First probable Australian cases of human infection with *Rickettsia felis* (cat-flea typhus)

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Human infection with Rickettsia felis *has been reported in most parts of the world, and* R. felis *has recently been confirmed in cat fleas in Western Australia. The clinical presentations of* R. typhi *and* R. felis *are similar, and in the past, the incidence of* R. felis *infection may have been underestimated. We describe the first reported cases of probable human* R. felis *infection in Australia. Two adults and three children in Victoria contracted a rickettsial disease after exposure to fleas from kittens. Molecular testing of fleas demonstrated the presence of* R. felis *but not* R. typhi. *(MJA 2011; 194: 41-43)*

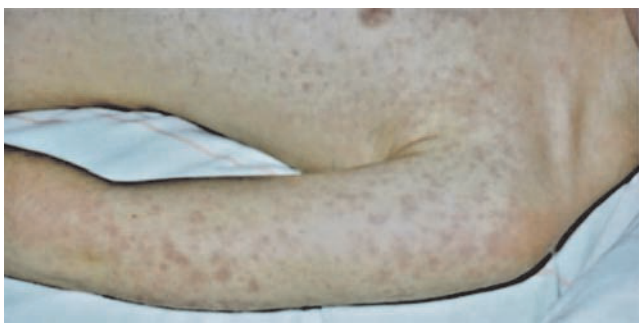
Clinical records

Patient B, a previously well 9-year-old girl, was admitted to a children's hospital in Melbourne, Victoria, in April 2009 with severe abdominal pain, fevers to 39°C and a non-pruritic erythematous macular rash, initially present on the trunk and then spreading to the upper limbs and face (Box 1). The patient described a prodrome of 5 days of fever and malaise, with occasional vomiting and diarrhoea. She had been appropriately vaccinated, had no drug allergies, and did not regularly take any medication.

On initial examination, the girl appeared unwell, with pitting oedema of the ankles and a generalised macular rash. There was no hepatosplenomegaly or significant lymphadenopathy. Initial laboratory test results indicated leukopenia (white blood cell count, 3.0×10^9/L [reference range (RR), 4.5–13.5 $\times 10^9$/L]), lymphopenia (lymphocytes, 0.42×10^9/L [RR, 1.5–6.5 $\times 10^9$/L]), thrombocytopenia (platelet count, 38×10^9/L [RR, 150–400 $\times 10^9$/L]), hyponatraemia (Na+, 133 mmol/L [RR, 135–145 mmol/L]), hypoalbuminaemia (serum albumin, 19 g/L [RR, 33–47 g/L]), and elevated transaminase levels (aspartate aminotransferase, 168 IU/L [RR, < 55 IU/L]; alanine aminotransferase, 177 IU/L [RR, < 55 IU/L]). Treatment with ticarcillin–clavulanic acid and gentamicin was commenced. Urine and blood cultures were ordered, as well as serological tests for a range of infectious diseases.

The patient lived with her parents and two siblings in suburban Melbourne on a hobby farm next to a wooded reserve notable for stagnant water and mosquitoes. The family had many pets, including a dog, goat, ducks, budgerigars, mice and a domesticated rat. They had never travelled outside Australia, and had not recently had visitors from overseas. About 3 weeks before the onset of the illness, the family had acquired a pair of kittens (Cat 1 and Cat 2) from a farm in Lara, a rural suburb in Victoria, and had given Cat 2 to a neighbour.

1 Widespread erythematous macular rash, Patient B



Patient B had ongoing persistent fever and severe abdominal pain. Her platelet count remained low, and her hepatic function, coagulopathy, hyponatraemia and hypoalbuminaemia worsened. On Day 3 of her admission, she developed pulmonary oedema and required a short stay in the intensive care unit, during which she received azithromycin, albumin and frusemide, as well as intensive supportive therapy and monitoring. She was given intravenous immunoglobulin (IVIG) 2 g/kg for possible Kawasaki disease but showed no response.

Also on Day 3 of Patient B's hospitalisation, her 8-year-old sister (Patient C) presented with fevers to 40°C, mild abdominal pain and a rash on her torso. On examination, she appeared to be well, but had florid facial flushing, a macular rash spreading to the limbs, tender cervical lymph nodes and a mildly tender abdomen.

