

### Similarity of Chemokines Charge and the V3 Domain of HIV-1 env Protein

**To the Editor:** Most clinical HIV-1 isolates can infect CD4<sup>+</sup> peripheral blood T lymphocytes, monocytes, and cultured macrophages (macrophage or M-tropic) but not transformed T-cell lines. In contrast, HIV-1 strains adapted for growth in transformed T-cell lines (T-cell line or T-tropic) do not infect primary monocytes or macrophages. This difference in tropism appears to be a consequence of specific amino acid changes in the env protein. Changes in env responsible for an M- to T-tropism shift often involve the acquisition of multiple positively charged residues in the hypervariable V3 loop domain (1). However, some non-V3 determinants are also important for viral tropism. Although both types of viruses use CD4 as receptor, the CXCR4 chemokine receptor (previously designated LESTR/fusin) is the unique cofactor for entry of T-tropic HIV-1 strains (2). The CCR5 chemokine receptor was subsequently demonstrated to be the cofactor for M-tropic HIV-1 isolates (3). Although some direct evidence for cell surface association of the CD4-env complex and the CXCR4 coreceptor was obtained (4), little detail is available on the molecular forces responsible for these protein-protein interactions. In particular, there is no direct evidence to indicate that the V3 loop binds to the chemokine receptor.

Jiang (5) reported that the extracellular domain of the CXCR4 coreceptor for T-tropic HIV-1 isolates is more negatively charged than the CCR5 coreceptor. Because T-tropic isolates have evolved a positively charged V3 domain, it was suggested that coreceptor-env binding involves the interaction between oppositely charged residues. We now expand this analysis

by showing that the chemokines corresponding to the different receptors have a similarly unbalanced composition of charged amino acids. So far, the CXCR4 receptor has been demonstrated to bind only the SDF-1 chemokine (6). The CCR5 receptor binds more than one chemokine, including RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$  (7). The amino acid sequence of these four chemokines is presented in the Figure. We listed the number of positive residues (arginine [R] and lysine [K]) and negative residues (aspartic acid [D] and glutamic acid [E]), and calculated the net charge. SDF-1 appears to have the highest number of positive residues and the lowest number of negative residues, resulting in a net charge of +11. All other chemokines have much less positively charged amino acids, resulting in a net charge for MIP-1 $\alpha$  and MIP-1 $\beta$  of -2 and -1, respectively. The RANTES chemokine has an intermediate charge of +7, which may correlate with the unique receptor use of this chemokine (e.g., RANTES, but not MIP-1 $\alpha$  and MIP-1 $\beta$ , binds the CCR3 receptor [7]). These results are consistent with the idea that positive charges in SDF-1 interact with negative charges in the CXCR4 receptor, and this binding may thus resemble the HIV-1 env-CXCR4 interaction.

Early evidence that both the chemokines and HIV-1 bind to the same domain of the chemokine receptor comes from virus inhibition studies. Several  $\beta$ -chemokines suppress infection with M-tropic but not T-tropic HIV isolates (8), and SDF-1 specifically blocks entry of T-tropic isolates (6). Although direct blocking of the receptor may explain part of this chemokine-mediated inhibition, it has also been proposed that internalization of the receptor contributes to the antiviral effect (9). Irrespective of the precise antiviral mechanism, the combined results of this analysis

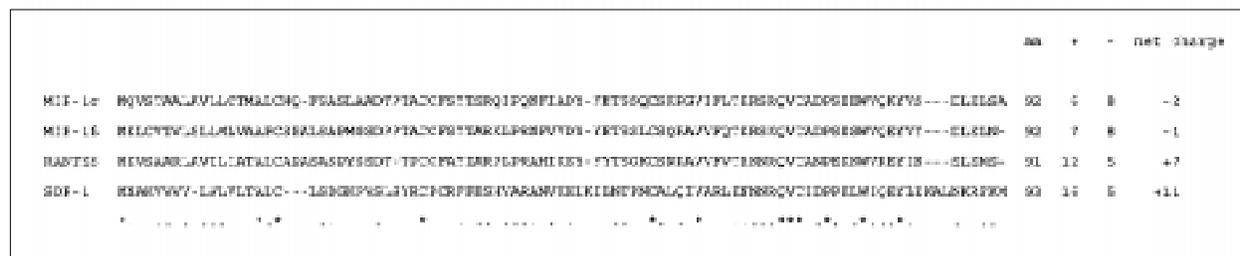


Figure. Amino acid alignment of the four chemokines was performed with the PC/Gene program. An overall identity and similarity of 12.4% and 40.2% was calculated, respectively. The initiator methionine contained within each sequence is removed in the processing of the chemokine. The SDF-1 form shown is the  $\beta$ -form; the  $\alpha$ -form lacks the C-terminal amino acids RFKM, thus reducing the net charge to +9.  
 \* = a perfectly conserved residue.  
 . = a conservative change.

