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Bartonella clarridgeiae, *B. henselae* and *Rickettsia felis* in fleas from Morocco

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A total of 554 fleas were collected in the Moroccan Casablanca and Tiznit regions from domesticated animals and ruminants between August 2007 and October 2008 and were tested for the presence of *Rickettsia* spp. and *Bartonella* spp. using molecular methods. For the first time in Morocco, we found *Rickettsia felis*, the agent of flea-borne spotted fever in *Ctenocephalides felis*; *B. henselae*, an agent of cat scratch disease; and *Bartonella clarridgeiae*, a cat pathogen and potentially a human pathogen.

INTRODUCTION

Fleas are insects in the order *Siphonaptera* and include ~2500 species and subspecies (Bitam *et al.*, 2010). Fleas are medically and economically important because they are vectors of several diseases relevant to human health, including plague (Bitam *et al.*, 2006a), murine typhus and bartonelloses (Bitam *et al.*, 2010). Flea-borne disease organisms are widely distributed throughout the world in endemic disease foci that contain components of the enzootic cycle (Bitam *et al.*, 2010).

In Morocco, studies of flea and human flea-borne infections have been limited, even though murine typhus, or 'endemic typhus', is prevalent and plague has been reported (Blanc *et al.*, 1936). During the French protectorate of Morocco in the 1930s, the Pasteur Institute of Morocco isolated the agent of the murine typhus, *R. typhi* (formerly *R. mooseri*) from rats of the city and port of Casablanca. Two years later, the first case of murine typhus in Morocco was confirmed (Blanc *et al.*, 1936). However, current data are unavailable regarding flea-borne rickettsioses in Morocco.

In this study, we analysed fleas collected in Morocco for evidence of *Rickettsia* spp. and *Bartonella* spp. infection to identify the possible aetiological agents and vectors for flea-borne agents that affect humans.

MATERIALS AND METHODS

Collection and Identification of Fleas

From August 2007 to October 2008, fleas were collected from sheep, cats and dogs in

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Casablanca as well as from sheep and goats in the Tiznit region in southern Morocco and were stored in 70% alcohol and transported to France. Using taxonomic keys (Beaucournu and Launay, 1990), one researcher (NB) identified the fleas morphologically, and a second researcher (JCB) reexamined 5% of each batch.

Polymerase Chain Reaction and Sequencing

All arthropod specimens were surface disinfected by 5-minute immersion in iodinated alcohol before they were rinsed with sterile distilled water for 10 minutes and dried. They were crushed individually in a sterile Eppendorf tube. DNA was extracted from each specimen using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Each sample was tested for the presence of *Rickettsia* spp. DNA by real-time semi-quantitative PCR (qPCR) in a Smartcycler, using primers and Taqman probes that targeted a partial sequence of the citrate synthase (*gltA*) gene (Berrelha *et al.*, 2009). Positive samples were further tested by real-time semi-quantitative PCR using primers and probes that targeted the *R. felis bioB* gene (Berrelha *et al.*, 2009). Each sample of DNA was screened for the *Bartonella* genus using qPCR with a Taqman probe that targeted the 16S/23S RNA intergenic spacer (ITS) (Varagnol *et al.*, 2009). In each test, we included a negative control of DNA extracted from uninfected laboratory ticks and a positive control of DNA from *R. felis* and *B. elizabethae* (ITS PCR) (one positive control for every 20 samples). The flea DNA samples that tested positive for *Bartonella* by qPCR were confirmed via standard PCR of the ITS gene fragments (Rolain *et al.*, 2003) using the GeneAmp PCR System 2400 and 9700 thermal cyclers (PerkinElmer, Waltham, MA, USA). Amplification products were analysed by electrophoresis on a 1% agarose gel that was stained with ethidium bromide. To identify the detected *Bartonella* spp., PCR products were purified and sequenced in duplicate (both forward and reverse) using the BigDye Terminator v1.1 Cycle Sequencing Kit (ABI PRISM, PE Applied Biosystems, Coignieres, France) and primers specific for the ITS fragments (Rolain *et al.*, 2003). The sequences were resolved on an ABI 3100 capillary sequencer (Applied Biosystems). All sequences were assembled and edited with the Auto Assembler software version 1.4 (Perkin Elmer). We used BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) to compare our sequences with those in the GenBank database.

