## Navigational Instinct: A Reason Not to Live Trap Deer Mice in Residences

**To the Editor:** Although the rodent that most often invades homes in North America is the house mouse, *Mus musculus*, the deer mouse, *Peromyscus maniculatus*, principal vertebrate host of Sin Nombre virus (SNV) (1), also invades homes (2), particularly in rural areas. Barring deer mice from human habitations would prevent domiciliary acquisition of SNV. Current recommendations (3) are to prevent wild rodents from entering homes or to snap trap (kill) them should they enter.

To conduct longitudinal studies of hantaviruses in southeastern Colorado on a former cattle ranch now returning to its natural condition as short-grass prairie, we often stay in an old bunkhouse, used by many research groups at irregular intervals. The house, furnished with beds and full kitchen facilities, is well maintained but has openings through which mice can pass to and from the outside. For safety and cleanliness, we removed mice we found inside the house, but between April 1996 and April 1998, we live trapped and released them rather than snap trapping them. Before release the rodents were identified to species; were measured and assessed regarding general appearance and health, sexual preparedness, and presence of wounds; were bled for antibody tests; and were ear-tagged. Nineteen deer mice and one pinyon mouse (a *P. truei*, which did not return) were examined and tagged. At first, we simply released these animals approximately 50 m from the house, but when we realized that they were returning, we released them at increasing distances (50 m to 1,500 m) from the house; the distances were measured by pace counts by at least two investigators.

Three deer mice had been captured multiple times in our test grid (as far as 250 m from the house) before they were first captured in the house. Once captured in the house, however, they were not captured in traps of the grid (i.e., outside the house). The mean distance traversed by the five deer mice that returned to the house was at least 394 m; one mouse returned after being released 500 m and 1,000 m, then 750 m, and 1,200 m from the house at consecutive daily trapping sessions of 3 days. Sometime within the subsequent 6 weeks, this mouse returned to the house from the 1,000-m release point and then from 750 m and 1,200 m away on consecutive days within our 3-day trapping period. Each of the mice returning to the house did so within 24 hours of release, two as few as 6 hours after release from 500 m and 750 m away. Nine mice were captured once; six of eight mice captured twice were captured at least once more; one was captured 10 times, one 7 times, one 6 times, one 4 times, and two 3 times. Equal numbers of male and female, adult and juvenile mice were captured in the house, but only adult mice (5 of 5) returned to the house. Returning deer mice maintained or gained weight between captures and grew in length at approximately the same rate as deer mice captured in the test grid.

Some rodents have been documented to move similar distances (e.g., 1,200 m), but they took more than 2 weeks to complete the trek (4). Homing ability, site fidelity, and navigational proficiency of rodents are well documented (5,6). Teferi and Millar (7) studied the homing ability of deer mice in Alberta, Canada; 50% of deer mice in that study returned to their home sites (a short-grass prairie habitat). The mice traveled 650 m to 1,980 m (mean 1,500 m) and had to cross a river and pass optimal habitat patches to reach their home sites. Deer mice with previous homing experience were more successful in returning home (100%) than inexperienced mice (60%) and faster in doing so (8). Teferi and Millar (7) suggest that these deer mice were able to navigate in a direct route to their home sites. We released mice in locations where they had no direct route to the house; they had to follow a winding road, climb over rocky outcroppings nearly 17 m high, or otherwise surmount obstacles and dangers, such as predators (7).

None of the mice we captured had immunoglobulin G (IgG) antibody to SNV. However, infected deer mice released and then returning to a house or uninfected deer mice released, infected, and then returning to a house would increase the likelihood of human contact with an SNV-infected mouse. The risk would be the same for other hantaviruses infecting other peridomestic rodents. Against current recommendations that rodents in homes be snap trapped, some homeowners live trap and release them outside their homes. Our data strongly support snap trapping mice in homes and provide evidence that released wild mice return and may place the residents at risk.