and the one presented by Jiang (5) suggest that both the SDF-1 chemokine and the V3 loop of T-tropic HIV-1 viruses use positively charged amino acids for an electrostatic interaction with the negatively charged CXCR4 receptor. To examine whether the similarity between the chemokine and env V3 domain is also apparent at the primary sequence level, we performed an amino acid alignment; however, we found no conserved motifs (data not shown). A detailed mutational analysis is required to further our understanding of the env-coreceptor interaction.

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### **Detection of Glycoprotein of *Burkholderia pseudomallei***

**To the Editor:** Melioidosis, a potentially fatal disease that is difficult to diagnose and treat, is common in areas with subtropical climate (e.g., Singapore, the southern provinces of China) and is hyperendemic in Thailand. The etiologic agent, *Burkholderia pseudomallei* (*Pseudomonas pseudomallei*), is widely distributed in Southeast Asia and northern Australia. The agent has the potential to become established in regions with similar climate conditions, particularly if animals infected with *B. pseudomallei* are imported from endemic-disease zones (1-3).

Rapid and reliable detection of *B. pseudomallei* and its antigens has many potential applications. Recently, we developed a monoclonal antibody immunoenzyme test system for the detection of minimal concentrations of a *B. pseudomallei* glycoprotein, which is considered one of the pathogenicity factors for this microorganism. This glycoprotein, called Ag8 by N.N. Piven and V.I. Ilyukhin (4), is present in different strains of *B. pseudomallei* and *B. mallei* but not in other *Burkholderia* spp. (*B. aeruginosa*, *B. putida*, *B. cepacia*, *B. malthophilia*, *B. fluorescens*, *B. pseudoalcaligenes*). Ag8 is composed of 10% protein and 90% carbohydrate, has molecular mass 800 kDa, and is localized in an extracellular capsulelike substance surrounding *B. pseudomallei* cells (5).

We developed an immunoenzyme test system with three monoclonal antibodies (Mab) to different epitopes of Ag8 (Mab 2A6-IgG3, Mab 2H7-IgG1, Mab 1G2-IgG2b) and one antibody to epitopes common for Ag8 and LPS of *B. pseudomallei* (mab 1ES-IgG2b). A sandwich enzyme-linked immunosorbent assay (ELISA) was used for the detection of Ag8 in different test samples (6). The sensitivity of the immunoenzyme test system was determined with a standard antigen sample. Minimal sensitivity (37 ng/ml of carbohydrate) was observed when polyclonal immunoglobulins were used as "catching" antibodies. Maximal sensitivity (0.37 ng/ml of carbohydrate) was noted when either Mabs 2A6 or mixtures of Mabs were used as catching antibodies.

The test system was further evaluated with samples of extracellular antigens (extracts of cultural media, fractions after gel chromatography of extracellular antigens) and bacterial suspensions of *B. pseudomallei* and *B. mallei* strains isolated in different regions of the world.

Levels of Ag8 in cultural media varied considerably depending on periods of cultivation of bacteria. Additionally, the level of Ag8 varied among strains of *B. pseudomallei* and *B. mallei*. Among 61 strains of *B. pseudomallei* from the museum collection (most of which were isolated in Southeast Asia and northern Australia), three had increased ability to produce Ag8. These strains had been isolated from clinical specimens (blood, abscesses of hospitalized melioidosis patients) in Vietnam. The strains gave results typical of *B. pseudomallei* species in all routine serologic tests (agglutination test, immunofluorescence assay, immunodiffusion test). In contrast, the *B. pseudomallei* glanders agent (16 strains from the museum collection) had reduced ability for Ag8 production; ELISA titers of Ag8 were a thousandfold less in culture fluids in these strains.

The ELISA technique not only facilitates diagnosis of disease but also provides a rational basis for selecting strains for vaccine production. It also has considerable utility for studying the pathogenicity of *B. pseudomallei*.

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### Forging New Perspectives on Disease Surveillance

**To the Editor:** Recognizing disease emergence as a paradigm uniquely influenced by human activity demands a reevaluation of traditional

disease surveillance systems. Part of a surveillance program should be focused on the areas of human activity where disease emergence is most likely to occur. A system that monitors areas known to be involved in disease emergence, such as development projects, agriculture, climate, and refugee movements, may greatly increase our ability to detect and prevent outbreaks.

