult	Female	No	-
2018	NW	EH2	Young	Male	No	-
2018	NW	EH5	Adult	Male	No	-
2018	NW	EI1	Adult	Female	Yes	Rectal swab
2018	NW	EI2	Adult	Female	No	-
2018	NW	EI3	Young	Female	Yes	-
2018	NW	EI4	Young	Female	Yes	-
2018	NW	EJ1	Young	Male	No	-
2018	NW	EJ2	Adult	Male	No	-
2018	NW	EJ3	Young	Female	Yes	-
2018	NW	EJ4	Adult	Female	No	-
2018	NW	EJ6	Adult	Female	No	-
2018	NW	EK1	Adult	Male	Yes	-
2018	NW	EL2	Adult	Male	No	-
2018	NW	EM1	Adult	Male	No	-
2018	NW	EM11	Adult	Female	Yes	-
2018	NW	EM12	Adult	Male	Yes	-
2018	NW	EM13	Adult	Male	Yes	-
2018	NW	EM15	Adult	Male	No	-
2018	NW	EM2	Young	Male	Yes	Second eye swab
2018	NW	EM3	Adult	Male	Yes	Second eye swab
2018	NW	EM4	Adult	Male	No	-
2018	NW	EM6	Young	Female	Yes	-
2018	NW	EN1	Adult	Male	No	-
2018	NW	EO1	Young	Male	No	-
2018	NW	EQ1	Adult	Female	No	-
2018	NW	EQ2	Adult	Female	No	-
2018	NW	ER1	Adult	Male	No	-
2018	NW	ES1	Adult	Male	Yes	-
2018	NW	ES2	Adult	Male	Yes	-
2018	NW	ET1	Young	Male	No	-
2018	NW	ET2	Adult	Female	No	-
2018	NW	EU2	Adult	Female	No	-
2018	NW	EV1	Young	Female	No	-
2018	NW	EV2	Young	Female	No	-
2018	NW	EW1	Adult	Female	No	-
2018	NW	EX1	Adult	Male	No	-
2018	NW	EY2	Young	Female	No	-
2018	NW	EY5	Young	Female	1	-
2018	NW	EZ1	Adult	Male	No	-
2018	NW	EZ2	Adult	Female	No	-

Data samples: Nanodrop

Animal ID	Sample ID	Position	DNA (ng/µl)	260/280	260/230
A18I	A18Ie	Eye	20.76	1.47	5.00
	A18Ir	Rectum	19.59	1.56	-7.98
A19AA	A19AAe	Eye	9.73	1.36	-1.55
	A19AAr	Rectum	14.18	1.41	-3.55
A19J	A19Je	Eye	34.51	1.84	7.66
	A19Jr	Rectum	21.33	1.64	-8.91
A25K	A25Ke	Eye	7.77	1.23	-1.03
	A25Kr	Rectum	38.92	1.61	1.56
A2F	A2Fe	Eye	27.74	1.53	5.36
	A2Fr	Rectum	34.3	1.64	4.87
A30E	A30Ee	Eye	9.97	1.24	-1.90
	A30Er	Rectum	6.72	1.28	-0.72
A3F	A3Fe	Eye	7.47	1.31	-0.86
	A3Fr	Rectum	24.6	1.55	2.22
A4F	A4Fr	Rectum	56.68	1.61	1.25
A4GN	A4GNe	Eye	8.43	1.64	-0.86
	A4GNr	Rectum	6.9	1.14	-0.72
A52BB	A52BBe	Eye	28.28	1.53	2.22
	A52BBr	Rectum	35.21	1.77	4.91
A54CC	A54CCe	Eye	7.96	1.24	-1.25
	A54CCr	Rectum	11.9	1.42	-3.02
A5F	A5Fe	Eye	4.99	1.65	-0.44
	A5Fr	Rectum	51.87	1.87	3.35
A6E	A6E.1e	Eye 1	51.26	1.69	4.11
	A6E.2e	Eye 2	108.15	1.83	2.88
	A6Er	Rectum	40.49	1.70	4.80
A75A	A75Ae	Eye	13.2	1.38	-2.63
	A75Ar	Rectum	9.87	1.21	-1.96
A76A	A76Ae	Eye	10.92	1.27	-2.5
	A76Ar	Rectum	117.59	1.94	2.51
A79B	A79Be	Eye	14.2	1.59	-6.5
	A79Br	Rectum	11.99	1.24	-2.79
A7E	A7E.1e	Eye 1	236.62	1.86	2.54
	A7E.2e	Eye 2	9.56	1.20	-1.71
	A7Er	Rectum	29.12	1.61	6.58
A80E	A80Ee	Eye	22.65	1.42	5.81
	A80Er	Rectum	16.32	1.41	-4.33
A8G	A8Ge	Eye	10.56	1.23	-1.9
	A8Gr	Rectum	15.29	1.47	-3.41
A90C	A90Ce	Eye	8.00	1.12	-1.25
	A90Cr	Rectum	12.92	1.52	-1.85
A9G	A9Ge	Eye	14.34	1.49	-2.79
	A9Gr	Rectum	17.68	1.53	-9.95
AC81	AC81e	Eye	7.99	1.12	-1.06
	AC81r	Rectum	9.76	1.20	-1.73
AC83	AC83e	Eye	16.76	1.53	-5.16
	AC83r	Rectum	35.11	1.69	4.03
AC92	AC92e	Eye	11.19	1.27	-2.08
	AC92r	Rectum	16.57	1.60	-7.73
ACC56	ACC56e	Eye	17.32	1.53	-5.59
	ACC56r	Rectum	57.3	1.85	3.31
AH24	AH24e	Eye	26.92	1.57	8.45
	AH24r	Rectum	44.16	1.74	2.54
AM44	AM44e	Eye	7.80	1.03	-1.00
	AM44.1r	Rectum	36.15	1.75	7.65
	AM44.2r	Rectum	110.36	1.87	2.58
AO47	AO47.1e	Eye 1	11.62	1.21	-2.67
	AO47.2e	Eye 2	9.13	1.44	-1.17
	AO47r	Rectum	51.77	1.73	3.33
BC2	BC2.1e	Eye 1	14.7	1.89	-41.68
	BC2.2e	Eye 2	14.35	1.77	-6.15
	BC2r	Rectum	83.57	1.81	2.67
BE1	BE1e	Eye	8.20	1.11	-1.18
	BE1r	Rectum	37.73	1.83	5.29
BF1	BF1.1e	Eye 1	78.25	1.78	3.47