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## *Bartonella quintana* in Body Lice Collected from Homeless Persons in Russia

**To the Editor:** Lice are obligate blood-feeding insects; three lice species (*Pediculus humanus* var *capitatis*, *P. humanus* var *corporis*, and *Phtirus pubis*) have been connected with humans throughout history. The body louse (*P. humanus corporis*) is the vector for three infectious diseases: epidemic typhus caused by *R. prowazekii*, trench fever caused by *B. quintana*, and relapsing fever caused by *Borrelia recurrentis* (1-3). Infestation with the body louse is associated with cold weather, poverty, and poor hygiene. In Russia, louse-transmitted diseases have caused more deaths than any other infectious disease in recent centuries (4). During the last decade, pediculosis (infestation with *P. humanus*) has increased markedly throughout the world (5,6), especially in developing countries and in areas (e.g., Eastern Europe, Russia) that have undergone vast social and economic changes. The incidence of pediculosis in Russia is approximately 220 to 300 cases per 100,000 inhabitants (7). Social and economic upheavals in the former Soviet Union have increased the number of homeless people, among whom pediculosis is highly prevalent (6).

A disease of the past, epidemic typhus, has reemerged as a public health concern after a 1996 outbreak in Burundi, the largest outbreak of the disease since World War II (5,8). During World War II, a huge typhus epidemic caused illness in more than 20,000,000 people in Russia. *R. prowazekii* infection can persist in a latent form in convalescent typhus patients, remanifesting itself in a recrudescent form (Brill-Zinsser disease) in patients under stress (1). Sporadic cases of Brill-Zinsser disease are reported every year in all regions of the former Soviet Union (9) and because most of the population has no immunity to *R. prowazekii,* the risk for a typhus outbreak is increased. In a recent outbreak in the Lipetsk region, 360 km from Moscow, 24 louse-infested, febrile patients in an unheated psychiatric institution had serologically diagnosed typhus (10).

The great epidemics of trench fever in Europe took place during World War I (2). However, recently a large outbreak of trench fever associated with epidemic typhus has been reported in Burundi (5). Sporadic cases of *B. quintana* infection have occurred during the last decade in Europe and the United States, mainly in HIV-infected patients, the homeless, and persons with chronic alcoholism; the infection has manifested itself as trench fever, bacteremia, bacillary angiomatosis, or endocarditis (11-16). Relapsing fever has not been reported in Russia for more than 50 years, despite a high prevalence after the 1917 revolution and during World War II (17).

We studied the presence of typhus, trench fever, and relapsing fever agents in body lice collected from homeless persons in Moscow. The lice were collected at the Moscow Municipal Disinfection Center, where the homeless wash and delouse themselves, as well as disinfect or change their clothes. Only participants who gave informed consent were included. Lice were collected from the participant's clothing (from the inner surface and seams of t-shirts, shirts, and sweaters); 3 to 25 lice were found on each volunteer. Lice were collected from May to October 1996 (214 samples) and from June to September 1997 (54 samples).

From June to September 1997, 300 homeless male attendees were examined; 57 (19%) had body lice or louse eggs (three had only eggs) on their clothing. Lice were identified as *P. humanus corporis,* according to standard taxonomic keys (6,18). Lice from each person were split into pools of three to eight insects, and DNA was extracted from each pool and tested for *R. prowazekii, B. quintana, and B. recurrentis* by polymerase chain reaction (PCR) analysis. Primers used for PCR analysis and conditions for DNA amplification have been described (5,19-22). Uninfected, laboratory-reared lice served as negative controls, and DNA of *R. rickettsii, B. elizabethae,* and *B. burgdorferi* were used as positive controls.

Results of each amplification were resolved in 1% agarose gels (type LE; Sigma-Aldrich Chimie, St. Quentin Fallavier, France) and were visualized under UV light after ethidium bromide staining. The sizes of amplicons were determined by comparison with the DNA molecular weight marker VI (Boehringer, Mannheim, Germany). To confirm the identity of amplicons, their nucleotide base sequence was determined by using an AmpliCycle sequencing kit (Perkin-Elmer Corp., Foster City, CA) according to the manufacturer's instructions.

PCRs incorporating rickettsia- and borrelia-specific primers did not yield products from any DNA extracts derived from the louse samples. Positive controls in both reactions yielded bands of the expected size. Thus, louse samples were not infected with *R. prowazekii* or *B. recurrentis*. Initial screening with PCR incorporating nonspecific primer pairs for *Bartonella* species yielded products of the estimated amplicon size of approximately 1,200 bp for 33 (12.3%) of the 268 louse samples. These results were confirmed by PCR incorporating primers (CS.443p–CS.979n) specific for the *gltA* gene. Products of this reaction were characterized by base-sequence determination. All 33 *Bartonella*-positive samples yielded a partial gltA sequence identical to that of *B. quintana* (22). Persons infested with infected lice were younger than 30 years to older than 60 years of age.

