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Review Article

Rickettsia felis is an emerging human pathogen associated with cat fleas: A review of findings in Taiwan

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Abstract *Rickettsia felis* is an emerging rickettsial agent principally associated with cat fleas (*Ctenocephalides felis*), formerly discovered in 1990. Since then, clinical cases of *R. felis* infection have been identified globally by specific DNA sequences in patients with undifferentiated febrile illness, including in Taiwan, but such evidence is limited. *R. felis* rickettsiosis is self-limiting and easily treated with doxycycline, but its diagnosis remains a challenge. Environmental risk factors for *R. felis* rickettsiosis have yet to be clearly demonstrated, and its transmission biology is incompletely understood. Cat fleas are naturally infected with *R. felis* at varying rates, and vector competence in the transmission of *R. felis* has been demonstrated in animal models, including dogs, which may serve as reservoir hosts. In northern Taiwan, despite ~20% of cat fleas infesting companion animals consistently found to be infected with *R. felis*, only a few cases of potential *R. felis* infection have been identified through a retrospective serological investigation, though without molecular confirmation. Ecological studies have identified divergent *R. felis*-like organisms in different arthropod hosts, but these strains appear to serve as nonpathogenic endosymbionts. Although its association with disease is limited, we believe cat flea-borne *R. felis* warrants increased recognition in an aging population due to immunosenescence and the proximity of companion animals to the elderly. Adopting a One Health approach involving collaboration and communication between clinicians, veterinarians, public health practitioners, and environmental scientists will improve our

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knowledge about this neglected pathogen and promote the prevention and control of vector-borne diseases.

Introduction

Rickettsia felis is an obligate intracellular alphaproteobacterium that infects diverse arthropods[1—3] but is principally associated with cat fleas (*Ctenocephalides felis*)[4] and is widely regarded as an emerging human pathogen[3,5,6] associated with a rickettsial disease referred to as flea-borne spotted fever, cat flea typhus, and *R. felis* rickettsiosis.[1,6] *R. felis* was formerly discovered in 1990, observed by transmission electron microscopy in various tissues of *C. felis* from the Elward Laboratory (Soquel, CA, USA) colony[4] and was referred to as the ELB agent until its designation as a novel species in 1996 on a genetic basis.[7] Historically, rickettsiae morphologically similar to *R. felis* were described in the coelomic cavity of cat fleas by Hertig and Wolbach in 1924 following a similar observation by Sikora in 1918.[8] After initial difficulty growing ELB agent in culture,[9] the reference type strain *R. felis* URRWXCal2 was isolated in Marseille, France, from cat fleas obtained from Flea Data Inc. (Freeville, NY, USA) and found to be genetically identical to ELB agent.[10,11] Phylogenetically, the *Rickettsia* genus is most commonly divided into four groups, in order of emergence, the tick-borne ancestral group (AG), flea- and louse-borne typhus group (TG), transitional group (TRG) with diverse arthropod hosts—to which *R. felis* belongs—and the largest, tick-borne spotted fever group (SFG).[12,13] Others have included TRG in the SFG,[14] and recently designated the TRG as SFGII among five phylogroups[15]; regardless, TRG rickettsiae consistently form a distinct clade.

Another flea-borne rickettsiosis (murine or endemic typhus) is caused by *Rickettsia typhi*, which belongs to the TG and is vectored by the rat flea *Xenopsylla cheopis* but is also capable of infecting cat fleas, including co-infection with *R. felis*.[16] Murine typhus is clinically similar to *R. felis* rickettsiosis and is a common rickettsial disease in Taiwan,[17] with endemic foci in western coastal districts near ports associated with the presence of rodents. Other species in the order Rickettsiales cause clinically related vector-borne rickettsioses that are co-endemic in Taiwan, including chigger mite-borne *Orientia tsutsugamushi*, the cause of scrub typhus, which is the most prevalent endemic vector-borne disease in Taiwan,[17] and tick-borne *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* which cause human monocytic ehrlichiosis and human granulocytic anaplasmosis.[18—20] To date, only a few cases of potential SFG rickettsioses have been identified in Taiwan,[21—23] despite the widespread detection of *Rickettsia* spp. in ticks.[24] Currently, notifiable or reportable diseases in Taiwan only include scrub typhus, murine typhus, and epidemic typhus (caused by louse-borne *Rickettsia prowazekii*, with no cases in Taiwan since World War II),[17] and the Taiwan Centers for Disease Control (TCDC) clinical guide to zoonoses groups *R. felis* rickettsiosis with SFG rickettsioses.[25]

