Subclinical Avian Influenza A(H5N1) Virus Infection in Human, Vietnam

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Laboratory-confirmed cases of subclinical infection with avian influenza A(H5N1) virus in humans are rare, and the true number of these cases is unknown. We describe the identification of a laboratory-confirmed subclinical case in a woman during an influenza A(H5N1) contact investigation in northern Vietnam.

In 2012, a debate was published in Science about the number of humans who have experienced subclinical infection with avian influenza A H5 and how this unknown denominator could affect the case-fatality rate reported by the World Health Organization (1,2). The controversy rests, to a large extent, on interpretation of serologic tests used to detect prior H5 infection and the paucity of virologically confirmed subclinical or mild cases. Here we describe a case of subclinical avian influenza A H5 infection, confirmed both virologically and serologically.

The Case

The subclinical case was detected in 2011 during a contact investigation of a 40-year-old man suspected of having influenza A(H5N1) virus infection. The man’s household had sick poultry that were consumed by household members. The chickens roamed close to the sleeping area of the household members. The index case-patient, his daughter, and his daughter-in-law were involved in slaughtering and household members. The index case-patient had fever, cough, dyspnea, and diarrhea that progressed over 2 days, preparing the chickens. The index case-patient had fever, and his daughter-in-law were involved in slaughtering and household members. The index case-patient, his daughter, and his daughter-in-law were involved in slaughtering and household members. The disease progressed, and he died 2 days later.

A throat swab taken from the index case-patient on day 3 of illness was tested by reverse transcription PCR, and results were positive for influenza A(H5N1) virus. Hemagglutination inhibition (HI) and microneutralization (MN) tests for H5N1-specific antibodies were negative in samples taken during the acute phase of illness (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/10/13-0730-Techapp1.pdf).

On day 5 of illness of the index case-patient, a contact investigation was initiated. Throat swab specimens were collected from 4 household members and 1 close contact of the index case-patient: his spouse (age 47 years), daughter (age 18 years), daughter-in-law (age 25 years), and grandson (age 1 year) and an unrelated man (age 43 years). None of the contacts had signs or symptoms. Infection control measures were initiated, and all household members were given oseltamivir (75 mg/d) for 1 week and instructed to seek immediate health care if fever or respiratory symptoms developed. Results of HI testing of serum samples collected during the acute illness phase of the index case-patient were negative.

The human throat swab samples were tested by conventional RT-PCR. The sample from the index case-patient’s daughter, collected 6 days after the woman had slaughtered a chicken, was positive for influenza A/H5 by real-time RT-PCR, and virus was recovered on day 10 of inoculation on MDCK cells (online Technical Appendix). The virus was identified by sequencing as influenza A/H5, clade 2.3.2.1. The woman had no signs or symptoms at the time the throat swab was collected, nor did she report any symptoms to health authorities during the subsequent week. Chickens were also tested, and 4 chickens in the commune tested positive for influenza A(H5N1) virus by RT-PCR of throat and cloacal swab specimens.

Repeat throat swab specimens collected from the 4 household contacts 6 days after the initial collection yielded negative test results for influenza/H5. Serologic testing showed seroconversion only in the woman with subclinical infection; her HI titer increased from <20 to 160 against both clade 2.3.4 and 2.3.2.1 viruses (Table 1). During a second contact investigation 1 month later, 20 other members of the commune were screened by RT-PCR of throat swab specimens and serologic testing. All results were negative for influenza A(H5N1) virus.

The full genome of the identified virus strain (A/CM32/2011) was sequenced (online Technical Appendix) and confirmed to be clade 2.3.2.1 by using the Highly Pathogenic H5N1 Clade Classification Tool (3). The open reading frames of the genes were translated and aligned with all clade 2.3.2.1 sequences available from leading to hospital admission. Despite intensive care and treatment with oseltamivir and antibiotics, the disease progressed, and he died 2 days later.
A(H5N1) virus in ferrets (5) are associated with airborne transmissibility of influenza.