	BF1.2e	Eye 2	13.31	1.34	-2.25
	BF1r	Rectum	11.38	1.32	-1.94
BI1	BI1e	Eye	5.76	1.19	-0.59

	BI1r	Rectum	64.21	1.89	3.46
BL4	BL4e	Eye	48.62	1.74	5.28
	BL4r	Rectum	20.56	1.53	-11.88
BO1	BO1e	Eye	10.61	1.22	-1.53
	BO1r	Rectum	10.1	1.33	-1.16
BO2	BO2e	Eye	9.75	1.15	-1.76
	BO2r	Rectum	5.63	1.10	-0.55
BT2	BT2e	Eye	14.84	1.32	-4.50
	BT2r	Rectum	13.16	1.22	-4.53
BX2	BX2.1e	Eye 1	11.61	1.16	-2.43
	BX2.2e	Eye 2	109	1.78	2.91
	BX2r	Rectum	21.39	1.60	-58.6
BY1	BY1.1e	Eye 1	8.93	1.10	-1.64
	BY1.2e	Eye 2	60.25	1.69	3.33
	BY1r	Rectum	8.65	1.06	-1.42
BY2	BY2.1e	Eye 1	36.49	1.69	5.89
	BY2.2e	Eye 2	46.57	1.71	4.11
	BY2r	Rectum	13.07	1.45	-2.42
BZ1	BZ1e	Eye	8.17	1.05	-1.13
	BZ1r	Rectum	12.9	1.30	-2.90
BZ2	BZ2.1e	Eye 1	9.13	1.12	-1.55
	BZ2.2e	Eye 2	8.03	0.97	-1.21
	BZ2r	Rectum	34.77	1.59	3.03
BZ3	BZ3e	Eye	8.15	1.14	-1.10
	BZ3r	Rectum	63.78	1.73	3.44
CB1	CB1.1e	Eye 1	7.75	1.29	-1.15
	CB1.2e	Eye 2	6.35	1.41	-0.76
	CB1r	Rectum	42.84	1.87	4.25
CB2	CB2e	Eye	8.14	1.32	-1.18
	CB2r	Rectum	9.20	1.28	-1.65
CD1	CD1.1e	Eye 1	9.32	1.31	-1.70
	CD1.2e	Eye 2	9.52	1.35	-1.74
	CD1r	Rectum	26.67	1.65	10.82
CD3	CD3.1e	Eye 1	7.28	1.38	-0.93
	CD3.2e	Eye 2	7.21	1.48	-0.82
	CD3r	Rectum	11.61	1.60	-1.84
CE2	CE2.1e	Eye 1	8.25	1.22	-1.22
	CE2.2e	Eye 2	5.33	1.32	-0.5
	CE2r	Rectum	12.00	1.69	-1.8
CE3	CE3e	Eye	9.84	1.28	-1.64
	CE3r	Rectum	41.94	1.76	2.67
CE4	CE4.1e	Eye 1	9.13	1.27	-1.39
	CE4.2e	Eye 2	8.29	1.45	-1.18
	CE4r	Rectum	8.76	1.52	-1.00
CE5	CE5e	Eye	7.46	1.17	-0.90
	CE5r	Rectum	10.63	1.36	-2.27
CE6	CE6e	Eye	7.20	1.32	-0.83
	CE6r	Rectum	12.94	1.54	-2.42
CS1	CS1.1e	Eye 1	6.46	1.36	-0.72
	CS1.2e	Eye 2	8.63	1.24	-1.31
	CS1r	Rectum	39.26	1.72	6.25
CS2	CS2.1e	Eye 1	20.41	1.57	-9.09
	CS2.2e	Eye 2	7.87	1.37	-0.97
	CS2r	Rectum	8.77	1.30	-1.37
CS4	CS4	Unknown	6.39	1.37	-0.65
CS5	CS5.1e	Eye 1	8.36	1.37	-0.98
	CS5.2e	Eye 2	7.02	1.59	-0.76
	CS5r	Rectum	15.06	1.66	-4.18
CS6	CS6	Unknown	11.84	1.41	-1.64
CS8	CS8.1e	Eye 1	7.03	1.27	-0.68
	CS8.2e	Eye 2	11.93	1.76	-1.6
	CS8r	Rectum	9.08	1.39	-1.32
CT1	CT1.1e	Eye 1	6.10	1.30	-0.59
	CT1.2e	Eye 2	9.68	1.19	-1.7
	CT1r	Rectum	8.53	1.22	-1.23
CW1	CW1e	Eye	5.53	1.15	-0.52
	CW1r	Rectum	18.39	1.59	-11.02
CW2	CW2e	Eye	9.47	1.33	-1.51
	CW2r	Rectum	277.83	1.80	1.47
CW4	CW4.1e	Eye 1	10.36	1.47	-1.59
	CW4.2e	Eye 2	10.38	1.27	-2.13

	CW4r	Rectum	12.77	1.43	-3.36
CW5	CW5e	Eye	7.13	1.25	-0.84
	CW5r	Rectum	125.25	1.83	2.74
CX1	CX1e	Eye	7.05	1.12	-0.82
	CX1r	Rectum	25.43	1.73	8.61
DA1	DA1e	Eye	8.98	0.18	0.121
	DA1r	Rectum	39.02	0.78	0.424
DC1	DC1e	Eye	30.57	0.61	0.342
	DC1r	Rectum	1696.81	33.94	18.183
DC2	DC2e	Eye	19.34	0.39	0.213
	DC2r	Rectum	89.72	1.79	0.981
DD1	DD1e	Eye	26.02	0.52	0.323
	DD1r	Rectum	35.85	0.72	0.373
DD2	DD2.1e	Eye 1	5.74	0.12	0.069
	DD2.2e	Eye 2	16.75	0.34	0.199
	DD2r	Rectum	98.33	1.97	1.00
DD3	DD3e	Eye	15.78	0.32	0.168
	DD3r	Rectum	45.62	0.91	0.466
DE1	DE1e	Eye	6.58	0.13	0.10
	DE1r	Rectum	8.21	0.16	0.098
DE10	DE10e	Eye	33.35	0.67	0.361
	DE10r	Rectum	7.06	0.14	0.096
DE11	DE11e	Eye	26.38	0.53	0.297
	DE11r	Rectum	12.05	0.24	0.139
DE2	DE2e	Eye	20.76	0.42	0.287
	DE2r	Rectum	64.29	1.29	0.679
DE3	DE3e	Eye	3.46	0.07	0.04
	DE3r	Rectum	9.57	0.19	0.104
DE5	DE5e	Eye	11.66	0.23	0.105
	DE5r	Rectum	16.49	0.33	0.165
DE6	DE6e	Eye	21.13	0.42	0.252
	DE6r	Rectum	22.19	0.44	0.258
DE9	DE9e	Eye	14.08	0.28	0.148
	DE9r	Rectum	11.05	0.22	0.124
DF1	DF1e	Eye	4.51	0.09	0.045