Phenotypic features that differentiate *R. felis* from SFG rickettsiae include host range, different culture requirements, and the possession of a truncated outer membrane A (OmpA/Sca0) protein and unique antigens that remain uncharacterized.[10,11,26] In fact, the cellular pathogenesis of *R. felis* is almost entirely unstudied. Host cell invasion by *Rickettsia* spp. occurs via an endocytic mechanism involving the outer membrane protein B (OmpB/Sca5) in concert with other surface cell antigen (Sca) proteins.[27] The genome of *R. felis* encodes the most diverse set of *sca* genes among available *Rickettsia* genomes,[28] most of which with unknown function. The cellular tropism of *R. felis* has yet to be determined. While *Rickettsia* spp. primarily infect endothelial cells, another member of the TRG and cause of rickettsialpox, mite-borne *Rickettsia akari* (undetected in Taiwan), also infects macrophages.[27] Once inside the cell, *R. felis* appears to associate with actin filaments[28] similar to SFG rickettsiae which polymerize actin for intracellular motility.[27] Intriguingly, *R. felis* possesses two forms of pili that have not been observed on other *Rickettsia* spp., one thought to be involved in conjugative plasmid transfer and the other to serve as a potential virulence factor involved in host cell adhesion.[28] While other *Rickettsia* spp. harbor one or more plasmids (absent in the TG),[15] only *R. felis* appears to have the capacity for conjugative plasmid transfer, supported by the presence of an additional host-specific plasmid (pLbAR) in divergent strains infecting non-blood-feeding booklice (*Liposcelis bostrychophila*).[29] Interestingly, the plasmid content of cat flea-associated *R. felis* strains has been shown to alter with increased passage number with the loss of the short form of pRF (pRFδ),[30] but it is unclear whether this alters gene expression and phenotype. In addition to functional studies, further genomic and transcriptomic characterization of diverse *R. felis* isolates may help elucidate its pathogenicity and host—cell interactions.

Global association with clinical disease

Molecularly confirmed cases

The first suspected case of human infection with *R. felis*, reported in 1994, was retrospectively identified by molecular methods in the blood of a patient suspected of having murine typhus in South Texas, USA, in 1991; specifically, nested PCR-restriction fragment length polymorphism analysis and Southern hybridization with a specific probe targeting the 17-kDa antigen gene was used to differentiate *R. felis* and *R. typhi*.[31] It was not until 2000 that *R. felis* DNA was identified in three patients with fever, headache, rash or skin lesions, and central nervous system involvement in Yucatán,

Mexico, by PCR amplification of the 17-kDa antigen gene with an identical sequence to *R. felis* URRWXCal2 from blood or skin biopsies.[32] An additional case from the Americas, in a Brazilian patient with an unexplained febrile rash, was reported in 2001 from Didier Raoult's laboratory in Marseille, identified by microimmunofluorescence (MIF) and supported by nested PCR amplification of a fragment of the gene encoding citrate synthase (*gltA*) from the patient's serum with a sequence identical to *R. felis* URRWXCal2.[10] In 2000, the Marseille team subsequently amplified a fragment of the Sca4 (PS120 protein) gene (*sca4*, formerly 'gene D') identical to *R. felis* URRWXCal2 in the acute-phase serum of a middle-aged woman in Germany suffering from high fever and rash.[33] Five years later, a group in Spain amplified partial *R. felis* DNA sequences (*gltA*, *sca0*/*ompA*, and *sca5*/*ompB*) in the acute-phase blood of a young couple in Spain who presented with suspected human trombiculiasis based on recent exposure history, one with fever, and reported that two of three gene products had 1-2 base pair mismatches with *R. felis* URRWXCal2.[34] Another case of suspected *R. felis* rickettsiosis was reported in 2006 by the investigators in Yucatán, but the 17-kDa gene sequence was divergent from *R. felis* URRWXCal2 (with 96.5% sequence identity reported),[35] and more likely represented a *R. felis*-like organism (RFLO) based on pairwise identities of sequences available on GenBank. Nucleotide sequence mismatches could also result from errors introduced during PCR or sequencing.