Large development projects entail ecologic upheavals that can facilitate disease emergence. Construction of a dam in 1987 in Mauritania resulted in increased mosquito breeding sites and in an explosion in the mosquito population; epidemics of Rift Valley fever quickly followed (1). The Southeastern Anatolia Irrigation Project on the Euphrates and Tigris Rivers in Turkey, which will provide irrigation for 1.7 million hectares, has already increased malaria and leishmaniasis cases in the local population (2). The massive Three Gorges Dam Project on the Yangtze River in China, which will create a reservoir 760 km long, must be evaluated for its impact on local disease. With knowledge of endemic diseases and their reservoirs and vectors in these areas of ecologic change, public health workers can anticipate disease epidemics and implement prevention measures.

Incorporating climate predictions into a disease surveillance system would supplement resources in an area known to affect disease emergence. The U.S. Agency for International Development's (USAID) Famine Early Warning System monitors the African continent for two major factors implicated in emergence: temperature and precipitation. Focused on countries at high risk for food shortages and famine, the early warning system is an example of a predictive and preventative surveillance system. Precipitation, temperature, and plant health data from satellites are evaluated as indicators of crop failure. These data are supplemented by information from field representatives who directly observe agricultural production. USAID's system and other global monitoring systems can provide a base level of surveillance that can add to our knowledge of climatologic influence on disease emergence.

The beginning of the Zairian refugee crisis in 1994 illustrates the need for surveillance among refugee populations. In July 1994, 500,000 to 800,000 Rwandan Hutus fled into the North Kivu region of Zaire. In the month between July 14 and August 14, 48,347 of these refugees died,

predominantly of infectious diseases (3). Similar infectious disease epidemics occurred among Kurdish refugees in 1991 (4), Somali refugees in 1992 (5), and Burundian refugees in Rwanda in 1993 (6). In the wake of intense political, social, or physical disruption, the movement of large numbers of people creates ideal conditions for disease outbreaks. When moving into new areas, refugees may not be equipped immunologically against endemic diseases. Most refugee camps are overcrowded, with inadequate sanitation or medical care (7). Refugees often have severe shock or stress, which in combination with poor nutrition, weakens immune defenses. Therefore, refugee populations are extremely vulnerable and should be closely monitored for infectious disease outbreaks. The United Nations High Commissioner for Refugees, the International Rescue Committee, and the Human Rights Watch can provide rapid notification about disease emergence in refugee populations.

Recognizing human involvement as a common critical factor in emergence creates the possibility of refining international disease surveillance. The Centers for Disease Control and Prevention, the World Health Organization, and national governments should foster relationships with organizations already placed to provide disease emergence information in populations and locations implicated in disease emergence. These relationships will increase the scope and efficiency of our efforts to prevent human disease.

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### Provide a Context for Disease Emergence

**To the Editor:** When a disease emerges, the trend is to assume that another important and spreading infection is about to devastate humans or animals. Some qualification of the term “emergence” is needed to put emerging diseases into a context for each target species. There may be a cause for alarm and further action or, alternatively, no real change except in knowledge. In Australia, for example, an old disease “emerged” in a new area, while in another, a disease new to the continent emerged. The two disease agents were Ross River virus (which causes fever and polyarthritis in humans) and bluetongue virus (which often causes fatal disease in sheep). Both causative viruses are insect-borne.

Ross River virus is probably of very ancient lineage as an infection transmitted between marsupials and indigenous mosquitoes and was on the Australian continent long before humans first entered (some tens of thousands of years ago). In 1975, the infection was not known to occur in Tasmania, the state separated from the Australian mainland by a wide stretch of sea. In that year, my group detected a clear-cut seroconversion to Ross River virus in sentinel cows in northern Tasmania (1). Cooperative investigations found antibody-positive sera first in marsupials and then in persons who had never left Tasmania. The existing clinical condition of polyarthritis was linked to Ross River virus only after the causative virus was recognized indirectly (2). The marsupial populations of mainland Australia and Tasmania were continuous until the seas rose at the end of the last ice age. Ross River disease had emerged in Tasmania, but only from obscurity.

