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REVIEW

An update on the detection and treatment of *Rickettsia felis*

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Abstract: *Rickettsia felis* was described as a human pathogen almost two decades ago, and human infection is currently reported in 18 countries in all continents. The distribution of this species is worldwide, determined by the presence of the main arthropod vector, *Ctenocephalides felis* (Bouché). The list of symptoms, which includes fever, headache, myalgia, and rash, keeps increasing as new cases with unexpected symptoms are described. Moreover, the clinical presentation of *R. felis* infection can be easily confused with many tropical and nontropical diseases, as well as other rickettsial infections. Although specific laboratory diagnosis and treatment for this flea-borne rickettsiosis are detailed in the scientific literature, it is possible that most human cases are not being diagnosed properly. Furthermore, since the cat flea infests different common domestic animals, contact with humans may be more frequent than reported. In this review, we provide an update on methods for specific detection of human infection by *R. felis* described in the literature, as well as the treatment prescribed to the patients. Considering advances in molecular detection tools, as well as options for as-yet-unreported isolation of *R. felis* from patients in cell culture, increased diagnosis and characterization of this emerging pathogen is warranted.

Keywords: *Rickettsia felis*, human cases, laboratory diagnosis, treatment

Introduction

Rickettsia felis is considered an emerging human pathogen and the etiologic agent of flea-borne rickettsiosis, also known as flea-borne spotted fever and cat flea typhus. Rickettsioses are arthropod-borne diseases caused by obligate rod-shaped, intracellular Gram-negative α-proteobacteria of the genus *Rickettsia*, which can infect humans and different animals.[1] The genus *Rickettsia* has been divided into three major groups based on their antigenic and genetic characteristics: (1) the spotted fever group (SFG), which includes several nonpathogenic as well as pathogenic species such as the etiological agents of Rocky Mountain spotted fever/Brazilian spotted fever (*Rickettsia rickettsii*), Mediterranean spotted fever (*R. conorii*), flea-borne spotted fever (*R. felis*), rickettsial pox (*R. akari*), *R. massiliae*, and *R. slovaca*; (2) the typhus group (TG), which includes the etiological agents of epidemic and endemic typhus (*R. prowazekii* and *R. typhi*); and (3) the ancestral group, containing *R. belli* and *R. canadensis*.[2–4] Although a fourth group has been proposed more recently, which separates *R. akari*, *R. australis*, and *R. felis* from the SFG and places them in a separate group called the transitional group,[5,6] the validity of a separate group for these species has been debated.[7,8]

R. felis was first observed by electron microscopy from midgut epithelial cells and other tissues of adult cat fleas (*Ctenocephalides felis felis*), and it was named "ELB"

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after El-Labs (Soquel, CA).[9] A close affinity of ELB to *R. typhi* was demonstrated initially by immunofluorescence assays.[9,10] Additional characterization of the ELB agent followed, and evidence from polymerase chain reaction (PCR) amplification, restriction fragment length polymorphism (RFLP) analyses, and sequencing of the 17 kDa protein and citrate synthase gene (*gltA*) fragments indicated that ELB was distinct from *R. typhi*.[10–12] Other studies confirmed this same fact, and description of the organism as *R. felis* was performed by Higgins et al in 1996.[13] Initial isolation and cultivation had been reported by Radulovic et al,[11] but maintenance in culture was not possible at the time, and contamination with *R. typhi* was suspected.[14] In 2001, Bouyer et al amplified the recombinant outer membrane protein A gene (*ompA*) by PCR, a gene present only in SFG rickettsiae.[14] Although previous evidence from analysis of other gene sequences suggested placement of *R. felis* in the SFG,[2,10] this evidence of *ompA* finally confirmed that the new *Rickettsia* was in fact a member of the SFG. *Rickettsia felis* was further characterized and redescribed, and descriptions were emended in 2002.[14,15]