The first case of *R. felis* rickettsiosis in Asia with molecular confirmation was reported in Taiwan by investigators at the TCDC in 2008, occurring in a 27-year-old female hospitalized in Kaohsiung in early 2005 who presented with fever, chills, headache, and fatigue.[36] Initial clinical diagnosis included suspicion of a rickettsial infection based on exposure history and peripheral numbness (though rarely seen in rickettsial infections), and confirmational diagnosis included amplification of 17-kDa antigen gene, 60-kDa heat-shock protein gene (*groEL*), and *sca5*/*ompB* fragments by quantitative PCR (qPCR) with DNA sequences identical to *R. felis* URRWXCal2.[36] However, the disease in this patient cannot be entirely ascribed to *R. felis* due to contemporaneous urinary tract infection and genital herpes. Even so, evidence of *R. felis* infection was additionally supported with serological confirmation by immunofluorescence assay (IFA) using whole-cell antigens of a local *R. felis* isolate genetically identical to *R. felis* URRWXCal2 (as later reported),[37] with a >4-fold rise in IgG/IgA/IgM antibody titers between paired sera with low titers against other *Rickettsia* spp. with known cross-reactivity.[36]

Detailed case descriptions from these reports indicate that *R. felis* rickettsiosis is a mild disease, presenting most consistently with fever, headache, and fatigue with rash or leukocytosis.[32,33,36] Similar clinical features have been reported from molecularly confirmed cases of *R. felis* rickettsiosis in Kenya[38,39] and Senegal.[40] Severe manifestations have only been reported in a few patients with molecular confirmation, including a case of respiratory compromise in Mexico[41] and two fatal cases of meningoencephalitis in Indonesia.[42] These instances may be attributed to failure to receive appropriate treatment. Doxycycline is the recommended antibiotic treatment for all rickettsioses.[43,44] The TCDC recommends doxycycline (20 mg/day) or a regimen of tetracycline, chloramphenicol, or ciprofloxacin to treat spotted fever rickettsioses.[25] However, a higher dose of doxycycline (200 mg/day) has been used to treat *R. felis* rickettsiosis in Germany,[33] Spain,[34] and Taiwan[36] with rapid resolution of symptoms.

Limited evidence for pathogenicity

Koch's postulates are generally considered the gold standard to establish causality between a putative pathogen and its associated disease, including the presence of the pathogen in diseased but not healthy individuals, isolation of the pathogen from a diseased individual, and reproduction of the disease in an animal model, as well as re-isolation of the pathogen.[45] Presenting a potential challenge to the first postulate, *R. felis* DNA has been reportedly detected in the blood of afebrile individuals in Kenya[39] and Gabon.[46] In Kenya, Maina et al. reported that eight afebrile patients were positive for a *Rickettsia* genus-specific *gltA* qPCR assay targeting a 74-bp fragment,[39] but confirmatory sequence data with a species-specific assay was not reported for these patients. In Gabon, Mourembou et al. found two afebrile children to be positive by at least one *R. felis*-specific qPCR assay.[46] These children were recruited while accompanying a clinically ill parent with whom they would share similar environmental exposures; thus, they could have been more recently infected and in an asymptomatic incubation period. The authors had apparently received criticism for inadequate controls to prevent potential contamination, as the issue was discussed at length. In addition to the points mentioned by Mourembou et al., we recommend collecting blank samples in the field for DNA extraction to ensure that contamination is not introduced during sample collection.

R. felis has yet to be isolated from a human.[43] Rickettsiae are fastidious organisms that are difficult to cultivate and require specialized biological safety level 3 facilities that are not universally available. As for culture requirements, media supplemented with tryptose phosphate broth is required for optimal growth of *R. felis* in mammalian cells at temperatures above 32 °C commonly used for rickettsial isolation.[47] Future attempts should be made using specimens collected before the administration of antibiotics[44] with rapid inoculation of shell-vials.[48]

Surprisingly few attempts have been made to study *R. felis* infection in animal models. C3H/HeN mice were previously challenged with *R. felis* based on susceptibility to *Rickettsia parkeri* but observed no detectable *R. felis* DNA in blood, even when challenged with 5×10^9 rickettsiae.[49] BALB/c mice have been used to study hypothetical modes of transmission by the Marseille team, including from artificially infected *Anopheles gambiae*, which did not produce signs of disease,[50] and immunodeficient BALB/c SCID mice were exposed to aerosols containing an unspecified amount of *R. felis* filtered from homogenized booklice, which also did not develop overt disease.[51] The former study by Dieme et al.[50] was performed to investigate the suspected role of mosquitoes as vectors of *R. felis* in sub-Saharan Africa,[5,52] and demonstrated the potential for horizontal transmission of *R. felis* from mosquitoes to mammals. Although there is some evidence for transovarial transmission based on *R. felis* infection of wild-caught male mosquitoes,[52–54] *R. felis* infection in wild-caught female mosquitoes is consistently low (e.g., 0.7% in Senegal[52] and 1.5%

throughout China).[53] Mediannikov et al.[51] sought to test the hypothesis that humans could be infected via inhalation of "infected booklice particles," as booklice are common in the environment in rural Senegal, including in dust samples from beds.[5] Further work is needed to demonstrate the pathogenic potential of booklice-associated *R. felis*, including a comparison with cat flea-associated *R. felis* to elucidate any differences in pathogenesis between host-specific strains.