Patient C's initial laboratory test results indicated mild leukopenia (white blood cell count, 4.5×10^9/L) and hyponatraemia (Na+, 132 mmol/L). Treatment with ticarcillin–clavulanic acid and gentamicin was commenced. Over 48 hours she became thrombocytopenic (platelet count, 77×10^9/L), with worsening abdominal pain and hyponatraemia (Na+, 132 mmol/L), and elevated alanine aminotransferase (75 IU/L). She was given IVIG 2 g/kg for possible Kawasaki disease. Her condition improved rapidly.

On Day 7 of Patient B's hospitalisation, Patient D, the girls' 4-year-old brother, presented with a fever of 39.6°C and five erythematous macules on his legs and trunk. He was otherwise well.

Laboratory test results for Patient D showed leukopenia (white blood cell count, 4.0×10^9/L), with no other abnormalities. He was admitted for observation without treatment.

The three siblings were discharged home on Day 11 of Patient B's hospitalisation, without definitive diagnoses. Patients C and D had episodes of fever for 1 week, but remained well otherwise.

A phone review on Day 18 found that the three children were well and afebrile. However, their maternal grandmother (Patient E) had had 3 days of fever and rigors and had been admitted to another hospital for observation. On advice from the children's doctor, Patient E's treating doctor administered doxycyline and her condition subsequently improved. It was also discovered that the neighbour who had been given Cat 2 (Patient A) had become unwell 2 days before Patient B, with a non-specific febrile illness

that had settled by the time Patient B was admitted to hospital. She was therefore the initial case in the cluster.

All patients had had extensive close contact with one or both of the cats. The children's parents had minimal contact with the cats and were asymptomatic. The family reported that both cats had flea (*Ctenocephalides felis*) infestations when they acquired them. Cat 1 no longer had fleas after having been treated topically with insecticide, but its serum was tested for typhus-group rickettsial species. Because it was unwell, Cat 2 had been euthanased before blood samples could be taken. As collecting fleas from the two kittens was not possible, fleas from other cats of the group into which they were born, including the kittens' mother, were collected for molecular analysis to identify any rickettsial species they carried.

Serological testing was performed using indirect microimmunofluorescence assay (IFA).[1,2]

Initial serological analysis (in April 2009) for the presence of both spotted-fever-group and typhus-group rickettsial antibodies was undertaken on Patients B and C. The results showed the presence of typhus-group but not spotted-fever-group rickettsial antibodies. A month later (May 2009), serological testing was repeated for Patients B and C, and initial testing was done for Patients D, E and A. The tests showed rising typhus-group rickettsial antibody titres in patients B, C and E and high titres in patients A and D. In addition, Patient C showed clear evidence of seroconversion (Box 2), while both parents were negative for rickettsial antibodies. Serological testing undertaken on Cat 1 also showed the presence of typhus-group rickettsial antibodies (Box 2).

DNA was extracted from the serum of Patient C (buffy coat [white cell layer] was not available), and Cat 1, and from pooled and crushed cat fleas that were collected from cats in the group that Cat 1 and Cat 2 had come from. A rickettsial real-time polymerase chain reaction (PCR) test was performed on the extracted DNA samples.[3] The fleas, but not the patient's or cat's serum, were positive for rickettsial DNA.

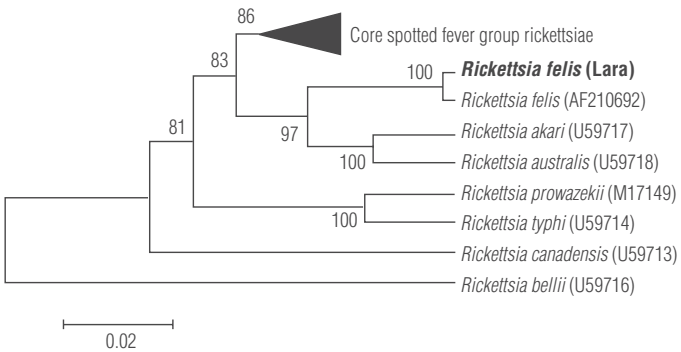
A 1077 base-pair fragment of the rickettsial citrate synthase gene was amplified and sequenced.[4] This sequence was compared with the validated rickettsial species[5] and showed closest phylogenetic similarity to *Rickettsia felis*, with a sequence similarity of 99.7% (1074/1077 base pairs). *Rickettsia typhi* DNA was not detected in the cat fleas. The citrate synthase gene (*gltA*) sequence analysis using the neighbour-joining algorithm is shown in Box 3.

