

was detected in 9 *P. irritans* fleas (7 male [6 unfed and 1 engorged] and 2 [engorged] female) from 3 houses, including the house where a confirmed human case of plague had occurred (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/20/8/13-0629-Techapp1.pdf>). Eight sequences (GenBank accession nos. KJ361938–KJ361945) were obtained and share 99% nucleotide homology with plasminogen activator genes of *Y. pestis* published in GenBank (accession nos. AF528537, AY305870). No *Y. pestis* was detected in the 24 *S. fonqueniei*, 9 *X. cheopis*, 10 *E. gallinacea*, or 1 *C. canis* fleas collected.

Although only *X. cheopis* and *S. fonqueniei* fleas had previously been described as plague vectors in Madagascar, *P. irritans* fleas were most commonly collected during this field study; engorged and unfed male and female *P. irritans* fleas carried *Y. pestis*. Other studies have found *P. irritans* fleas in the plague risk area in other countries in Africa (5,6); one study found that *P. irritans* fleas may play a role in plague epidemiology in Tanzania (5). Data on *P. irritans* fleas in rats make it unlikely that these fleas are involved in rat-to-human transmission of *Y. pestis* in Madagascar. During 1922–1995, a total of 118,608 rats were caught and examined in Madagascar, but only 148 *P. irritans* fleas were identified, and none have been found on rats since 1996 (<http://www.pasteur.mg/spip.php?rubrique124>). The high density of *P. irritans* fleas we observed in villages where plague outbreaks occurred in late 2012 and early 2013 (<http://www.pasteur.mg/spip.php?rubrique124>) supports the possibility that *P. irritans* fleas played a role in domestic human-to-human transmission of *Y. pestis* during these outbreaks.

**Jocelyn Ratovonjato,  
Minoarisoa Rajerison,  
Soanandrasana Rahelinirina,  
and Sébastien Boyer**

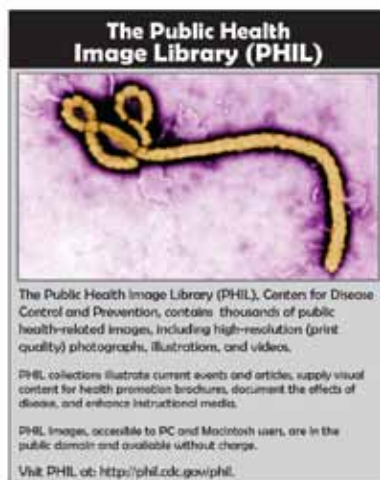
Author affiliations: Institut Pasteur de Madagascar, Antananarivo, Madagascar (J. Ratovonjato, S. Boyer); and World Health Organization Collaborating Centre, Institut Pasteur de Madagascar, Antananarivo (M. Rajerison, S. Rahelinirina)

DOI: <http://dx.doi.org/10.3201/eid2008.130629>

## References

1. Bulter T. Plague and other *Yersinia* infections. In: Greenough WB III, Merigan TC, editors. Current topics in infectious disease. New York: Plenum; 1983. p. 71–92.
2. Brygoo ER. Epidemiology of the plague at Madagascar [in French]. Arch Inst Pasteur Madagascar. 1966;35:7–147.
3. World Health Organization. Report on global surveillance of epidemic-prone infectious diseases—plague [cited 2013 Apr 15]. [http://www.who.int/csr/resources/publications/plague/CSR\\_ISR\\_2000\\_1/en/index5.html](http://www.who.int/csr/resources/publications/plague/CSR_ISR_2000_1/en/index5.html)
4. Hinnebusch J, Schwan TG. New method for plague surveillance using polymerase chain reaction to detect *Yersinia pestis* in fleas. J Clin Microbiol. 1993;31:1511–4.
5. Laudisoit A, Leirs H, Makundi RH, Van Dongen S, Davis S, Neerinx S, et al. Plague and the human flea, Tanzania. Emerg Infect Dis. 2007;13:687–93. <http://dx.doi.org/10.3201/eid1305.061084>
6. Eisen RJ, Gage KL. Transmission of flea-borne zoonotic agents. Annu Rev Entomol. 2012;57:61–82. <http://dx.doi.org/10.1146/annurev-ento-120710-100717>