In a groundbreaking study, investigators in Australia challenged four healthy puppies at 14 weeks subcutaneously with 1×10^6 *R. felis*.[55] None of the dogs developed overt disease, and only two had hematological abnormalities with mild neutrophilia and transient lymphocytosis.[55] Notably, *R. felis* was reportedly re-isolated from the buffy coat obtained on day 8 post-inoculation from two dogs, and *R. felis* was detected in the blood by PCR for up to 100 days.[55] Unfortunately, limited data was included in the publication to support these observations. Findings in this study should be confirmed with *R. felis* URRWXCal2. Prolonged asymptomatic bacteremia in mammal hosts is not unprecedented for *Rickettsia* spp., for example, as demonstrated by experimental infection of laboratory rodents with *R. typhi*.[56] It is reasonable that as a natural host of cat fleas, the dog immune system has coevolved with cat flea-associated bacteria and could play a role in the maintenance of *R. felis*.

Challenges with serodiagnosis

Serological assays are the most frequently used methods for diagnosing rickettsial infections, namely MIF and IFA, typically considered the gold standard.[48] However, these methods lack specificity for the diagnosis of *R. felis* infection due to cross-reactivities with *R. typhi*, which has not been adequately characterized, and SFG rickettsiae, including *Rickettsia conorii* (the agent of Mediterranean spotted fever) against the 120-kDa antigen[10,26]; thus, co-endemic *Rickettsia* spp. must be included in MIF and IFA assays with *R. felis*. Typically, the infecting agent is identified as that with an antibody titer $\geq$2 serial dilutions higher than other rickettsial antigens.[57] For validation or in ambiguous cases, Western blot and cross-adsorption assays may be used to identify the infecting agent, but these methods are laborious and have only been conducted in Marseille for this purpose.[57—59]

Serodiagnosis is determined by either seroconversion or a $\geq$4-fold increase in antibody titer between acute- and convalescent-phase sera.[43,44] Seroconversion is not required to indicate acute infection, as patients may have had previous exposure to an antigenically related agent, and additional diagnostic criteria include sufficiently high IgM or IgG titer in acute phase serum (e.g., $>$1:32 for IgM and $>$1:64 for IgG).[57] On the other hand, some molecularly confirmed cases have failed to develop detectable antibodies,[60,61] for which a satisfactory explanation remains elusive. Fortunately, nested PCR and qPCR provide far more sensitive methods for the direct molecular detection of *R. felis* during the acute phase (ideally obtained before administration of antibiotics that would reduce the amount of *R. felis* found in peripheral circulation), even using serum instead of whole blood, underscoring the importance of collecting blood samples for diagnosis soon after onset of fever.

Indirect enzyme-linked immunosorbent assay (ELISA) has been used as an alternative for *R. felis* serodiagnosis.[62] Unlike MIF and IFA, which use whole-cell antigens, indirect ELISA utilizes recombinant protein antigen(s). ELISA also relies on the interpretation of quantitative fluorescence values rather than the direct visualization of fluorescence to determine positivity, requiring a determination of an optical density cutoff based on prior evaluation of patients with known infection status. This is a challenge for *R. felis*, as so few cases have been molecularly confirmed; thus, it is only possible to compare ELISA values with MIF or IFA results and is therefore limited in its utility. In addition, cross-reactivity with antigenically related species still needs to be carefully examined (e.g., with monoclonal antibodies directed against the same target antigen of related species), and antigenic variation may be observed at the strain level that could affect assay sensitivity in different regions (e.g., as observed for *R. felis* OmpA).[60]