Bluetongue viruses occur widely in southern and eastern Asia (3). This general picture has been established only since the discovery of bluetongue virus in Australia. Overt disease occurs in sheep on the fringes of the endemic-disease region and in susceptible sheep imported into various countries within the region (3). In

contrast, at least eight members of the bluetongue group of viruses have entered Australia and some of these the Pacific countries; they must have arrived after ruminant populations were introduced in New Guinea, Australia, and the Pacific islands. They spread through the ruminant-dependent *Culicoides* species vectors, also introduced (4). Emergence of bluetongue in Australia has so far meant one sheep dead of bluetongue disease in 1989 (in a population of 100 million sheep) since the discovery of the presence of bluetongue virus in Australia in 1977 (4). However, the potential for a major epidemic remains and the discovery caused major trade difficulties. In both these situations the disease was emergent but its potential was very different.

Since the latter part of 1994, two newly recognized zoonotic viruses have been reported from Australia: paramyxovirus (equine morbillivirus), which caused deaths in horses and humans (5), and lyssavirus (closely related to rabies virus), which has also caused a human death in Australia (6). In both instances, strong evidence indicates that bats are the maintenance hosts. Bats are probably the oldest form of placental mammal in Australia, with fossil evidence from the Middle Miocene era, circa 15 million years ago (7). Some species of bats migrate between various countries of southern Asia and the Pacific; probably they migrated more in the ice ages when sea distances were shorter. This past continuity of the bat populations and the inadequate study of the Microchiroptera in Asia for rabies viruses led me to forecast in 1989 that rabies (or a rabieslike virus) was established in bats in Australia (8). These recently recognized disease agents are both emergent but have not become important for humans or animals.

As a hypothetical example, Ebola virus has been shown by Swanepoel and others to multiply well in bats of three species (9). Two of the species used experimentally belong to the genus *Tadarida*, which is well represented in Africa, Asia, and Australia (10). Evidence that bats are the reservoir hosts of Ebola virus (Reston), which is known to infect monkeys in the Philippines should be sought in the Philippines and for its silent presence in bats in Australia. If such evidence were found, Ebola would be labeled as emerging in Australia and other countries between Australia and the Philippines, although no cases of disease might ever occur east of the

line where monkeys are indigenous.

The scientist who looks for evidence of a disease agent in a country or region and the regulator who has to deal with the public health or economic consequences of a new or newly recognized disease have conflicting interests. The national and international reaction to the discovery of many agents in countries where they had not been found does not take into account the measure of risk for the disease. The term "emergent" disease needs some qualifiers to diminish fear and overreaction.

One way would be to rate the risk for the disease on a scale of 1 to 5; another would be to provide a context that notes the capacity of the agent to spread and cause illness and death.

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### Resistance to Dryness of *Escherichia coli* O157:H7 Strains from Outbreak in Sakai City, Japan, 1996

**To the Editor:** A large outbreak of *Escherichia coli* O157:H7 with more than 6,000 cases occurred in Sakai City, Osaka Prefecture, Japan, in July 1996 (1); after the outbreak, more than 1,000 secondary infections occurred in the families of the patients (2). We studied the resistance of *E. coli* O157:H7 to dryness because the survival on surfaces of inert materials under dry conditions may be related to the transmissibility of the strains.

*E. coli* O157:H7 strains grown on 3.0% nutrient broth with 1.5% agar for 20 to 22 hours at 37°C were suspended at a concentration of approximately  $5 \times 10^8$  cfu/ml in a 10% skim milk, 0.5% NaCl solution. Aliquots (10  $\mu$ l) of bacterial suspensions were spread to approximately 10 cm<sup>2</sup> on plastic petri plates for bacterial culture and dried under the air flow of a clean bench until no aliquots were evident. After storage in the dark at room temperature, bacteria were harvested with saline and gauze and the viable number was counted (n = 3).

The log reductions 12 hours after drying were employed to show the resistance levels of *E. coli* O157:H7 to dry stress. The log reductions of the three strains from the Sakai City outbreak (RIMD0509950, RIMD0509894, and RIMD0509951) were  $0.04 \pm 0.34$ ,  $0.14 \pm 0.06$ , and  $0.20 \pm 0.60$ , respectively (mean = 0.13), whereas those of the *E. coli* O157:H7 strains from the other cases varied from  $0.71 \pm 0.18$  to  $4.57 \pm 1.02$ . (RIMD0509861,  $0.71 \pm 0.18$ ; ATCC35150,  $1.15 \pm 0.35$ ; RIMD0509826,  $1.23 \pm 0.60$ ; RIMD0509742,  $1.28 \pm 0.18$ ; ATCC43890,  $1.31 \pm 0.17$ ; RIMD0509933,  $1.48 \pm 0.19$ ; RIMD0509932,  $1.48 \pm 0.22$ ; RIMD0509765,  $3.60 \pm$

$0.24$ ; RIMD0509764,  $4.57 \pm 1.02$ ; mean = 1.87). Although the strain from the Sakai City outbreak (RIMD0509950) survived for at least 35 days under the dry conditions, the strains from the other cases had no viable cells after 7 days. The log reductions of *E. coli* JM109 and DH10B strains were more than 10, and no viable cells were detected in 12 hours.