RESULTS

A total of 554 fleas were collected, including 391 *Ctenocephalides felis*, 130 *Pulex irritans* and 33 *Ctenocephalides* spp. In all qPCR and PCR experiments, the expected results were obtained for the positive and negative controls. *R. felis* DNA was detected in 112 (20%) of 554 fleas. *Bartonella* DNA was detected by qPCR in 24 (4.3%) of 554 fleas. Seven of the 24 positive fleas (seven *Ctenocephalides felis* collected in the Casablanca and Tiznit regions) tested positive for *Bartonella* spp. using the standard ITS PCR. Sequencing identified *Bartonella clarridgeiae* (100% similarity) in three *C. felis* samples collected from cats in Casablanca and *Bartonella henselae* in four *C. felis* (99.08% similarity) samples collected from goats and sheep in the Tiznit region. Table summarizes our results, and Fig. 1 shows a map of the Moroccan regions used in this survey.

DISCUSSION

Our results provide the first evidence of *R. felis*, *B. henselae* and *B. clarridgeiae* in Morocco, and they suggest the presence of the related flea-borne diseases.

R. felis is the agent of flea-borne spotted fever. *R. felis* was first detected (as '*R. ctenocephali*') in *C. felis* in Europe in 1918,

TABLE. *Flea collection and detection of* Rickettsia *spp.* and Bartonella *spp., Morocco 2007–2008*

Flea collection					Results				
					Detection of *Rickettsia* spp.		Detection of *Bartonella* spp.		
Collection sites (number of fleas collected)		Host	Species of fleas collected	Fleas number	Positive samples using qPCR of a partial sequence of *gltA*	Positive samples using qPCR of a *R. felis* gene	Positive samples using PCR of the ITS	Identification by gene sequence (% similarity with the corresponding sequence available in GenBank)	Co-infection
Tiznit	Douar Afaibork (367)	Goats and sheep (3 herds)	*Ctenocephalides* spp.	22	0	0	0	*Bartonella henselae*	
			C. felis	277	4	4	4	(99.08 % with L35101.1)	
			Pulex irritans	68	0	0	0		
	Douar Lahlate (48)	Goats and sheep (1 herd)	*Pulex irritans*	48	0	0	0		
	Douar Tazmourt (25)	Sheep (1 herd)	*Pulex irritans*	14	0	0	0		
			Ctenocephalides spp.	11	0	0	0		
Casablanca	(114)	Dogs and sheep	*C. felis*	105	102	102	0		
		Cats	*C. felis*	9	6	6	3	*Bartonella clarridgeiae* (100% with AF312497.1)	2

