



# Micro Morphs

## West Nile Virus Update: WNV in blood products and transplanted organs

*From the Centers for Disease Control and Prevention West Nile Virus homepage*

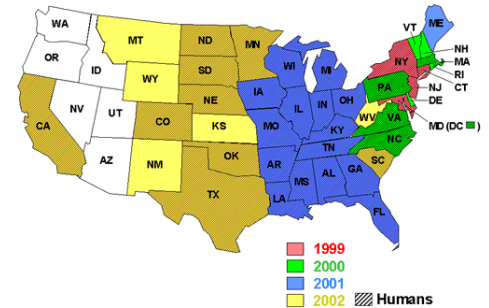
As of 2 October, the total number of West Nile virus cases reported to CDC reached 2,530 with 125 deaths. Thirty two states and Washington D. C. have reported human cases of West Nile virus in 2002.

The CDC, the Food and Drug Administration, the Health Resources and Services Administration, in collaboration with blood collection agencies and state and local health departments, continue to investigate WNV infections in recipients of blood products and organ transplantation. CDC has received reports from 10 states of 15 patients with confirmed West Nile infection diagnosed after receiving blood products within 1 month of illness onset.

It is likely that not all of the 15 patients were infected via blood products; all lived in areas with active West Nile virus activity and thus may have been infected via mosquito bites.

To recap the investigation so far, West Nile virus transmission from blood products has been confirmed in three patients. In a Mississippi investigation, West Nile virus was cultured

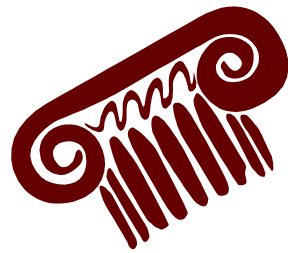
West Nile Virus in the United States, 1999 - 2002



Courtesy CDC website

from a blood product associated with a blood donor of one of the cases. In a Michigan investigation, two patients tested positive for WNV infection after receiving different blood products derived from a single blood donation subsequently found to have evidence of WNV.

The CDC and the Michigan Department of Community Health (MDCH) are continuing to investigate the West Nile Virus infection in a woman who received a blood transfusion who later became ill from WNV. She had been breastfeeding and her breast milk was shown to have evidence of WNV genetic material. Attempts to culture WNV from the breast milk are still underway.



### Inside this issue:

West Nile Virus Update

Malaria in Virginia

Resistant malaria

emicrocase

## Call for Abstracts

**2003 ASCLS Annual Meeting  
July 22-26, 2003  
Philadelphia, PA  
Abstracts must be submitted according to instructions provided in the ASCLS website and must be postmarked by January 15.**

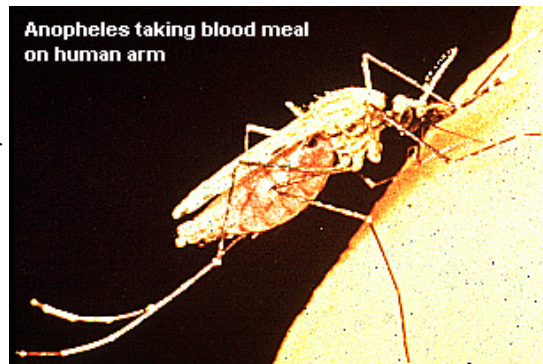
## Locally acquired malaria, Virginia, U.S.A.

In late August 2002, two (2) cases of locally acquired *Plasmodium vivax* malaria were diagnosed in Loudoun County, Virginia. Neither patient had any history of recent international travel, blood transfusion, organ transplant, injection drug use, or other risk factors for malaria. The majority of cases of malaria in the United States are imported from regions of the world where malaria is endemic.

Of the 1,402 malaria cases reported in the United States in 2000, two were blood-borne infections and 2 were congenitally acquired. The Centers for Disease Control and Prevention (CDC) is assisting Loudoun County health officials with the investigation. In addition, local health authorities are carrying out enhanced surveillance and mosquito control measures.

Meanwhile, search for malaria infections widens. Testing of mosquitoes to determine how widespread the problem continues in Fairfax county and Alexandria, Virginia and in Maryland. A military team of malaria experts has found two pools of malaria-carrying mosquitoes on a Maryland island in the Potomac River near Loudoun County, providing the first independent confirmation that the infected insects appear to be in the Washington area.

