European Journal of Epidemiology **10**: 695–698, 1994.

Serologic evidence for human ehrlichiosis in Africa

P. Brouqui[1], C. Le Cam[2], P.J. Kelly[2], R. Laurens[3], A. Tounkara[4], S. Sawadogo[5], Velo-Marcel[6], L. Gondao[7], B. Faugere[8], J. Delmont[8], A. Bourgeade[8] & D. Raoult[1]

[1] *Unité des Rickettsies, Faculté de Médecine de la Timone, Marseille, France;* [2] *Faculty of Veterinary Science, University of Zimbabwe, Mount Pleasant, Harare, Zimbabwe;* [3] *Mission de Coopération Francaise, Luanda, Angola;* [4] *Laboratoire National de Santé Publique, Bamako, Mali;* [5] *Centre de Transfusion Sanguine, Ouagadougou, Burkina Faso;* [6] *Ministére de la Santé, Moroni, Comores;* [7] *Centre de Transfusion Sanguine, Bangui, République Centrafricaine;* [8] *Centre de Recherche et de Formation en Médecine et Santé Tropicale, Hôpital Félix Houphöuet Boigny, Marseille, France*

Accepted in revised form 31 March, 1994

Abstract. Human ehrlichiosis is a recently recognized rickettsial disease. It is caused by *Ehrlichia chaffeensis*, an intraleucocytic Gram-negative, obligate intracellular bacterium, grouped within the genus *Ehrlichiae*. Most human cases of ehrlichiosis have been diagnosed in the USA. Two cases have been reported outside of the USA, one in Europe and one in Africa. From 1 January to 30 June 1992, 765 sera from blood donors or other asymptomatic subjects in 8 African countries, including Ivory Coast, Burkina Faso, Mali, Central African Republic, Angola, Zimbabwe, Mozambique and Commores Islands, were tested by indirect immunofluorescence for the presence of *E. chaffeensis* antibodies. Positive sera were confirmed by Western immunoblotting. Only two of 765 sera tested were positive. One serum obtained from Burkina Faso had an IgG titer of 1:200 and one from Mozambique had an IgG titer of 1:80. Human ehrlichiosis seems to occur infrequently in Africa, although many more sera from additional African countries need to be evaluated.

Key words: Africa, Epidemiology, *Ehrlichia chaffeensis*, Human ehrlichiosis, Seroprevalence, Western immmunoblot

Introduction

Human ehrlichiosis is a rickettsial disease which is caused by an intraleucocytic gram-negative bacterium of the genus *Ehrlichiae*. Most cases are probably due to infection by *Ehrlichia chaffeensis*, a species recently isolated only once from human blood [1]. This species is closely related to *E. canis*, which causes disease in dogs and occurs worldwide [2]. Most cases of human ehrlichiosis have been diagnosed in the USA, where the disease was first recognized [1, 3]. However, two cases have been reported outside of the USA, one in Europe [4] and one in Africa [5]. Patients usually present with a history of tick bite, high grade fever, headache, malaise, arthralgia and myalgia. Laboratory findings include leucopenia, thrombocytopenia, and elevated hepatic transaminases. Severe forms of the disease have been reported, including meningitis [6], acute renal failure [3], and acute respiratory distress syndrome [7]. Most patients recovered with tetracycline therapy, but some fatal cases have been reported [8]. In order to further our understanding of the epidemiology of this disease, we investigated the seroprevalence of anti-*E. chaffeensis* antibodies in humans in Africa by using indirect immunofluorescence testing and Western immunoblotting.

Patients and methods

Patients. From 1 January to 30 June 1992, the Research and Formation Center for Medicine and Tropical Health in Marseille, in collaboration with the WHO and African health organizations, collected 765 sera from either blood donors (when possible) or asymptomatic subjects in 8 African countries (Table 1). Sera obtained from subjects in Ivory Coast, Burkina Faso, Mali, Central African Republic, Angola, Zimbabwe, Mozambique and Commores Islands were investigated. Sera were stored at –80 °C prior to submission to the National Center for Rickettsial References and Research in Marseille, France.

Serology. All sera were tested by indirect immunofluorescence (IFA) and IFA-positive sera were confirmed by Western immunoblotting.