Serological findings in Taiwan

In Taiwan, two retrospective serological studies have been performed to investigate the prevalence of *R. felis* rickettsiosis among other rickettsioses. Lai et al. investigated 413 patients at E-Da Hospital in Kaohsiung between 2004 and 2009 with suspected zoonotic and vector-borne diseases (Q fever, scrub typhus, murine typhus, leptospirosis, and dengue fever), 158 cases with diagnoses from TCDC and 255 cases without a diagnosis.[21] They used customized MIF slides from Fuller Laboratories (USA) to detect IgM and IgG against *R. felis*, *R. conorii*, and *Rickettsia japonica*.[21] To the best of our knowledge, *R. felis* LSU (isolated from a cat flea colony maintained at Louisiana State University, USA)[63] was used as an antigen in this assay,[64] which could lack sensitivity compared to a local isolate. Identification of acute infection was determined by a $\geq$4-fold increase in IgG titer between acute- and convalescent-phase sera or positive IgM reaction ($\geq$1:64), and the infecting agent was identified as that with an antibody titer $\geq$2 serial dilutions higher than other antigens.[21] These criteria are consistent with those previously used for serodiagnosis of rickettsial infection. Lai et al. determined three patients to be acutely infected with *R. felis*, but one patient had IgG titers with only one serial dilution difference in convalescent-phase serum between *R. japonica* (1:2048) and *R. felis* (1:4096) and for IgM titers between *Rickettsia rickettsii* (1:128) and *R. felis* (1:256),[21] and should not have been identified as acute *R. felis* infection based on predefined criteria. The other two cases had confirmational diagnoses from TCDC of Q fever (a zoonotic disease similar to rickettsioses caused by the intracellular bacterium *Coxiella burnetii*) and scrub typhus.[21] Co-infection makes it impossible to attribute the disease observed in these individuals to *R. felis*. Despite insufficient evidence to confirm infection with *R. felis*, this study illustrates the presence of multiple endemic zoonotic and rickettsial diseases in southern Taiwan that should be considered in differential diagnosis.

Yang et al. investigated 122 patients at National Taiwan University (NTU) Hospital in Taipei between 2009 and 2010,

initially suspected to have either Q fever, scrub typhus, or murine typhus with negative tests from TCDC.[23] MIF slides were prepared in-house to test for IgM and IgG antibodies against *R. felis*, *R. typhi*, and *R. japonica*.[23] Regrettably, the identity of the strains used in this study was not reported but included a local isolate of *R. felis* from a cat flea,[37] and other heat-inactivated whole-cell antigens were type strains provided by the rickettsial laboratory (Unité des Rickettsies) in Marseille. Identification of the infecting agent was defined by a ≥2-fold difference in antibody titer between antigens, and acute infection was indicated by a ≥4-fold increase in IgG titer between acute- and convalescent-phase sera or positive IgG (≥1:64) or IgM (≥1:32) reactions and four patients were determined to be acutely infected with *R. felis*.[23] Similar to Lai et al., substantial cross-reactivity was observed between *R. felis* and *R. japonica* but not *R. typhi*.[23] Yang et al. also found one patient to have one serial dilution difference in IgG titer of convalescent-phase serum between *R. japonica* (1:512) and *R. felis* (1:1024), the other patients with ≥2 serial dilutions difference in antibody titer between antigens.[23] If we interpret findings from these two studies equally, we may not consider *R. felis* as the infecting agent in the first patient that developed acute respiratory distress syndrome after doxycycline was stopped and, unfortunately, expired at day 30 despite intensive supportive care.[23] This case had recently arrived from Guangzhou, China, where other potentially cross-reactive rickettsiae may be endemic. Two of the other three patients (females 26 and 47 years of age) presented with fever and skin rashes that led to suspicion of rickettsial infections.[23] The third patient was a 77-year-old male with diabetes and chronic kidney disease who presented with general malaise without fever and abnormal liver function.[23] After failure to treat suspected *Escherichia coli* bacteremia with deteriorating liver function, physicians suspected scrub typhus (it was later found that the patient did not have antibodies against *O. tsutsugamushi*) and administered a 7-day course of levofloxacin and the patient recovered.[23] Oral fluoroquinolones, including levofloxacin, are an effective alternative treatment for less severe rickettsioses in adults.[44] This may represent the first documented case of *R. felis* rickettsiosis in an elderly person and is especially concerning given the rapid aging of Taiwan's population[65] and the common detection of *R. felis* cat fleas (reviewed in Section 4). The gradual decline of immunity with age (i.e., immunosenescence) increases susceptibility to infection and poorer outcomes, especially with the coexistence of multiple comorbidities, as seen in influenza.[66]