The strains from the Sakai City outbreak also showed marked acid resistance. Acid resistance of *E. coli* O157:H7 has been reported to depend on *rpoS*, which is induced in a stationary phase (3). Since the *E. coli* O157:H7 strains in a stationary phase were more resistant to dry and acid stresses than those in a log phase, *rpoS* may also be associated with resistance to dry stress. However, no deletions of *rpoS* were detected by polymerase chain reaction analysis in the *E. coli* O157:H7 strains used in this experiment. Further study on the mechanism of resistance will be needed to establish new strategies for eradicating the bacteria.

A case-control study by the Ministry of Health and Welfare of Japan showed that uncooked radish sprouts were the vehicle of the largest outbreak in Sakai City (4). In two small outbreaks of *E. coli* O157:H7 in March 1997, the vehicle of infection might have been radish sprouts; therefore, the possible contamination of white radish seeds with *E. coli* O157:H7 has been discussed (5). If such contamination was present, dry resistance might be involved in the survival on or in white radish seeds because the bacteria were exposed to dry conditions for a long period before sprouting. We propose that dry resistance be considered an important factor in infection.

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### Irradiation Pasteurization of Solid Foods

**To the Editor:** Osterholm and Potter have made a strong case for irradiation pasteurization of solid foods that enter kitchens as raw agricultural commodities, such as meat, poultry, and seafood (1). Irradiation pasteurization was advocated to protect against foodborne diseases caused by common pathogens such as *Campylobacter*, *Cryptosporidium*, *Escherichia coli*, *Listeria*, *Salmonella*, and *Toxoplasma* (2). An additional rationale for irradiation pasteurization is bacterial resistance to antimicrobial drugs, a major health concern, which will undoubtedly increase in magnitude unless new approaches become available (3). The widespread use of antibiotics in animal husbandry may be the cause of some of this resistance, for example, in vancomycin-resistant enterococci associated with the agricultural use of glycopeptide antibiotics (4,5). Furthermore, resistance to glycopeptide antibiotics can be transferred from enterococci to other gram-positive organisms, at least in the laboratory (6). Thus, resistant bacterial strains from animal sources may enter the human population through contaminated food without necessarily causing immediate disease but resulting in expanded human reservoirs of antimicrobial resistance through horizontal gene transfer (7). When such bacterial strains are subsequently transmitted to a susceptible person, serious disease could result, which would be exceedingly difficult to treat (8). Irradiation pasteurization of solid foods could reduce the magnitude of transfer of resistance genes through contaminated foods.

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### Emerging Infectious Diseases in Brazil

**To the Editor:** Hooman Momen's update on emerging infectious diseases in Brazil (1) appears to be based solely on notifiable disease data, which cannot adequately describe the current situation. Additional data in several areas may be useful.

**Parasitic diseases:** Dr. Momen's update restricts itself to protozoal diseases and does not distinguish between mucocutaneous and visceral leishmaniasis. Visceral leishmaniasis is in fact expanding in many suburban and urban areas in the northeast. Mucocutaneous leishmaniasis, after a small retreat following extensive deforestation, has made a comeback; and in many suburban areas in Rio de Janeiro and São Paulo, in the southeast, transmission is occurring, probably because of changes in sandfly ecology (1).

A helminthic disease of interest is mansoni schistosomiasis, which has been expanding its area of transmission, reaching over to Santa Catarina, in the south, to Pará in the north, expanding also westward, to Mato Grosso and Mato Grosso do Sul. The number of cases, as well as the associated illness, has possibly been reduced, but there is no doubt that the disease can be found in a much larger area than 20 years ago. Other emerging helminthiases of interest, albeit not of public health concern, are

onchocerciasis, still restricted to the Yanomami group in Roraima, bordering Venezuela; *Angiostrongylus costaricensis* infection (2), found in the south, Rio Grande do Sul; and some cases of lagochilascariasis, reported from Pará.

Viral diseases: As Dr. Momen pointed out, dengue is by far the most serious emerging viral disease in Brazil, and the area occupied by *Aedes aegypti* is expanding. Dengue hemorrhagic fever has occurred occasionally, but no outbreaks have been recorded. However, measles is no longer a problem; the outbreaks have been controlled.