IFA testing: *Ehrlichia chaffeensis* (Arkansas strain) and *Ehrlichia canis* (Oklahoma strain) (both

Table 1. Seroprevalence of human ehrlichiosis in Africa

Countries	No. *E. chaffeensis* positive serum* No. serum tested (%)	
Angola	0/113	(0%)
Comoros	0/93	(0%)
Ivory Coast	0/94	(0%)
Mali	0/100	(0%)
Central African Republic	0/100	(0%)
Zimbabwe	0/150	(0%)
Burkina Faso	1/100	(1%)
Mozambique	1/15	(6.6%)

* IFA titer > 1:50

from J. Dawson, CDC, Atlanta GA) were grown in the canine malignant histiocytic cell line DH 82 [9] in the presence of 12.5% fetal calf serum and 5% L-glutamine in MEM. When 50% of the cells were infected, as determined by Diff-Quik (DADE, Düdingen, Germany) staining of slide-centrifuged-infected cells, the supernatant was discarded and the adherent cells were removed with 0.02% EDTA/phosphate buffered saline (PBS), and resuspended in 0.1% sodium azide/PBS at a concentration of 10^6 cells/ml. As positive control, we used serum obtained from a patient convalescent from human ehrlichiosis which had a titer of 1:1600, kindly provided by J.S. Dumler, University of Maryland School of Medicine, Baltimore. All sera were tested for the presence of IgG and IgM antibodies. Prior to IgM-IFA, the sera were absorbed for rheumatoid factor. Anti-*Ehrlichia* antibodies were revealed with an anti-human IgG+IgM FITC-labeled globuline (Immunoteck, Marseille, France). According to previous IFA studies [10] the sera were considered positive if titers were greater than 1:50 for IgG or IgM.

Western immunoblotting: *Ehrlichia chaffeensis* or *E. canis*-infected cells were disrupted mechanically through a 27 gauge needle and non-disrupted cells and debris were separated from free *Ehrlichia* by centrifugation at 100 *g*. *Ehrlichia*-rich supernatant was centrifuged at 7,000 *g* in a Beckman L8-80 ultracentrifuge through a 25% sucrose gradient. The pellet was resuspended in sucrose-phosphate-glutamate buffer, assayed for protein concentration, and dissolved in final sample buffer (FSB: 4% 2-mercaptoethanol, 10% glycerol, 2% sodium-dodecyl-sulfate, SDS, and 0.8% bromophenol blue in 62.5 mM Tris buffer, pH 6.8) at a final concentration of 1 mg/ml of protein. SDS-polyacrylamide slab gel electrophoresis was performed by a modification of the Laemmli method by using a 12.5% polyacrylamide separating gel [11]. Gels were electrophoresed at 20 mA overnight in 25mM Tris buffer (pH 8.3) containing 192 mM glycine and 0.1% SDS. Proteins were then electrotransferred to nitrocellulose membranes (Sartorius, Vangard International, Neptune, NJ) for 2 hours at 27 volts in 25 mM sodium phophate buffer (pH 7.4). Transfer membranes were blocked overnight at 4 °C in blocking buffer (5% nonfat dry milk in PBS with 0.05% Tween 20 and 3% bovine serum albumin) and then were incubated for 2 hours at room temperature with serum diluted 1:80 in blocking buffer. Immunoblots were subsequently washed in three changes of PBS with 0.05% Tween 20, and incubated for 1 hour at room temperature in anti-human (IgG+IgM) peroxidase-labeled secondary antibody (Immunotech, Marseille, France) diluted 1:200 in PBS. The immunoblots were washed again as described above, and bound peroxidase activity was detected by using 4-chloro-1-naphthol as the substrate.

Results and discussion

A total of 765 sera obtained from all over the African continent were tested. By IFA, only two were positive for *E. chaffeensis* antibodies. One submitted from Burkina Faso had an IgG titer of 1:200 and one from Mozambique had an IgG titer of 1:80. No IgM were found. Both serum were also negative for *E. canis* and for other anti-rickettsiae antibodies, including *Rickettsia conorii*, *Coxiella burnetii*, and *Rickettsia typhi*.

Western immunoblot analysis of the serum from the Burkina Faso patient showed that it reacted strongly against some of the *Ehrlichia chaffeensis* SDS-denatured antigenic proteins, especially a 45 kDa proteine (Figure 1, line 1). The fact that the antigenic profile revealed by this patient's serum is not identical to the positive control may indicate that the *Ehrlichia* involved here is not *Ehrlichia chaffeensis* but a closely related *Ehrlichia* spp. When assayed with *E. canis* antigen, the patient's serum also cross-reacted with *E. canis* SDS-denatured antigenic proteins. This discrepancy between *E. chaffeensis* and *E. canis* IFA and Western immunoblot data may be explained by the denaturation of critical conformation-dependant epitopes in SDS, as reported previously between *Ehrlichia sennetsu* and *Ehrlichia risticii* [11].