	DF1r	Rectum	52.34	1.05	0.52
DF2	DF2e	Eye	15.13	0.30	0.177
	DF2r	Rectum	25.51	0.51	0.282
DF3	DF3e	Eye	4.02	0.08	0.052
	DF3r	Rectum	33.49	0.67	0.367
DF4	DF4e	Eye	66.06	1.32	0.703
	DF4r	Rectum	311.5	6.23	3.24
DG1	DG1e	Eye	26.23	0.53	0.306
	DG1r	Rectum	29.01	0.58	0.304
DG2	DG2e	Eye	28.38	0.57	0.309
	DG2r	Rectum	28.24	0.57	0.305
DG3	DG3e	Eye	59.59	1.192	0.681
	DG3r	Rectum	68.52	1.37	0.767
DH1	DH1e	Eye	40.64	0.813	0.449
	DH1r	Rectum	14.02	0.28	0.159
DH4	DH4e	Eye	22.48	0.45	0.278
	DH4r	Rectum	258.47	5.169	2.886
DH5	DH5e	Eye	35.16	0.703	0.373
	DH5r	Rectum	27.64	0.553	0.283
DH6	DH6e	Eye	8.19	0.164	0.094
DI1	DI1e	Rectum	32.27	0.645	0.359
	DI1r	Eye	25.93	0.519	0.281
DJ1	DJ1e	Rectum	25.71	0.514	0.285
	DJ1r	Eye	167.26	3.345	1.786
DK1	DK1.1e	Eye	9.59	0.192	0.105
	DK1.2e	Eye	23.76	0.475	0.269
	DK1.3e	Eye	16.47	0.329	0.19
	DK1r	Rectum	133.23	2.665	1.418
DL3	DL3e	Eye	32.37	0.647	0.359
	DL3r	Rectum	5.67	0.113	0.072
EA1	EA1e	Eye	7.51	1.44	1.07
	EA1r	Rectum	48.93	1.70	1.12
EA2	EA2e	Eye	8.55	1.57	1.06
	EA2r	Rectum	27.73	1.79	1.53
EA3	EA3e	Eye	9.28	1.56	1.08
	EA3r	Rectum	37.58	1.72	1.58

EB1	EB1e	Eye	26.79	1.62	1.34
	EB1r	Rectum	54.95	1.77	1.63
EB2	EB2e	Eye	10.57	1.32	1.19
	EB2e	Rectum	25.69	1.62	1.11
EB3	EB3e	Eye	10.07	1.72	1.18
	EB3r	Rectum	23.13	1.73	1.47
EB4	EB4e	Eye	8.63	1.85	1.11
	EB4r	Rectum	64.24	1.85	1.53
EB5	EB5.1e	Eye 1	9.27	1.48	1.02
	EB5.2e	Eye 2	8.23	1.47	1.11
	EB5r	Rectum	34.00	1.65	1.37
EC1	EC1e	Eye	10.69	1.31	1.07
	EC1r	Rectum	129.01	1.88	1.85
EC6	EC6e	Eye	27.75	1.58	1.40
	EC6r	Rectum	36.41	1.71	1.48
ED5	ED5e	Eye	18.27	1.51	1.00
	ED5r	Rectum	50.18	1.8	1.47
ED6	ED6e	Eye	24.42	1.61	1.22
	ED6r	Rectum	21.26	1.69	1.28
ED7	ED7.1e	Eye 1	23.55	1.71	1.40
	ED7.2e	Eye 2	20.66	1.65	1.38
	ED7r	Rectum	23.70	1.65	1.37
ED8	ED8.1e	Eye 1	179.59	1.88	2.01
	ED8.2e	Eye 2	135.15	1.87	2.00
	ED8r	Rectum	79.56	1.83	1.67
EE1	EE1e	Eye	17.5	1.57	1.3
	EE1r	Rectum	41.44	1.95	1.69
EF1	EF1e	Eye	5.99	1.48	0.83
	EF1r	Rectum	29.32	1.66	1.10
EF2	EF2e	Eye	13.52	1.44	1.13
	EF2r	Rectum	88.81	1.93	1.68
EF3	EF3e	Eye	22.92	1.55	1.21
	EF3r	Rectum	35.85	1.74	1.47
EG2	EG2e	Eye	9.13	1.25	0.91
	EG2r	Rectum	22.51	1.56	1.12
EG3	EG3e	Eye	11.47	1.41	1.06
	EG3r	Rectum	63.34	1.89	1.72
EG4	EG4e	Eye	11.84	1.55	0.94
	EG4r	Rectum	55.81	1.97	1.90
EH1	EH1e	Eye	8.05	1.49	0.98
	EH1r	Rectum	63.39	1.83	1.71
EH2	EH2e	Eye	11.03	1.49	0.95
	EH2r	Rectum	150.42	1.88	1.21
EH5	EH5e	Eye	5.21	1.48	0.88
	EH5r	Rectum	82.06	1.87	1.69
EI1	EI1.1e	Eye 1	18.85	1.61	1.09
	EI1.2e	Eye 2	15.3	1.71	1.28
	EI2e	Rectum	14.65	1.37	1.05
EI2	EI2r		24.85	1.89	1.47
	EI3.1e		87.11	1.85	1.88
EI3	EI3.2e	Eye 1	191.33	1.93	2.07
	EI3r	Eye 2	32.74	1.87	1.75
	EI4.1e	Rectum	84.97	1.89	1.93
EI4	EI4.2e	Eye 1	294.84	1.94	2.19
	EI4r	Eye 2	32.93	1.92	1.70
	EJ1e	Rectum	27.32	1.53	1.04
EJ1	EJ1r	Eye	53.37	1.83	1.49
	EJ2e	Rectum	20.24	1.52	1.32
EJ2	EJ2r	Eye	59.96	1.89	1.32
	EJ3.1e	Rectum	9.39	1.20	0.85
EJ3	EJ3.2e	Eye	12.21	1.59	0.99
	EJ3r	Rectum	80.97	1.97	1.60
EJ4	EJ4e	Eye	10.12	1.27	0.91
	EJ4r	Rectum	37.56	1.79	1.47
EJ6	EJ6e	Eye	12.25	1.49	1.14
	EJ6r	Rectum	17.92	1.65	1.25
EK1	EK1.1e	Eye 1	30.6	1.78	1.39
	EK1.2e	Eye 2	226.23	1.91	1.94
	EK1r	Rectum	34.78	1.75	1.21
EL2	EL2e	Eye	8.82	1.33	0.95
	EL2r	Rectum	59.35	1.83	1.52

EM1	EM1e	Eye	28.01	1.55	1.44
	EM1r	Rectum	31.53	1.86	1.50
EM11	EM11.1e	Eye 1	62.85	1.90	1.83
	EM11.2e	Eye 2	38.75	1.78	1.61
	EM11r	Rectum	40.32	1.97	1.51
