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A comparative study of three methods of detection of *Borrelia crocidurae* in wild rodents in Senegal

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Abstract

In a rural area in Sénégal with a high incidence of tick-borne relapsing fever in humans, *Borrelia crocidurae* was studied in the blood and brain of wild rodents (*Mastomys erythroleucus, Arvicanthis niloticus* and *Rattus rattus*) using 3 methods: (i) direct examination of thick blood films; (ii) intraperitoneal inoculation of blood into white mice; (iii) intraperitoneal inoculation of homogenized cerebral tissue into white mice. Of the 82 rodents examined, the proportion of infected animals was respectively 2·4%, 7·3% and 14·6% for each method, and 18·3% for all 3 methods combined. Of the 12 animals with infected cerebral tissue, only 3 were found to have infected blood. These results suggest that isolated infections of the brain occur frequently in Senegalese wild rodents. Measurement of the real prevalence of *B. crocidurae* should therefore take into account these infections in addition to blood infections.

Introduction

In West Africa, tick borne relapsing fever is due to the spirochaete *Borrelia crocidurae* (LÉGER, 1917). Small mammals act as the reservoir for infection and the only known vector is the ornithodorean tick *Alectorobius sonrai* (see DURIEUX, 1932; MATHIS & DURIEUX, 1934; MATHIS *et al.*, 1934). However, since case reports are rare, this borreliosis has remained poorly studied and few epidemiological data are available. We have recently shown that *B. crocidurae* is a major cause of morbidity in rural areas in Dakar (TRAPE *et al.*, 1991). In contrast to *B. duttoni*, the agent of tick-borne relapsing fever in East Africa, the usual rarity of *B. crocidurae* in the blood of patients explains why the disease is generally not diagnosed. This spirochaete could constitute an important cause of morbidity in the Sahel and Sudan regions of West Africa (TRAPE *et al.*, 1991 and unpublished data).

Small wild mammals are the reservoir host of *B. crocidurae*. In particular, 8 species of rodents were found infected in Senegal (MATHIS & DURIEUX, 1934; BOIRON, 1949; TRAPE *et al.*, 1991). As in humans, it is usually difficult to find *B. crocidurae* in small mammals. The absence of spirochaetes in a thick blood film does not exclude blood infection, as shown by the higher prevalence rate demonstrated after intraperitoneal inoculation of blood into white mice (BOIRON, 1949; TRAPE *et al.*, 1991). Furthermore, isolated infections of internal organs such as brain and spleen are known to occur (BOIRON, 1949).

The present study was conducted in a Senegalese village where there is a high incidence of relapsing fever in humans (Trape *et al.*, paper submitted for publication). The objective was to compare the prevalence of blood and cerebral infections in wild rodents in an area where preliminary investigations by direct examination of thick blood films had failed to demonstrate any blood infection in rodents.

Materials and Methods

The study was conducted in September 1991 in Dielmo (13°45'N, 16°25'W), a village of 250 inhabitants in the Sine-Saloum region of Senegal. The climate and the vegetation are typically Sudanese. Rainfall occurs over a four-month period, from late June to mid-October. Average annual rainfall is 900 mm according to readings made in the region since the beginning of the century. However, over the last 20 years, this figure has rarely been reached and it was only 611 mm during the year of the study.

Rodents were trapped alive inside dwellings with lattice-work traps baited with peanut butter or onions. The captured animals were anaesthetized with chloroform and 0·8 mL of blood was drawn by cardiac puncture. Using this blood, a thick film was made and stained with Giemsa's stain for direct examination for *B. crocidurae* and 2 white mice were given intraperitoneal injections of about 0·3 mL each. The brains of the anaesthetized rodents were aseptically removed, individually ground in mortars, and diluted with 5 mL of physiological saline; 0·3 mL of the suspension was inoculated intraperitoneally to each of 2 white mice. Three and 6 d after inoculation with blood or cerebral tissue suspension, thick blood films were prepared from the mice to look for the presence of *B. crocidurae*; 200 oil-immersion fields were systematically examined (equivalent to about 0·5 µL of blood).

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Results

Eighty-two rodents were captured and examined: 73 *Mastomys erythroleucus* (multimammate rat), 8 *Arvicanthis niloticus* (Nile rat), and 1 *Rattus rattus* (black rat). Fifteen (18·3%) of these rodents were found to be infected with *B. crocidurae* by at least one of the 3 methods (Table).

Table. Comparison of three methods for detection of *Borrelia crocidurae* in wild rodents

Species	No. examined	No. positive: Total	Direct blood examination	Blood inoculation[a]	Brain inoculation[a]
Mastomys erythroleucus	73	12	1	5	10
Arvicanthis niloticus	8	3	1	1	2
Rattus rattus	1	0	0	0	0
Total	82	15	2	6	12

[a]Intraperitoneal inoculation to white mice.