There is no evidence to support that hepatitis B is declining because of vaccination. Vaccination is still restricted to areas of high prevalence. Other states are beginning vaccination programs in newborns, but it will be some time before these programs have any effect on prevalence. As to hepatitis C, because diagnostic testing is only recently becoming widespread, we are probably experiencing an increase in detection rather than in incidence.

Other notable agents are Mayaro and Oropouche viruses, which are arthropod-borne and among the most common causes of febrile illness in the Amazon region. *Aedes albopictus*, found all over the country, could be a potential vector (3). Apart from HIV, other retroviruses are cause for concern: HTLV-I and HTLV-II screening is recommended for blood banks, and enough data exist to conclude that the infection is widespread in the country but with a low prevalence (0.4% and 0.1%, respectively). Clusters of disease have not been identified, but adult T-cell leukemia/lymphoma is far from a curiosity (4).

Bacterial diseases: Brazilian purpuric fever, caused by *Haemophilus influenzae* biogroup *aegyptius*, was first reported in outbreaks in the central-south part of the country (western São Paulo, eastern Mato Grosso do Sul, and northwestern Paraná) about 10 years ago, causing a syndrome much like meningococcemia (5). For enteric infections, the limited data available present interesting trends. *Salmonella* Enteritidis is rising and *S. Typhimurium* is declining in São Paulo and the southern states. These trends may reflect improved sanitation and increased use of industrialized foods and contaminated animal feeds (6).

Fungal diseases are not reportable, but many epidemiologic studies have been conducted. Paracoccidioidomycosis (South American blastomycosis) was unheard of in the Amazon region,

never being found in native habitants; however, because of environmental and socioeconomic changes, the infection is now being identified (7).

Antimicrobial resistance is a serious problem, not only within hospitals, but also in the community. Penicillin-resistant pneumococcus is not yet a widespread problem, but it has been detected (8); the same situation exists with regard to *Mycobacterium tuberculosis* (9).

The problem of emerging infectious disease is gaining increasing attention in Brazil, and published reports together with notifiable disease data underline the main points of concern.

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### Reply to L.J. da Silva

**To the Editor:** Dr. da Silva's letter raises several important points. My article, however, was never intended to be comprehensive. The choice of which emerging infectious diseases to include was difficult, especially in a country where many endemic infections continue at a high prevalence and others, thought to be controlled, are reemerging.

As Dr. da Silva states, many reports (in Portuguese and English) discuss infectious diseases in Brazil; however, this information is rarely current. The information about measles in my article is a case in point. At the time of my article, an outbreak causing national concern was occurring in Brazil; it has since been controlled. A further problem is that the most detailed and reliable studies are generally of only a regional or local nature, for example, the recent excellent report by Merchan-Hamann (1) on the situation of endemic diseases in north and northeastern Brazil and other references cited by Dr. da Silva. To obtain current information at the national level and provide numerical data rather than merely discuss current trends, I focused on notifiable diseases.

As Dr. da Silva states, schistosomiasis has continued to decrease both in the number of cases and associated illness. Onchocerciasis has been restricted to a small focus in northern Brazil for many years, and a recent report of a new focus in the state of Goias has yet to be confirmed. In my opinion, neither infection could be considered emerging. An important helminthiasis that perhaps should be mentioned is Bancroftian filariasis with a main focus in Recife and minor foci in Belem and Alagoas. Because of traditional and novel control strategies, the number of cases is declining in all foci.

The information I used about hepatitis is confirmed by the National Reference Center on Viral Hepatitis of the Ministry of Health. Febrile illnesses in the Amazon are the great enigma and probably provide the cover for many new diseases that may still emerge. For example, only approximately 20% of blood slides taken from suspected malaria patients in the Amazon are confirmed as positive, which leaves at least one million cases of febrile illness per year undiagnosed. I am unaware of any data that show Mayaro and Oropouche viruses as the most

common cause of these illnesses. Dr. da Silva's letter provides useful additional information on bacterial diseases, antimicrobial resistance, and a number of low-prevalence diseases that may in time prove to be important emerging infections.

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### A Brief Update on Rabbit Hemorrhagic Disease Virus

**To the Editor:** We read with interest the paper by A. Smith et al. (*Emerg Infect Dis* 1998; 1:13-20) on calicivirus emergence from ocean reservoirs. Our attention was drawn particularly to the data and comments regarding rabbit hemorrhagic disease (RHD), a recently emerged and devastating disease of just one rabbit species, *Oryctolagus cuniculus*. We have been involved in RHD research and diagnosis since 1989. Like D. Gregg's laboratory at the Foreign Animal Diseases, U.S. Department of Agriculture, Greenport, USA, our laboratory at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia, Italy, was in 1991 designated a Reference Laboratory for RHD by the International Office of Epizootics (OIE), Paris, France. Although other aspects of the article by Dr. Smith and colleagues appear unclear (e.g., the fact that feline calicivirus is classified among human pathogens like Norwalk virus), we will confine our comments to a few main points concerning RHD virus (RHDV).