A recent report indicates that 11% of the homeless in Russia are infested with lice (23); in our limited study, we observed a prevalence as high as 19%. With widespread louse infestation and overcrowding, a single case of Brill-Zinsser disease can cause an outbreak of epidemic typhus. A patient more than 50 years of age with Brill-Zinsser disease was the suspected primary source of typhus infection during the 1997 Lipetsk outbreak. Presence of lice in the hospital permitted disease dissemination (10). Although our data showed that none of the 268 louse pools were infected with *R. prowazekii*, the serious threat of an outbreak requires continued surveillance. No samples were found to contain *B. recurrentis* DNA, yet dissemination of body lice could also cause relapsing fever to reemerge.

Interest in bartonellosis has recently increased, particularly in association with HIV infection, because *Bartonella* species can cause bacteremia in the immunocompromised (15). Recent investigations have demonstrated that *B. quintana* cause bacillary angiomatosis, lymphadenopathy (16), endocarditis (24), and infections of the central nervous system (25,26) in healthy persons. Recent reports of *B. quintana* infection outbreaks in the United States (14,27), Africa (5), and Europe (11,13,28) suggest either greater awareness or a reemergence of this infection. Persons who are homeless or alcoholic are particularly at risk (11-13,27,29). In all recently reported cases, the role of a possible arthropod vector has remained unclear (30,31), although lice exposure, together with homelessness, is a risk factor for *B. quintana*-induced bacillary angiomatosis (15). The fact that 12.3% of studied lice samples were *B. quintana*-positive confirms the role of this arthropod vector in the contemporary life cycle of the agent. A similar prevalence of *B. quintana* in body lice was reported in Burundi (5) and has been observed in France (D. Raoult, unpub. data). On the basis of data from our study, Moscow should be considered an area at high risk for an outbreak of bartonellosis.

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## Tick-Transmitted Infections in Transvaal: Consider *Rickettsia africae*

**To the Editor:** We report a case of African tick-bite fever (ATBF) in a 54-year-old French hunter returning to France on 21 April 1997, after a 15-day visit to Transvaal, South Africa. While traveling in the veld, the hunter removed (but did not keep) two ticks from his left leg. Two days later, he observed eschars at the bite sites. Within 5 days, he had high fever (39.5°C) and headache and decided to fly back to France, where he was admitted to the Infectious Diseases Department in the Hotel Dieu Hospital in Clermont-Ferrand. The patient's clinical symptoms were persistent fever, severe headache, and two inflammatory eschars on the left leg. Laboratory results were normal. On 22 April, an acute-phase serum sample and eschar biopsy were sent to our laboratory. The patient was treated with 200 mg per day doxycycline for 10 days. His symptoms resolved. A second serum sample was collected on 13 May.

Microimmunofluorescence was performed as previously described (1). Although the acute-phase serologic results were negative, the convalescent-phase serum exhibited anti–*R. africae* and anti–*R. conorii* titers of 16 for immunoglobulin (Ig) G and 8 for IgM. Sera were adsorbed with *R. conorii* and *R. africae* antigens (2), and serologic testing and Western blot analysis (1) were performed on the resultant supernatants. Cross-adsorption of the convalescent-phase serum caused the homologous and heterologous antibodies to disappear when adsorption was performed with *R. africae* antigens; only homologous antibodies disappeared when adsorption was performed with *R. conorii*. Western immunoblot, performed with the same adsorbed serum, indicated *R. africae* infection by demonstrating a specific reactivity pattern with *R. africae*–specific antigens in the 110-kDa to 145-kDa region (2). An inoculation eschar biopsy specimen was injected into human embryonic lung fibroblasts, according to the centrifugation shell-vial technique (3). After 6 days' incubation at 32°C, a Gimenez staining of methanol-fixed human embryonic lung fibroblasts showed rickettsialike bacilli. The strain was identified by direct immunofluorescence performed on the cells with an anti–*R. africae* monoclonal antibody (4). Moreover, DNA was extracted from the ground eschar biopsy specimen and from 200 µL of shell-vial supernatant, by using a QIAmp Tissue kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instructions. These extracts were used as templates with primers complementary to a portion of the coding sequence of the rOmpA encoding gene in a polymerase chain reaction (PCR) assay (5), and the base sequences of the resulting PCR products were determined (5). The sequence obtained by both methods was the same as the *R. africae* sequence in Genbank (100% similarity).