Cat fleas as the principal vector of *R. felis*

Exposure to companion animals and their ectoparasites, especially fleas, is commonly associated with *R. felis* rickettsiosis.[32–34,36,41,57,59,67] Direct contact with flea-infested dogs has been reported in several cases,[33,34,57] and a patient described flea bites four days before fever onset.[41] Cat fleas are the only ectoparasite with experimentally demonstrated vector competence (i.e., capacity for vertical and horizontal transmission of a pathogen) for *R. felis*,[49] and are naturally infected with *R. felis* at varying rates[1,3] due to less than 100% efficient transovarial transmission.[68] Cat fleas develop a disseminated infection with *R. felis*, including in the salivary glands, after feeding on an infected blood meal[69] and horizontally transmit *R. felis* to cats[70] and dogs[55] through feeding. It has not been ruled out whether *R. felis* remains infectious in cat flea feces in which transcriptionally active *R. felis* has been detected.[71] Dogs are suspected of serving as reservoir hosts for *R. felis* in light of the previously discussed experimental evidence[55] and preferential detection of *R. felis* DNA in asymptomatic over clinically ill dogs.[55,72]

In Taiwan, several studies have examined *R. felis* prevalence in *C. felis* collected from dogs and cats, primarily conducted in northern Taiwan (Table 1).[37,73–76] While most sequences were identical to *R. felis* URRWXCal2, a subset of fleas were potentially infected with RFLOs in two studies[73,75] and *Candidatus* Rickettsia senegalensis sequences were detected in a flea in New Taipei.[76] These data provide a baseline *R. felis* infection rate of ~20% of cat fleas infesting stray and companion animals in northern Taiwan. In contrast, cat fleas collected from companion

Table 1 Prevalence of *Rickettsia felis* infecting cat fleas (*Ctenocephalides felis*) collected from dogs and cats throughout Taiwan.[37,73–76]

Host	Prevalence	Location	Study period	Reference
Laboratory-reared cats	18% (7/40)	Taipei	1991	[74]
Companion dogs	5% (1/19)[MIR]		2002–2007	[37]
Companion cats	0% (0/5)			
Stray dogs	9% (7/78)[MIR]			
Stray cats	20% (13/64)			
Stray dogs & cats	16% (70[†]/451)[MIR]		2006	[73]
Stray dogs	23% (53/231)		2007	[74]
Stray cats	20% (37/189)			
Companion dogs & cats	19% (22/116)	New Taipei	2012	[76]
	4% (6/155)	Hualien	2015	
	4% (8/222)		2019–2021	
Companion dogs	33% (3[†]/9)	Taipei, Taoyuan, Changhua,	2017–2018	[75]
Companion cats	36% (5[†]/14)	Pingtung, Hualien		

MIR = minimum infection rate; [†]includes potential *Rickettsia felis*-like organisms (i.e., *gltA* <99.9% identical to *R. felis* URRWXCal2).

dogs and cats in eastern Taiwan, in Hualien, had a much lower prevalence rate of *R. felis* infection, remaining stable at 4% in 2015 and from 2019 to 2021.[76] Of note, *R. felis* prevalence in cat fleas meets or exceeds that observed for *Bartonella* spp., including *Bartonella henselae* and *Bartonella clarridgeiae* associated with cat scratch disease, and coinfection with *R. felis* and *Bartonella* spp. was detected in 1—2% of cat fleas examined in northern and eastern Taiwan.[74,76] Whether co-infection with *R. felis* occurs in cases of bartonellosis needs to be clarified.

Limited data is available elsewhere in Taiwan, but about one-third of cat fleas collected from companion dogs and cats around Taiwan were infected with *R. felis* or RFLOs, albeit from a limited sample.[75] Despite several cases of potential *R. felis* rickettsiosis being identified in southern Taiwan, *R. felis* prevalence in cat fleas has not been assessed there, necessitating expanded molecular surveillance. On the other hand, based on environmental surveillance data, we may predict a lower prevalence of *R. felis* rickettsiosis in eastern Taiwan.

Divergence in other arthropod hosts

As introduced, *R. felis* also infects booklice, with genomic characterization of one culture isolate to date, *R. felis* LSU-Lb, isolated at LSU.[77] In asexual (parthenogenic) *L. bostrychophila* populations, *R. felis* appears to be an obligate mutualist required for the early development of oocytes, is maintained by 100% transovarial transmission,[78,79] and its unique plasmid pLbAR encodes a toxin—antitoxin module that may induce parthenogenesis.[80] Phylogenomic analysis indicates that the acquisition of *R. felis* from cat fleas has occurred independently in geographically disparate *L. bostrychophila* populations,[29] but a natural mode of horizontal transmission of *R. felis* between these hosts (e.g., by larval predation on *R. felis*-infected eggs or consumption of feces containing viable *R. felis*) has yet to be experimentally demonstrated. Healy et al. demonstrated the potential for acquisition of *R. felis* LSU-Lb infection in cat fleas via bloodmeal, which was transmitted between cofeeding fleas in an artificial feeding system.[81]