Direct examination of the thick blood films revealed only 2 infected animals (2·4%); 6 (7·3%) were found by blood inoculation, and 12 (14·6%) were detected by inoculation of cerebral tissue ($P<0·05$ by Cochran's Q test). Only 3 of the 15 infected animals had both blood and brain infections (one *A. niloticus* and 2 *M. erythroleucus*).

Discussion

Because of their abundance and close contact with humans in rural areas, *M. erythroleucus* and *A. niloticus* are, epidemiologically speaking, the most important reservoir of *B. crocidurae* in Senegal. Our findings show that, to assess the real prevalence of infection among these 2 species, it is necessary to combine examination of both blood and cerebral tissue, as was demonstrated in urban areas of Dakar for *R. rattus*, *R. norvegicus*, *Cricetomys gambianus* and *Mus musculus* (see BOIRON, 1949; DURIEUX & BOIRON, 1950). However, since only blood infections are able to infect the vector ticks, brain infections may have epidemiological importance only if spirochaetes can reappear in the blood from the brain or other internal organs. To our knowledge, this has never been investigated in any of these rodent species. In white

mice, *B. crocidurae*, can, in general, no longer be isolated from the blood 2 or 3 months after inoculation, whereas infection of the cerebral tissue can last for 7 months (MATHIS & DURIEUX, 1930).

Due to the low sensitivity of direct thick blood film examination, the frequent dissociation of blood and brain infections, and the low prevalence of *B. crocidurae* in rodents within our study area (in spite of a high incidence of relapsing fever in humans), we believe that, for studies investigating the rodent reservoir of infection, a variety of diagnostic methods should be used in combination, especially when the number of animals studied is low.

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Short Report

Specificity of Cholera Screen™ test during an epidemic of cholera-like disease due to *Vibrio cholerae* O139 synonym Bengal

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Prompt diagnosis of cholera in the field is important for quick medical intervention, and for institution of appropriate measures for control of the disease. Cultural methods of diagnosis of the causative organism, *Vibrio cholerae* O1, take at least 24 h. However, laboratory facilities for outbreak diagnosis are not available in remote areas of developing countries vulnerable to outbreaks. A rapid test with the availability of results in a few minutes, and which can be used for field diagnosis, was recently developed. This test, known as Cholera Screen™, is a monoclonal antibody-based coagglutination test, developed and marketed by New Horizons Diagnostics Corporation, Columbia, Maryland, USA (COLWELL *et al.*, 1992). The test was previously evaluated with laboratory cultures and a small number of clinical samples, and was found to be highly specific and sensitive in comparison with cultural methods of diagnosis of *V. cholerae* O1 infection. However, further evaluation of the kit with clinical samples is desirable.

Clinical cholera due to a *V. cholerae* non-O1 serogroup* broke out in parts of India and Bangladesh towards the end of 1992 and since has spread to almost the entire Indian subcontinent (ALBERT *et al.*, 1993a) and other parts of Asia (CHONGSA-NGUAN *et al.*, 1993). The outbreak serotype is *V. cholerae* O139, with the suggested name 'Bengal' referring to its first isolation from the coastal areas of the Bay of Bengal. The organism in all respects closely resembles *V. cholerae* O1 El Tor, but it is not agglutinated by *V. cholerae* O1 antiserum. This outbreak provided a valuable opportunity to evaluate the specificity of the Cholera Screen™ test.

The Cholera Screen™ test kit consists of (i) *V. cholerae* O1 detection reagent containing monoclonal antibody adsorbed to heat-killed *Staphylococcus aureus* cells, (ii) specimen control reagent containing mouse immunoglobulin G, adsorbed to heat-killed *S. aureus cells*, and (iii) a small filtering device consisting of a squeezable 2 mL plastic tube fitted with a 1 μm pore-size membrane filter that allows bacterial cells to pass through while retaining faecal or other extraneous material. The kit also contains heat-killed *V. cholerae* O1 and non-O1 cells as positive and negative controls respectively. One mL of a liquid stool sample is transferred to the filtering device, and 2 drops of filtrate are placed in each of 2 circles marked on a slide. One drop of cholera detection reagent is added to one of the circles (test circle) and a drop of specimen control reagent is added to the other (control circle); the slide is then gently agitated either by hand or by a circular shaker. Agglutination in the test circle and its lack in the control circle within 2 min indicates the presence of *V. cholerae* O1.

One stool sample from each of 57 suspected cholera cases admitted to the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka between February and July 1993 was tested using the Cholera Screen™ test. The stool samples were simulta-

Table. Results of culture and Cholera Screen ™ testing of diarrhoeal stool samples

Culture result	Cholera Screen™ test Positive	Negative	Total
V. cholerae O1	4	0	4
V. cholerae O139	0	47	47
No vibrio	0	6	6
Total	4	53	57

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*See also the paper by Kuruvilla, A., Jesudason, M. V., Mathai, D., John, L. & John, T. J. (1994). The clinical pattern of diarrhoeal illness caused by the new epidemic variant of non-O1 *Vibrio cholerae*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 438.