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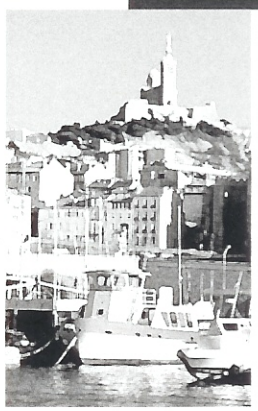

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

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P095 First detection of Rickettsia felis in fleas from Laos

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Background: Rickettsia felis is the agent of flea-borne spotted fever, an emerging disease that has been associated with several flea species throughout the world. However, in Asia, R. felis has been identified in Thailand and Indonesia only. Also, the epidemiology of rickettsioses has been poorly studied in the Lao People's Democratic Republic.

Objectives: The aim of this work is to detect rickettsiae in fleas collected in Laos and analyzed by molecular methods.

Methods: In December 2007, in Phu Khaokhoay (around 100 km from Vientiane), a total of 22 fleas were collected on dogs, including 16 Ctenocephalides orientis, 3 Ct. canis, and 3 Ct. felis felis. A specimen of Ct. orientis was also collected on a cat. DNA was extracted from each specimen. Each sample was screened by a specific real time polymerase chain reaction (RT-PCR) done in a LightCyclerTM instrument using primers and TaqmanTM probes targeting a fragment of the gltA gene of spotted fever group rickettsiae. Positive samples were then tested by a RT-PCR using TaqmanTM probes targeting specifically R. felis.

Results: A total of 13 fleas were found to be infected by R. felis. These results provide the first evidence of R. felis in Laos, and suggest the presence of flea-borne spotted fever in this country.

P096 Rickettsia felis and R. massiliae in Ivory Coast, Africa

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Background: Spotted fever group rickettsioses have been poorly studied in sub-Saharan Africa. This is the case in Ivory coast, where Rickettsia africae, the agent of African tick-bite fever, is the sole SFG rickettsia known to be prevalent in the country.

Objectives: The aim of this work is to detect spotted fever group rickettsiae in fleas and ticks collected in Ivory Coast, Africa.

Methods: In 2007, ticks and fleas were collected from rodents and dogs near Bouaké. Each arthropod was identified using morphological criteria and when doubtful using molecular methods. Rickettsiae were detected in the DNA samples extracted from each specimen. The detection of Rickettsia spp was made by polymerase chain reaction (PCR) amplification and DNA sequencing for ticks, and by a specific real time polymerase chain reaction (RT-PCR) done in a LightCycler* instrument using primers and Taqman* probes for fleas.

Results: A total of 13 fleas and 25 ticks were tested. R. massiliae was identified in a Rhipicephalus sp. tick collected from a dog. R. felis was detected in a Ctenocephalides canis specimen collected from dog.

Conclusion: This is the first detection of R. felis, the agent of the emerging flea-borne spotted fever, and R. massiliae, an emerging tick-borne pathogen. These results have both epidemiological and clinical consequences.

P097 Mediterranean spotted fever and other Rickettsial diseases in Tunisia: report of 174 cases

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Background: Between human rickettsial infections in Tunisia, Mediterranean spotted fever (MSF) is the most frequent and it's commonly admitted that the disease is benign.

Objective: To study epidemiological and clinical features of rickettsioses and to precise clinical presentation of each rickettsial infection in order to improve management of those diseases.

Methods: Retrospective study of rickettsial infections cases treated between January 1992 and December 2006 in the department of infectious diseases of Rabta Hospital in Tunis. Case's definition was based on clinical criteria and/ or positive rickettsial serology.

Results: 174 patients were included, 119 males and 55 females with mean age near 35 years. 47% of patients were living in rural area and 66% had a domestic dog. Fever was present in 99% of patients and the dissociation between heart pulsation and body temperature in 38% of them. Headache and skin eruption were presents respectively in 66% and 55% of patients. "Tache noire" was detected in 70% of cases and often localized in leg and thigh (30%). Biochemical laboratory tests revealed: normal white blood cell count in 109 cases (63%), thrombocytopenia in 88 cases (51%) and hepatic cytolysis in 109 cases (63%). A high level of lactate dehydrogenase is noted in 98 patients (56%). X-chest radiography was performed for 134 cases; it was pathological in 13% of cases. The most frequent complications are respiratory (10%) and neurological (9%). Eight patients had electric modifications of the electrocardiogram. Serodiagnostic was performed for 117 patients. Antibody titers, detected by indirect immunofluorescence are positive in only 46 patients (39%). 85% of patients were infected by Rickettsia conorii and 13% had significant level of antibody against Rickettsia typhi. Doxycyclin was frequently used in the treatment. (60% of cases) Clinical outcome was favourable for 172 patients and tow patients died.

