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研究報告の概要	<p>呼吸器感染は、最も広範なヒトの感染症を構成しており、その相当な割合が未知の病原体によって引き起こされている。レオウイルス (respiratory enteric orphan viruses) は、1950年代初めにヒトから初めて分離され、それまでに知られている如何なる疾患とも関係していなかったことから、そう名付けられた。ここで我々は、マレーシアの Melaka において 39歳の男性患者から分離された、以前には知られていなかったレオウイルス (Melaka ウイルスと名付けられた) について報告するが、患者はウイルス分離の際、高熱と急性呼吸器疾患に苦しんでいた。患者の家族の内の2名が1週間以内に同様の症状を呈し、同じウイルスに感染していることを示す血清学的証拠を有していた。</p> <p>疫学的追跡調査により、その父親が臨床的症状を発現する1週間以内に、その家族がコウモリに曝露されていたことが明らかとなった。遺伝子配列分析によって、1999年にマレーシアの Tioman 島のオオコウモリから分離されたレオウイルスである Pulau ウイルスと Melaka ウイルスの間に、緊密な遺伝子的関係があることが示された。この島のポランディアからの血清のスクリーニングから、109人中14人 (13%) が Pulau と Melaka の両ウイルスに陽性であることが明らかとなった。これは、ヒトの急性呼吸器疾患と関係したオルソレオウイルスについての最初の報告である。Melaka ウイルスは、ヒトに無症候性に感染することが知られている哺乳動物の種々のレオウイルスとは血清学的に関係していない。</p>					<p>使用上の注意記載状況・ その他参考事項等</p> <p>代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。</p> <p>2. 重要な基本的注意</p> <p>(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT (GPT) 値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査 (NAT) を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セフデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理 (ナノフィルトレーション) 及び pH 3.9~4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。</p>
	報告企業の意見				今後の対応	
<p>ヒトの急性呼吸器疾患と関係したオルソレオウイルスについての最初の報告である。Melaka ウイルスはレオウイルス科に属し、エンベロープを有しない、直径 69~84nm の RNA ウイルスである。これまで血漿分画製剤によって Melaka ウイルスが伝播したとの報告はない。原料血漿に Melaka ウイルスは混入し得るのか否か、混入する場合どの程度混入し得るのか等の情報が得られていないため、現時点では安全性評価は困難であり、今後も情報収集に努める。</p>				<p>Melaka ウイルスに関連する情報については、今後も注視することとする。</p>		





# A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans

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Respiratory infections constitute the most widespread human infectious disease, and a substantial proportion of them are caused by unknown etiologic agents. Reoviruses (respiratory enteric orphan viruses) were first isolated from humans in the early 1950s and so named because they were not associated with any known disease. Here, we report a previously unknown reovirus (named "Melaka virus") isolated from a 39-year-old male patient in Melaka, Malaysia, who was suffering from high fever and acute respiratory disease at the time of virus isolation. Two of his family members developed similar symptoms  $\approx$ 1 week later and had serological evidence of infection with the same virus. Epidemiological tracing revealed that the family was exposed to a bat in the house  $\approx$ 1 week before the onset of the father's clinical symptoms. Genome sequence analysis indicated a close genetic relationship between Melaka virus and Pulau virus, a reovirus isolated in 1999 from fruit bats in Tioman Island, Malaysia. Screening of sera collected from human volunteers on the island revealed that 14 of 109 (13%) were positive for both Pulau and Melaka viruses. This is the first report of an orthoreovirus in association with acute human respiratory diseases. Melaka virus is serologically not related to the different types of mammalian reoviruses that were known to infect humans asymptotically. These data indicate that bat-borne reoviruses can be transmitted to and cause clinical diseases in humans.

respiratory infection | zoonosis | human-to-human transmission | orthoreovirus | Pulau virus

Respiratory tract illness (RTI) accounts for a large portion of public health spending worldwide. Although several new etiologic agents of RTI have been identified in the past few decades, a substantial proportion of these illnesses remain undiagnosed. Recently, three previously unknown coronaviruses have been identified as the causative agents for mild to very severe RTI, including the novel coronavirus responsible for the global outbreak of the severe acute respiratory syndrome (SARS) (1–3).