A study by Merhej et al showed that most genes of *R. felis* genome place it in the SFG clade.[8] However, phylogenetic analyses of *R. felis* genes revealed that some of them come from a variety of origins, as has been shown for other bacteria like *Escherichia coli*, which demonstrates that not all genes show vertical inheritance during evolutionary history and that horizontal gene transfer probably occurs. *Rickettsia felis* can acquire new genes horizontally, since it has been shown that this species is present in many different hosts,[16–28] and concomitant infections by more than one intracellular bacterium may lead to recombination events.[8] It has also been demonstrated that *R. felis* can have one, two, or no plasmids, which were probably acquired through horizontal exchange by conjugation.[5,29–31] Although studies have recognized all genes in the different *R. felis* studied, their function is not all clear. It would be important in future to determine if those newly acquired genes could change characteristics like tropism or antigenicity.

R. felis infection in invertebrate and vertebrate animals

The ecology of *R. felis* has been reviewed previously,[22,32–34] and although it is not the focus of this review, some general considerations are presented concerning infection and detection in vertebrate and invertebrate hosts.

The cat flea, *C. felis felis*, is considered the primary vector and reservoir of *R. felis*. Detection of *R. felis* DNA in these fleas has been successful everywhere it has been investigated. Given that the cat flea is cosmopolitan in distribution, the presence of *R. felis* follows this same pattern, and has been already reported in every continent except Antarctica.[10,17,35–41] However, *R. felis* is not restricted to *C. felis*, and molecular evidence of infection, although less frequent, has been reported in other species of arthropods, such as fleas, ticks, and mites, including the familiar species *Ctenocephalides canis*, *Xenopsylla cheopis*, *Pulex irritans*, *Tunga penetrans*, *Echidnophaga gallinacea*, *Rhipicephalus sanguineus*, *Amblyomma cajennense*, chiggers (Trombiculidae), and even in nonbiting insects.[19,20,22–28,34,35]

In most of these cases, the presence of *R. felis* in arthropods has been confirmed by detection and sequencing or RFLP analyses of rickettsia-specific gene fragments, the most common being *gltA*, *htrA* (17 kDa protein), *ompA*, and *ompB*.[22] Quantitative real-time PCR (qPCR) assays to detect *R. felis* DNA in fleas have also been developed and are useful in determining infection load and kinetics.[42,43] Conversely, successful isolation and culture of *R. felis* directly from cat fleas has been reported only from laboratories in France, the US, Brazil, and Costa Rica using cell lines of vertebrate (XTC-2 and Vero) and arthropod (ISE6 and C6/36) origin.[36,44–46] No isolation of *R. felis* from vertebrates has been reported. The conditions of cultivation and growth of *R. felis* in different cell lines are described later in this review.

Rickettsia felis is maintained in flea populations mainly by transovarial transmission.[10,47] Evidence also suggests horizontal transmission from other infected fleas or infection through a rickettsemic blood meal is likely.[48,49] Although there is no evidence of fitness loss or increased mortality in infected *C. felis*, results of some studies suggest that *R. felis* may actually increase fitness to facilitate transmission to the next generation of fleas or a vertebrate host.[43]

Infection of vertebrates probably occurs during blood feeding of infected fleas, although transmission through infective flea feces is possible.[47,50] Various domestic and peridomestic animals may exhibit evidence of *R. felis* natural infection. Antibodies against *R. felis* can be present in animals, including dogs, cats, and opossums, and the presence of specific DNA fragments has also been detected in animals.[51–62] Since acquisition of *R. felis* from blood meal and transmission from fleas to animals has been demonstrated in laboratory experiments,[47,49] cats, dogs, and opossums have been considered possible reservoirs.[13,53,57,62]

Symptomatic disease caused by *R. felis* infection in domestic or wild animals may vary, but a direct causal association has not been proven. One study showed no statistical association between presence of *R. felis* antibodies and

illness in cats,[56] and another report mentions a PCR-positive dog with fatigue and digestive symptoms.[54] In addition, an experimental infection of opossums with *R. felis* resulted in antibody response, although bacteremia was undetectable.[61] Given that isolation of *R. felis* directly from sick animals has not been performed so far and that prevalence of infection and/or rickettsemia may not be high,[56,63–65] there is no conclusive evidence at this time to confirm the role of these animals as reservoirs or victims of disease.