EM12	EM12.1e	Eye 1	16.64	1.84	1.50
	EM12.2e	Eye 2	15.06	1.59	1.33
	EM12r	Rectum	97.6	1.90	1.97
EM13	EM13.1e	Eye 1	72.66	1.90	1.85
	EM13.2e	Eye 2	306.49	1.93	2.04
	EM13r	Rectum	16.4	1.74	1.36
EM15	EM15e	Eye	9.37	1.36	0.97
	EM15r	Rectum	68.75	1.81	1.53
EM2	EM2.1e	Eye 1	missing		
	EM2.2e	Eye 2	5.33	1.74	1.00
	EM2r	Rectum	104.48	1.95	2.05
EM3	EM3.1e	Eye 1	missing		
	EM3.2e	Eye 2	84.53	1.86	1.81
	EM3r	Rectum	6.36	1.55	0.97
EM4	EM4e	Eye	355.93	1.90	2.07
	EM4r	Rectum	159.53	1.87	1.89
EM6	EM6.1e	Eye 1	30.47	1.82	1.63
	EM6.2e	Eye 2	36.92	1.75	1.56
	EM6r	Rectum	10.36	1.57	1.15
EN1	EN1e	Eye	14.14	1.78	1.05
	EN1r	Rectum	31.38	1.80	1.58
EO1	EO1e	Eye	11.44	1.27	1.1
	EO1r	Rectum	46.07	1.79	1.38
EQ1	EQ1e	Eye	15.24	1.71	1.41
	EQ1r	Rectum	272.89	1.90	1.53
EQ2	EQ2e	Eye	31.63	1.83	1.52
	EQ2r	Rectum	18.39	1.69	1.37
ER1	ER1e	Eye	6.74	1.62	0.88
	ER1r	Rectum	39.44	1.98	1.71
ES1	ES1.1e	Eye 1	7.84	1.63	1.08
	ES1.2e	Eye 2	60.11	1.93	1.77
	ES1r	Rectum	17.95	1.72	1.32
ES2	ES2.1e	Eye 1	14.87	1.96	1.21
	ES2.2e	Eye 2	46.59	1.70	1.40
	ES2r	Rectum	14.49	1.71	1.16
ET1	ET1e	Eye	9.87	1.43	0.99
	ET1r	Rectum	42.01	1.85	1.57
ET2	ET2e	Eye	10.65	1.46	0.93
	ET2r	Rectum	42.54	1.81	1.48
EU2	EU2e	Eye	10.77	1.31	1.07
	EU2r	Rectum	50.94	1.8	1.46
EV1	EV1e	Eye	8.07	1.44	1.07
	EV1r	Rectum	44.25	1.75	1.58
EV2	EV2e	Eye	24.87	1.62	1.21
	EV2r	Rectum	51.69	1.94	1.76
EW1	EW1e	Eye	6.82	1.43	0.95
	EW1r	Rectum	21.73	1.74	1.42
EX1	EX1e	Eye	10.04	1.37	0.99
	EX1r	Rectum	74.23	1.87	1.48
EY2	EY2e	Eye	25.85	1.62	1.23
	EY2r	Rectum	73.79	1.86	1.83
EY5	EY5.1e	Eye 1	20.51	1.51	1.12
	EY5.2e	Eye 2	10.8	1.38	0.95
	EY5r	Rectum	112.01	1.89	1.68
EZ1	EZ1e	Eye	11.51	1.31	0.96
	EZ1r	Rectum	91.77	1.92	1.86
EZ2	EZ2e	Eye	9.98	1.43	0.96
	EZ2r	Rectum	93.92	1.93	1.60

Data samples: *Chlamydiaceae* PCR, Array Mate, Sequencing

Animal ID	Sample ID	Position	RT-PCR for *Chlamydiaceae*				*Chlamydia* species	
			CT1	CT2	Mean CT	Result	ArrayMate	Sequencing
A18I	A18Ie	Eye	ud	ud	ud	Negative		
	A18Ir	Rectum	ud	ud	ud	Negative		
A19AA	A19AAe	Eye	ud	ud	ud	Negative		
	A19AAr	Rectum	ud	ud	ud	Negative		
A19J	A19Je	Eye	ud	ud	ud	Negative		
	A19Jr	Rectum	ud	ud	ud	Negative		
A25K	A25Ke	Eye	ud	ud	ud	Negative		
	A25Kr	Rectum	ud	ud	ud	Negative		
A2F	A2Fe	Eye	ud	ud	ud	Negative		
	A2Fr	Rectum	ud	ud	ud	Negative		
A30E	A30Ee	Eye	ud	ud	ud	Negative		
	A30Er	Rectum	ud	ud	ud	Negative		
A3F	A3Fe	Eye	ud	ud	ud	Negative		
	A3Fr	Rectum	ud	ud	ud	Negative		
A4F	A4Fr	Rectum	ud	ud	ud	Negative		
A4GN	A4GNe	Eye	ud	ud	ud	Negative		
	A4GNr	Rectum	ud	ud	ud	Negative		
A52BB	A52BBe	Eye	ud	ud	ud	Negative		
	A52BBr	Rectum	ud	ud	ud	Negative		
A54CC	A54CCe	Eye	ud	ud	ud	Negative		
	A54CCr	Rectum	ud	ud	ud	Negative		
A5F	A5Fe	Eye	ud	ud	ud	Negative		
	A5Fr	Rectum	ud	ud	ud	Negative		
A6E	A6E.1e	Eye 1	24.88	25.07	24.975	Positive	*C. felis*	
	A6E.2e	Eye 2	24.04	23.98	24.01	Positive	*C. felis*	
	A6Er	Rectum	ud	ud	ud	Negative		
A75A	A75Ae	Eye	ud	ud	ud	Negative		
	A75Ar	Rectum	24.95	24.82	24.885	Positive	*Negative*	*No sequence*
A76A	A76Ae	Eye	ud	ud	ud	Negative		
	A76Ar	Rectum	ud	ud	ud	Negative		
A79B	A79Be	Eye	ud	ud	ud	Negative		
	A79Br	Rectum	ud	ud	ud	Negative		
A7E	A7E.1e	Eye 1	36.59	35.6	36.095	Positive	*Chlamydiaceae*	*C. felis*
	A7E.2e	Eye 2	ud	ud	ud	Negative		
	A7Er	Rectum	ud	ud	ud	Negative		
A80E	A80Ee	Eye	ud	ud	ud	Negative		
	A80Er	Rectum	ud	ud	ud	Negative		
A8G	A8Ge	Eye	ud	ud	ud	Negative		