Address for correspondence: Jocelyn Ratovonjato, Medical Entomology Unit, Institut Pasteur de Madagascar, BP 1274, Antananarivo 101, Madagascar; email: [ratov@pasteur.mg](mailto:ratov@pasteur.mg)



## Serologic Surveillance for West Nile Virus in Dogs, Africa

**To the Editor:** West Nile fever is caused by the West Nile virus (WNV), a mosquito-borne member of the genus *Flavivirus*. Birds are the natural reservoir of the virus, which is maintained in nature in a mosquito–bird–mosquito transmission cycle. WNV has been detected in many regions worldwide, including North America, Europe, Africa, the Near East, and Asia (1). WNV has been shown to cause meningoencephalitis in humans and horses. In the United States, seroconversion in dogs was detected 6 weeks before a human case was reported (2). Thus, dogs could be considered as sentinels for WNV infection, but their role as reservoir is unlikely because of short-term and low levels of viremia (3). In this study, we determined the seroprevalence of WNV in dogs living close to humans in different environments to assess their role as sentinels of this potentially severe zoonosis.

During 2003–2012, blood samples were collected from 753 adult dogs from France and 6 countries in Africa (Table). Samples were centrifuged within 24 h after collection, separated, frozen at  $-20^{\circ}\text{C}$ , and sent to the virology laboratory of the Institut de Recherche Biomédicale des Armées (Marseille, France). Each sample was systematically tested for IgG against WNV by using an in-house ELISA with inactivated WNV as antigen. Serum samples were considered positive if the optical density at 450 nm was  $>3$ -fold the mean of that for negative antigen. Because of the antigenic cross-reactivity among flaviviruses, all positive samples were further tested by Western blot for WNV-specific antibodies (4); seroprevalence was calculated on the basis of Western blot–confirmed cases only.

Table. Prevalence of West Nile virus antibodies in dog populations, France and Africa, 2003–2012

Country and area	No. dogs, N = 753	No. positive for IgG by ELISA	No. results confirmed by Western blot	Prevalence, % (95% CI)
France				
Corsica	35*	3	0	0 (0–10)
Var	25*	3	3	12.0 (2.5–31.2)
Gard	11*	1	1	9.1 (0.2–41.3)
Imported from				
Germany/the Netherlands	9*	0	0	0 (0–33.6)
Hungary	24*	6	3	12.5 (2.7–32.4)
Djibouti	47*	8	6	12.8 (4.8–25.7)
N'Djamena, Chad	50*	13	12	24.0 (13.1–38.2)
	5	5	5	100.0 (47.8–100.0)
Senegal				
Dakar	11*	0	0	0 (0–28.5)
	16†	3	3	18.7 (4.1–45.6)
Siné-Saloum	33	6	1	3.0 (0.1–15.8)
Casamance	81	3	3	3.7 (0.8–10.4)
Abidjan, Côte d'Ivoire	137	7	3	2.2 (0.5–6.3)
Kinshasa, Democratic Republic of the Congo	24	4	3	12.5 (2.7–32.4)
Haut-Ogooué, Gabon	245	0	0	0 (0–1.5)

\*French military working dogs.

†Senegalese gendarmerie working dogs.

For the statistical analysis, we used the exact binomial method to calculate 95% CIs of the proportions and the Fisher exact test to calculate *p* values and compare the seroprevalence rates between countries; significance was set at *p*<0.05.

Seropositive dogs were found in all portions of Africa and France surveyed except northeastern Gabon and Corsica (Table). Seroprevalence of WNV in native dogs was significantly higher in Chad than in the Democratic Republic of the Congo (DRC) (*p*<0.001), Senegal (*p*<0.00001), Côte d'Ivoire (*p*<0.000001), and Gabon (*p*<0.000001). Seroprevalence was low in Kinshasa, DRC (12.5%), and Dakar, Senegal (11.1%), but in N'Djamena, Chad, all 5 native dogs tested had specific antibodies against WNV.