Since first described in Africa in 1910, tick-transmitted rickettsioses have been imputed to a single rickettsial species, *Rickettsia conorii*, although two distinct clinical illnesses have been observed (6): an urban form in patients in contact with dogs and their ticks (*Rhipicephalus* spp.) characterized by fever, headache, myalgia, cutaneous rash, and a lesion at the site of the tick bite (7), and a rural form in patients in contact with cattle or game and their ticks (*Amblyomma* spp.) characterized by mild signs and frequent lack of rash (8).

Although *R. africae* was initially isolated from *Amblyomma* cattle ticks in 1973, the first evidence of its pathogenic role in humans was seen in 1992 in a patient who, after a tick bite, had fever, an inoculation eschar, regional lymphadenopathy, but no cutaneous rash (9). Since then, an additional 20 cases of *R. africae*–related infections have been reported in travelers returning from Zimbabwe and South Africa (2,10).

*R. conorii* has long been considered the only African spotted fever group rickettsia, responsible for both Mediterranean spotted fever and ATBF. Since the first case was described (9), most of the 20 reported cases of ATBF occurred as outbreaks (2,10) in Europeans returning from Zimbabwe and South Africa. The occurrence of concomitant ATBF cases is unusual since Mediterranean spotted fever is generally sporadic and is likely related to the biologic characteristics of the recognized vector of *R. africae*, *Amblyomma* spp. ticks. While both are nonnidicolous ticks, *Amblyomma* spp. and *Rhipicephalus* spp. exhibit very different host-seeking behavior (11). *Amblyomma* spp. are ticks of cattle and wild ungulates, are not host-specific, and can readily feed on humans; they are "hunter ticks" and exhibit an "attack strategy" (in response to stimuli they specifically converge on nearby hosts). *Rhipicephalus* spp. are dog ticks and vectors of *R. conorii*; very host-specific, they exhibit an "ambush strategy" (they are passive and remain quiescent in their habitat until a vertebrate host passes). Up to 72% of *A. hebraeum* are infected with *Rickettsia*-like organisms, in particular *R. africae* (12); *Amblyomma* spp. are widely distributed in rural areas in sub-Saharan Africa (13) and prevalence of *A. hebraeum* ticks, incidence of ATBF cases, and prevalence of *R. africae* antibodies have been strongly linked (14). Rural Africans are also commonly infected with *R. africae*, usually at a young age (14). In Zimbabwe, Kelly et al. (15) demonstrated that 55% of the tested human sera had antibodies against *R. africae*.

ATBF usually has specific clinical features: shorter incubation period than for Mediterranean spotted fever, multiple inoculation eschars (related to the host-seeking behavior and host-specificity of *Amblyomma* spp. ticks, which are "attack ticks" [15]), regional lymphadenopathies, frequent lack of cutaneous rash or a pale vesicular eruption, and absence of complications (2). Although only 22 proven cases have been described so far (including the present case), ATBF has been recognized as a commonly encountered disease in southern Africa since 1900 (8,16). Epidemiologic and clinical features indicate that several cases previously diagnosed on the basis of serology results only as *R. conorii*-caused may have been caused by *R. africae*.

Given the serologic cross-reactivity among spotted fever group rickettsiae, microimmunofluorescence, the easiest serologic method, may not be sufficient for the etiologic diagnosis of a rickettsial spotted fever. A definitive diagnosis of ATBF requires either additional serologic procedures, such as cross-adsorption or Western blot, or the use of PCR or culture. As for PCR, rOmpA-amplification possesses sufficient sequence heterogeneity among the spotted fever group rickettsiae to be used as an identification tool (5). The centrifugation-shell vial-cell culture (3), used routinely in our laboratory, reliably isolates strictly intracellular bacteria, including rickettsia, from blood and tissue specimens, especially eschar biopsies (the specimen of choice for isolation procedures or genomic detection). We noted cross-reactions between *R. africae* and *R. conorii.* Cross-adsorption between anti–*R. africae* and anti–*R. conorii* antibodies and Western blots confirmed that the antibodies we detected were directed specifically at *R. africae*. Furthermore, both PCR and cell culture confirmed the diagnosis of *R. africae* infection.