Although human ehrlichiosis probably occurs as frequently as Rocky Mountain spotted fever in the USA [7], very few cases have been reported elsewhere. We tested more than 5000 sera from the south of France and were unsuccessful in detecting a serologic response in any patient (unpublished data). One case has been reported in Portugal [4], one in Mali [5] and one possible case in Spain [12]. The clinical presentation of human ehrlichiosis is non-specific [7]; when compared with non-case patients or with patients with rickettsial or viral diseases, only absolute lymphopenia, headache and exposure to tick bites were significantly more frequent in ehrlichiosis patients [13, 14]. In addition, isolation of the agent

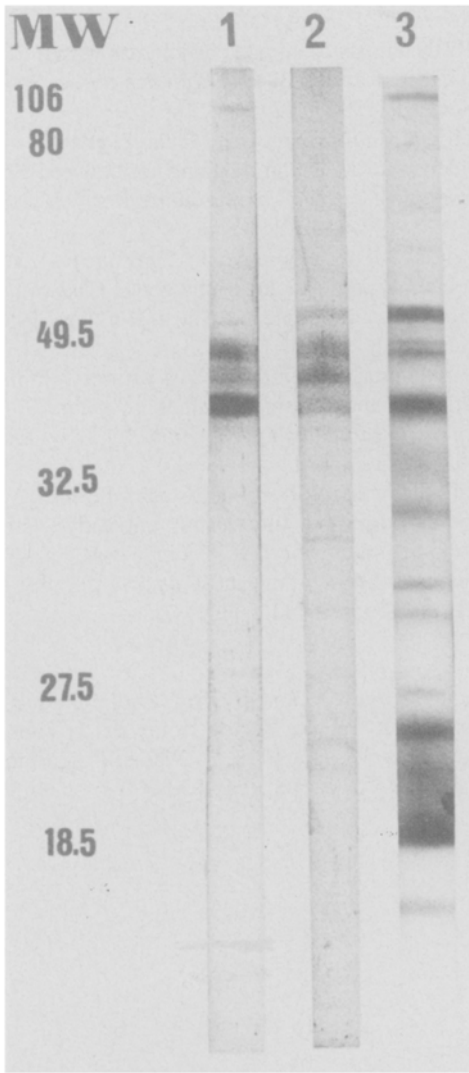

Figure 1. Western immunoblots of *Ehrlichia chaffeensis* (line 1) and *Ehrlichia canis* (line 2) revealed with 1:80 dilution of the serum from the Burkina Faso patient. *Ehrlichia chaffeensis* positive control (line 3) revealed with 1:80 dilution of an IFA positive patient (1:1600) [Courtesy of Dr J.S. Dumler].

in tissue culture is difficult and requires a specialized laboratory; in fact, *Ehrlichia chaffeensis* has only been isolated from humans once [1]. In some patients, the ehrlichiae in infected tissues have been demonstrated by immunohistologic techniques, thus confirming the serologic data [6, 8]. In fact, the diagnosis of ehrlichiosis is currently based upon serology. Indirect immunofluorescence is the most frequently used method for serology [15], but clearly lacks specificity [16]. Of 85 patients considered to have human ehrlichiosis based upon seroconversion or a four-fold rise or fall in titer against *E. canis*, 31 also had titers considered diagnostic for one or more rickettsial infections (*R. rickettsii*, *R. typhi*, and *C. burnetii*) and 14 of those patients had a four-fold or greater change in titer [16]. Among 16 serologically confirmed patients, Rohrbach reported that two had other serologically confirmed diagnoses, one hepatitis A and one Epstein-Barr virus infection [14].

Moreover, there is significant serologic cross-reactivity among members of the genus Ehrlichieae especially with *E. canis*, *E. chaffeensis*, and *Cowdria ruminantum* [17]. In order to improve the specificity of the serology, antigenic characterization of different *Ehrlichia* species has been attempted by using Western immunoblotting [11, 18, 19]. When Kelly et al. compared Western immunoblotting and IFA for diagnosis of canine ehrlichiosis in Zimbabwe, it was found that Western immunoblots were much more specific [20]. However, the positive predictive value of an isolated positive serology is likely to be low when the test is performed in an area where the seroprevalence is less than 1%, and thus a specific serologic diagnosis cannot be rendered under these circumstances.

In conclusion, human ehrlichiosis in Africa is infrequent although many sera from additional African countries need to be evaluated before definitive conclusions can be drawn. Because of the strong antigenic cross-reactivity between *Ehrlichia* species, care must be exercised for interpretation of *Ehrlichia*-positive IFA serologic results, especially in low endemic areas. Thus IFA should be systematically confirmed by Western immunoblotting, immunohistologic studies, PCR techniques and in vitro isolation, when these techniques and appropriate specimens are available.

