First sequence-confirmed case of infection with the new influenza A(H1N1) strain in Germany

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Here, we report on the first sequence-confirmed case of infection with the new influenza A(H1N1) virus in Germany. Two direct contacts of the patient were laboratory-confirmed as cases and demonstrate a chain of direct human-to-human transmission.

A patient in his 30s was admitted to the department of internal medicine of a district hospital in southern Germany, on 24 April 2009 with influenza symptoms. Two days earlier, he had returned from a vacation in Mexico. He presented with fever up to 40° C, cough and dyspnoea. Headache and myalgia were not present. In addition, this patient had an unrelated, previously undiagnosed chronic disease. He was isolated on the morning of 27 April, and fever and dyspnoea resolved during that day. Since the evening of that day, he has been treated with oseltamivir. Because of his underlying medical condition he was transferred to the University Medical Centre on 28 April, where he has been in stable condition until present, with no further clinical signs of influenza.

Laboratory analysis

On 27 April 2009, the Laboratory of Medical Microbiology and Hygiene at the University of Regensburg Medical Center received a nose and throat swab of the patient for influenza PCR, because an infection with the new influenza A(H1N1) strain was suspected [1,2].

TaqMan-PCR for an 86 bp fragment of the M1 matrix protein gene was performed on the same day and was negative for influenza B, but weakly positive for influenza A (10^2-10^3 copies from 1 ml of swab extraction buffer). The two involved hospitals and health authorities were informed immediately. The primers used (set A, see Table) were part of an in house TaqMan-PCR system designed for conventional influenza A strains.

Sequencing of this PCR product on 28 April showed that 45 bp excluding the primers were identical to the California 04/2009 H1N1 isolate from the current outbreak (GenBank entry FJ966085.1). The 45 bp sequence differed in three nucleotide

Table

Oligonucleotide primers used for amplification and sequencing

A	Influenza A-specific TaqMan PCR system	forward primer (InflA-MA2-1b) 5'-GYT CTC ATG GAR TGG CTA AAG ACA-3'	backward primer (InflA-MA2-2) 5'-GGC ACG GTG AGC GTG AA-3'	TaqMan probe (InflA-MA-So-2b) FAM-5'-ACC AAT CCT GTC ACC TCT GAC TAA GGG GA-3'-TAMRA	
B	Primers targeting M1 gene sequence	outer forward primer (MA-c_1) 5'-ACC GAG GTC GAA ACG TA-3'	outer backward primer (MA-c_2) 5'-CGA TCA AGA ATC CAC AAT ATC-3'	inner forward primer (MA-c_3) 5'-CAG AGA CTT GAA GAT GTC TTT G-3'	inner backward primer (MA-c_4) 5'-TTC TGR TAG GYC TGC AAA TT-3'
C	Primers targeting HA gene sequencing	outer forward primer (H1N1_HA_F1) 5'-CCG CAA ATG CAG ACA CAT TA-3'	outer backward primer (H1N1_HA_R1) 5'-CCC ATT AGA GCA CAT CCA GAA-3'	inner forward primer (H1N1_HA_F2) 5'-TGC GAA CAA TTC AAC AGA CA-3'	inner backward primer (H1N1_HA_R2) 5'-CCC AGG GAG ACT ACC AGT ACC-3'
D	Novel Influenza A(H1N1)-specific TaqMan PCR system [3]	forward primer (H1SWS) 5´-CAT TTG AAA GGT TTG AGA TAT TCC C-3´	backward primer (H1SWAs1) 5´-GGA CAT GCT GCC GTT ACA CC-3´	TaqMan probe (H1SWP) FAM-5`-ACA AGT TCA TGG CCC AAT CAT GAC TCG-3´-BBQ	

TaqMan PCR system A had been designed for conventional influenza A strains. Due to the two distinct nucleotides in the probe region, this assay may slightly underestimate the viral load of the novel influenza A(H1N1) strain. Primer system B had been designed for conventional influenza A strains. It does not exactly fit the novel influenza A(H1N1) strain in several positions, but worked well for sequencing the first German isolate.

positions from conventional human influenza A strains, two of them within the TaqMan probe region. This was considered a strong indication for infection with the new virus.