Reoviruses (respiratory enteric orphan viruses) are a large and diverse group of nonenveloped viruses with segmented dsRNA genomes that are taxonomically classified into 10 genera in the family *Reoviridae* (4, 5). Members of the genus *Orthoreovirus* contain 10 genome segments and have been isolated from a broad range of mammalian, avian, and reptilian hosts. Orthoreoviruses are divided into two subgroups, fusogenic and nonfusogenic, based on the ability of the virus to induce cell–cell fusion and syncytium formation (6, 7). The mammalian orthoreoviruses (MRV) are nonfusogenic, whereas the remaining members of the genus are fusogenic, including avian orthoreoviruses, baboon orthoreoviruses, reptilian orthoreoviruses, and Nelson Bay orthoreovirus (NBV). Since the first isolation of MRV from humans in 1951, it has been shown that MRV infection is quite common in the human population (8). How-

ever, although many diseases in animals have been attributed to orthoreovirus infection, from neurological symptoms in baboons and snakes to pneumonia and death in chickens, infections in humans are generally benign with very rare cases of mild upper respiratory tract illness or enteritis in infants and children (7).

Bats, probably the most abundant, diverse, and geographically dispersed vertebrates on earth, have recently been shown to be the reservoir hosts of a variety of zoonotic viruses responsible for severe human disease outbreaks, some with very high mortality (9). In the period from 1994 to 1999, four new viruses in the family *Paramyxoviridae* were discovered, and all appeared to have bats as a reservoir host. Hendra virus emerged in Queensland, Australia, in 1994, killing one human and 14 horses (10), and was responsible for at least four other sporadic outbreaks involving horse and human cases between 1994 and 2006 (11). The closely related Nipah virus (NiV) emerged in 1998–1999 in Peninsular Malaysia, resulting in the death of >100 people and the culling of >1 million pigs (12). Since then, several NiV outbreaks have been recorded in Bangladesh and India (11). Fruit bats in the genus *Pteropus* (flying foxes) are the natural reservoir of both Hendra virus and NiV. NiV is present in fruit bat populations in Indonesia, Thailand, Malaysia, and Cambodia (9). In 1997, another new paramyxovirus, Menangle virus (MenPV), emerged as the cause of a disease outbreak in pigs causing stillbirth and abortion in a commercial piggery near Sydney, Australia (13). Two workers who were exposed to infected pigs developed a flu-like illness with rash and high titers of antibodies to MenPV (14). Seropositive flying foxes were found in a colony near the piggery, although MenPV was not isolated. Two years later, the fourth new paramyxovirus from bats, Tioman virus, was isolated from pteropid bat urine samples from Tioman Island off the east coast of Peninsular Malaysia (15). Tioman virus is related to MenPV, but its disease-causing

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Abbreviations: CPE, cytopathic effect; MDCK, Madin–Darby canine kidney; MeIV, Melaka virus; MRV, mammalian orthoreoviruses; NBV, Nelson Bay orthoreovirus; NiV, Nipah virus; PuIV, Pulau virus; RTI, respiratory tract illness; SARS, severe acute respiratory syndrome; TCID<sub>50</sub>, tissue culture 50% infective dose.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession nos. EF026043–EF026046).

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status in animals and humans remains unknown. During the same period (1994–1999), Australian bat lyssavirus (ABLV) spilled over from bats to humans, resulting in two fatal infections (9, 16). Recently, we and another group independently identified horseshoe bats (genus *Rhinolophus*) as the reservoir host for a group of genetically diverse SARS-like coronaviruses (17, 18), suggesting that the etiological agent responsible for SARS had a bat origin. Although the exact origin of Ebola virus is still unknown, recent serological and molecular studies suggested a potential link between Ebola virus and bats (19).

