Chikungunya Disease Outbreak, Reunion Island

To the Editor: A serious outbreak of chikungunya disease recently occurred on Reunion Island (population \( \approx 770,000 \)) (1). Between March 1, 2005, and April 30, 2006, \( \approx 255,000 \) cases were reported in this French territory in the Indian Ocean. Most cases occurred after mid-December 2005, with a maximum of 45,000 cases during the week of January 29 to February 4, 2006 (2). Surveillance figures were confirmed by a serosurvey that found a prevalence of 18% of recent infection markers in pregnant women in March 2006.

Chikungunya is a self-limiting febrile viral disease characterized by arthralgia or arthritis. Symptoms may last for several months, but recovery was, until now, considered universal (3). However, in January 2006, the health authorities on this island started receiving death certificates mentioning chikungunya as a cause of death, either direct or indirect. By the end of April, 213 death certificates with this finding had been received. To assess the affect of chikungunya disease, we compared the crude death rate (CDR) observed during the outbreak period with an expected death rate computed from the 2002–2004 historical data.

The study included the period January 1, 2005, through April 30, 2006. The expected number of deaths (all causes) for 2005 and 2006 was the number of deaths by sex and age observed during 2002–2004 modified by an estimation of the population size for 2005–2006. The details of this method, which was used during the heat wave in France in 2003, have been reported (4). The number of deaths in Reunion was obtained daily from 13 of 24 computerized registry offices throughout the island and represented 87% of the deaths on the island.

During 2005, the monthly CDR remained within expected range of statistical variation. From January through April 2006, respectively, monthly CDRs were 7.1%, 34.4%, 25.2%, and 8.3% higher than expected rates (\( p<0.01 \) for February and March). This corresponded to 226 excess deaths reported by the 13 offices participating in the study and 260 excess deaths when data were extrapolated to the entire population of the island (an increase of 18.4%) (Figure). The 260 excess deaths is a crude figure that includes potentially all causes of death. This figure leads to a rough estimate of the case-fatality rate for chikungunya disease of \( \approx 1/1,000 \) cases. Excess deaths were observed mainly in persons \( \geq 75 \) years of age.

CDRs began to exceed the expected range during the last week of January 2006 and remained elevated until the end of the study period. This situation closely matched the kinetics of the epidemic curve of chikungunya disease. CDR is a stable variable in time for a defined population. Only a massive phenomenon can have an effect on it, and no other abnormal health event affected the island at this time. Thus, the outbreak of chikungunya disease was likely responsible for most of the excess deaths observed in Reunion during the first 4 months of 2006.

Deaths associated with chikungunya disease have been rarely reported. This outbreak in Reunion is the first with such a high incidence in a setting where real-time death reporting is a standard procedure. In such settings, CDR monitoring should be considered syndromic surveillance and should be implemented when an abnormal health phenomenon affects large populations.

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References

Figure. Expected and observed number of deaths reported by 13 computerized registry offices in Reunion Island, France, January 2005–April 2006.
A virus subtype H5N1 (mentally and naturally with influenza) ed in persons infected both experi-
raminidase active site has been report-
(Y) at position 274 of the neu-	ution from histidine (H) to tyrosine
caused by a single amino acid substi-	virus infection. Oseltamivir resistance
treatment and prevention of influenza
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Assay to Detect H5N1 Oseltamivir Resistance

To the Editor: Oseltamivir is a
neuraminidase inhibitor approved for
treatment and prevention of influenza
virus infection. Oseltamivir resistance
cau sed by a single amino acid substi-
tion from histidine (H) to tyrosine
(Y) at position 274 of the neur-
aminidase active site has been report-
ed in persons infected both experi-
mentally and naturally with influenza
A virus subtype H5N1 (1,2). Evidence
suggests that using lower doses of
oseltamivir or shorter-than-recommen-
ded treatment periods can con-
tribute to emergence of viral resist-
ance (1,3) Currently, oseltamivir is
being used in several countries that
may be affected by epidemics of
H5N1. Therefore, monitoring for
oseltamivir-resistant strains of H5N1
during oseltamivir administration is
essential for outbreak management
and prevention.