Human cases of flea-borne spotted fever

Human infection with *R. felis* has already been reported in the US,[66] Mexico,[67–69] Brazil,[36] France,[36,70] Germany,[52] Spain,[54,71] Sweden,[72] Israel,[73] South Korea,[74] Taiwan,[75] Thailand,[76] Laos,[77] Tunisia,[78,79] Egypt,[80] Australia,[81] Senegal,[82] Kenya,[83,84] and New Zealand.[85]

Clinical findings for *R. felis* infection may be confused with infection due to other rickettsial agents like *R. typhi* and some members of the SFG, as well as other infectious diseases like dengue, malaria, brucellosis, leptospirosis, or even other clinical conditions like Kawasaki disease.[69,77,81,83] One example of misdiagnosis is a case reported as murine typhus diagnosed by serology in 2008, which in 2010 was confirmed by PCR as an infection by *R. felis* and not *R. typhi*, using the patient's same frozen serum.[73]

Fever (greater than 38°C), headache, myalgia, and maculopapular rash are the most common symptoms.[66] The presence of a cutaneous eschar at the bite site is possible, although it may be infrequent.[52,70] Respiratory and digestive symptoms, including cough, pulmonary edema, pneumonia, nausea, vomiting, and diarrhea, have been reported.[35,67,70,86] Neurological signs have also been documented, such as the reports of infection in patients presenting subacute meningitis and acute polyneuropathy-like symptoms from Sweden and Taiwan, respectively.[72,75] Although *R. felis* infection in most cases has been observed as a mild to moderate illness, respiratory, neurologic, and visceral affections can occur, leading to complications such as those reported in severe cases from Mexico.[69] Although no deaths attributed to *R. felis* infection are reported in the literature, the first two cases reported from Brazil presented stupor, and one of them coma.[36]

During *R. felis* infection, laboratory results for tests like hematocrit and hemoglobin are usually in the normal range, but some patients have severe thrombocytopenia and elevated bilirubin (2.7–3.1 mg/dL), which presents as jaundice.[69] The most common abnormalities are associated with increased aminotransferase levels: aspartate aminotransferase (85–108 U/L) and alanine aminotransferase (135–160 U/L).[69,70,81]

Knowledge of epidemiological context, clinical history, signs, symptoms, and general laboratory tests are important for diagnosis of rickettsial diseases. Since infection with *R. felis* can cause illness anywhere from mild to moderate to severe, it may be confused with signs and symptoms of other infectious and noninfectious diseases. Therefore, diagnosis of flea-borne spotted fever requires specific laboratory tests to detect *R. felis* infection.

Laboratory detection of *R. felis* infection in humans

Methods for detection of *R. felis* infection in humans are derived from the general methods used in diagnosis for rickettsial diseases. Although the general principles and applications of these methods have been reviewed previously,[3,87,88] the following section describes their applications in detection of specific *R. felis* infection.