The first reovirus of bat origin, NBV, was isolated in 1968 from the heart blood of a flying fox (*Pteropus poliocephalus*) in New South Wales, Australia (20). NBV was the first mammalian orthoreovirus to display the fusogenic properties characteristic of avian orthoreoviruses (21). In 1999, during a search for NiV in pteropid bats on Tioman Island, a novel orthoreovirus, Pulau virus (PuIV), was isolated from *Pteropus hypomelanus* (22, 23). Serological and sequence characterization revealed that PuIV was closely related to NBV. It is not known whether these bat orthoreoviruses are capable of infecting and causing disease in animals or humans.

Here, we report the discovery and characterization of Melaka virus (MeIV), the third virus in the NBV species group, and its isolation from a human patient with fever and acute respiratory illness. Our data indicate that not only was the virus capable of human-to-human transmission, but also that its origin was probably bats. Furthermore, serological survey data suggest that the related bat-derived PuIV is also capable of infecting humans, raising the possibility that PuIV might have caused respiratory illness that was undiagnosed or misdiagnosed in the past.

## Results

**Virus Isolation from a Patient with Acute Respiratory Disease.** On March 20, 2006, a 39-year-old male patient (MRA) with a 1-day history of high fever, cough, and sore throat was treated in a government outpatient clinic in the suburb of Melaka, the capital city of the state of Melaka, in Malaysia. His high-grade fever was associated with headache, myalgia, malaise, loss of appetite, generalized body weakness, and severe prostration. There was no associated giddiness, blurring of vision, photophobia, skin eruption, conjunctivitis, or arthritis or related episode of abdominal pain, nausea, vomiting, or diarrhea. The high fever and associated symptoms persisted for 4 days. The patient was treated by different doctors on three occasions during his illness. His cough was initially mild and associated with coryza. Over the subsequent week, his cough worsened progressively with production of yellowish mucoid sputum. However, there was no associated dyspnea, tachypnea, or hemoptysis, although there was one episode of epistaxis. His sore throat, described as severe in nature, persisted for 4 days and was associated with a sensation of tightness in the pharynx and neck, which led to some degree of difficulty in swallowing both liquid and solid food. He continued to feel weak and lethargic for nearly a fortnight after defervescence.

Physical findings at first examination showed MRA to be a Malay man of medium build who appeared unwell and tired. His oral temperature was 39°C and vital signs were stable, with a recorded blood pressure of 120/80 mmHg (1 mmHg = 133 Pa) and pulse rate of 80 beats per min. He was not cyanosed, jaundiced, or in respiratory distress. There was no cutaneous rash or petechiae. His throat displayed injection and hyperemia with slight enlargement of the tonsils but was free of white exudates. Air-entry into his lungs was equal and breath sound was bronchovesicular without other adventitious sounds. The abdominal examination was normal and hepatosplenomegaly was not noted. A mobile, slightly tender cervical lymph node ( $\approx 1 \times 1.5$  cm) was palpable in each anterior triangle of his neck. Other systemic examinations were essentially normal, and a provisional clinical diagnosis of influenza-like illness was made