ATBF appears to be an important emerging disease in visitors to rural areas of southern Africa. *R. africae* should be considered a potential pathogen in patients returning from such areas who have fever, headache, multiple inoculation eschars, or regional lymphadenopathy after a tick bite.

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## Extended-Spectrum Beta-Lactamase-Producing *Salmonella* Enteritidis in Trinidad and Tobago

**To the Editor:** *Salmonella* Enteritidis, a predominantly localized pathogen of the human gastrointestinal tract, can become invasive in very young, very old, malnourished, and immunocompromised patients. In recent years, *S.* Enteritidis has emerged as a major intestinal pathogen in Trinidad and Tobago (population 1.2 million); in 1997, *S.* Enteritidis caused 79 (66%) of 119 culture-confirmed salmonella infections, in contrast to 18 (18%) of 99, 48 (47%) of 102, and 107 (61%) of 178 in 1994, 1995, and 1996, respectively. Increased incidence of *S.* Enteritidis infections has been reported worldwide (1,2). Of 216 human *S.* Enteritidis isolates tested for antimicrobial susceptibility between 1994 and 1996 in Trinidad, none were resistant to cephalosporins, aminoglycosides, ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, and norfloxacin/ciprofloxacin by the Kirby-Bauer disk diffusion method, which uses the National Committee for Clinical Laboratory Standards (NCCLS) breakpoints (3).

Here we report an unusual isolate of *S.* Enteritidis resistant to all penicillins and cephalosporins—including third-generation cephalosporins, gentamicin, tobramicin, and trimethoprim-sulphamethoxazole—by the Kirby-Bauer disk diffusion method. Amoxicillin-clavulanate and piperacillin-tazobactam disks gave zone sizes of 15 mm and 19 mm, respectively, which are classified as intermediate in the NCCLS guidelines. This isolate was recovered from the blood culture of a febrile, nonneutropenic patient with multiple myeloma on two occasions 24 hours apart in March 1998. The isolate was sensitive only to ofloxacin and imipenem. Admitted to the hospital with compressed fracture of the spine for physiotherapy in December 1997, the patient had several febrile episodes and received several courses of multiple empirically prescribed antibiotics (cefotaxime, gentamicin, and piperacillin). The patient had not traveled abroad during the previous 6 months.

Because cephalosporin resistance in salmonellae has not been reported before in the Caribbean, we investigated the mechanism behind this third-generation cephalosporin resistance further. Using amoxicillin-clavulanate in combination with ceftazidime, ceftriaxone, and aztreonam, we performed the double disk synergy test to determine whether this strain was an extended-spectrum beta-lactamase producer as described elsewhere (3); augmentation of the zone at the junction of amoxicillin-clavulanate and aztreonam/ceftriaxone/ceftazidime zones confirmed that indeed it was.

In the past few years, third-generation cephalosporin resistance in *S.* Enteritidis has been described in Europe (4), the United States (5), Turkey (6), India (7,8), and Argentina (9). Few reports exist of extended-spectrum beta-lactamase–mediated third-generation cephalosporin resistance in *Salmonella* spp. To our knowledge, this is the first report of this type of resistance among *S.* Enteritidis in the Caribbean. This patient was treated with ciprofloxacin for 1 week; subsequent blood cultures were negative.

This unusual isolate highlights the need to establish an antimicrobial resistance surveillance network for Salmonella isolates, including *S.* Enteritidis, to monitor the trends and new types of resistance mechanisms in the Caribbean. An epidemiologic study of *S.* Enteritidis infections is being planned to describe the extent of the problem and to define risk factors and vehicles of human infections in three Caribbean countries, including Trinidad and Tobago.

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## New *emm* (M Protein Gene) Sequences of Group A Streptococci Isolated from Malaysian Patients

**To the Editor:** We analyzed the M-type–specific *emm* gene sequences of 24 random *Streptococcus pyogenes* isolates from sterile- and nonsterile-site clinical specimens of Malaysian patients. In contrast to isolates in the United States, which rarely have new *emm* sequences, 6 of these 24 Malaysian isolates had new *emm* gene sequences, which suggests a large reservoir of group A streptococci expressing new M-type specificities in Malaysia.