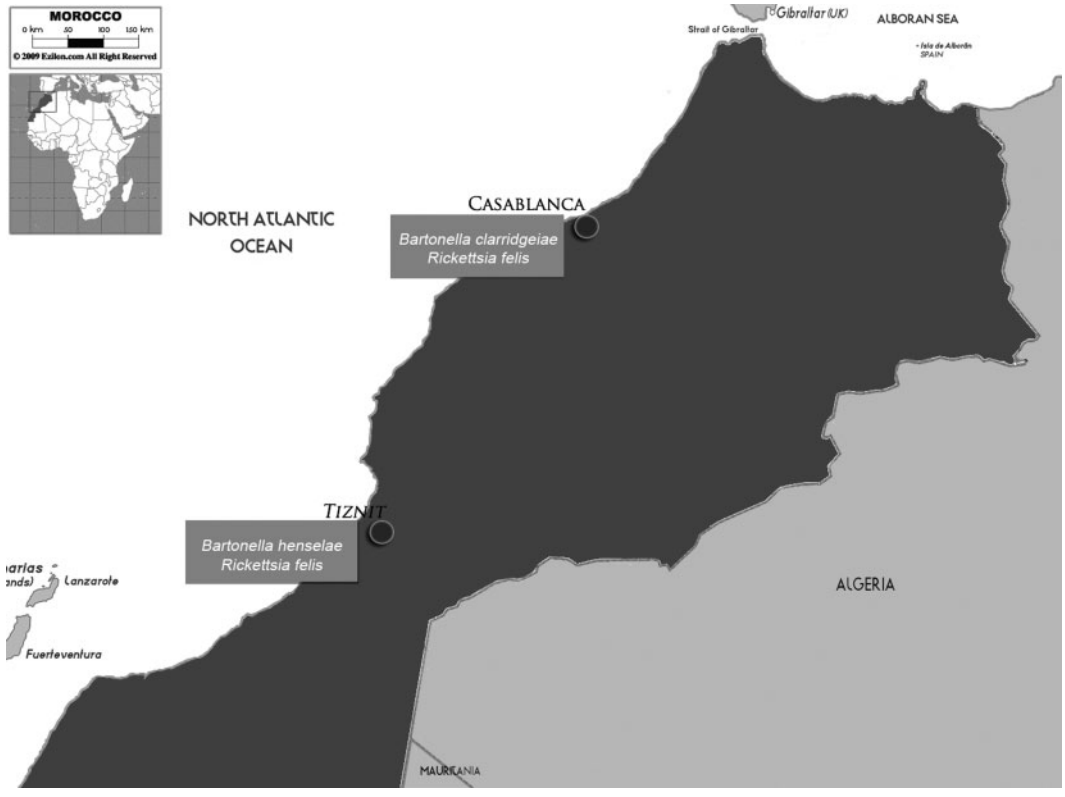

FIG. 1. Map of Moroccan regions of the fleas collection.

was rediscovered in 1990 and was definitively characterized in 2002 (Bitam *et al.*, 2010). This rickettsia has been associated with a large variety of flea species; however, *C. felis* is currently the only known biological vector of *R. felis* and serves as the main reservoir. This flea species, likely plays a central role in transmitting human illness (Pérez-Osorio *et al.*, 2008; Reif and Macaluso, 2009). In North Africa, *R. felis* has been identified in fleas from Algeria (Bitam *et al.*, 2006b). Flea-borne spotted fever is an emerging disease with few confirmed cases throughout the world. The first human case of *R. felis* infection was reported from Texas in 1994 (Reif and Macaluso, 2009). Since then, additional human cases have been reported from Spain, Germany, France, Egypt, Brazil, Mexico, Thailand, Taiwan, South Korea, Laos, Tunisia and Australia (Parola *et al.*, 2003; Kaabia *et al.*, 2006; Znazen *et al.*, 2006; Williams *et al.*, 2011). More recently, a study conducted over 9 months in two Senegalese villages, found that up to 6% of 134 indigenous febrile non-malaria patients were infected with *R. felis* (Socolovschi *et al.*, 2010). The major clinical symptoms of these patients were fever associated with weakness, headache with sleep disorders and digestive and respiratory symptoms. The lack of cutaneous rash or inoculation eschar was noted; however, a cutaneous rash might be imperceptible in patients with pigmented skin.

In Morocco, eight different rickettsiae of the spotted fever group have been identified, including seven pathogens: *R. conorii, R. aeschlimannii*, *R. massiliae*, *R. slovaca*, *R. monacensis*, *R. raoultii* and *R. helvetica* (Sarih *et al.*, 2008; Boudebouch *et al.*, 2009a; Boudebouch *et al.*, 2009b).

Our results revealed an important *R. felis* infestation of *Ctenocephalides* fleas, which

were collected from cats and dogs as well as from a herd of sheep from the same location. *Pulex irritans* were not found infected, although these fleas have already been found elsewhere to be infected by *R. felis* (Azad *et al.*, 1997; Sackal *et al.*, 2008).

Our results suggest that *R. felis* infections are prevalent in Morocco and that fleas are potential vectors of flea-borne spotted fever in this country. Further studies are needed to elucidate and describe the epidemiology of *R. felis* infections (Pérez-Osorio *et al.*, 2008). However, clinicians in Morocco, and those elsewhere who may see patients who have travelled to Morocco, need to be aware of possible *R. felis* infections in patients who present signs of spotted fever and/or an eschar. Although it may be misdiagnosed as and treated similarly to tick-borne Mediterranean spotted fever, the risk of exposure and methods of prevention of flea-transmitted diseases are different and warrant consideration.