The researchers found infected mosquitoes on Selden Island in Montgomery County Maryland using a different testing method previously employed in Loudoun by a contractor. Robert A. Wirtz, chief of the Entomology Branch at the Centers for Disease Control and Prevention, said researchers in Atlanta plan to re-test the latest results. But Wirtz said the polymerase chain reaction test used in



Anopheles taking blood meal on human arm

Maryland, in which genetic material is amplified to make it easier to find the malaria parasite's DNA, has long proven reliable.

Wirtz also explained that although it is unlikely that the results will be true positives, it is possible that people who picked up malaria in areas where the disease is endemic (overseas) live in the area and that they had infected the mosquitoes. Some of them may remain asymptomatic, however. Also, while it is rare to see so many malaria-carrying mosquitoes in one place in the United States, it could happen, he added

SOURCES: Morbidity and Mortality Weekly Report 2002;51:921-923.

Washington Post  
Tuesday, October 8, 2002;

For more information concerning locally acquired malaria cases, see

Zucker R.J., Changing patterns of autochthonous malaria transmission in the United States: a review of recent outbreaks. *Emerg Infect Dis*, 1996;2:37-43.

Centers for Disease Control and Prevention. Malaria Surveillance - United States, 2000. *MMWR Morb Mortal Wkly Rep* 2002;51(SS-05):9-21.

Of the 1,402 malaria cases reported in the United States in 2000, two were blood-borne infections and 2 were congenitally acquired.

## Clinical Study Confirms Single Gene Change in Chloroquine-Resistant Malaria

*From the National Institute of Allergy and Infectious Diseases*

A team of U.S. and African medical researchers has developed a molecular marker that can be used to diagnose individuals with and survey populations for malaria parasites that are resistant to the drug chloroquine. The marker may not only help doctors select the best therapy for their patients, it may also assist public health officials determine countrywide treatment guidelines.

The results of their study, reported in this week's *New England Journal of Medicine*, puts a confirmatory clinical stamp on the recent laboratory discovery that tiny mutations in a single gene of the malaria parasite confer resistance to the drug. In the new study, the marker was found 100 percent of the time in clinical cases of chloroquine-resistant malaria.

A safe, inexpensive and highly effective treatment, chloroquine was the mainstay antimalarial drug worldwide in the latter half of the 20th century until overuse pressured *Plasmodium falciparum*, the most deadly malaria parasite, to develop ways to evade its effects. Doctors in South America and Southeast Asia have largely given up using the drug. Resistant parasites continue to spread, especially in Africa where 90 percent of malaria deaths, primarily among young children, now occur. Although the problem also seriously undercuts malaria control efforts in Africa -- most dramatically in sub-Saharan Africa -- chloroquine remains the treatment of choice for many African countries because affordable alternatives do not exist, and partial immunity among older children and adults is widespread and helps the drug work.

The *NEJM* study, led by Christopher V. Plowe, M.D., Abdoulaye Djimde, Pharm.D., and their colleagues at the University of Maryland School of Medicine in Baltimore, the University of Mali in Bamako, and the National Institute of Allergy and Infectious Diseases (NIAID), is the first of several ongoing field studies to confirm laboratory findings published last October by Thomas E. Wellems, M.D., Ph.D., and his col-

leagues in NIAID's Laboratory of Parasitic Diseases. The NIAID group reported that small changes in the *pfcr* gene of chromosome 7 of *P. falciparum* associate completely with chloroquine resistance in parasite lines from Asia, Africa and South America.

"Our clinical data strongly support that this *pfcr* mutant is responsible for chloroquine resistance," states Dr. Djimde, "but the level of resistance may be modulated by other factors or other genes."

"The clinical validation of this laboratory marker is good news for diagnosing chloroquine resistance in the field as well as in clinics and hospitals," says NIAID Director Anthony S. Fauci, M.D. "Importantly, public health officials in malaria-endemic countries may use this tool to survey their populations for increases or decreases in chloroquine-resistant parasites, helping them make informed decisions about front-line malaria therapy."