The M protein is a surface-exposed principal virulence factor of group A streptococci (GAS) and a potential vaccine candidate. The hypervariable M-type–specific N-terminal portion of the M molecule extends from the cell wall and evokes protective antibodies. Approximately 75 M antigenic types of GAS are recognized, and several provisional types have been proposed (1). Formulation of a universally effective vaccine is complicated by the M-type–specific nature of protective anti-GAS antibodies, temporal and geographic variations in GAS M-type prevalence (2), and lack of information on GAS M types from areas where rheumatic fever and rheumatic heart disease, sequelae of GAS pharyngitis, are endemic (3). The lack of specific M-typing antisera is also a limiting factor in determining type distribution. Recently, Beall and colleagues (4,5) demonstrated that sequence analysis of the hypervariable portion of the *emm* gene encoding M-type specificity (*emm* typing) was an alternative when M-typing antisera were not available.

Attempts to type selected Malaysian strains of GAS by M protein status have yielded poor results. Fewer than 16% of strains were typable with standard M-typing antisera (6). The existence of new M types in Southeast Asia was suggested as an explanation. To investigate this possibility, we subjected 27 selected strains (6 from blood, 15 from pharyngitis, 3 from pus, and 3 pharyngeal carrier cultures) collected between January 1994 and December 1996 to *emm* typing. Initial isolation, serogrouping, T typing, and determination of opacity factor production were performed in Kuala Lumpur, by standard techniques, commercial media, reagents, and antisera (7). Strains were transported to the Centers for Disease Control and Prevention in Atlanta, Georgia, USA, for *emm* typing, where serogrouping, T typing, and opacity factor determinations were repeated, and *emm* typing was performed (4,5). DNA sequences were subjected to homology searches against all known *emm* sequences by Genetics Computer Group Software, Version 9. (Most sequences in this database were found in strains isolated from patients living in Europe and North America.)

Of the 27 cultures analyzed, 24 were GAS, 2 were group G streptococci, and 1 was nongroupable Streptococcus. Ten of the 24 GAS strains were standard *emm* types *emm*3, *emm*12, *emm*22, *emm*60, and *emm*76 (encoding the classic M types M3, M12, M22, M60, and M76, respectively); 4 were the provisional *emm* types *pt180*, *pt2841*, and *pt5757*; and 3 were previously identified *emm* sequence types *st64/14* and *st2034* (GenBank accession numbers X72932 and U74320, respectively). The *st2034* sequence, originally identified in children from Papua New Guinea, has also been found in Brazil, California, and Hawaii (B. Beall, R. Facklam, unpub. data). One GAS had a sequence previously found in group G streptococci (*emmLG6*, accession number U25741). Finally, 6 were of five new *emm* sequence types discovered in this study (*st4529*, *st4547*, *st4532*, *st4545*, and *st3018,* with accession numbers AF060368, AF052426, AF077666, AF077668, and AF077669, respectively). The newly found group A *st4545* sequence was more similar to various group G streptococcal *emm* sequences than to known group A *emm* sequences. One group G isolate had a previously found group G 5' *emm* sequence (*stLG6*, accession number U25741). The nongroupable Streptococcus had an *emm* sequence previously associated with group L Streptococcus (Beall and Facklam, unpub. data). These results demonstrate the usefulness of *emm* typing in areas where specific M-typing antisera are not available.

Identifying 6 (25%) of 24 GAS with new *emm* types provides further evidence of new M serotypes of GAS in Malaysia. The deduced amino acid sequences of the mature hypervariable N termini of ST4529, ST4532, ST4547, and ST3018 ranged from 43% to 82% identity to M proteins of known sequence (data not shown). The M nontypability of these isolates suggests unique serologic specificity. ST4547, ST4532, and ST3018 had 70% to 82% identity over the first 55-variable-region amino acids, with their closest matching known M proteins (M70, M27, and M22, respectively), but whether antibodies against any of these proteins would cross-protect against strains expressing these M proteins is unknown. Even though the M70 protein is 70% identical over its first 50 variable N terminal amino acids to the M33 protein, antibodies against the M70 and M33 proteins do not cross-protect, which suggests that no cross-protection would occur. The new deduced M protein with the lowest similarity to any known M protein was ST4529, whose closest match had a 43% identity over the N-terminal 55 residues of the M5 protein. *st4529* likely encodes a new serospecifically unique M protein.