Is RHDV a calicivirus or a parvovirus? RHD is caused by a calicivirus (1-3). The articles cited by Dr. Smith date back to 1991 and are part of a book review promoted and edited by OIE (4). This landmark review includes papers from China and the United States supporting the parvovirus hypothesis and papers from Europe concluding that RHDV is a calicivirus. A retrospective reading of those articles may explain the reasons for the misinterpretation of some results. However, this occurred in 1991 and, after 7 years, more than 50 published articles consider RHDV a

calicivirus. Actually, RHDV is one of the best characterized caliciviruses, and the publication of its full genome sequence in 1991 was the first of a *Caliciviridae* member (5).

Diagnostic tools have been developed by our and other laboratories (3,4,6). Thanks also to specific monoclonal antibodies produced towards RHDV and European brown hare syndrome virus (EBHSV) by our colleague E. Brocchi, we standardized different enzyme-linked immunosorbent assays (ELISAs) for the diagnosis of related diseases (4,6-8). In particular, we developed five different ELISAs for serology that allow the detection of antibodies specific for RHDV or EBHSV or that are cross-reactive. In addition, we can define the antibody response in rabbits and hares in terms of isotype-involved immunoglobulin M (IgM), IgA, and IgG (9). Today the main difficulty is the qualitative distinction between RHDV and rabbit calicivirus (RCV, a recently identified nonpathogenic calicivirus) antibodies because of the close antigenic profiles of these viruses (6). Finally, RHDV- and EBHSV-specific polymerase chain reaction has been developed in at least five laboratories besides ours. We have sent these reagents and/or diagnostic methods to at least 19 laboratories outside Italy, including Australia, New Zealand, and the United States.

Does RHDV infect humans? This question has arisen together with the prospect of using RHDV as a biologic control agent in countries like Australia and New Zealand, when they were free of RHDV. In Europe, where the disease naturally occurred and quickly spread, no particular control on human health was planned. In Italy only, between 1987 and 1990, hundreds of millions of rabbits died of RHD in regions where the average density of humans is very high. As a consequence of the use of the vaccine since 1991, the incidence of RHD among breeding rabbits decreased drastically and quickly. Nevertheless, the disease is still endemic, mainly in small farms and among wild rabbits. EBHS also is endemic in wild hares, and hunters are highly exposed to the virus since hares are their main target. However, neither in humans nor in animal species other than rabbits and hares have any diseases similar to RHD ever been reported. In relation to the likelihood of mild or inapparent infections, we used 100 human sera randomly selected from blood donors to carry out a preliminary

standardization of an RHD-ELISA that has been periodically used to control the sera of the RHD laboratory staff. Very recently, we tested nine sera from laboratory personnel exposed to RHDV; again no positive result was noted by RHD-ELISA. These findings have limited epidemiologic value, but considering the high level of exposure of part of the sample, it is evident that RHDV infection in humans is unlikely to be the rule.

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### Rabbit Hemorrhagic Disease

**To the Editor:** The recent article on calicivirus by Smith et al. (1) is misleading in its use of the study concerning human health aspects of rabbit hemorrhagic disease (RHD) by Mead et al. (2).

The RHD exposure categories of “low” and “high” used by Mead et al. and mentioned in the first column of page 18 (1) are not related to the categories of “low” and “high” given in the same paragraph at the top of the second column. The reader might easily assume that it was Mead et al. who considered that Jul–Dec 1995 was “a low exposure period.” This is not so—such a classification is made by Smith et al.

Further, the reader might assume that it was the study by Mead et al. that concluded “that exposure to RHD virus remains a plausible explanation for increased disease incidence.” Again this is an inference drawn by Smith et al. and is the opposite of the conclusion of Mead et al.

The basis of exposure in the study by Mead et al. is at an individual level—the respondents were chosen either because they had been handling rabbits or as controls in determining the level of disease. In contrast, Smith et al. consider exposure at a broad environmental level and disregard whether the respondents had been handling infected rabbits or not. Actually, more contact with rabbits occurred during the first half of the study than during the second.