Although real-time PCR or
pyrosequencing is more rapid than
high-throughput assays for mutation
detection (4,5), the conventional PCR
technique can be applied to detect
drug-resistant mutation (6) in areas
lacking real-time PCR or pyrosequ-
encing capabilities. Therefore, to
discriminate between oseltamivir-
sensitive and oseltamivir-resistant
strains, we developed a simple
method, based on PCR, which takes
advantage of the H274Y substitution.
The forward primer was designed
from the conserved region common to
both wild-type and mutant strains; the
reverse primers were designed specifi-
cally for wild-type and mutant
strains, respectively, derived from the
3’ terminal base of each primer. The
primers consisted of a forward primer
N1f (nt 517-534: 5’-GGGCTGTGC
GCTGTATTG-3’) and reverse primer
H274r (nt 759-784: 5’-GGATAA-
CAGGAGCAYTCTCCATAGT
-3’) for wild-type strain detection or
Y274r (nt 759-784: 5’-GGATAACA-
GGAGCAYTCTCATAGTA
-3’) for mutant strain detection. (Note: Under-
lined letters represent differences in
nucleotides between plus vs. minus
primers.) Both strains yielded prod-

ucts of ~267 bp; hence, the assay con-
sisted of 2 separate reactions for
detecting wild-type and mutant
strains, respectively.

For each reaction, 1.0 µL cDNA
was combined with a reaction mixture
that contained 10 µL 2.5× MasterMix
(Eppendorf, Hamburg, Germany), for-
ward and reverse primers at a final
concentration of 0.15 µM, and nucle-
ase-free water to a final volume of 20
µL. Thermocycling conditions com-
pri
definal denaturation at 94°C for
3 min and 35 cycles of amplification
including denaturation (94°C, 30 s),
annealing (65°C, 50 s), extension
(72°C, 45 s), and final extension
(72°C, 7 min). Subsequently, 10 µL of
the amplified products was ana-
lized by using 2% agarose gel
electrophoresis.

To optimize the assay, we per-
formed PCR-based H274Y mutagene-
sis of the N1 fragment of the H5N1
virus (primers on request). The result-
ing mutagenic and wild-type products
were cloned into the pGEM-T Easy
Vectors (Promega, Madison, WI,
USA), confirmed by direct sequenc-

ing, and then used as positive con-
trols. Preliminary results showed that
the wild-type primer was specific for
the oseltamivir-sensitive strain,
whereas the mutant primer can be
used to detect the oseltamivir-resistant
strain exclusively because no signifi-
cant cross-amplification had been
observed.

To establish sensitivity, serial 10-
fold dilutions of the standard N1 plas-
mids (wild-type and mutant) ranging
from 10⁶ to 10¹ copies/µL were used
as a template. The threshold concen-
trations for detection of wild-types
and mutants were 10² copies/µL. To
provide semiquantitative data to
detect subpopulations of the resistant
variants, the 2 control plasmids were
mixed at wild-type:variant ratios of
10⁶:10², 10⁷:10³, 10⁸:10⁴, 10⁹:10⁵, 10⁶:
10², and 10⁵:10³. The result
showed that the density of the expect-
ed bands depended on the amount of
DNA templates (Figure B). However,
the mixing experiments indicated that
the predominant mixtures of wild-
type:resistant variant were 80:20,
which is the lowest ratio of resistant
variants that the assay can reliably
detect (data not shown). To assess
specificity, human DNA and viral
cDNA extracted from other subsotypes
of influenza A virus (N2–N9) were
subjected to this assay. No cross-reac-
tion occurred with human DNA or
other subsotypes of influenza A virus.

We further validated the assay by
testing 3 specimens from hosts treat-
ed with oseltamivir and 17 specimens
from untreated hosts; infection with
H5N1 was detected by using multi-
plex real-time PCR (7). The speci-
nmens from oseltamivir recipients
were isolated from a Vietnamese
patient on the third day after he
received a prophylactic dose (1) and
from 2 tigers (CU-T7 and KU-11) (8). The
specimens from untreated hosts
were isolated from the plasma of an
H5N1-infected human (9) and from
virus-containing allantoic fluid iso-
lated from infected chickens, ducks,