These findings potentially affect vaccine development. Although new *emm* sequences were identified in a survey in the United States (5), the percentage of new strains with new *emm* sequences was much lower (6%) than was found with these Malaysian isolates. *emm* typing of a larger number of strains from rheumatic fever– and rheumatic heart disease–endemic areas is required to deduce amino acid sequences for the development of a suitable M protein–based vaccine.

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## Mutant Chemokine Receptor (CCR-5) and Its Relevance to HIV Infection in Arabs

**To the Editor:** Approximately 10% of HIV-infected patients may remain AIDS-free for a long time; moreover, some persons do not become infected with HIV despite multiple high-risk sexual exposures (1,2). Factors responsible for this relative resistance to infection and disease include cytotoxic T cells, neutralizing antibodies, high concentrations of certain chemokines (e.g., RANTES, MIP-1), human leukocyte antigen haplotype, mannose-binding protein, and tumor necrosis factor alpha, C4, and TAP polymorphism (2-4). One of the chemokine receptors, CCR-5, which along with CD4 acts as co-receptor for HIV entry into macrophages, provides upon mutation a genetic restriction to HIV infection in homozygous persons and control of disease progression in heterozygous persons (5,6). Thus a 32bp deletion in the open reading frame of the region encoding the second extracellular loop of this receptor causes synthesis of a highly truncated protein that fails to express on the cell surface, leading to loss of HIV-1 co-receptor activity.

Studies in healthy Caucasian Europeans and North Americans show that approximately 1% of the population are homozygous for this deletion (Δ32), whereas 15% to 20% are heterozygous (5-9); surprisingly, a higher percentage (up to 20%) of persons at high risk for HIV but HIV-negative are homozygous for this deletion. However, no such mutation is seen in Japanese, Native Americans, Chinese, Africans, and Tamil Indians, which suggests that in these groups either resistance to HIV infection is not present or factors other than mutated CCR-5 are in operation. African-Americans and Hispanics show a low rate of mutation, possibly because of intermarriage with Caucasians (4). The low frequency of CCR-5 mutation in Arabs with close contacts with Turks in the Eastern Province of Saudi Arabia may also be due to intermarriage. However, certain persons with mutated CCR-5 can become HIV-infected (10); in such cases other chemokine receptors (e.g., CXCR-4, CCR-2, and CCR-3) are believed to facilitate infection.

HIV infection in Saudi Arabia (population 18 million) is uncommon; the World Health Organization has so far (1985 to 1997) documented 334 cases of AIDS in this region (11). We, therefore, studied for the first time the mutation of CCR-5 in Arabs residing in Saudi Arabia. DNA was isolated from the peripheral blood mononuclear cells of 105 male blood donors not infected with HIV and nine HIV-infected patients (seven male and two female). The latter were divided into three groups, according to published criteria (2): four persons whose infection did not progress over the long term and who showed only modest decline of CD4 count after several years of infection, one person whose infection progressed normally, and four persons whose infection progressed rapidly and CD4 count fell below 100/µl within 2 years. Primers flanking 32 nucleotide deletion of CCR-5 were used to generate wild type (W) and deleted (Δ32) fragments of 189 bp and 157 bp, respectively (5). Amplification was done in a Perkin-Elmer thermal cycler 9,600, by a 20 µl reaction mixture that contained 0.25mM of dNTPs, 20 pM of each primer ('5-CAAAAAGAAGGTCTTCATTACACC-3,5-CCTGTGCCTCTTCTTCTCATTTCG-3'), and 0.5 units of Taq polymerase in 1x reaction buffer. All reagents were obtained from Pharmacia (Sweden). The amplified product was separated on 2% agarose at 120 V for 45 min and examined under UV light. Of the uninfected blood donors, 104 (99%) were homozygous for the wild type, and 1 (0.96) was heterozygous for the mutation. None of the HIV-infected patients had the mutation. Thus, the mutation is present, albeit infrequently, in Arabs. A review of 68 HIV-infected patients in our files showed that, as in Caucasians, infection progressed rapidly in 8%, did not progress over the long term in 6%, and progressed normally in 86% (2). Therefore, other hitherto unknown protective factors must be operative in Arabs.

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