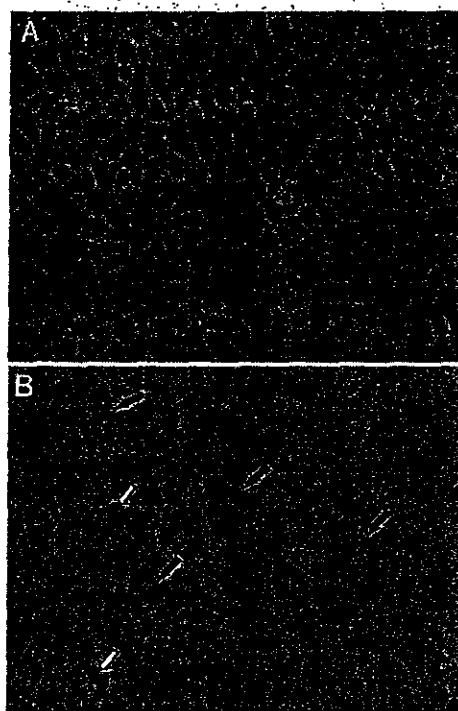


Fig. 1. Syncytia formation in MeIV-infected MDCK cells. (A) Mock-infected. (B) MeIV-infected. Two types of syncytial cells were observed: cells still attached to culture flask surface (filled arrows) and cells detaching from the surface (open arrows).

at first examination. Neither chest x-ray nor venous blood was taken, but a throat swab was collected and transported in viral transport medium to a laboratory for virus isolation.

A virus causing a syncytial cytopathic effect (CPE) in Madin-Darby canine kidney (MDCK) cells (Fig. 1) was isolated after 48 h incubation at 37°C. The virus was named “Melaka virus” (MeIV) after the location of the index case. After two passages in MDCK cells, MeIV replicated and caused syncytial CPE in all types of mammalian cell lines available in the laboratory and also in mosquito-derived C6/36 cells [see supporting information (SI) Table 2].

MeIV-infected cells failed to react to antiserum against most known respiratory viruses, including adenovirus, influenza A and B, parainfluenza 1, 2, and 3, and respiratory syncytial virus. PCR analyses of the original throat swab by using primers against these viruses were also negative (data not shown).

**Morphological and Serological Characterization.** Transmission electron microscopical examination of ultrathin sections from MDCK and Vero cells exhibiting a syncytial CPE and associated negative-stained particles from the supernatant revealed icosahedral viruses resembling those of the family *Reoviridae*, genus *Orthoreovirus*. The particles possessed a mean diameter of  $74.20 \pm 5.02$  nm ( $n = 43$ ) in negative-stained preparations and  $78.86 \pm 5.31$  nm ( $n = 37$ ) for individual viruses in ultrathin sections. The viruses were naked and possessed a double capsid with conspicuous “spikes” or “turrets” situated on the inner core. Within ultrathin sections, viruses were observed in the cytoplasm as individual particles in paracrystalline arrays and associated with semi-electron-dense inclusion bodies (Fig. 2).

The growth characteristics of MeIV and PuIV were similar in a number of cell lines, although subtle differences existed in the degree of syncytial CPE that each virus induced (SI Table 2).

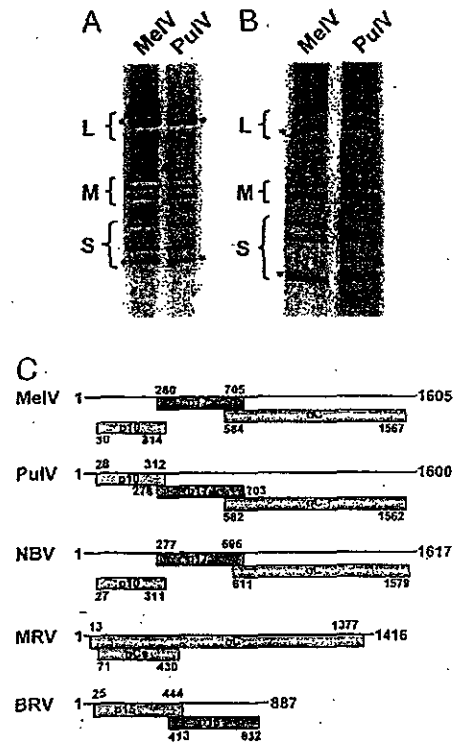
Results obtained from cross-neutralization studies (SI Table 3)



**Fig. 2.** Electron micrographs of MeIV. (A) Negative-stained MeIV. (Scale bar: 200 nm.) (B) Image of an ultrathin section of a MeIV-infected multinucleated Vero cell. N, nucleus; arrows, paracrystalline viral arrays (inclusion bodies not shown). (Scale bar: 5  $\mu$ m.) (C) Higher magnification view of a paracrystalline array. (Scale bar: 200 nm.)

indicated that serum from the patient (MRA) neutralized both MeIV and PuIV with equal efficacy. Similarly, rabbit anti-PuIV serum cross-neutralized MeIV but not any of the three MRV types. Vice versa, none of the three MRV-type sera used in this study was able to neutralize either MeIV or PuIV. All members of the family appeared to have preexisting anti-MRV2 antibodies that showed no correlation with their anti-MeIV antibody status.