Aside from booklice, *R. felis* DNA has been detected in various tick, mite, louse, and mosquito species worldwide,[1,3,53,54,82] and has been isolated from an *Ixodes ricinus* tick in Slovakia.[83] Cofeeding transmission (i.e., transfer of a pathogen from an infected to uninfected arthropod feeding within proximity on the same host) of *R. felis* has been experimentally demonstrated between cat fleas and from cat fleas to other flea species, ticks, and mosquitoes in the absence of systemic infection of the vertebrate host.[84,85] However, the capacity for wild mammals from which *R. felis*-infected ectoparasites have been recovered (including small mammals, ruminants, and monkeys)[1,3] to serve as reservoirs has yet to be determined. In addition, *R. felis* was detected in the household case-bearer moth in Brazil,[86] representing the first report of *R. felis* in a non-blood-feeding insect other than *L. bostrychophila*, but was only supported by a partial *gltA* sequence with failure to amplify *Rickettsia* genus-specific *sca0*/*ompA* or *sca5*/*ompB* fragments. In the absence of a culture isolate, molecular observations are better supported by sequence data for multiple gene targets in the multilocus sequencing typing (MLST) scheme put forth by Fournier et al. (including 16S rRNA/*rrs*, *gltA*, *sca0*/*ompA*, *sca4*, and *sca5*/*ompB*).[87] For culture isolates, genome sequence-based parameters have been established to delineate *Rickettsia* species.[13]

RFLOs have been demarcated as those with gene sequences in the MLST scheme that are divergent from *R. felis* URRWXCal2 (i.e., with sequence identities below maximal intra-species identities; e.g., <99.9% for *gltA*).[3] A potential point of confusion, the term RFLO has remained associated with the newly described species *Rickettsia asembonensis* and *Ca.* R. senegalensis, both isolated from *Ctenocephalides* spp. and of unknown pathogenicity.[88] RFLOs have been molecularly identified in diverse arthropods throughout the world, including fleas, ticks, mites, tsetse flies, wasps, and mosquitoes.[2,3,54,78,89] It is evident that the genetic diversity of RFLOs in arthropods has yet to be unearthed, and their characterization will improve our understanding of rickettsial evolution and transmission dynamics.

In Taiwan, several studies have detected *R. felis* in fleas collected from rodents and shrews, including *X. cheopis* in the Kaohsiung-Pingtung area,[90] and *Acropsylla episema* and *Stivalius aporus* in Hualien.[91] While these are individual observations, *Ixodes granulatus* ticks parasitizing rodents in Kinmen are consistently found to be infected with *R. felis*,[24,92] and a RFLO (*Rickettsia* sp. TwKM03) has been detected in *I. granulatus* from a shrew in Matsu and *Leptotrombidium deliense* chigger mites and Mesostigmata sp. mites collected from rodents in Kinmen and Hualien, respectively.[93] It has yet to be experimentally determined whether *I. granulatus* is a competent vector of *R. felis*, which would be relevant to the broader Asia-Pacific region where *I. granulatus* and *R. felis* are co-endemic.[1,3,94] To this point, transovarial transmission of *R. felis* was experimentally demonstrated in the American dog tick *Dermacentor variabilis* but without subsequent transstadial transmission.[95] DNA sequences of *R. felis* and RFLOs have also been detected in the organs of rodents around Taiwan and its offshore islands (Taoyuan, Taitung, Kinmen, Matsu, and Penghu).[96] RFLOs have also been detected in *Rhipicephalus sanguineus* ticks collected from stray dogs in Kaohsiung,[97] which also vectors anaplasmosis, ehrlichiosis, and babesiosis. We found that some studies did not include the study period or location, the type of host from which ectoparasites were collected, or sequence data. These data are critical for interpretation, and others may look to Tsui et al.[93] for an excellent example of what information to include.

In addition to these observations, we have identified a divergent *R. felis* strain (tentatively named *R. felis* NTU-Lb) infecting an asexual *L. bostrychophila* colony maintained at NTU (COX1 gene deposited in GenBank with the accession number OM932502) (Fig. 1). Compared to other *R. felis* strains, for which data is scarce, *R. felis* NTU-Lb possesses three unique SNPs in *gltA* each causing a missense mutation. Following the previously mentioned criteria, *R. felis* NTU-Lb may be regarded as a RFLO but requires further genetic characterization for its classification as a novel species.