In addition, Dr. Djimde notes, the tool can also be used at the individual level to diagnose chloroquine-resistant malaria in non-immune individuals, for example, travelers or people who live in places where malaria occurs only sporadically, such as deserts or highlands within endemic countries. Non-immune individuals are at greater risk for the severe complications of malaria when they are infected.

Currently, it takes 14 days to diagnose a chloroquine-resistant infection in an individual, and two to three days to detect resistance in laboratory strains of the parasite. The molecular tool developed by the University of Maryland and University of Mali groups, which relies in part on the ultrasensitive diagnostic technique called polymerase chain reaction (PCR), takes only a few hours.



A child with malaria. Malaria kills an African child every 30 seconds.

"Our ultimate goal," says Dr. Djimde, "is to develop a biochemical method to detect the presence of the mutant gene in a few minutes, but we're not there yet."

The investigators carried out their study in the towns of Mopti and Bandiagara in central eastern Mali, areas with a low level of resistance to chloroquine. They invited community members age 2 years or older who had malaria symptoms to be examined by a physician at their NIAID-funded clinic. Individuals who met the study criteria and agreed to participate in the study received chloroquine treatment and were followed by the study team for 14 days afterwards.

Dr. Plowe and his colleagues collected blood samples before and after treatment and analyzed them for specific mutations in two different *P. falciparum* genes, *pfcr* and *pfmdr 1*, based on earlier work suggesting that these genes might harbor mutations important to chloroquine resistance.

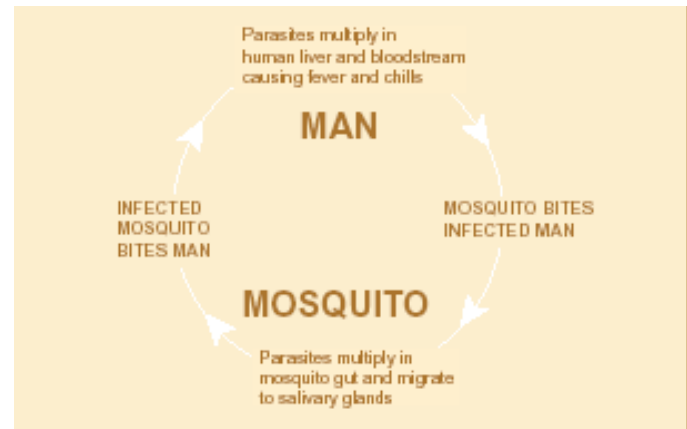
Among patients in the study, they found the parasite T76 *pfcr* mutation in 100 percent of 60 people with infections that failed to respond to chloroquine treatment versus 41 percent of 116 infected people who were randomly sampled prior to receiving treatment. The Y86 *pfmdr 1* mutation had a statistically significant but weaker link with chloroquine resistance. Based on their findings, the researchers believe that the *pfcr* mutant is responsible for chloroquine resistance but that the *pfmdr 1* mutant may help modulate the level of resistance.

Some people who carried the resistant parasite did clear their infections after chloroquine therapy. To determine what role prior immunity may have played in this outcome, the investigators compared infection clearances in children younger than 10 years old to those among the older individuals. In the younger group, 69 percent of pre-treatment infections with the T76 *pfcr* mutation failed chloroquine treatment compared with only 34 percent of such pre-treatment infections in the older group.

One current research focus, says Dr. Djimde, is to understand which immunological events contribute to clearing resistant parasites. Using their new tool, they will compare patients who can clear the resistant parasites with those who cannot, and examine the immunological differences I

these two populations.

"Malaria is the number one killer in Mali and one of the leading causes of death in Africa," says Dr. Djimde. "Our goal in Mali is to do applied research to help our health authorities combat malaria and to ease the burden of the disease on our people."



*Man and mosquito play complementary roles in the malaria cycle.*

#### References:

1. A Djimde et al. A molecular marker for chloroquine-resistant *falciparum* malaria. *New England Journal of Medicine* 344:257-63 (2001).
2. D Warhurst. A molecular marker for chloroquine-resistant *falciparum* malaria. *New England Journal of Medicine* 344:299-301 (2001).
3. DA Fidock et al. Mutations in the *Plasmodium falciparum* digestive vacuole transmembrane protein *PfCRT* and evidence for their role in chloroquine resistance. *Molecular Cell* 6(4):861-71 (2000).