	A8Gr	Rectum	ud	ud	ud	Negative		
A90C	A90Ce	Eye	ud	ud	ud	Negative		
	A90Cr	Rectum	ud	ud	ud	Negative		
A9G	A9Ge	Eye	ud	ud	ud	Negative		
	A9Gr	Rectum	ud	ud	ud	Negative		
AC81	AC81e	Eye	ud	ud	ud	Negative		
	AC81r	Rectum	ud	ud	ud	Negative		
AC83	AC83e	Eye	ud	ud	ud	Negative		
	AC83r	Rectum	ud	ud	ud	Negative		
AC92	AC92e	Eye	ud	ud	ud	Negative		
	AC92r	Rectum	ud	ud	ud	Negative		
ACC56	ACC56e	Eye	ud	ud	ud	Negative		
	ACC56r	Rectum	ud	ud	ud	Negative		
AH24	AH24e	Eye	ud	ud	ud	Negative		
	AH24r	Rectum	ud	ud	ud	Negative		
AM44	AM44e	Eye	ud	ud	ud	Negative		
	AM44.1r	Rectum	ud	ud	ud	Negative		
	AM44.2r	Rectum	ud	ud	ud	Negative		
AO47	AO47.1e	Eye 1	ud	ud	ud	Negative		
	AO47.2e	Eye 2	35.0519	36.641	35.84645	Positive	*Chlamydiaceae*	*C. felis*
	AO47r	Rectum	ud	ud	ud	Negative		
BC2	BC2.1e	Eye 1	35.1719	34.9677	35.0698	Positive	*C. felis*	
	BC2.2e	Eye 2	35.0067	34.7748	34.89075	Positive	*C. felis*	
	BC2r	Rectum	ud	ud	ud	Negative		
BE1	BE1e	Eye	ud	ud	ud	Negative		
	BE1r	Rectum	ud	ud	ud	Negative		
BF1	BF1.1e	Eye 1	31.5552	31.3297	31.44245	Positive	*C. felis*	
	BF1.2e	Eye 2	36.7327	35.4988	36.11575	Positive	*Negative*	*C. felis*
	BF1r	Rectum	ud	ud	ud	Negative		

BI1	BI1e	Eye	ud	ud	ud	Negative		
	BI1r	Rectum	ud	ud	ud	Negative		
BL4	BL4e	Eye	ud	ud	ud	Negative		
	BL4r	Rectum	ud	ud	ud	Negative		
BO1	BO1e	Eye	ud	ud	ud	Negative		
	BO1r	Rectum	ud	ud	ud	Negative		
BO2	BO2e	Eye	ud	ud	ud	Negative		
	BO2r	Rectum	ud	ud	ud	Negative		
BT2	BT2e	Eye	34.3094	34.5487	34.42905	Positive	*C. felis*	
	BT2r	Rectum	ud	ud	ud	Negative		
BX2	BX2.1e	Eye 1	37.9373	36.5315	37.2344	Positive	*C. felis*	
	BX2.2e	Eye 2	32.7982	32.8616	32.8299	Positive	*C. felis*	
	BX2r	Rectum	ud	ud	ud	Negative		
BY1	BY1.1e	Eye 1	ud	ud	ud	Negative		
	BY1.2e	Eye 2	34.2376	33.5535	33.89555	Positive	*C. felis*	
	BY1r	Rectum	ud	ud	ud	Negative		
BY2	BY2.1e	Eye 1	31.1352	31.496	31.3156	Positive	*C. felis*	
	BY2.2e	Eye 2	ud	ud	ud	Negative		
	BY2r	Rectum	32.1063	33.9275	33.0169	Positive	*C. felis*	
BZ1	BZ1e	Eye	ud	ud	ud	Negative		
	BZ1r	Rectum	ud	ud	ud	Negative		
BZ2	BZ2.1e	Eye 1	ud	ud	ud	Negative		
	BZ2.2e	Eye 2	ud	ud	ud	Negative		
	BZ2r	Rectum	ud	ud	ud	Negative		
BZ3	BZ3e	Eye	ud	ud	ud	Negative		
	BZ3r	Rectum	ud	ud	ud	Negative		
CB1	CB1.1e	Eye 1	ud	ud	ud	Negative		
	CB1.2e	Eye 2	ud	ud	ud	Negative		
	CB1r	Rectum	ud	ud	ud	Negative		
CB2	CB2e	Eye	ud	ud	ud	Negative		
	CB2r	Rectum	ud	ud	ud	Negative		
CD1	CD1.1e	Eye 1	ud	ud	ud	Negative		
	CD1.2e	Eye 2	ud	ud	ud	Negative		
	CD1r	Rectum	ud	ud	ud	Negative		
CD3	CD3.1e	Eye 1	ud	ud	ud	Negative		
	CD3.2e	Eye 2	ud	ud	ud	Negative		
	CD3r	Rectum	ud	ud	ud	Negative		
CE2	CE2.1e	Eye 1	ud	ud	ud	Negative		
	CE2.2e	Eye 2	ud	ud	ud	Negative		
	CE2r	Rectum	ud	ud	ud	Negative		
CE3	CE3e	Eye	ud	ud	ud	Negative		
	CE3r	Rectum	ud	ud	ud	Negative		
CE4	CE4.1e	Eye 1	ud	ud	ud	Negative		
	CE4.2e	Eye 2	ud	ud	ud	Negative		
	CE4r	Rectum	ud	ud	ud	Negative		
CE5	CE5e	Eye	ud	ud	ud	Negative		
	CE5r	Rectum	ud	ud	ud	Negative		
CE6	CE6e	Eye	ud	ud	ud	Negative		
	CE6r	Rectum	ud	ud	ud	Negative		
CS1	CS1.1e	Eye 1	ud	ud	ud	Negative		
	CS1.2e	Eye 2	ud	ud	ud	Negative		
	CS1r	Rectum	ud	ud	ud	Negative		
CS2	CS2.1e	Eye 1	26.1261	25.8848	26.00545	Positive	*C. felis*	
	CS2.2e	Eye 2	32.0345	32.0445	32.0395	Positive	*C. felis*	
	CS2r	Rectum	ud	ud	ud	Negative		
CS4	CS4	Unknown	29.7162	29.8017	29.75895	Positive	*C. felis*	