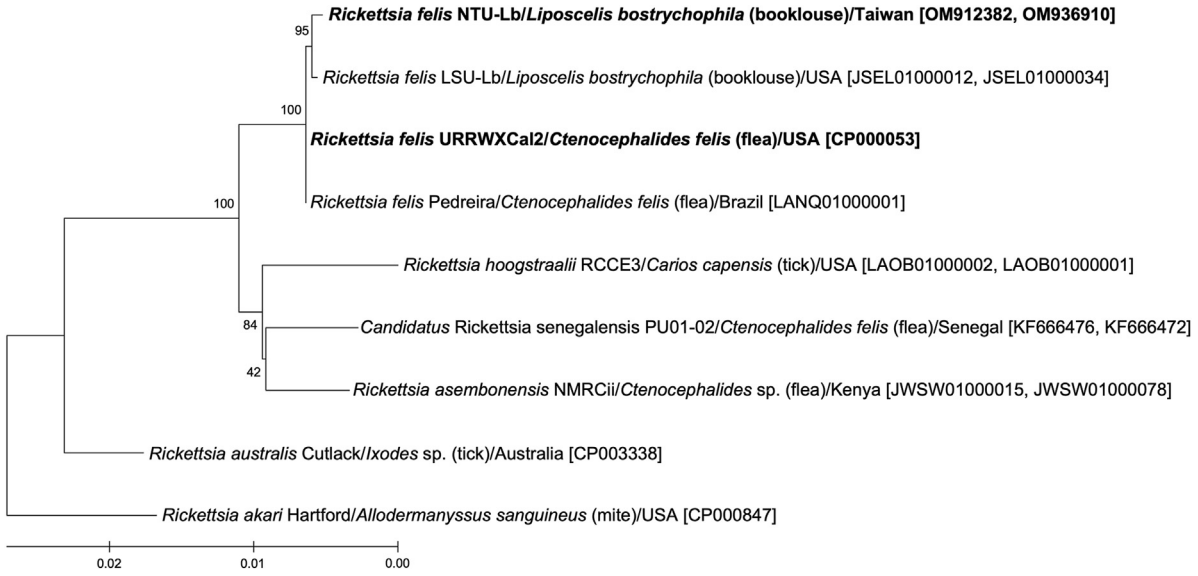

Figure 1. Phylogenetic relationship of transitional group (TRG) *Rickettsia* spp. based on concatenated nucleotide sequences of 16S rRNA (*rrs*) and citrate synthase gene (*gltA*) inferred using the Neighbor-Joining method with Kimura 2-parameter distances in MEGA11.[101] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The scale bar indicates the number of base substitutions per site. There were 2659 positions (*rrs* = 1409; *gltA* = 1250) in the final dataset. Taxa names include host and country with GenBank accession numbers in brackets.

One Health interface

As reviewed, cat fleas are the principal vector of *R. felis*, and their maintenance hosts cohabitate with humans; thus, contact with flea-infested companion animals is a likely risk factor for *R. felis* infection. In a recent study, veterinarians recovered *Ctenocephalides* spp. from 20% of companion cats and 9% of dogs around Taiwan.[98] Ectoparasite prevention and control through regular grooming and chemoprophylaxis is needed to reduce flea burden and the potential to maintain *R. felis*. To this end, veterinarians play an essential role in educating companion animal caretakers. Since stray animals have higher flea infestation rates and flea indices in the absence of flea control, especially cats,[74] individuals who care for them should be aware of the potential risk of *R. felis* infection. Due to frequent contact with companion animals and their ectoparasites, veterinarians are likely at increased risk for infection with *R. felis*. In Australia, 16% of 131 veterinarians had past exposure to *R. felis*, and 35% had exposure to either *R. felis* or *R. typhi*, as assessed by MIF.[99] Other occupations that come into frequent contact with domestic animals or have potential contact with rodents (e.g., animal husbandry and agricultural sectors) may also be at increased risk for infection with *R. felis*. In Spain, exposure to *R. felis* assessed by IFA was significantly higher among these occupations compared to the general population.[100] General practitioners and occupational health physicians can raise awareness among these populations. Implementation of clinical One Health practice necessitates communication and collaboration between physicians and veterinarians and has been assessed to require a significant culture change in Australia.[102] In Taiwan's current landscape, public health professionals are tasked with raising awareness among clinicians and veterinarians about neglected vector-borne rickettsiae, including *R. felis*. To this end, it will be helpful to evaluate the current knowledge, attitudes, and practices of human and animal health professionals toward vector-borne diseases in Taiwan, among which rickettsioses constitute most of the burden. Additionally, an integrated surveillance system for vector-borne diseases could help facilitate interprofessional communication and collaboration and encourage One Health practice.

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