Conclusion: MSF is the dominant rickettsial infection in Tunisia and it is often benign. Human infection by other "emerging or re-emerging" rickettsia will be probably reported in the future.

P098 Rickettsia conorii Malish and Israeli spotted fever strains causing disease in Portuguese Dogs

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Objectives: Mediterranean spotted fever (MSF) has the highest incidence in Portugal compared with other endemic countries. However, few studies have been performed to understand the disease in dogs, one of the most important hosts of Riphicephalus sanguineus ticks, vector of Rickettsia conorii strains. Our main objective was to identify R. conorii as a cause of disease in dogs with clinical suspicion of tick-borne disease.

Methods: MSF was investigated in dogs with clinical suspicion of tick-borne disease in Algarve, Portugal. Fifty five dogs were subjected to physical evaluation, and blood samples were collected. Serological assay, detection of rickettsial DNA by PCR, and isolation attempts performed by shell-vial technique were done to confirm the diagnosis of infection by R. conorii in dogs. Differential diagnosis was performed for Anaplasma spp, Ehrlichia canis, Borrelia burgdorferi s.l and Babesia canis.

Results: From a total of 55 dogs, 28 (51%) had antibodies against spotted fever group. R. conorii Malish DNA was amplified from six blood samples and R. conorii Israeli spotted fever strain from another two. The illness of the eight dogs with confirmed canine MSF, with a recent history of exposure to ticks, was documented based on clinical manifestations and laboratory findings. The most common signs and symptoms found were fever (100%), anorexia (100%), and prostration (100%). Digestive signs such as vomit were found in 37.5% of the dogs, and diarrhea in 25%. One dog presented epistaxis and another petechial rash. Additionally, the presence of thrombocytopenia (100%), leucopenia (50%) and anemia (50%) were reported. R. sanguineus ticks attached to three different dogs at the time of admission were negative to the presence of rickettsial DNA when they were tested by PCR assay.

Conclusion: To our knowledge this study reports the detection of R. conorii Malish and R. conorii Israeli tick typhus strains associated with canine disease for the first time in Portugal. An interesting aspect of this study is that the symptoms presented by the dogs are similar to those described in human cases. The petechial rash found in one dog may well be an indication of severe disease as is the case in humans.

P099 First report of Ehrlichia ewingii detected by molecular investigation in dogs from Brazil

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The genus Ehrlichia encompass a group of obligate intracellular bacteria that infect phagocytes and are transmitted by ticks to dogs, equines, ruminants, felines and human. Canine granulocitic ehrlichiosis, caused by Ehrlichia ewingii, is a moderately severe disease causing polyarthrite in chronically infected dogs. It has already been described in dogs from the United States and Cameroon. This work aimed to verify incidence of E. ewingii in dogs from Viçosa, Minas Gerais state, Brazil. For this, 100 samples of blood were obtained from dogs that had arrived at the Veterinary Hospital of Federal University of Viçosa for accomplishment of complete blood count. These samples were tested for nested-PCR with genus-specific primers (ECB/ECC) and specie-specific primers for E. ewingii (EE5/HE3). Five dogs infected by E. ewingii were found by nested-PCR. This is the first report of E. ewingii infecting dogs in South America. In North America, E. ewingii is mainly transmitted by Amblyomma americanum, been the ticks Riphicephalus sanguineus and Dermacentor variabilis found infected with this bacterium, suggesting these tick species as a potential vectors. As these species, except R. sanguineus, are not common in South America, other species of ticks should be involved in the transmission of E. ewingii to dogs in Brazil. Therefore, further studies should be undertaken to provide a better understanding of E. ewingii ecology in Brazil.

P100 First molecular detection of Ehrlichia canis in cats from Brazil

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Ehrlichia species that naturally infected felines were not been characterized yet. Furthermore inclusions Ehrlichia-like have been observed in monocytes, lymphocytes and granulocytes of cats with fever symptoms and throbocytopenia. Serological evidence were reported in Spain, France and USA. Molecular evidences confirm the presence of Ehrlichia canis in cats with compatible clinical signs of monocitic ehrlichiosis in North America.