We further report the first detection of *Bartonella clarridgeiae* and *B. henselae* in fleas from Morocco. qPCR appeared to be more sensitive for the detection of these bacteria compared with regular PCR. *Bartonella* are fastidious, gram-negative bacteria that have been identified on a variety of domestic and wild mammals. Currently, more than 20 known species or subspecies of *Bartonella* are known. Among the 13 species and subspecies that are known or suspected to be human pathogens, four have been isolated from cats (Chomel and Kasten, 2010). Fleas appear to play an important role in the maintenance and transmission of many *Bartonella* species among cat and rodent populations (Chomel *et al.*, 2009). *B. henselae* is the causative agent of cat scratch disease (CSD), which is characterized by benign regional lymphadenopathy, low-grade fever, malaise and aching. In addition, headache, anorexia and splenomegaly can occur, while abscessed lymph nodes are reported occasionally. Five to nine percent of CSD cases may develop atypical manifestations, including Parinaud's oculoglandular syndrome, encephalitis, endocarditis, hemolytic anemia, hepatosplenomegaly, glomerulonephritis, pneumonia, relapsing bacteremia and osteomyelitis (Chomel and Kasten, 2010). CSD is more frequently observed in persons who are younger than 20 years old and in people who own a young cat or who have been scratched or bitten by a cat (Chomel and Kasten, 2010). *B. henselae* is also involved in other clinical situations, such as endocarditis, bacillary angiomatosis and peliosis hepatitis in immunocompromised patients (Rolain *et al.*, 2003). Domestic cats are the principal reservoir for B. henselae. Several serotypes have been identified, such as the two main genotypes designated Houston-1 and Marseille. The respective prevalences of these two genotypes vary considerably among cat populations in different geographical areas (Bouchouicha *et al.*, 2009; Angelakis *et al.*, 2010; Chomel and Kasten, 2010).

Bartonella clarridgeiae is known to infect domestic cats. It was first isolated in the USA from the pet of an HIV-positive patient (Chomel and Kasten, 2010). It has been suspected to be an agent of CSD in humans, but its pathogenic role in humans has yet to be definitively demonstrated (Anderson and Neuman, 1997). The transmission of *B. clarridgeiae* is associated with the inoculation of wounds with infected cat flea (*Ctenocephalides felis*) feces (Breitschwerdt and Kordick, 2000).

These results suggest the presence of infection with these organisms; however, more studies are needed to precise the prevalence of these agents in arthropods in Morocco to estimate the risk of human exposure.

The incidence of flea-borne diseases in the world is much greater than is generally recognized by physicians and health authorities. As a result, diagnosis and treatment are often delayed by health care professionals who are unaware of the presence of these infections and thus do not take them into consideration during diagnosis.

REFERENCES

Anderson, B. E. & Neuman, M. A. (1997). Bartonella spp. as emerging human pathogens. *Clinical Microbiology Reviews*, **10**, 203–219.

Angelakis, E., Billeter, S. A., Breitschwerdt, E. B., Chomel, B. B. & Raoult, D. (2010). Potential for tick-borne bartonelloses. *Emerging Infectious Diseases*, **16**, 385–391.

Azad, A. F., Radulovic, S., Higgins, J. A., Noden, B. H. & Troyer, J. M. (1997). Flea-borne rickettsioses: ecologic considerations. *Emerging Infectious Diseases*, **3**, 319–327.

Beaucournu, J. C. & Launay, H. (1990). *Les Puces de France et du Bassin Méditerranéen Occidental. Faune de France, Vol. 76*, Paris: FFSSN.

Berrelha, J., Briolant, S., Muller, F., Rolain, J. M., Marie, J. L., Pagés, F., Raoult, D. & Parola, P. (2009). *Rickettsia felis* and *Rickettsia massiliae* in Ivory Coast, Africa. *Clinical Microbiology and Infection*, **15**, 251–252.

Bitam, I., Baziz, B., Rolain, J. M., Belkaid, M. & Raoult, D. (2006a). Zoonotic focus of plague, Algeria. *Emerging Infectious Diseases*, **12**, 1975–1977.

Bitam, I., Dittmar, K., Parola, P., Whiting, M. & Raoult, D. (2010). Fleas and flea-borne diseases. *International Journal of Infectious Diseases*, **14**, e667–e676.

Bitam, I., Parola, P., De La Cruz, K. D., Matsumoto, K., Baziz, B. & Rolain, J. M. (2006b). First molecular detection of *Rickettsia felis* in fleas from Algeria. *American Journal of Tropical Medicine and Hygiene*, **74**, 532–535.

Blanc, G., Pouponneau, A., Baltazard, M. & Carbou, A. (1936). Existence du Typhus murin chez l'homme au Maroc: première observation faite à Casablanca. *Maroc Médical*, 165.

Bouchouicha, R., Durand, B., Monteil, M., Chomel, B. B., Berrich, M., Arvand, M., Birtles, R. J., Breitschwerdt, E. B., Koehler, J. E., Maggi, R., Maruyama, S., Kasten, R., Petit, E., Boulouis, H. J. & Haddad, N. (2009). Molecular epidemiology of feline and human *Bartonella henselae* isolates. *Emerging Infectious diseases*, **15**, 813–816.