Detection of antibodies

Specific methods for the diagnosis of rickettsial diseases of the SFG in humans started in the late 1960s utilizing serologic tests, the immunofluorescent antibody assay being the reference method for detection of specific antibodies to SFG rickettsiae.[89,90] The most important limitation of serologic tests is the cross-reaction that occurs between species of rickettsiae within the same group and sometimes even between groups. Although this cross-reaction is common between species,[91–93] immunofluorescence is considered the reference method for diagnosis of rickettsial infection.[3,87,88] It is also the first step towards the diagnosis and screening of rickettsial diseases for mainly nonendemic geographic areas.[94] Twofold serial dilutions of the sera should be performed to determine an end titer using antigens from one or more species of rickettsiae. Absorption of sera with complementary rickettsiae can be useful when cross-reactivity occurs, and Western blot may also aid in species identification.[3,95]

Detection of antibodies to SFG or TG rickettsiae in human infections with *R. felis* has been performed by immunofluorescence methods in some of the cases reported, although species confirmation has been determined by other means (Table 1). A general guideline used for identification of the rickettsial agent responsible is mentioned in several of the reports.[3] According to this, if cross-reactivity occurs, a higher titer of antibodies to *R. felis* in comparison to other species (usually by two or more serial dilutions) would suggest specific infection by *R. felis* or a very similar species.[36,76]

Table 1 Summary of *Rickettsia felis*–specific diagnostic/confirmatory methods and treatment reported in human infection

Country	Year of publication	N cases confirmed	*R. felis*-specific detection and identification methods*	Specific *R. felis* treatment and outcome	Reference
USA	1994	1	PCR 17-kDa protein gene fragment RFLP Southern hybridization	Doxycycline	66
Brazil	2001	2	MIF antibody titers to *R. felis* higher by two or more dilutions Nested PCR *gltA* gene fragment, sequencing (1 patient)	NI	36
Mexico	2000	3	PCR 17-kDa protein gene fragment; sequencing	Doxycycline 2 weeks (one patient), recovered	67
	2006	1	PCR 17-kDa protein gene fragment, sequencing	Doxycycline, discharged after 1 week Chloramphenicol	68
	2009	2	PCR *gltA*, *ompA*, *ompB*, gene fragments, sequencing and RFLP	(IV 75 mg/kg per day for 10 days), both recovered within 5 days	69
France	2001	2	MIF antibody titers to *R. felis* higher by two or more dilutions	NI	36
	2009	1	MIF, *R. felis* confirmed by Western blot with cross-adsorption	Doxycycline, rapid improvement	70
Germany	2002	1	Seroconversion, MIF antibody titers to *R. felis* higher by two or more dilutions, species confirmed by Western blot Nested PCR for PS120 protein gene fragment	Doxycycline (200 mg/day for 7 days), recovered within 3 days	52
Thailand	2003	1	MIF antibody titers to *R. felis* higher by two or more dilutions, species confirmed by Western blot	Doxycycline (200 mg/day for 7 days)	76
South Korea	2005	3	Nested PCRs *ompB and gltA* gene fragments, RFLP and sequencing	NI	74
Spain	2005	5	MIF antibody titers to *R. felis* higher by two or more dilutions, species confirmed by Western blot with cross-adsorption	NI	54
	2006	2	Nested PCRs *gltA* and *ompB* gene fragment, seminested PCR *ompA*, sequencing	Doxycycline (200 mg/day for 10 days), recovered within 2 days	71
Tunisia	2006	8	MIF, Western blot with cross-adsorption	NI	78
	2009	1	MIF, Western blot with cross-adsorption	Tetracycline and doxycycline	79
Laos	2006	1	MIF, Western blot with cross-adsorption	NI	77
Egypt	2007	1	Quantitative real-time PCR specific for *R. felis ompB* gene fragment	NI	80
Israel	2010	1	Quantitative real-time PCR specific for *R. felis ompB* gene fragment	Doxycycline	73
Kenya	2010	6	Quantitative real-time and nested PCR 17 kDa protein gene fragments, sequencing Quantitative real-time PCR specific for *R. felis ompB* gene fragment Nested PCR *ompB* gene fragment, sequencing	NI	83
	2012	21	PCR *R. felis* plasmid PCR 17-kDa protein gene, *ompB*, *R. felis* plasmid gene fragments, sequencing Quantitative real-time PCR specific for *R. felis ompB* gene fragment	NI	84
Australia	2011	5 (probable)	MIF, high titers or seroconversion to TG rickettsiae PCR *gltA* gene fragment from cat fleas, sequencing	Doxycycline (one patient), improved	81
Senegal	2010	8	Quantitative real-time and nested PCR *gltA* gene fragments, sequencing Quantitative real-time PCR biotin synthase *R. felis*-specific gene fragment	NI	82