**Molecular and Phylogenetic Characterization.** Comparison of genome segments by gel electrophoresis revealed almost identical electropherotypes for MeIV and PuIV (Fig. 3A and B). Sequence analysis of the four small (S) segments indicated a very close genetic relationship between the two viruses. One S class genome segment of orthoreoviruses may be polycistronic, and its coding arrangement is characteristic of a particular species group (24). As shown in Fig. 3C, the S1 segment of MeIV has an almost identical coding arrangement to that of PuIV and NBV. Another genetic marker often used for species differentiation of orthoreoviruses is the highly conserved genome terminal sequences at the 5' end of the positive-sense RNA (7). For MeIV, the consensus sequence is 5'-GCUUwA (w = U or A), which is identical to that of PuIV and NBV (SI Fig. 5). The deduced protein products encoded by the four S segments of the two



**Fig. 3.** Comparison of genome segments and coding strategy of the polycistronic S-class genome segments. (A) Genome segments of MeIV and PuIV separated on a 1% agarose gel. (B) Genome segments of MeIV and PuIV separated on a 10% SDS-polyacrylamide gel. The classes of genome segments (L, large; M, medium; S, small) are labeled on the left, and the asterisks indicate comigrating bands where more than one segment is present. (C) Coding arrangement of the polycistronic S segments of three bat orthoreoviruses in comparison with two other mammalian orthoreovirus species. The line on top represents the RNA genome and the shaded boxes underneath depict protein-coding regions in reading frames 1–3 (top to bottom). Numbers refer to size, in base pairs, of the genome segments and to the first and last nucleotides of the individual ORFs (excluding the stop codons). The names of the encoded proteins are indicated within the shaded boxes.

viruses are very similar in size and share high levels of sequence identity (SI Figs. 6–9).

To establish the evolutionary relationship among MeIV, PuIV, and other known orthoreoviruses, phylogenetic trees were constructed based on deduced amino acid sequences of the major outer capsid protein (Fig. 4A) and the major inner capsid protein (Fig. 4B), respectively. In both phylogenetic trees, MeIV was most closely related to PuIV and was placed in the species group III together with NBV. Similar trees were obtained based on other proteins encoded by the S genome segments (data not shown).

**Epidemiological Investigation.** MRA presently holds the rank of sergeant in the Malaysian Army and works as an accounts clerk in a military base in Melaka. He has not been involved in jungle training or overseas field trips for the last 2 years. However, an unusual event occurred  $\approx$ 1 week before the onset of his fever. On that day, a bat flew through the main door into his living room at  $\approx$ 8 p.m. while he was watching television. The bat flew “frantically” for  $\approx$ 2–3 min in the living room without making any “squeaky” noise or alighting on the ceiling and subsequently managed to fly out through the same door through which it entered.

MRA is married with five children aged 12, 11, 8, 6, and 2, and his wife was 8 months pregnant at the time of his illness. Approximately 6 days after the onset of his fever, his second

