Serological Typing of Spotted Fever Group Rickettsia Isolates from Zimbabwe

PATRICK J. KELLY1* AND PETER R. MASON2

Faculties of Veterinary Science1 and Medicine,2 University of Zimbabwe, Harare, Zimbabwe

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Eight rickettsialike organisms were isolated in tissue culture from ticks of dogs and cattle from various areas of Zimbabwe. These isolates and a reference strain, Rickettsia conorii Simko, were tested by microimmunofluorescence against homologous and heterologous antisera raised in mice. From the titers obtained by this method, specificity differences (SPDs) were calculated between each of the rickettsiae. Only small serological differences were detected among the isolates from ticks obtained from dogs (mean SPD, 0.5) and also among the isolates from ticks obtained from cattle (mean SPD, 0.3). However, when isolates from ticks obtained from dogs and cattle were compared, the serological differences were greater (mean SPD, 1.3). The isolates from ticks obtained from dogs were found to be very similar serologically to the Simko strain of R. conorii (mean SPD, 0.8), while three of four isolates from ticks obtained from cattle were different enough (SPD, ≥3) to be identified as separate serotypes. These findings indicate that there is a high degree of antigenic heterogeneity among the tick-transmitted spotted fever group rickettsiae in Zimbabwe.

Within rickettsiae of the spotted fever group (SFG) there are large numbers of pathogenic and nonpathogenic strains, only one of which, Rickettsia conorii, has reliably been reported to occur in Africa. Infection with this organism causes tick-bite fever in humans. Some 50 years ago, Gear (2) commented on two clinically different forms of the disease in South Africa, a mild rural form associated with ticks of cattle and game animals and a more severe urban form associated with ticks of dogs. At the time, however, Gear was unable to demonstrate antigenic differences in the rickettsiae associated with these clinical versions. Later, Gear et al. (3) reported antigenic differences in rickettsial strains from South Africa, but details of how these findings were obtained were not given.

Antigenic differences in R. conorii have been reported from elsewhere in the world. Using cross-inhibition indirect fluorescent-antibody tests, Goldwasser et al. (5) demonstrated serological differences between Indian and Casablanca strains of R. conorii. These strains also differed serologically from rickettsiae isolated in the West Indies and Israel. In Sicily, marked antigenic polymorphism of local isolates of SFG rickettsiae has been demonstrated by biological studies on pathogenicity in guinea pigs and reactivity with monoclonal antibodies (9).

The purpose of this study was to determine the serological differences between isolates of SFG rickettsiae from Zimbabwe.

MATERIALS AND METHODS

Ticks collected from various areas of Zimbabwe were examined by the hemolymph test (1) for rickettsialike organisms (RLOs). Ticks positive for RLOs were identified and then washed for 5 min in 10% hydrogen peroxide followed by 5 min in 80% ethanol. Each tick was then triturated in a sterile pestle and mortar with 1 ml of sterile, ice-cold brain heart infusion broth. The triturate was aspirated into a tuberculin syringe through a 27-gauge needle. Male Hartley guinea pigs seronegative for antibodies to SFG rickettsiae were injected intraperitoneally with 0.2 ml of the triturate. They were subsequently examined on a daily basis for scrotal swelling, and the rectal temperature was determined during that examination. At 2 weeks post inoculation, the guinea pigs were bled by cardiac puncture, and their sera were tested for SFG rickettsial antibodies by an indirect immunofluorescence assay by using R. conorii Simko antigen.

The remaining triturate was added to a monolayer of Vero cells in a 75-ml tissue culture flask from which the growth medium had been decanted. The cells were incubated at 35°C for 4 h before they were washed three times with sterile tissue culture medium (M199 with 2 mM glutamine, 10 mM sodium bicarbonate, 1% fetal bovine serum, and 50 μg of sulfacetamide per ml). Following the last washing, 10 ml of medium was added to each flask, which was then returned to the incubator. After 5 days, the presence of RLOs was demonstrated by scraping off a few cells from the bottom of the flask and staining them with Gimenez stain (4).

Cells were detached by three freeze-thaw (−20°C, 37°C) cycles and centrifuged at 10,400 × g for 30 min. The supernatant was decanted, and the remaining pellet was suspended in 2 ml of K36 buffer (10). Trypsin (200 μl of a 5% solution) was added, and the mixture was placed in a shaking water bath (150 rpm) at 30°C for 45 min. At the end of the shaking period, 2 ml of brain heart infusion broth was added to stop the reaction and the mixture was centrifuged at 17,300 × g for 15 min. The supernatant was decanted, and the remaining pellet was suspended in 0.5 ml of K36 buffer. Antigen slides for microimmunofluorescence (MIF) were prepared as described previously (7).

A known strain of R. conorii Simko, originally isolated in Ethiopia (6) was obtained from R. Swanepoel, National Institute for Virology, Johannesburg, South Africa, where it was maintained in egg yolk sac cultures. Organisms were purified and used in the preparation of antigen slides for MIF by the methods described above.

Species-specific antibodies to both R. conorii Simko and the local RLOs were raised by intraperitoneal inoculation of BALB/c laboratory mice with 0.2 ml of the egg yolk sac or tissue culture suspensions, respectively, prior to purifica-
tion. Three mice were inoculated with each isolate. The mice were bled at day 10 postinfection, and their sera were tested by MIF against homologous and heterologous antigens in three separate tests using sera from the three mice that were inoculated with each isolate, with each test being read independently by P.J.K. and P.R.M. The final titer was obtained by taking the average of six individual titers and rounding that value off to the nearest dilution used in the tests. The specificity difference (SPD) was calculated from these final titers by subtracting the sums of the heterologous test titers for each isolate from the corresponding homologous test titers after first converting each titer to a numerical value, such that 1/16 = 1, 1/32 = 2, 1/64 = 3, etc., as described by Philip et al. (8).