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contains the N170D (4) samples compared with the avian samples. A/CM32/2011

pared with influenza A(H5N1) virus samples from Asia.

tween the clade consensus and A/CM32/2011 were com-
changes specific to human infection, the differences be-

phylogenetic analysis of clade 2.3.2.1 from Vietnam se-
wwwnc.cdc.gov/EID/article/19/10/13-0730-T2.htm). A

no acid changes are summarized in Table 2 (Appendix,
the Influenza Research Database (www.fludb.org). Ami-
no acid changes are preferentially seen in human
samples compared with the avian samples. A/CM32/2011
contains the N170D (4) and T172A mutations in HA that
are associated with airborne transmissibility of influenza
A(H5N1) virus in ferrets (5); these mutations are found in
most avian influenza A(H5N1) clade 2.3.2.1 samples
(Table 2, Appendix). The results indicate that the virus is
a typical influenza A(H5N1) clade 2.3.2.1 virus, with no
remarkable changes.

Conclusions

We report subclinical infection with avian influenza
A(H5N1) virus in a human in Vietnam, confirmed by RT-
PCR, virus isolation from throat swab, and detection of
specific antibodies. A subclinical case was also reported
from Pakistan in 2008 (6). Sequence analysis of the Viet-
am case showed that the infecting virus belonged to influ-

ence A(H5N1) clade 2.3.2.1. This clade was first detected in
poultry in northern Vietnam in early 2010 and replaced
clade 2.3.4 in that area, whereas clade 1 remains predomi-
nant in southern Vietnam, with 4 confirmed cases reported
in early 2012 (7). The recent clade 2.3.2.1 has evolved from
clad e 2.3.2 viruses that has circulated among poultry
in eastern Asia since 2005 and has become predominant in
several Asian countries. Since clade 2.3.2.1 viruses were
initially detected in Vietnam, prevalence has increased in
poultry, but no associated rise in detection of human cases
has been observed (7). Similarly, clade 2.3.2.1 virus has
been circulating in poultry in India, but no human cases
have been reported (8). The HA sequence of this virus is
similar to an influenza A(H5N1) virus detected by RT-
PCR in a 3-year-old patient with influenza-like illness in-
vestigated as part of the National Influenza System Sur-
veillance in 2010 (M.Q. Le, unpub. data). This patient had
mild symptoms and survived, which raises the possibility
that this strain represents a less virulent form of influenza
A(H5N1) in humans.

In our investigation, the case-patient with subclinical
infection was treated with oseltamivir while she was as-
ymptomatic, which may explain why she did not develop
clinical disease. Studies using human volunteers indicate
that seasonal influenza virus shedding may occur ≈24
hours before symptom onset in 25%–30% of patients (9).
Likewise, community cohort studies show presumpt-
omenic shedding and asymptomatic shedding in 15%–20% of
patients infected with seasonal influenza viruses and with
influenza A(H1N1)pdm09 virus (10–12). Oseltamivir is
known to prevent disease when given before inoculation
in human volunteers and to shorten duration and lessen
the severity of illness in natural infection (13), but we found
no evidence in clinical and volunteer studies from the lit-

erature suggesting that oseltamivir may prevent clinical
illness once detectable infection has been established, as
we found in this subclinical case (13,14). The patient we
investigated probably was exposed during slaughtering of
a chicken 6 days before her positive throat swab was col-
lected. However, because chickens in the commune tested
positive at the time of the contact investigation, ongoing
exposure to influenza A(H5N1) cannot be excluded as
the source of infection. Furthermore, the patient may not
have reported symptoms to the health authorities for
personal reasons.

Thus far, evidence of subclinical influenza A(H5N1)
 virus infections has been collected on the basis of sero-
logic testing only (1), but it is unclear whether serologic
testing reliably detects subclinical cases. According to the
World Health Organization, MN titers ≥80 are indicative
of infection but must be confirmed by a second serologic
test because of the possibility of cross-reactivity (7). The
interpretation of results from a single serum sample is lim-
ited by the specificity or sensitivity of serologic tests, and
viral shedding times may mean that infected cases may be
missed. Estimating the incidence of asymptomatic influ-
nenza A(H5N1) virus infection in humans exposed to sick
poultry or human case-patients requires further careful
study using early collection of swab samples and paired
acute and convalescent serum samples.

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References


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