As part of the study, we tested 50 military dogs from France twice, before and after a 4-month mission in Chad; 12 (24.0%) became seropositive after the stay. In addition, 12.5% of military working dogs in France imported from Hungary were seropositive on initial testing. We also found that, in France, dogs are the sentinels of WNV circulation in the Var (12.0%) and Gard (9.1%) departments. All dogs we tested that were positive for

IgG were negative for IgM, a finding that indicates infection by the virus did not occur recently.

The results and the statistical analysis reveal notable differences in the seroprevalence rates, according to the geographic area. N'Djamena, Chad, where all native dogs tested positive for WNV, is located at the confluence of the Chari and Logone Rivers and is an area with high densities of residential and migratory birds. In contrast, the northeastern region (Haut-Ogooué) of Gabon, where no native dogs tested positive for WNV, is an ecosystem of wet forests without migratory birds, unfavorable to virus circulation. In Dakar, 18.7% of native dogs were seropositive. In these central parts of Senegal, characterized by a semi-arid climate and vegetation composed of steppe plants and bluegrass, several WNV strains have been isolated from birds and mosquitoes. The seroprevalence was lower (0%–12.5%) in the sub-Saharan area, including Côte d'Ivoire, Gabon, DRC, and Senegal (Siné-Saloum and Casamance), where the humid or semihumid climate is linked with tropical rain forests or woodland savannah known to favor sedentary birds (5).

In a large proportion of the human and animal population of Africa,

immunity to WNV has developed (1). A serologic survey of dogs from the Highveld region of South Africa showed that 37% (138/377) had neutralizing antibodies against WNV (6). Similarly, seroprevalence of antibodies against WNV is high among dogs in the United States, for example, 55.9% (218/390) in the Gulf Coast region (7). In Turkey, an area where many birds stop over during migration, seroprevalence among dogs was high (37.7%, 43/114) (8).

Our study highlights the role of dogs as sentinels for WNV circulation, particularly in southeastern France (Gard and Var departments), where WNV epidemics and epizootics occurred in 2000 and 2003. In addition, we observed that military working dogs purchased from Hungary, where WNV infection is common (9), may be seropositive. Seroprevalence in dogs returning from short missions in WNV-endemic countries such as Chad was also observed. Therefore, our data emphasize the usefulness and convenience of WNV seroprevalence surveys in dogs for studying WNV epidemiology and circulation. It is possible that dogs living close to humans could attract infected mosquitoes, thereby reducing human infection.

## Acknowledgments

We thank Olivier Bourry, José Gomez-Peñate, and Hubert Bassene for their help during the conduct of field work, Jean-Paul Durand, and all the persons who contributed to the study, especially the French military veterinarians. We thank also the team of the virology laboratory of the Institut de recherche biomédicale des armées (William Daries, Patrick Gravier, and Olivier Merle) for processing the samples.

Financial support was provided in part by the French Defense Medical Service.

**Bernard Davoust,  
Isabelle Leparç-Goffart,  
Jean-Paul Demoncheaux,  
Raphaël Tine,  
Mamadou Diarra,  
Grégory Trombini,  
Oleg Mediannikov,  
and Jean-Lou Marié**

Author affiliations: Groupe de Travail en Épidémiologie Animale du Service de Santé des Armées, Toulon, France (B. Davoust, J.-P. Demoncheaux, G. Trombini, J.L. Marié); Unité de Recherche sur les Maladies Infectieuses et Tropicales Émergentes (IRD 198), Dakar, Senegal (B. Davoust, O. Mediannikov); Centre National de Référence des Arbovirus—Institut de Recherche Biomédicale des Armées, Marseille, France (I. Leparç-Goffart); and Services Vétérinaires de la Gendarmerie Nationale, Dakar (R. Tine, M. Diarra)