In Brazil the first serological evidence of E. canis in free-ranging felids was reported in 2006. This work aimed to verify incidence of E. canis in cats from Viçosa, Minas Gerais state, Brazil. For this, 15 samples of blood were obtained from cats that had arrived at the Veterinary Hospital of Federal University of Viçosa for accomplishment of complete blood count. These samples were tested for nested-PCR with genus-specific primers (ECB/ECC) and specie-specific primers for E. canis (ECAN5/HE3). Three cats infected with E. canis were found by nested-PCR. Only one animal showed thrombocytopenia. This is the first molecular report of E. canis infecting cats in South America. The transmission route of ehrlichial diseases in cats is not known yet. Furthermore, the increase of the number of reported cases of Ehrlichia infections in these animals, reinforce the necessity of more studies to define which species are infecting cats and the role of cats in the epidemiology of ehrlichiosis in Brazil.

P101 Spotted Fever Rickettsiae, Ehrlichia and Anaplasma in Ticks from Peridomestic Environments in Panama

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Objectives: Rickettsiae and rickettsial diseases have been known in Panama since the 1950; however, the spectrum of rickettsial agents, their arthropod vectors and geographic distribution remain poorly known. The purpose of this work was to assess the presence and prevalence of Rickettsia and Anaplasmataceae in ticks from peridomestic sites in Panama.

Methods: 185 ticks of the genus Amblyomma (90), Anocentor (71), Boophilus (20) and Rhipicephalus (4) were collected from horses and dogs from 5 peridomestic sites and from a tapir from a local Zoo (El Valle), and a cow from Cerro Chucantí. DNA was extracted from individual ticks and screening was conducted. SYBR-Green PCR assays detecting a fragment of the OmpA gene and 16S rRNA gene, respectively, were used for detection of DNA of the spotted fever group rickettsiae (SFG) and Anaplasmataceae. TaqMan targeting gltA was used for detection of DNA of R. prowazekii. Positive results were verified by direct sequencing of the PCR products.

Results: In total, 61.6% ticks were found positive for SFG rickettsiae; including Amblyomma (90%), Anocentor (42%), and Boophilus (15%). No R. prowazekii DNA was detected. DNA of Anaplasmataceae was detected in 40% Boophilus, 16.9% Anocentor and 13% Amblyomma. Rickettsia amblyommii, Ehrlichia chaffeensis, E. ewingii, Anaplasma phagocytophilum, and A. marginale were identified by DNA sequencing.

Conclusions: Although Rocky Mountain spotted fever is endemic in Panama, R. rickettsii was not detected during this study. Exposure to R. amblyommii may be responsible for the high rates of SFG seropositivity previously determined in Panama. The unexpected high prevalence of diverse Anaplasmataceae in ticks from peridomestic environments suggests that the veterinary and public health impact of these agents in Panama needs further evaluation.

P102 Clearance of Ehrlichia canis after doxycycline treatment initiated during different phases of experimental canine monocytic Ehrlichiosis

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Ehrlichia canis is a tick-borne monocytotropic member of the rickettsial family Anaplasmataceae, and the primary etiologic agent of canine monocytic ehrlichiosis (CME). This relatively well-characterized disease offers unique advantages and opportunities to probe interactions among monocytotropic Ehrlichia spp. and their mammalian and tick hosts, and such studies can help to elucidate potential roles of dogs as sentinels, reservoirs and models for tick-borne zoonoses. Experimentally induced CME sequentially consists of acute, subclinical and chronic phases, and doxycycline is the current antibiotic of choice for treating all phases of this disease. Although doxycycline effectively alleviates clinical CME, some studies report that E. canis infections persisted after doxycycline treatments of naturally and experimentally infected dogs during the post-acute phases of illness, which suggests development of a host carrier state that could lead to recrudescence and opportunity for infection of tick vectors. Conversely, other studies report clearance of E. canis infections from experimentally infected dogs treated during acute and subclinical phases of CME, indicating that doxycycline is efficacious against both the infection and the disease.