CS5	CS5.1e	Eye 1	33.8283	33.5082	33.66825	Positive	*C. felis*	
	CS5.2e	Eye 2	ud	ud	ud	Negative		
	CS5r	Rectum	36.6511	36.2742	36.46265	Positive	*Negative*	*No sequence*
CS6	CS6	Unknown	31.2052	31.4902	31.3477	Positive	*C. felis*	
CS8	CS8.1e	Eye 1	33.3891	33.011	33.20005	Positive	*C. felis*	
	CS8.2e	Eye 2	29.7418	29.804	29.7729	Positive	*C. felis*	
	CS8r	Rectum	ud	ud	ud	Negative		
CT1	CT1.1e	Eye 1	ud	ud	ud	Negative		
	CT1.2e	Eye 2	ud	ud	ud	Negative		
	CT1r	Rectum	ud	ud	ud	Negative		
CW1	CW1e	Eye	ud	ud	ud	Negative		
	CW1r	Rectum	ud	ud	ud	Negative		
CW2	CW2e	Eye	ud	ud	ud	Negative		
	CW2r	Rectum	ud	ud	ud	Negative		
CW4	CW4.1e	Eye 1	ud	ud	ud	Negative		

	CW4.2e	Eye 2	ud	ud	ud	Negative	
	CW4r	Rectum	ud	ud	ud	Negative	
CW5	CW5e	Eye	ud	ud	ud	Negative	
	CW5r	Rectum	ud	ud	ud	Negative	
CX1	CX1e	Eye	ud	ud	ud	Negative	
	CX1r	Rectum	ud	ud	ud	Negative	
DA1	DA1e	Eye	ud	ud	ud	Negative	
	DA1r	Rectum	ud	ud	ud	Negative	
DC1	DC1e	Eye	ud	ud	ud	Negative	
	DC1r	Rectum	ud	ud	ud	Negative	
DC2	DC2e	Eye	ud	ud	ud	Negative	
	DC2r	Rectum	ud	ud	ud	Negative	
DD1	DD1e	Eye	ud	ud	ud	Negative	
	DD1r	Rectum	ud	ud	ud	Negative	
DD2	DD2.1e	Eye 1	ud	ud	ud	Negative	
	DD2.2e	Eye 2	36.90	36.10	36.50	Positive	*C. abortus*
	DD2r	Rectum	ud	ud	ud	Negative	
DD3	DD3e	Eye	ud	ud	ud	Negative	
	DD3r	Rectum	ud	ud	ud	Negative	
DE1	DE1e	Eye	ud	ud	ud	Negative	
	DE1r	Rectum	ud	ud	ud	Negative	
DE10	DE10e	Eye	ud	ud	ud	Negative	
	DE10r	Rectum	ud	ud	ud	Negative	
DE11	DE11e	Eye	ud	ud	ud	Negative	
	DE11r	Rectum	ud	ud	ud	Negative	
DE2	DE2e	Eye	ud	ud	ud	Negative	
	DE2r	Rectum	ud	ud	ud	Negative	
DE3	DE3e	Eye	ud	ud	ud	Negative	
	DE3r	Rectum	ud	ud	ud	Negative	
DE5	DE5e	Eye	ud	ud	ud	Negative	
	DE5r	Rectum	ud	ud	ud	Negative	
DE6	DE6e	Eye	ud	ud	ud	Negative	
	DE6r	Rectum	ud	ud	ud	Negative	
DE9	DE9e	Eye	ud	ud	ud	Negative	
	DE9r	Rectum	ud	ud	ud	Negative	
DF1	DF1e	Eye	ud	ud	ud	Negative	
	DF1r	Rectum	ud	ud	ud	Negative	
DF2	DF2e	Eye	ud	ud	ud	Negative	
	DF2r	Rectum	ud	ud	ud	Negative	
DF3	DF3e	Eye	ud	ud	ud	Negative	
	DF3r	Rectum	ud	ud	ud	Negative	
DF4	DF4e	Eye	ud	ud	ud	Negative	
	DF4r	Rectum	ud	ud	ud	Negative	
DG1	DG1e	Eye	ud	ud	ud	Negative	
	DG1r	Rectum	ud	ud	ud	Negative	
DG2	DG2e	Eye	ud	ud	ud	Negative	
	DG2r	Rectum	ud	ud	ud	Negative	
DG3	DG3e	Eye	ud	ud	ud	Negative	
	DG3r	Rectum	ud	ud	ud	Negative	
DH1	DH1e	Eye	ud	ud	ud	Negative	
	DH1r	Rectum	ud	ud	ud	Negative	
DH4	DH4e	Eye	ud	ud	ud	Negative	
	DH4r	Rectum	ud	ud	ud	Negative	
DH5	DH5e	Eye	ud	ud	ud	Negative	
	DH5r	Rectum	ud	ud	ud	Negative	
DH6	DH6e	Eye	ud	ud	ud	Negative	
DI1	DI1e	Rectum	ud	ud	ud	Negative	
	DI1r	Eye	ud	ud	ud	Negative	
DJ1	DJ1e	Rectum	ud	ud	ud	Negative	
	DJ1r	Eye	ud	ud	ud	Negative	
DK1	DK1.1e	Eye	ud	ud	ud	Negative	
	DK1.2e	Eye	ud	ud	ud	Negative	
	DK1.3e	Eye	ud	ud	ud	Negative	
	DK1r	Rectum	ud	ud	ud	Negative	
DL3	DL3e	Eye	ud	ud	ud	Negative	
	DL3r	Rectum	ud	ud	ud	Negative	
EA1	EA1e	Eye	ud	ud	ud	Negative	
	EA1r	Rectum	ud	ud	ud	Negative	
EA2	EA2e	Eye	ud	ud	ud	Negative	
	EA2r	Rectum	ud	ud	ud	Negative	
EA3	EA3e	Eye	ud	ud	ud	Negative	

	EA3r	Rectum	ud	ud	ud	Negative		
EB1	EB1e	Eye	ud	ud	ud	Negative		
	EB1r	Rectum	ud	ud	ud	Negative		
EB2	EB2e	Eye	ud	ud	ud	Negative		
	EB2e	Rectum	ud	ud	ud	Negative		
EB3	EB3e	Eye	ud	ud	ud	Negative		
	EB3r	Rectum	ud	ud	ud	Negative		
EB4	EB4e	Eye	ud	ud	ud	Negative		
	EB4r	Rectum	ud	ud	ud	Negative		
EB5	EB5.1e	Eye 1	ud	ud	ud	Negative		
