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Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome

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Severe acute respiratory syndrome (SARS) is thought to be caused by a novel coronavirus, SARS-associated coronavirus. We studied viral shedding of SARS coronavirus to improve diagnosis and infection control. Reverse-transcriptase PCR was done on 2134 specimens of different types. 355 (45%) specimens of nasopharyngeal aspirates and 150 (28%) of faeces were positive for SARS coronavirus RNA. Positive rates peaked at 6–11 days after onset of illness for nasopharyngeal aspirates (87 of 149 [58%], to 37 of 62 [60%]), and 9–14 days for faeces (15 of 22 [68%], to 26 of 37 [70%]). Overall, peak viral loads were reached at 12–14 days of illness when patients were probably in hospital care, which would explain why hospital workers were prone to infection. Low rate of viral shedding in the first few days of illness meant that early isolation measures would probably be effective.

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A new disease entity known as severe acute respiratory syndrome (SARS) appeared in Guangdong Province, People’s Republic of China, in late 2002, and then in Hong Kong, Vietnam, Singapore, and Canada, in March, 2003.1 A novel coronavirus, SARS-associated coronavirus, was identified as the putative cause.2 This disease proved to be caused by a novel coronavirus, SARS-associated coronavirus, was

| Days after onset | Nasopharyngeal aspirates | Other upper respiratory tract | Faeces | Serum | Urine | Lower respiratory tract |
|-----------------|--------------------------|-------------------------------|--------|-------|-------|------------------------|
|                 | Positive N               | Positive N                   | Positive N | Positive N | Positive N | Positive N | Positive N |
| 0-2             | 66 (35%)                 | 191                           | 32 (30%) | 107   | 3 (13%) | 24               | 4 (19%) | 21   | 0   | 3   | 0   | 1   |
| 3-5             | 140 (45%)                | 310                           | 34 (32%) | 105   | 15 (28%) | 53               | 7 (14%) | 43   | 1 (33%) | 3   | 1 (100%) | 1   |
| 6-8             | 87 (58%)                 | 149                           | 23 (47%) | 54    | 23 (47%) | 49               | 4 (33%) | 12   | 0   | 5   | 11 (92%) | 12  |
| 9-11            | 37 (60%)                 | 62                            | 6 (32%) | 19    | 26 (70%) | 37               | 1 (29%) | 4   | 1 (25%) | 5   | 4 (100%) | 4   |
| 12-14           | 13 (42%)                 | 31                            | 6 (33%) | 12    | 15 (68%) | 22               | 0   | 3   | 8   | 2 (67%) | 3   |
| 15-17           | 9 (39%)                  | 23                            | 4 (26%) | 16    | 13 (54%) | 24               | 1 (33%) | 3   | 0   | 6   | 0   | 0   |
| 18-20           | 1 (13%)                  | 8                             | 6 (35%) | 17    | 10 (39%) | 26               | 0   | 3   | 1 (14%) | 7   | 2 (67%) | 3   |
| 21-23           | 1 (20%)                  | 5                             | 1 (11%) | 9     | 14 (48%) | 29               | 0   | 0   | 2   | 1 (100%) | 1   |
| >23             | 1 (10%)                  | 10                            | 8 (8%)  | 150   | 31 (12%) | 268              | 0   | 0   | 3 (2%) | 159  | 1 (25%) | 4   |
| Total           | 355 (45%)                | 789                           | 116 (24%) | 489   | 150 (28%) | 540               | 20 (23%) | 89   | 6 (3%) | 198  | 22 (78%) | 29  |

n=Number of patients. N=total number of specimens in period. *p<0·0001 for variation in positive rate. †Other upper respiratory tract specimens consisted of throat and nasal swabs (216), throat swabs (164), nasopharyngeal swabs (47), and nasal swabs (62). ‡Lower respiratory tract specimens consisted of bronchoalveolar lavage (3), tracheal aspirates (18), and sputum (8).