2 Serology results of five seropositive patients and a cat exposed to rickettsial infection, 2009

Patient /cat	Sex, age in years	Day of onset*	Status in family	Rickettsia group	Serum antibody titre April	May	June
A	F, 63	–2	Neighbour	SFG	nd	< 1/128	nd
				TG	nd	1/16 384	nd
B	F, 9	1	Child	SFG	1/128	1/128	nd
				TG	1/1024	1/8192	nd
C	F, 8	3	Child	SFG	1/128	1/256	nd
				TG	< 1/128	1/16 384	nd
D	M, 4	7	Child	SFG	nd	1/128	nd
				TG	nd	1/16 384	nd
E	F, 59	15	Grandmother	SFG	nd	< 1/128	< 1/128
				TG	nd	1/1024	1/2048
Cat 1	F, < 1	na	Pet	SFG	nd	nd	< 1/128
				TG	nd	nd	1/512

na = not applicable. nd = not done. SFG = spotted-fever group (ie, *Rickettsia australis* and *R. honei*). TG = typhus group (ie, *R. prowazekii* and *R. typhi*).
* Compared with Patient B's admission (Day 1).

3 Condensed phylogenetic tree comparing the DNA fragment sequenced in this analysis ("*Rickettsia felis* [Lara]") with validated rickettsial species



Relationship of a 1077 base-pair fragment of the gltA *gene of* Rickettsia felis *(Lara) among other validated rickettsial species, with the core spotted-fever-group rickettsiae truncated. The tree was prepared using the neighbour-joining algorithm.* Bootstrap values are indicated at each node. The scale bar represents a 2% nucleotide divergence.*

* Molecular Evolutionary Genetics Analysis (MEGA) software, version 4.0, 2007 [free internet download].

Discussion

The five patients described here are the first reported cases of probable human *R. felis* infection in Australia, and the analyses provide the first molecular evidence of *R. felis* in cat fleas in Victoria. It has been previously detected in cat and dog fleas in Western Australia by molecular analysis.[6] Human infection with *R. felis* has been reported in most other parts of the world.[7-10]

While genetically a member of the spotted-fever rickettsia group, *R. felis* behaves clinically and serologically like a typhus-group rickettsia and is transmitted by fleas. Antibodies induced by *R. felis* react with typhus-group rickettsiae in serological tests, rather than with spotted-fever-group rickettsiae. A petechial rash is an infrequent sign of infection, and a macular or maculopapular rash is present in only 50% of patients (Box 1). The high attack rate and severity of infection noted in this cluster may be due to the heavy flea infestation that was reported.

Resolution without therapy is well described in rickettsial infection. Only two patients (B and C) received antimicrobial therapy with known activity against rickettsial species. The five patients showed a strong positive result for the presence of typhus-group antibodies. Patient C's clear seroconversion was consistent

with recent acute *R. felis* or *R. typhi* infection.[7] While exposure to either *R. felis* or *R. typhi* could have led to Cat 1 producing typhus-group antibodies, only *R. felis* DNA was detected in the cat fleas. It is common for blood from cats infected with *R. felis* to be negative for rickettsial DNA,[8] as in this case. Cat 1 still had antibodies to *R. felis* but either had cleared the infection, or the organism was present in tissues other than peripheral blood. In a previous experimental exposure of cats to *R. felis*-positive fleas, 13 of 16 cats were positive by serological testing using IFA, but only five of the 16 were positive by PCR.[11]

The human cases reported in this study were only identified serologically, and as the clinical presentations of *R. typhi* and *R. felis* are similar, *R. typhi* cannot be completely ruled out as the causative agent. However, given the molecular data from the cat fleas, *R. felis* is the more likely causative agent. In the past, the incidence of *R. felis* infection in patients with raised typhus group antibody levels may have been underestimated, with the causative agent probably reported as *R. typhi* when it may have been *R. felis* — a confusion that has been seen in other studies.[8,9]

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Competing interests

None identified.

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