	EB5.2e	Eye 2	ud	ud	ud	Negative		
	EB5r	Rectum	ud	ud	ud	Negative		
EC1	EC1e	Eye	ud	ud	ud	Negative		
	EC1r	Rectum	ud	ud	ud	Negative		
EC6	EC6e	Eye	ud	ud	ud	Negative		
	EC6r	Rectum	ud	ud	ud	Negative		
ED5	ED5e	Eye	ud	ud	ud	Negative		
	ED5r	Rectum	ud	ud	ud	Negative		
ED6	ED6e	Eye	ud	ud	ud	Negative		
	ED6r	Rectum	ud	ud	ud	Negative		
ED7	ED7.1e	Eye	37.677	37.9634	37.8202	Positive	*Chlamydiaceae*	*C. felis*
	ED7.2e	Eye 2	35.862	36.817	36.3395	Positive	*Chlamydiaceae*	*C. felis*
	ED7r	Rectum	ud	ud	ud	Negative		
ED8	ED8.1e	Eye 1	32.031	32.04	32.0355	Positive	*C. felis*	
	ED8.2e	Eye 2	32.478	33.064	32.771	Positive	*C. felis*	
	ED8r	Rectum	ud	ud	ud	Negative		
EE1	EE1e	Eye	ud	ud	ud	Negative		
	EE1r	Rectum	ud	ud	ud	Negative		
EF1	EF1e	Eye	ud	ud	ud	Negative		
	EF1r	Rectum	ud	ud	ud	Negative		
EF2	EF2e	Eye	ud	ud	ud	Negative		
	EF2r	Rectum	ud	ud	ud	Negative		
EF3	EF3e	Eye	ud	ud	ud	Negative		
	EF3r	Rectum	ud	ud	ud	Negative		
EG2	EG2e	Eye	ud	ud	ud	Negative		
	EG2r	Rectum	ud	ud	ud	Negative		
EG3	EG3e	Eye	ud	ud	ud	Negative		
	EG3r	Rectum	ud	ud	ud	Negative		
EG4	EG4e	Eye	ud	ud	ud	Negative		
	EG4r	Rectum	ud	ud	ud	Negative		
EH1	EH1e	Eye	ud	ud	ud	Negative		
	EH1r	Rectum	ud	ud	ud	Negative		
EH2	EH2e	Eye	ud	ud	ud	Negative		
	EH2r	Rectum	ud	ud	ud	Negative		
EH5	EH5e	Eye	ud	ud	ud	Negative		
	EH5r	Rectum	ud	ud	ud	Negative		
EI1	EI1.1e	Eye 1	28.5	28.409	28.4545	Positive	*C. felis*	
	EI1.2e	Eye 2	29.69	29.844	29.767	Positive	*C. felis*	
	EI1r	Rectum	Sample missing					
EI2	EI2e	Eye	30.635	30.877	30.756	Positive	*C. felis*	
	EI2r	Rectum	ud	ud	ud	Negative		
EI3	EI3.1e	Eye 1	27.87	27.83	27.85	Positive	*C. felis*	
	EI3.2e	Eye 2	26.051	25.894	25.9725	Positive	*C. felis*	
	EI3r	Rectum	28.373	28.355	28.364	Positive	*Chlamydiaceae*	*C. felis*
EI4	EI4.1e	Eye 1	25.278	24.976	25.127	Positive	*C. felis*	
	EI4.2e	Eye 2	22.159	22.36	22.2595	Positive	*C. felis*	
	EI4r	Rectum	32.924	32.742	32.833	Positive	*Negative*	*C. felis*
EJ1	EJ1e	Eye	ud	ud	ud	Negative		
	EJ1r	Rectum	ud	ud	ud	Negative		
EJ2	EJ2e	Eye	ud	ud	ud	Negative		
	EJ2r	Rectum	ud	ud	ud	Negative		
EJ3	EJ3.1e	Eye 1	ud	ud	ud	Negative		
	EJ3.2e	Eye 2	ud	ud	ud	Negative		
	EJ3r	Rectum	ud	ud	ud	Negative		
EJ4	EJ4e	Eye	ud	ud	ud	Negative		
	EJ4r	Rectum	ud	ud	ud	Negative		
EJ6	EJ6e	Eye	ud	ud	ud	Negative		
	EJ6r	Rectum	ud	ud	ud	Negative		
EK1	EK1.1e	Eye 1	34.623	33.791	34.207	Positive	*C. felis*	
	EK1.2e	Eye 2	29.968	31.016	30.492	Positive	*C. felis*	
	EK1r	Rectum	ud	ud	ud	Negative		

EL2	EL2e	Eye	ud	ud	ud	Negative	
	EL2r	Rectum	ud	ud	ud	Negative	
EM1	EM1e	Eye	28.804	28.757	28.7805	Positive	*C. felis*
	EM1r	Rectum	ud	ud	ud	Negative	
EM11	EM11.1e	Eye 1	ud	ud	ud	Negative	
	EM11.2e	Eye 2	ud	ud	ud	Negative	
	EM11r	Rectum	ud	ud	ud	Negative	
EM12	EM12.1e	Eye 1	31.017	31.356	31.1865	Positive	*C. felis*
	EM12.2e	Eye 2	34.206	33.842	34.024	Positive	*C. felis*
	EM12r	Rectum	ud	ud	ud	Negative	
EM13	EM13.1e	Eye 1	ud	ud	ud	Negative	
	EM13.2e	Eye 2	27.217	27.195	27.206	Positive	*C. felis*
	EM13r	Rectum	ud	ud	ud	Negative	
EM15	EM15e	Eye	ud	ud	ud	Negative	
	EM15r	Rectum	ud	ud	ud	Negative	
EM2	EM2.1e	Eye 1	*Sample missing*				
	EM2.2e	Eye 2	ud	ud	ud	Negative	
	EM2r	Rectum	ud	ud	ud	Negative	
EM3	EM3.1e	Eye 1	Sample missing				
	EM3.2e	Eye 2	25.609	25.791	25.7	Positive	*C. felis*
	EM3r	Rectum	ud	ud	ud	Negative	
EM4	EM4e	Eye	28.48	28.635	28.5575	Positive	*C. felis*
	EM4r	Rectum	ud	ud	ud	Negative	