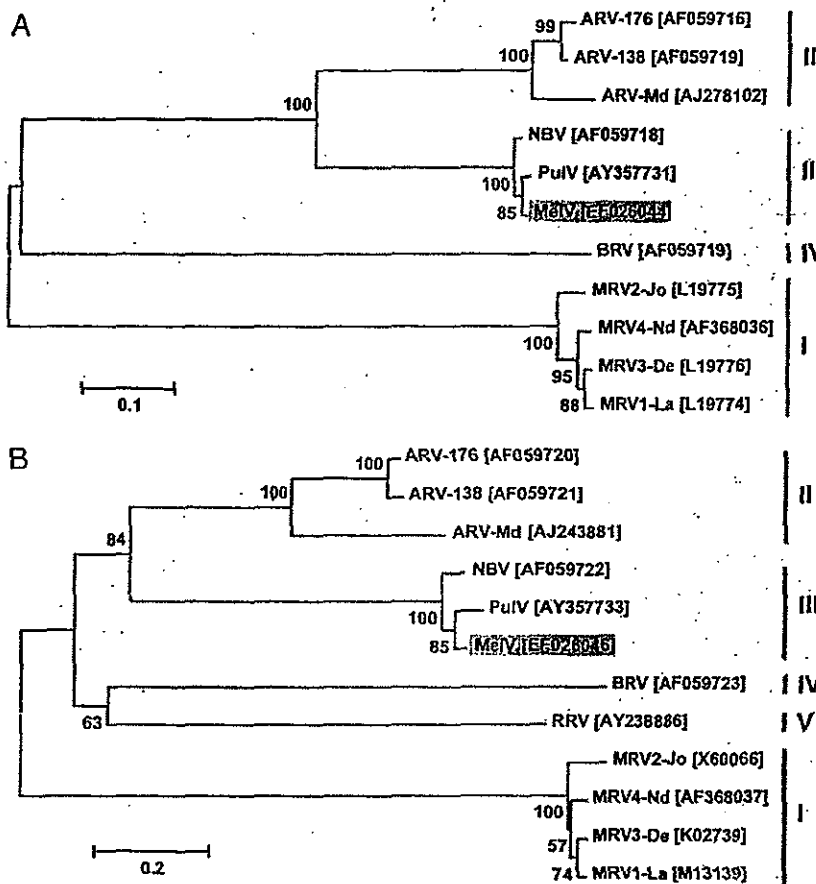


Fig. 4. Phylogenetic trees of orthoreoviruses based on deduced amino acid sequence of the major inner (A) and outer (B) capsid proteins. The GenBank accession number for each sequence is given in bracket next to the abbreviated virus name. ARV, avian reovirus; BRV, baboon reovirus; RRV, reptilian reovirus. Numbers at nodes indicate levels of bootstrap support calculated from 1,000 trees.

child (his 11-year-old daughter) developed a high fever; his fourth child (his 6-year-old son) developed a high fever the next day. Both children were lethargic for 1 day but did not have a cough, sore throat, or skin rashes, although the fourth child had one episode of vomiting. His wife did not experience any illness or subjective feeling of being unwell. She gave birth to a normal, healthy baby girl on April 16, 2006.

MRA and his family live in a single-story linked house in a residential area of 30 houses situated in the suburb of Melaka. The family did not keep any pets or rear any poultry in the house compound, although a neighbor who lived five houses away kept a few parrots.

On follow-up investigation, venous blood samples were taken from MRA on three separate occasions. Venous blood samples were also taken from his family members on the third occasion. The sera from the patient and his family members were tested for the presence of antibodies against MeIV, and the results are summarized in Table 1. Similar results were also obtained when the sera of the different family members were examined by immunogold electron microscopy. Whereas the sera of MRA, his wife, his 11-year-old daughter, and 6-year-old son were able to specifically react to MeIV, the sera from his other sons were negative (data not shown).

**Seroprevalence.** To test the prevalence of MeIV or other closely related reoviruses in the human population, we used a panel of human sera collected in 2001–2002 from volunteers on Tioman Island during serological surveillance for NiV (25). This panel of

sera was chosen because a large number of fruit bats were present on the island and several isolates of PuIV were made from bat urine specimens collected on the island at the time. The sera were tested against both PuIV and MeIV. The data obtained indicate that  $\approx 13\%$  of the 109 sera tested neutralized both viruses. Some sera neutralized both viruses equally well, but several showed a slightly higher neutralizing activity against PuIV than MeIV (see SI Table 4).