DOI: <http://dx.doi.org/10.3201/eid2008.130691>

## References

1. Dauphin G, Zientara S, Zeller H, Murgue B. West Nile: worldwide current situation in animals and humans. *Comp Immunol Microbiol Infect Dis*. 2004;27:343–55. <http://dx.doi.org/10.1016/j.cimid.2004.03.009>
2. Resnick MP, Grunenwald P, Blackmar D, Hailey C, Bueno R, Murray KO. Juvenile dogs as potential sentinels for West Nile virus surveillance. *Zoonoses Public Health*. 2008;55:443–7.
3. Kile JC, Panella NA, Komar N, Chow CC, MacNeil A, Robbins B, et al. Serologic survey of cats and dogs during an epidemic of West Nile virus infection in humans. *J Am Vet Med Assoc*. 2005;226:1349–53. <http://dx.doi.org/10.2460/javma.2005.226.1349>
4. Cabre O, Grandadam M, Marié JL, Gravier P, Prangé A, Santinelli Y, et al. West Nile virus in horses, sub-Saharan Africa. *Emerg Infect Dis*. 2006;12:1958–60. <http://dx.doi.org/10.3201/eid1212.060042>
5. Traore-Lamizana M, Zeller HG, Mondo M. Isolations of West Nile and Bagaza viruses from mosquitoes (Diptera: *Culicidae*) in Center Senegal (Ferlo). *J Med Entomol*. 1994;31:934–8.
6. Blackburn NK, Reyers F, Berry WL, Shepherd AJ. Susceptibility of dogs to West Nile virus: a survey and pathogenicity trial. *J Comp Pathol*. 1989;100:59–66. [http://dx.doi.org/10.1016/0021-9975\(89\)90090-X](http://dx.doi.org/10.1016/0021-9975(89)90090-X)
7. Levy JK, Lappin MR, Glaser AL, Birkenheuer AJ, Anderson TC, Edinboro CH. Prevalence of infectious diseases in cats and dogs rescued following Hurricane Katrina. *J Am Vet Med Assoc*. 2011;238:311–7. <http://dx.doi.org/10.2460/javma.238.3.311>
8. Ozkul A, Yildirim Y, Pinar D, Akcali A, Yilmaz V, Colak D. Serological evidence of West Nile Virus (WNV) in mammalian species in Turkey. *Epidemiol Infect*. 2006;134:826–9. <http://dx.doi.org/10.1017/S0950268805005492>
9. Paz S, Semenza JC. Environmental drivers of West Nile epidemiology in Europe and Western Asia—a review. *Int J Environ Res Public Health*. 2013;10:3543–62. <http://dx.doi.org/10.3390/ijerph10083543>

Address for correspondence: Bernard Davoust, Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE) CNRS UMR 7278 IRD 198 INSERM U1095 Aix-Marseille Université, Faculté de Médecine, 27 bd Jean Moulin, 13385 Marseille CEDEX 5, France; email: [bernard.davoust@gmail.com](mailto:bernard.davoust@gmail.com)

## Another Dimension

EID publishes thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

## Severe Encephalitis Caused by Toscana Virus, Greece

**To the Editor:** In late June 2012, a previously healthy, 49-year-old woman was admitted to the emergency department of Trikala General Hospital in Trikala, Greece, with confusion and delirium. A few hours before admission, she had had a grand mal seizure; she had experienced gastroenteritis with fever (38°C) 5 days earlier. On admission, she was intubated and transferred to the intensive care unit, where she underwent mechanical ventilation and sedation.

The patient was a resident of Genesi village (350 m altitude), 22 km west of Trikala in the Thessaly region. She had not traveled abroad or to other area of Greece. Results of blood and cerebrospinal fluid (CSF) laboratory testing were unremarkable except slight leukocytosis (leukocytes 11,330 cells/mm<sup>3</sup>, 92% neutrophils) and slightly elevated serum lactate dehydrogenase level (240 U/L). Brain imaging showed edema (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/20/8/14-0248-Techapp1.pdf>), which resolved 48 hours after admission. The patient was awakened on day 3 of hospitalization and extubated on day 4. Treatment included anticonvulsants, mannitol, antimicrobial drugs (vancomycin and ceftriaxone), acyclovir, and corticosteroids. The patient fully recovered and was discharged from the hospital on day 12 with short-term antiepileptic medication.

Because West Nile virus (WNV) infections emerged in 2010 in Greece and outbreaks have recurred (1), serum and CSF samples from the patient were sent for testing to the National Reference Centre for Arboviruses. Antibodies against WNV were not detected. Reverse transcription nested PCR was conducted by using generic primers for flaviviruses,