Smith et al. do not mention the conclusions of Mead et al.: These neither showed any significant difference between levels or types of illness in those exposed and those not exposed to RHD virus nor demonstrated any association between the exposure to RHD and number of episodes of illness in the subsequent 1 to 2 months.

The results of the study by Mead et al. may be summarized by noting that the average number of episodes of illness over the 13-month reporting period was 2.6 for respondents who had not been exposed to RHD virus, 2.2 for those classified as having a low level of exposure, and 2.3 for those classified as having a high level.

The study by Mead et al. concluded that, on the basis of the health survey and the lack of any serologic reaction of the respondents, there was considerable support to the view that RHD virus is not associated with infection or disease in humans. The results of the study have been submitted for publication in a scientific journal.

Reference 31 should refer to the Bureau of Resource Sciences (not Studies).

### C. Mead

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## Reply to Drs. Capucci, Lavazza, and Mead

**To the Editor:** We are aware of Capucci and Lavazza's excellent work. Indeed, one of the best characterized calicivirus genomes is that detected in rabbit hemorrhagic disease (RHD); however, the virus' infectivity, pathogenesis, modes of transmission, reservoirs, survival in nature, host of origin, virulence factors, number of neutralization serotypes, and multispecies infectivity are poorly characterized. Propagating this virus in vitro could provide insight for addressing questions relevant to caliciviruses that cannot be propagated in vitro.

We are unclear about the confusion regarding Norwalk virus and feline calicivirus (FCV). Both are caliciviruses. Norwalk virus is a human pathogen. FCV is in a different genus (1) that includes strains infecting humans (2). We know of no documented FCV infections in humans nor of detailed studies to search for such occurrences, although some evidence suggests the possibility (3).

Capucci and Lavazza's remaining questions address the etiology of RHD, diagnostic reagents, and possible human infection. They report nine laboratory workers as antibody negative but do not report test results on persons at high risk, such as rabbit farm workers, nor do they mention having positive control human or primate sera. Koch's postulates have been fulfilled for RHD: a parvovirus was isolated in vitro and was cell-passaged 15 times; at a second laboratory, the parvovirus was identified in materials causing RHD (4,5). In Europe the parvovirus etiology for RHD was deemed hypothetical but has not been refuted on a scientific basis. The calicivirus consistently identified in European materials has not been isolated in vitro, and Koch's postulates have not been fulfilled. Are the parvovirus-associated outbreaks of RHD in Mexico and China (4,5) and the calicivirus-associated RHD

outbreaks in Europe identical disease manifestations of two different viruses? Is RHD multifactorial requiring two or more agents? Is RHD caused by only a calicivirus or only a parvovirus? A calicivirus and a parvovirus can be isolated in vitro from the same fecal sample of a sick rabbit (N. Keefer, D.E. Skilling, A.W. Smith, unpub. data).

Our comments on RHD diagnostic assays referred to those used in Australia (6,7) to screen humans and experimentally infected animals to support legalizing the spread of RHD in Australia and New Zealand.

Public health protection requires prudent avoidance of pathogens associated with risk of adverse outcome, not necessarily proof of causation (8). In this context, human health risk for RHD goes largely unaddressed. The deliberate introduction of a new disease agent (RHD) known to cause death in mammals requires prudence rather than proof of human illness, especially when the scientific literature includes reports that the agent has induced antibody reactions in a wide range of mammalian and avian species (6).

Mead et al. (9) conclude, "No significant association between exposure to RCV and subsequent bouts of sickness could be demonstrated." Their recorded data do not support a statistically significant risk of illness because sample sizes in the monthly groups were too small for any meaningful interpretation. Mead et al. (9) state a "lack of any serologic reaction of the respondents," but a 50% cut-point was used for the competitive ELISA test, and some individual sera were repeated up to six times with percent inhibition reactions ranging from approximately 1% to 44% in one instance and 12% to 100% in another. Results were selected from these laboratory data and reported "lack of serologic reaction."

We derived our findings from data obtained under a freedom of information request. Mead et al. used the same data to support an opposite conclusion. Opposing conclusions "red flag" the quality of the study. In summary, the reporting of negative results of such a study cannot be used to support the important biologic, health, and political conclusion that humans are not at risk from infection with RHD.

We encourage a well-designed longitudinal

study of persons at high risk of RHD exposure to answer conclusively whether RHD has infected humans. If "the rule" means that most humans exposed to RHD would become infected, we agree with Dr. Capucci "that infection is unlikely to be the rule," but transmission of equine morbillivirus, Rift Valley fever, and H5N1 influenza to humans is also "unlikely to be the rule" (10).

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