#### Discussion

The last decade has experienced a surge in the discovery of emerging viruses of bat origin, several of which have had significant impact on human and animal health, tourism, and trade (26–29). Isolation of MeIV from a human patient and demonstration of human infection by the closely related PuIV serve as the latest examples of this emerging trend. Although we cannot discount the role of modern detection technology in facilitating identification of new viruses, it is believed that ecological changes, which result in greater interaction of human and wildlife animals, are mainly responsible for the increased spillover events observed in recent years (26–29).

A number of viruses recently emerged from bats display a “promiscuous” host range. Such viruses may have coevolved with bats, which are considered to be among the most ancient mammalian species, and use cell-surface receptors that are conserved across a range of animal species. This is certainly the case for henipaviruses, which naturally infect >10 different species in six mammalian taxonomic orders, an unusual obser-

**Table 1. Serological responses of the patient and his family members to MeIV**

Person (age)	First bleed (April 3)	Second bleed (May 12)	Third bleed (June 9)
Immunofluorescence antibody tests titer IgM/IgG			
MRA (39)	1:80/1:640	1:40/1:2,560	1:10/1:2,560
Wife (36)	NA	NA	1:20/1:2,560
Son (12)	NA	NA	-ve/-ve
Daughter (11)	NA	NA	1:10/1:320
Son (8)	NA	NA	-ve/-ve
Son (6)	NA	NA	1:10/1:80
Son (2)	NA	NA	-ve/-ve
Virus neutralization test titer			
MRA (39)	1:80	1:320	1:160
Wife (36)	NA	NA	1:40
Son (12)	NA	NA	-ve
Daughter (11)	NA	NA	1:160
Son (8)	NA	NA	-ve
Son (6)	NA	NA	1:40
Son (2)	NA	NA	-ve

NA, sera not available for testing; -ve, negative.

vation for paramyxoviruses whose host ranges are usually limited (11). Ephrin B2 has been identified as the main receptor for henipaviruses (30, 31), and its wide distribution and status as a member of a conserved family of cell-surface glycoprotein ligands (32) may help to account for the wide host range of henipaviruses *in vivo* and *in vitro*. Similarly, the receptor for the SARS coronavirus, angiotensin-converting enzyme 2 (33), is also conserved among different animals (34), an observation consistent with the finding that SARS coronavirus seems to be able to infect most mammalian species tested under experimental conditions (35). In this regard, it is worth noting that MeIV and PulV are also capable of replicating in a wide range of cell lines, including one derived from mosquitoes (see SI Table 2).

The risk of virus spillover from bats as a result of increasing encroachment by animals and humans into bat habitats is enhanced by the genetic diversity observed among newly emergent bat-borne viruses. For henipaviruses, four to five genetically distinct virus strains have been detected with different virological and biological properties. For example, the NiV Bangladesh strains, but not the NiV Malaysian strains, are capable of human-to-human transmission (11). This is also true for the SARS-like coronaviruses in bats. A limited search among horseshoe bats in China already uncovered at least five different genetic variants (17, 18), and the number of variants is expected to increase dramatically when a systematic survey is conducted across different countries and geographic areas. The same applies to the NBV species group, which has three different members so far. Considering the wide distribution of pteropid bats in India, Southeast Asia, Australia, and the Pacific, one can expect that more bat-borne reoviruses in this group will be discovered in the future. It should be noted that although the epidemiological tracing data and the close genetic relationship of MeIV to PulV and NBV strongly suggest that MeIV originated from bats, at this stage we do not have experimental evidence to prove the bat origin of MeIV and the direct bat-to-human transmission. Field surveillance of bat populations in Melaka will be necessary to resolve this issue.