NIAID is a component of the National Institutes of Health (NIH). NIAID supports basic and applied research to prevent, diagnose, and treat infectious and immune-mediated illnesses, including HIV/AIDS and other sexually transmitted diseases, tuberculosis, malaria, autoimmune disorders, asthma and allergies. NIH is an agency of the U.S. Department of Health and Human Services.

# emicro case 1

The patient was a 58-year-old Hispanic male who was admitted through the Emergency Room with complaints of bilateral leg pains. The pain started with his right lower calf. Within hours of onset, the pain increased in intensity and was accompanied with swelling. The patient was also febrile with chills. He denied vomiting or diarrhea.

Fifteen days prior to admission, the patient underwent an upper GI endoscopy which showed duodenitis. Medical history includes Laennec's cirrhosis, gastritis, upper GI bleeding. The patient also drank 3 cases of beer/week and smoked heavily. He was previously treated for cellulitis of the left leg.

Admitting diagnosis: Staphylococcal or streptococcal cellulitis of the leg.

## POINTS TO CONSIDER:

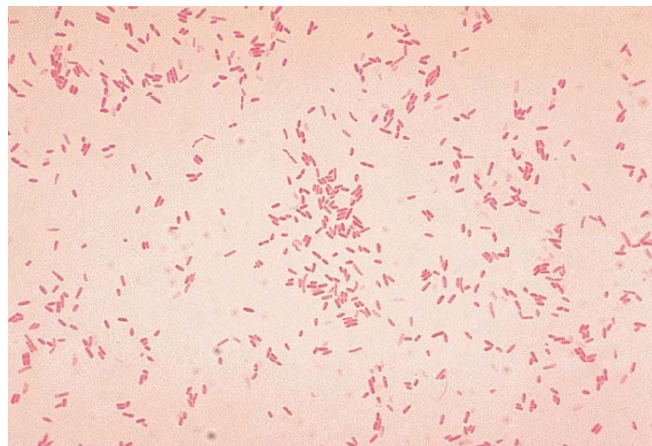
- Possible etiologic agents of necrotizing cellulitis
- Risk factors presented by the patient
- Possible sources of infection
- Procedures for maximum recovery and differential laboratory methods

Urine, blood and exudates from blisters were collected for culture. Initial therapy with oxacillin was started. However, the patient deteriorated rapidly within few hours of his admission. The blisters progressed to his trunk. His therapy was changed from oxacillin to multiple broad spectrum antimicrobials. Evidence of septic shock and DIC were apparent and the patient was placed on mechanical ventilator. He remained unresponsive to therapy and expired within 48 hours from his admission.

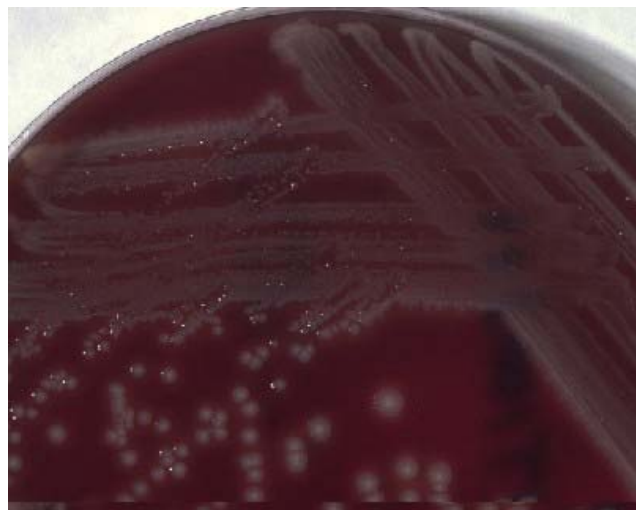
## Laboratory Identification

Urine, blood, and exudates grew oxidase positive Gram negative bacilli, glucose fermenter but non-lactose fermenter on MacConkey. The isolate was motile, produced catalase and indole. The organism produced growth in nutrient broth with 0% NaCl and no growth in 6.5% NaCl.

Case discussion will be posted on the next issue of *Micromorphs*.



Microscopic morphology: Gram stain



Colonial morphology on sheep blood agar.



If you would like to send your responses and comments regarding the case, email

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