EM6	EM6.1e	Eye 1	ud	ud	ud	Negative	
	EM6.2e	Eye 2	ud	ud	ud	Negative	
	EM6r	Rectum	ud	ud	ud	Negative	
EN1	EN1e	Eye	ud	ud	ud	Negative	
	EN1r	Rectum	ud	ud	ud	Negative	
EO1	EO1e	Eye	ud	ud	ud	Negative	
	EO1r	Rectum	ud	ud	ud	Negative	
EQ1	EQ1e	Eye	ud	ud	ud	Negative	
	EQ1r	Rectum	ud	ud	ud	Negative	
EQ2	EQ2e	Eye	ud	ud	ud	Negative	
	EQ2r	Rectum	ud	ud	ud	Negative	
ER1	ER1e	Eye	ud	ud	ud	Negative	
	ER1r	Rectum	ud	ud	ud	Negative	
ES1	ES1.1e	Eye 1	ud	ud	ud	Negative	
	ES1.2e	Eye 2	ud	ud	ud	Negative	
	ES1r	Rectum	ud	ud	ud	Negative	
ES2	ES2.1e	Eye 1	ud	ud	ud	Negative	
	ES2.2e	Eye 2	32.175	31.391	31.783	Positive	*C. felis*
	ES2r	Rectum	ud	ud	ud	Negative	
ET1	ET1e	Eye	ud	ud	ud	Negative	
	ET1r	Rectum	ud	ud	ud	Negative	
ET2	ET2e	Eye	ud	ud	ud	Negative	
	ET2r	Rectum	ud	ud	ud	Negative	
EU2	EU2e	Eye	ud	ud	ud	Negative	
	EU2r	Rectum	ud	ud	ud	Negative	
EV1	EV1e	Eye	ud	ud	ud	Negative	
	EV1r	Rectum	ud	ud	ud	Negative	
EV2	EV2e	Eye	ud	ud	ud	Negative	
	EV2r	Rectum	ud	ud	ud	Negative	
EW1	EW1e	Eye	ud	ud	ud	Negative	
	EW1r	Rectum	ud	ud	ud	Negative	
EX1	EX1e	Eye	ud	ud	ud	Negative	
	EX1r	Rectum	ud	ud	ud	Negative	
EY2	EY2e	Eye	ud	ud	ud	Negative	
	EY2r	Rectum	ud	ud	ud	Negative	
EY5	EY5.1e	Eye 1	ud	ud	ud	Negative	
	EY5.2e	Eye 2	ud	ud	ud	Negative	
	EY5r	Rectum	ud	ud	ud	Negative	
EZ1	EZ1e	Eye	ud	ud	ud	Negative	
	EZ1r	Rectum	ud	ud	ud	Negative	
EZ2	EZ2e	Eye	ud	ud	ud	Negative	
	EZ2r	Rectum	ud	ud	ud	Negative	

Sequencing results

Animal ID	Sample ID	Primer	Quality	Result	Comment
A75A	A75Ar	16S-IGF	No sequence	-	
		16S-IGR	No sequence		
A7E	A7E.1e	16S-IGF	Moderate, 18.5 %	*C. felis*	99% nucleotide identity with strain NS9 16S ribosomal RNA gene, partial sequence (JN606073.1) => 1N in sequence; 99% identity to all other *C. felis*
		16S-IGR	Moderate, 34.8 %		
AO47	AO47.2e	16S-IGF	Moderate, 31.1 %	*C. felis*	100% nucleotide identity with strain NS9 16S ribosomal RNA gene, partial sequence (JN606073.1); 100% identity with 10 C. felis sequences, 99% nucleotide identity with 3 *C. felis* clones (AY334534.1)
		16S-IGR	Moderate, 38.3 %		
BF1	BF1.2e	16S-IGF	Moderate, 28. 6 %	*C. felis*	100% nucleotide identity with strain NS9 16S ribosomal RNA gene, partial sequence (JN606073.1); 100% identity with 10 C. felis sequences, 99% nucleotide identity with 3 *C. felis* clones (AY334534.1)
		16S-IGR	Poor, 8.6 %		
CS5	CS5r	16S-IGF	No sequence	-	
		16S-IGR	No sequence		
ED7	ED7.1e	16S-IGF	Moderate, 40.4 %	*C. felis*	100% nucleotide identity with strain NS9 16S ribosomal RNA gene, partial sequence (JN606073.1) => 1N in sequence; 99% identity to all other *C. felis*
		16S-IGR	Moderate, 33.0 %		
ED7	ED7.2e	16S-IGF	Moderate to good, 64.6 %	*C. felis*	100% nucleotide identity with strain NS9 16S ribosomal RNA gene, partial sequence (JN606073.1) => 1N in sequence; 99% identity to all other *C. felis*
		16S-IGR	Moderate to good, 79.4 %		
EI3	EI3r	16S-IGF	Moderate to good, 66.7 %	*C. felis*	100% nucleotide identity with strain NS9 16S ribosomal RNA gene, partial sequence (JN606073.1) => 1N in sequence; 99% identity to all other *C. felis*
		16S-IGR	Moderate to good, 68.4 %		
EI4	EI4r	16S-IGF	Poor to moderate, 8.7 %	*C. felis*	99% nucleotide identity with strain NS9 16S ribosomal RNA gene, partial sequence (JN606073.1) => 1N in sequence; 99% identity to all other *C. felis*
		16S-IGR	Poor to moderate, 6.1 %		