A larger 600 bp PCR fragment of the matrix protein gene was sequenced on the same evening, using primer set B (see Table). In a BLAST search it was 100% identical over a stretch of 597 nucleotides with the above mentioned California isolate, but differed in at least 5% from annotated human influenza A(H1N1) strains. Conventional porcine influenza H3N2, but also H1N1 strains, were generally more closely related to our sequence than human strains.

Therefore, we considered this isolate as the first proven case of the new influenza A(H1N1) in Germany. Health authorities and physicians were immediately informed. The 597 nucleotide sequence was submitted to GenBank on the same day (FJ970928*).

In parallel, a 1,446 bp fragment of the haemagglutinin gene was amplified and sequenced using primer set C (see Table), and submitted to GenBank (FJ974021) on 30 April. This sequence was identical to two California strains (GenBank FJ969511 and FJ966952) isolated in the current worldwide outbreak, with the exception of only one nucleotide mismatch. In addition a 1,109 bp sequence of the neuraminidase gene of our first isolate has meanwhile been deposited in GenBank (FJ984953) and in the database of the Global Initiative on Sharing Avian Influenza Data (GISAID). It had 100% similarity with the Texas/04 and Texas/05 isolates (FJ981614 and FJ966969).

Contact testing

In the presumed incubation time, the index patient had several contacts of varying closeness and duration before his admission to hospital. Contact tracing and testing through the public health authorities was started immediately. Detailed data on the contacts of the index patient before entering the hospital will be reported by the public health authorities.

Before the patient was isolated on the morning of 27 April under suspicion of new influenza, he had an estimated 19 close contacts among staff at the district hospital and one patient who stayed in the same twin room as the index case in the district hospital.

One of the nurses who had close contact with him has so far tested positive for the new influenza A (H1N1), and had influenza symptoms for a period of two days on 26-27 April. This case received oseltamivir treatment and stayed isolated at home until 5 May when she had tested negative for the new influenza A(H1N1) strain.

All other contacts among the district hospital staff and additional hospital staff who had not had contact with the index patient (a total of 32 people) were tested one or two times and have remained PCR-negative and healthy as of 7 May.

All 32 were tested and offered oseltamivir prophylaxis, but only a minority accepted the treatment. Contacts among the staff at the University Medical Centre remained healthy and were not tested, because the isolation care of the patient was continued without interruption from the beginning.

A sputum sample of the patient sharing the room with the index case was found positive on 29 April in an influenza A-specific TaqMan-PCR assay (set A, see Table). A second TaqMan-PCR assay, specific for the new influenza A(H1N1) strain (primer set D, see Table), was found positive on the same day. The patient was isolated and treated with oseltamivir in the afternoon of 29 April, and health authorities were informed. He suffered only minor influenza-like symptoms.

Both this and the index patient have since tested negative for the new influenza A(H1N1) strain three times and have been released from isolation. Due to additional chronic diseases unrelated to influenza, however, they have not been released from the hospital yet.

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*Authors' correction:

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References

1. Centers for Disease Control and Prevention (CDC). Outbreak of swine-origin influenza A (H1N1) virus infection --- Mexico, March--April 2009. MMWR Morb Mortal Wkly Rep. MMRW 2009; 58(Dispatch):1-3. Available from: www.cdc.gov/mmwr/preview/mmwrhtml/mm58d0430a2.htm
2. Centers for Disease Control and Prevention (CDC). Swine influenza A (H1N1) infection in two children---Southern California, March--April 2009. MMWR Morb Mortal Wkly Rep. 2009;58(15):400-2. Available from: www.cdc.gov/mmwr/preview/mmwrhtml/mm5815a5.htm
3. Schweiger B, Biere B. TaqMan real-time PCR zur Detektion von porcinen Influenza A/H1N1-Viren. [TaqMan real-time PCR for the detection of porcine influenza A/H1N1 viruses. [In German]. Berlin: Robert Koch-Institute; 2009 May 3. Available from: http://www.rki.de/cln_100/nn_200120/DE/Content/InfAZ/I/Influenza/IPV/Schweinegrippe__PCR,templateId=raw,property=publicationFile.pdf/Schweinegrippe_PCR.pdf

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