(*Continued*)

Table 1 (*Continued*)

Country	Year of publication	N cases confirmed	*R. felis*-specific detection and identification methods*	Specific *R. felis* treatment and outcome	Reference
Taiwan	2008	1	Quantitative real-time PCR 17-kDa protein, *groEL*, *ompB* gene fragments, sequencing	Doxycycline (oral 100 mg every 12 hours for 5 days)	75
Sweden	2010	2	Quantitative real-time PCR *gltA*; nested PCR 17-kDa protein and *ompB* gene fragments, sequencing	Nonspecific antibiotics	72
New Zealand	2012	2	MIF, *R. felis* confirmed by Western blot with cross-adsorption	NI	85

Note: *PCRs not specific for *R. felis* unless otherwise stated.
Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; MIF, microimmunofluorescence; NI, not indicated or not applicable.

In addition, confirmation of *R. felis* antibodies has been performed by Western blot and/or cross-adsorption analyses.[70,71,76–78] However, these methods may not determine the species of *Rickettsia* responsible in every case.[52,71,78,85]

The presence of immunoglobulin G (IgG) antibodies in humans, which probably represent past infection with *R. felis*, has been demonstrated and may be relatively frequent.[85,93,96] Considering that the presence of IgG antibodies to *R. felis* does not necessarily mean current infection, demonstration of specific seroconversion to *R. felis* is required and has been used to confirm the presence of *R. felis* using immunological methods.[52] However, this is not without limitations, since seroconversion for IgG may appear a month or more after rickettsial infection.

Molecular methods

Rickettsia felis infection has been frequently diagnosed by PCR amplification of targeted genes. Samples are usually whole blood or serum, although highly sensitive nested and/or real-time PCR assays may be required to detect very low concentrations of rickettsial DNA present in serum. In a recent report from Sweden, *R. felis* DNA was detected in cerebrospinal fluid from two patients.[72] The genes most commonly amplified are *gltA, ompB,* and *htrA*. The *ompA* gene has also been used, although detection can be variable.[54,69] Several of the published reports indicate that *R. felis* was detected by amplifying more than two genes, and amplicons were confirmed as *R. felis* by sequencing in most cases (Table 1).

Sequencing of PCR products is usually necessary in order to get a definitive identification, considering that these genes are present in all SFG rickettsiae and only specific variations in each sequence allow differentiation. It has been difficult to properly standardize qPCR to separate between different SFG rickettsiae; nevertheless, real-time PCR methods have been developed specifically for *R. felis* gene fragments, including *ompB* and the biotin synthase gene.[42,82,97] This approach has been used to detect *R. felis*-specific infection in humans, which eliminates the need for sequencing (Table 1).[73,80,82,83]

Isolation in cell culture

Isolation of *R. felis* from human cases in cell culture has not been reported; it has only been documented from invertebrates. The best samples for isolation attempts, as is true for other SFG rickettsiae, would be blood and skin biopsies, mainly from the eschar zone if present.[3,87] Although different cells like Vero (primate), XTC-2 (amphibian), C6/36 (*Aedes albopictus*), ISE6 (tick), Aa23 (*A. albopictus*), Sua5B (*Anopheles gambiae*), L929 (mouse), and HUVEC (human) have been shown to support *R. felis* growth,[11,36,44–46,98–101] the cell lines have either not been successful for isolation of *R. felis* from human samples, or this has not been attempted.