References

1. Dawson JE, Anderson BE, Fishbein DB, et al. Isolation and characterization of an *Ehrlichia* from a patient diagnosed with human ehrlichiosis. J Clin Microbiol 1991; 29: 2741–2745.
2. Rikihisa, Y. The tribe *Ehrlichiae* and ehrlichial diseases. Clin Microbiol Rev 1991; 4: 286–308.
3. Maeda K, Markowitz N, Hawley RC, Ristic M, Cox D, McDade J. Human infection with *Ehrlichia canis*, a leukocytic rickettsia. N Engl J Med 1987; 316: 853–856.
4. Morais JD, Dawson JE, Greene C, Filipe AR, Galhardas LC, Bacellar F. First European case of ehrlichiosis. Lancet 1991; 338: 633–634.
5. Uhaa IJ, Maclean JD, Greene CR, Fishbein DB. A case of human ehrlichiosis aquired in Mali: Clinical and laboratory findings. Am J Trop Med Hyg 1992; 46: 161–164.
6. Dunn BE, Monson TP, Dumler JS, et al. Identification of *Ehrlichia chaffeensis* morulae in cerebrospinal fluid mononuclear cells. J Clin Microbiol 1992; 30: 2207–2210.
7. Dumler JS, Walker DH. Human ehrlichiosis. Current Opinion in Infectious Disease 1991; 4: 597–602.
8. Dumler JS, Brouqui P, Aronson J, Taylor JP, Walker DH. Identification of *Ehrlichia* in human tissue. N Engl J Med 1991; 325: 1109–1110.
9. Wellman MI, Krakowka S, Jacobs RM, Kociba GJ.

A macrophage-monocyte cell line from a dog with malignant histiocytosis. In Vitro Cell Dev Biol 1988; *24*: 223–229.

10. Brouqui P, Raoult D, Vidor E. Lack of co-transmission of *Rickettsia conorii* and *Ehrlichia canis* in human beings in the south of France. Eur J Epidemiol 1989; 5: 110–112.
11. Brouqui P, Dumler JS, Raoult D, Walker DH. Antigenic characterization of ehrlichiae: protein immunoblotting of *Ehrlichia canis*, *Ehrlichia sennetsu*, and *Ehrlichia risticii*. J Clin Microbiol 1992; 30: 1062–1066.
12. Guerrero A, Fishbein DB, Mesa E, Escudero E. Human infection by *Ehrlichia canis* in Spain. Med Clin 1991; 96: 236–237.
13. Fishbein DB, Kemp A, Dawson JE, Greene NR, Redus MA, Fields DH. Human ehrlichiosis: Prospective active surveillance in febrile hospitalized patients. J Infect Dis 1989; 160: 803–809.
14. Rohrbach BW, Harkess JR, Ewing SA, Kudlac J, McKee GL, Istre GR. Epidemiologic and clinical characteristics of persons with serologic evidence of *E. canis* infection. Am J Public Health 1990; 80: 442–445.
15. Dawson JE, Rikihisa Y, Ewing SA, Fishbein DB. Serologic diagnosis of human ehrlichiosis using two *Ehrlichia canis* isolates. J Infect Dis 1991; 163: 564–567.
16. Dawson JE, Fishbein DB, Eng TR, Redus MA, Greene NR. Diagnosis of human ehrlichiosis with the indirect fluorescent antibody test: Kinetics and specificity. J Infect Dis 1990; 162: 91–95.
17. Kelly P, Matthewman LA, Mahan S, et al. Serological evidence for antigenic relationships between *Ehrlichia canis* and *Cowdria ruminantium*. Res Vet Sci 1994; 56: 170–174.
18. Nyindo MBA, Kakoma I, Hansen R. Antigenic analysis of four species of the genus *Ehrlichia* by use of protein immunoblot. Am J Vet Res 1991; 52: 1225–1230.
19. Shankarappa B, Dutta SK, Mattingly-Napier BL. Antigenic and genomic relatedness among *Ehrlichia risticii*, *E sennetse*, and *E canis*. Int J Syt Bacteriol 1991; 42: 127–132.
20. Matthewman LA, Kelly PJ, Mahan S, et al. Western blot and indirect fluorescent antibody testing for antibodies reactive with *Ehrlichia canis* in sera from apparently healthy dogs in Zimbabwe. J South African Vet Ass 1993; 64: 111–115.

Address for correspondence: Prof. Didier Raoult, Unité des Rickettsies, Faculté de Médecine de la Timone, 27 Boulevard Jean Moulin, F-13385 Marseille-5, France
Phone: (91) 385 517; Fax: (91) 830 390