**RESULTS**

Altogether, eight rickettsial isolates were obtained. Strain A was isolated from *Rhipicephalus simus*; and strains B, D, and H were isolated from *Haemaphysalis leachi*. These ticks were all collected from dogs in the Harare area. Strains F, K, AM, and N were isolated from bont ticks (*Amblyomma hebraeum*) that were collected from cattle in the south of Zimbabwe.

All of the guinea pigs inoculated with tick suspensions developed antibody to *R. conorii* Simko. The guinea pigs infected with isolates from ticks obtained from dogs showed fever and scrotal reactions typical of those seen in animals with rickettsial infections, while none of the animals infected with isolates from *A. hebraeum* ticks showed scrotal swelling; only one isolate (isolate K) caused a mild febrile response (39.9°C on day 6).

All of the RLOs isolated induced antibody responses in mice, with homologous titers ranging from 1/128 to 1/1,024. Heterologous titers varied from 1/64 to 1/2,048. The SPDs varied from 0 to 4 (Table 1). In general, it was found that the SPD between RLOs isolated from ticks obtained from dogs was low (mean ± standard deviation, 0.5 ± 0.9), and the same was true for the SPDs between RLOs from *A. hebraeum* ticks (0.3 ± 0.8). A much higher mean SPD was calculated when comparing titers of sera from mice infected with isolates from ticks obtained from dogs against *A. hebraeum* RLO antigen (1.3 ± 0.7).

The mean SPD between *R. conorii* Simko and isolates from ticks obtained from dogs was 0.8 ± 1.0, while that for *R. conorii* Simko and isolates from *A. hebraeum* ticks was 2.8 ± 1.3.

**TABLE 1. SPDs between rickettsiae isolated in Zimbabwe and *R. conorii* Simko**

<table>
<thead>
<tr>
<th>Mouse anti-serum*</th>
<th>SPD of indicated rickettsial strain* isolated from ticks obtained from:</th>
<th>SPD of strain SPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dogs</td>
<td>Cattle</td>
</tr>
<tr>
<td></td>
<td>A B D H AM F N K</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0 0 -1 2 1 2 1 1</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0 0 1 0 1 0 0 0</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>-1 1 0 1 2 2 0 0</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>2 0 1 0 1 2 2 2</td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>2 1 2 1 0 0 0 3</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1 0 1 1 1 1 0 0 3</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>2 1 2 2 2 0 0 0 4</td>
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</tr>
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<td></td>
</tr>
<tr>
<td>S</td>
<td>1 0 0 2 3 3 4 1 0</td>
<td></td>
</tr>
</tbody>
</table>

* See text for explanation of strain designations.
* S. Reference strain *R. conorii* Simko.

**DISCUSSION**

It has been shown previously (8) that MIF is a reliable technique for antigenic differentiation of SFG rickettsiae. With this test, the titers for homologous and heterologous antisera are used to calculate the SPD between isolates. An SPD of less than 3 is regarded as indicative of strain variation within a serotype, while a SPD of 3 or more indicates a different serotype (8).

All eight isolates of RLOs collected from ticks obtained from dogs and cattle in various areas of Zimbabwe stimulated antibodies in mice that reacted with *R. conorii* Simko antigens at a titer of 1/128 or greater, indicating that there is considerable antigenic homogeneity between these strains. The results of the SPD calculations, however, showed that three of four of the isolates from ticks obtained from cattle were sufficiently serologically distinct to be classified by the above scheme as SFG serotypes separate from the serotype of *R. conorii*. This was not the case, however, with the isolates from ticks obtained from dogs. We note, however, that all of the ticks from dogs were obtained from the Harare, Zimbabwe, area, while bont ticks from cattle were endemic only in the south of the country. Thus, our findings could also be explained by geographical variation in antigenicity.

Although some degree of heterogeneity was detectable among the isolates from ticks obtained from dogs, these differences were consistent with strain variations within a serotype. The same was also true for the isolates from ticks obtained from cattle. When isolates from ticks obtained from dogs and cattle were compared, however, the SPDs generally indicated greater strain variation between the two groups than within either group.

The results of the guinea pig inoculation experiments indicated that the isolates from ticks obtained from dogs caused symptoms typical of those of *R. conorii* infections, mainly fever and scrotal swelling. On the other hand, the isolates from ticks obtained from cattle were generally of low pathogenicity to guinea pigs. This appears to be in accord with the observation of Gear (2) that there may be two clinical forms of presentation of human infections, with infections in urban areas resulting from the bites of ticks from dogs being more severe. All isolates, however, stimulated the production of antibodies to *R. conorii* Simko.

Our findings suggest that a spectrum of serologically related strains of SFG rickettsiae may occur in Zimbabwe. Different strains had different pathogenicities in guinea pigs. Some strains, particularly but not exclusively those isolated from ticks from dogs, showed close antigenic homogeneity with a recognized strain of *R. conorii*. Other isolates, particularly but not exclusively those isolated from ticks from cattle, were serologically distinct from *R. conorii* and, to a lesser extent, those isolated from ticks from dogs. The significance of this in terms of the epidemiology, the clinical presentation, and the diagnosis of tick-bite fever needs further study.

A recent study (9) in which monoclonal antibody techniques were used has documented that considerable heterogeneity exists within SFG rickettsiae isolated in Europe, where only *R. conorii* has been reported to occur. There appears to be a similar situation in Africa, although further studies with monoclonal antibodies and Western immunoblotting, in addition to MIF, are needed to confirm this.
ACKNOWLEDGMENTS

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LITERATURE CITED