MeIV is a unique orthoreovirus capable of infecting and causing disease in humans. The infection of multiple members in the same family and the delayed onset (by 1 week) of clinical symptoms of the two children strongly suggest human-to-human transmission. It is not clear to us how and when the wife became infected and why she displayed no clinical symptoms. Fusoge-

nicity is a known important virulence marker for many of the well characterized respiratory viruses, such as respiratory syncytial virus, henipaviruses, influenza, and parainfluenza viruses. Although widely present among human populations, none of the MRVs are fusogenic, an observation highlighting the potential importance of cell-cell fusion for the pathogenesis of orthoreoviruses in humans.

In a recent Foresight ([www.foresight.gov.uk](http://www.foresight.gov.uk)) report entitled "Infectious Diseases: Preparing for the Future," eight categories of infectious diseases were identified for which improved detection systems would make a difference over the next 10–25 years (36). Of the eight categories, MeIV represents categories one, three, and six: new diseases, zoonoses, and acute respiratory infections. With the advent of multiplexed diagnostic technologies both at the nucleic acid and protein levels, classic exclusion diagnosis will eventually be replaced with differential diagnosis. When a severe RTI patient presents at a hospital, it is important not only to exclude SARS or highly pathogenic avian influenza, but also to accurately determine the causative agent so that a targeted treatment regimen can be implemented. In this regard, identification of novel RTI agents will play a key role in shaping the future of RTI diagnosis, and the discovery of MeIV, its relationship with PulV and NBV, and the potential involvement of fusogenic reoviruses as causes of RTI will undoubtedly make a significant contribution to the improvement of RTI diagnosis and treatment.

#### Materials and Methods

**Virus Isolation and Purification.** The throat swab sample in viral transport medium was treated with antibiotics (100,000 units/ml penicillin and 100 µg/ml streptomycin). After 1 h of treatment, the sample was inoculated in duplicates (100 µl and 200 µl, respectively) into freshly confluent monolayers of MDCK (ATCC, CCL-34), Vero (ATCC, CCL-81), and Hep-2 (ATCC, CCL-23) cells cultured in a 24-well cell culture plate. The plate was incubated at 37°C in 5% CO<sub>2</sub> and examined daily for the presence CPE in cultured cells. After two passages in MDCK, the virus (MeIV) was passaged once in Vero cells in the presence of ciprofloxacin (10 µg/ml) to control mycoplasma contamination. The virus was purified by three consecutive limiting dilution passages in Vero cells in the presence of ciprofloxacin. After purification, the virus was passed twice at low multiplicity of infection in Vero cells and harvested 72 h postinfection to generate a working stock for analysis. Stock preparations of MeIV had average titers of 4 × 10<sup>6</sup> TCID<sub>50</sub> (tissue culture 50% infective dose)/ml. PulV was previously plaque-purified (23) and grown in Vero cells to a titer of 1 × 10<sup>6</sup> TCID<sub>50</sub>/ml. Virus preparations were purified for further analysis according to standard protocols by using Dounce homogenization and ultracentrifugation.

**Electron Microscopy.** Vero cells displaying syncytial CPE were processed through to resin blocks and ultrathin sections examined at 120 kV in a Philips CM120 transmission electron microscope (Philips, Eindhoven, The Netherlands). For negative staining, paraformaldehyde-fixed (2%) purified MeIV and stocks of PulV were adsorbed onto carbon-coated parlodion-film nickel grids (5 min), stained with 2% (wt/vol) phosphotungstic acid (pH 6.8) (1 min), and viewed as described above.

**Antibody Tests.** Immunofluorescence antibody tests (IFAT) were conducted as follows. A freshly confluent monolayer of MDCK was inoculated with MeIV at high multiplicity of infection and incubated at 37°C in 5% CO<sub>2</sub>. At full CPE, the infected cells were harvested, washed four times, and suspended in sterile PBS at a cell concentration of 3,000 cells per ml. Ten microliters of the infected cell suspension was carefully spotted onto each well of Teflon-coated slides, followed by air-drying over a warm plate and subsequent fixation in cold acetone for 10 min. Five micro-