Rickettsia conorii Malish and Israeli spotted fever strains causing disease in Portuguese Dogs

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Introduction: Dogs are the most important hosts of *Rhipicephalus sanguineus* ticks, the main vector and reservoir of *Rickettsia conorii* strains. In Portugal Mediterranean spotted fever is caused by *R. conorii* Malish and *R. conorii* Israeli tick typhus, that have been isolated from *R. sanguineus* ticks and MSF patients (Bacellar *et al*. 1999, Sousa *et al.* 2006). The role of the dog in *R. conorii* cycle is still not conclusive and the fact that dogs can develop disease with this agent has been a controversial subject.(Solano-Galego *et al*. 2005). Here we report, acute rickettsial illness caused by different strains of *R.conorii,* in dogs with clinical suspicion of tick-borne disease.

Methods: MSF was investigated in dogs with clinical suspicion of tick-borne disease. Fifty-five dogs from the Algarve region were subjected to physical evaluation between February and October 2004, and blood samples were collected for laboratory diagnostic. Imunofluorescence assay (IFA), detection of rickettsial DNA by PCR, and isolation attempts were performed to confirm the diagnosis of infection by *R. conorii*. Differential diagnosis was performed for *Anaplasma* spp., *Ehrlichia canis*, *Borrelia burgdorferi* s.l and *Babesia canis*.

Figure. a) Ticks attached b) Petechial rash

Results: From a total of 55 dogs, 28 (51%) had antibodies against spotted fever group. *R. conorii* Malish DNA was detected from six blood samples and *R. conorii* Israeli spotted fever strain from another two. The illness of the eight dogs with confirmed canine MSF, with a recent history of exposure to ticks, was documented based on clinical manifestations and laboratory findings. The most common signs and symptoms found in dogs were fever (100%), anorexia (100%), prostration (100%), vomit (37.5 %) and diarrhea (25%). One dog presented epistaxis and another petechial rash. Additionally, the presence of thrombocytopenia (100%), leucopenia (50%) and anemia (50%) were reported. *R. sanguineus* ticks attached to three different dogs at the time of admission were negative for the presence of rickettsial DNA when tested by PCR.

Discussion and conclusions: To our knowledge this study reports, for the first time in Portugal, the detection of *R. conorii* Malish and *R. conorii* Israeli tick typhus strains, and their association with canine disease. This finding corroborates also the recently descriptions of canine disease in Italian dogs caused by *R. conorii* Malish strain (Solano-Galego et al. 2005) To this date, the evidence of *R. conorii* infection in dogs in our country had been solely based on the detection of antibodies. Additionally, we were able to confirm that the identified strains of *R. conorii* were the same that cause the disease in humans.

Clinical signs and symptoms presented by the dogs are similar to those described in human MSF cases. Moreover, the petechial rash found in one dog could be an indication of severe disease as is it occurs in humans.

Table 1. Epidemiological, clinical and laboratory data from dogs with confirmed canine MSF

Case No.	Sex	Age	Breed	Clinical signs	Buffy coat smear	Hematologic abnormalities	IFA		PCR	
							R. conorii	*E. canis*	*R. conorii*	*E. canis*
1	F	3 y	Undetermined	Fever (40,4 ºC), anorexia, vomit	-	L (2,18 x10^3/µl), T (77 x10^3/µl)	+	-	Malish	-
2	M	2 y	Dalmatian	Fever (41, 0 ºC), anorexia, prostation	-	L (4,08 x10^3/µl), A (4,84 x10^3/µl), T (133 x10^3/µl)	+	-	Malish	-
3	F	3 m	Serra da estrela	Fever (39,8 ºC), anorexia	-	A (3,91 x10^3/µl), T (52 x10^3/µl)	-	-	Malish	-
4	M	3 y	Undetermined	Fever (40,5ºC), anorexia, epistaxis, weight lost	-	L (5,62 x10^3/µl), A (3,29 x10^3/µl), T (90 x10^3/µl)	+	+	Malish	-
5	F	4 y	Toy poodle	Fever (40,5 ° C), anorexia, vomit, diarrhea	-	L (4,9 x10^3/µl), T (102 x10^3/µl)	+	-	Malish	-
6	M	4 m	Undetermined	Fever (41, 1ºC), anorexia, prostation	*B. canis merozoites*	Lc (21,8 x10^3/µl), A (1,46 x10^3/µl), T (58 x10^3/µl)	-	-	Malish	-
7	F	2 y	Toy poodle	Fever (39, 8ºC), anorexia, petechiae	-	T (46 x10^3/µl)	+	-	Israeli tick typhus	-
8	M	5 y	Pincher	Fever (39,8 ºC), anorexia, vomit, diarrhea	-	T (67 x10^3/µl)	+	-	Israeli tick typhus	-

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