Successful isolates from fleas reported, for instance, that *R. felis* was detected in XTC-2 cells after 14 days in initial isolation and after 6 days in subsequent passages, while growth was half the rate in Vero cells.[36] Initial detection of *R. felis* growth in cell culture is usually determined by Giménez stain. Growth is optimal at 28°C in XTC-2 cells, and growth has been demonstrated at 28°C and 32°C in Vero, room temperature in Aa23 and Sua5B, 25°C and 28°C in C6/36, and 32°C in ISE6 cell lines.[36,44–46,98] Plaque production is reported at 9 and 18 days in XTC-2 and Vero cells, respectively,[15] while almost 100% infection is reported in Aa23 and Sua5B cells within 7 days of passaging.[98]

Isolation and propagation reports show that *R. felis* grows better at lower temperatures, in agreement with the usual conditions of their invertebrate host. Since optimal temperature for growth of mammalian cells is usually higher, replication of *R. felis* may be reduced or does not occur. Nevertheless, Saisongkorh et al report the establishment of *R. felis* for up to ten passages in mammalian cells (Vero and L 929) at 28°C, enhanced by using 4% of tryptose phosphate broth

as a supplement in minimum essential medium (MEM) cell culture medium with 2% fetal bovine serum.[101]

Growth of *R. felis* in these various vertebrate and invertebrate cell lines is possible, although isolation from human or other vertebrates has not been reported in the literature. In other species such as *R. rickettsii*, different strains have shown varying virulence depending on the vector or host species of isolation.[102,103] Therefore it is of utmost importance to attempt isolation of the bacterium, especially from human cases with apparent disease. If culture is successful, isolates of *R. felis* from symptomatic patients would allow further characterization of virulence factors, pathogenic potential, and course of infection of these pathogenic strains.

Clinical treatment

Whenever signs and symptoms suggest rickettsial disease, treatment should be started immediately, even before laboratory diagnosis is complete. Doxycycline (200 mg per day) is the antibiotic of choice for spotted fever rickettsioses.[104–106] These general guidelines have also been applied in flea-borne rickettsiosis (Table 1). For pregnant patients or patients who are allergic to this drug, disease may be treated with chloramphenicol. In severe cases, intravenous antibiotic is recommended for at least 24–48 hours after defervescence of fever. As with other rickettsioses, doxycycline is the antibiotic of choice for complicated cases of flea-borne typhus, although chloramphenicol has been used successfully to treat severe cases.[69] Recently, josamycin, a macrolide antibiotic, and fluoroquinolones have been used in other rickettsioses,[3,107] and they could also be effective against *R. felis*.

Although infection with *R. felis* may be self-limiting, disease should be treated due to the possibility of severe illness and complications.[62,72,75] The prompt and specific laboratory diagnosis of the diseases is very important, not only because it will help the patient's condition, but also in order to avoid using other antibiotics that may lead to selection of resistant bacteria, or other useless therapies like intravenous immunoglobulin in cases where Kawasaki disease has been suspected.[81]

Conclusion

The present review endorses the importance of *R. felis* as a pathogen to be considered in human cases presenting clinical symptoms that are common to many infectious diseases caused by different rickettsial species and other microorganisms. Human cases of flea-borne spotted fever have been described to date in almost 20 countries around the world. Since the main vector and reservoir, *C. felis felis*, is a common ectoparasite of dogs and cats globally, infection by *R. felis* is probably more common than reported. Misdiagnosis may be frequent in many cases due to poor awareness and information, as well as minimum or no availability of specific laboratory testing required to implicate *R. felis* directly. Although symptomatic cases are usually mild, there are reports of severe disease where treatment is essential. Considering that *R. felis* infections can be treated in the same manner as other rickettsiae (doxycycline is the drug of choice), timely diagnosis and treatment is important to prevent complications and severe outcomes. Therefore, public health authorities should increase awareness and diagnosis of *R. felis*, especially in developing countries, in order to recognize the presence of this global emerging disease.

Disclosure

The authors report no conflicts of interest in this work.

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