Boudebouch, N., Sarih, M., Socolovschi, C., Amarouch, H., Hassar, M., Raoult, D. & Parola, P. (2009a). Molecular survey for spotted fever group rickettsiae in ticks from Morocco. *Clinical Microbiology and Infection*, **15**, 259–260.

Boudebouch, N., Sarih, M., Socolovschi, C., Fatihi, T., Chakib, A., Amarouch, H., Hassar, M., Rolain, J. M., Parola, P. & Raoult, D. (2009b). Spotted fever group rickettsioses documented in Morocco. *Clinical Microbiology and Infection*, **15**, 257–258.

Breitschwerdt, E. B. & Kordick, D. L. (2000). *Bartonella* infections in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clinical Microbiology Reviews*, **13**, 428–438.

Chomel, B. B., Boulouis, H. J., Breitschwerdt, E. B., Kasten, R. W., Vayssier-Taussat, M., Birtles, R. J., Koehler, J. E. & Dehio, C. (2009). Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. *Veterinary Research*, **40**, 29.

Chomel, B. B. & Kasten, R. W. (2010). Bartonellosis, an increasingly recognized zoonosis. *Journal of Applied Microbiology*, **109**, 743–750.

Kaabia, N., Rolain, J. M., Khalifa, M., Ben Jazia, E., Bahri, F., Raoult, D. & Letaïef, A. (2006). Serologic study of rickettsioses among acute febrile patients in central Tunisia. *Annal New York Academy of Science*, **1078**, 176–179.

Parola, P., Sanogo, Y. O., Lerdthusnee, K., Zeaiter, Z., Chauvancy, G., Gonzalez, J. P., Miller, R. S., Telford, S. R. 3rd, Wongsrichanalai, C. & Raoult, D. (2003). Identification of *Rickettsia spp.* and *Bartonella spp.* in fleas from the Thai-Myanmar border. *Annal New York Academy of Science*, **990**, 173–181.

Pérez-Osorio, C. E., Zavala-Velázquez, J. E., Arias León, J. J. & Zavala-Castro, J. E. (2008). *Rickettsia felis* as emergent global threat for humans. *Emerging Infectious Diseases*, **14**, 1019–1023.

Reif, K. E. & Macaluso, K. R. (2009). Ecology of *Rickettsia felis*: a review. *Journal of Medical Entomology*, **46**, 723–736.

Rolain, J. M., Franc, M., Davoust, B. & Raoult, D. (2003). Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. *Emerging Infectious Diseases*, **9**, 338–342.

Sackal, C., Laudisoit, A., Kosoy, M., Massung, R., Eremeeva, M. E., Karpathy, S. E., Van, W. K., Gabitzsch, E. & Zeidner, N. S. (2008). *Bartonella spp.* And *Rickettsia felis* in fleas, Democratic Republic of Congo. *Emerging Infectious Diseases*, **14**, 1972–1974.

Sarih, M., Socolovschi, C., Boudebouch, N., Hassar, M., Raoult, D. & Parola, P. (2008). Spotted fever group rickettsiae in ticks, Morocco. *Emerging Infectious Diseases*, **14**, 1067–1073.

Socolovschi, C., Mediannikov, O., Sokhna, C., Tall, A., Diatta, G., Bassene, H., Trape, J.-F. & Raoult, D. (2010). *Rickettsia felis*-associated uneruptive fever, Senegal. *Emerging Infectious Diseases*, **16**, 1140–1142.

Varagnol, M., Parola, P., Jouan, R., Beaucournu, J.-C., Rolain, J. M. & Raoult, D. (2009). First detection of *Rickettsia felis* and *Bartonella clarridgeiae* in fleas from Laos. *Clinical Microbioly and Infection*, **15**, 334–335.

Williams, M., Izzard, L., Graves, S. R., Stenos, J. & Kelly, J. J. (2011). First probable Australian cases of human infection with *Rickettsia felis* (cat-flea typhus). *Medical Journal of Australia*, **194**, 41–43.

Znazen, A., Rolain, J. M., Hammami, A., Jemaa, M. B. & Raoult, D. (2006). *Rickettsia felis* infection, Tunisia. *Emerging Infectious Diseases*, **12**, 138–140.