Table 1: Variation in RT-PCR positive rates for SARS coronavirus in different specimens with day after onset of illness
nasopharyngeal aspirates, and faeces. Upper respiratory tract specimens, serum, and urine had the lowest positive rates. RT-PCR positive rate also varied with day after onset of disease when the specimen was taken. In nasopharyngeal aspirates, the positive rate in the first 2 days was only about a third, and rose to nearly 60% in 6–11 days, after which it declined. In faeces, the positive rate was fairly low in the first 5 days (up to 28%), but rose gradually to peak at around 70% at 9–14 days, with very high titres (table 2). Positive rates in faeces fell gradually, but remained high even after 23 days; one specimen was positive after 69 days. Results for other upper respiratory tract specimens, serum, and urine mirrored those of nasopharyngeal aspirates, although we received the bulk of specimens within 5 days of onset.

Our results show that the rate of viral shedding is low in the initial few days of illness, but in nasopharyngeal aspirates, faeces, and upper respiratory tract specimens, it rises significantly after 6 days to peak at 12–14 days after onset of disease. This viral load profile had been reported previously.\(^1\)\(^-\)\(^4\) Since patients are unlikely to be highly infectious in the first few days of illness, early isolation measures would probably be effective in prevention of transmission. Maximum viral shedding that was attained after 12–14 days of onset would explain why hospital transmission. Maximum viral shedding that was attained after 12–14 days of onset would explain why hospital measures would probably be effective in prevention of transmission.

Our study also showed that specimens taken in the first few days of illness were less likely to have detectable SARS coronavirus RNA than were those taken at least 6 days after onset. Therefore, we recommend that repeat specimens be taken after 6 days, should the initial specimens on admission be negative.

We showed that the detection rate of SARS coronavirus RNA differed widely between various types of body secretions, and with day of illness. Although lower respiratory tract specimens had the highest positive rate, the sample size was small and there was a risk to health-care workers through aerosol generation. Although nasopharyngeal aspirates are much more sensitive to RT-PCR testing than are other upper respiratory tract specimens, they also carried a risk of aerosol generation. Faecal positive rates also proved sensitive; the low overall rate (28%) was distorted by collection of a large number of specimens after 23 days of illness, to assess whether recovered patients were still secreting virus. The presence of SARS coronavirus RNA in serum meant there was a possibility that the virus could be transmitted by the blood-borne route.

The finding that the viral load in faeces is much higher than that in nasopharyngeal aspirates accords with the hypothesis that faeces may have an important role in the transmission of SARS coronavirus. Continued detection of SARS coronavirus RNA in faeces for long periods raises the possibility that patients who have recovered from SARS are infectious after discharge. Our data would help in the development of an appropriate testing strategy and transmission control measures for SARS.

### Table 2: Variation in geometric mean titre (GMT, copies per μL) for SARS coronavirus with day after onset of illness

| Days after onset | Nasopharyngeal aspirates | Faeces |
|------------------|--------------------------|--------|
|                  | Number of specimens | GMT   | Number of specimens | GMT   |
| 0–2              | 8                        | 7·7    | 0                   | --    |
| 3–5              | 10                       | 9·7    | 4                   | 76·0  |
| 6–8              | 10                       | 15·3   | 4                   | 338·1 |
| 9–11             | 9                        | 4·7    | 3                   | 68389·1 |
| 12–14            | 5                        | 179·4  | 5                   | 89389·1 |
| 15–17            | 5                        | 59·3   | 5                   | 214·0 |
| 18–20            | 0                        | --     | 2                   | 227·5 |
| 21–23            | 0                        | --     | 5                   | 133·0 |
| >23              | 0                        | --     | 6                   | 51·2  |
| **Total**        | 47                       | 13·8   | 34                  | 676·1 |

### Conflict of interest statement

None declared.

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### Genomic imprinting in disruptive spermatogenesis

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The possibility of imprinting disease transmission by assisted reproductive technologies has been raised after births of children with Angelman’s and Beckwith-Wiedemann’s syndromes. To investigate whether imprinting defects were associated with disturbed spermatogenesis, we studied two oppositely imprinted genes in spermatozoan DNA from normozoospermic and oligozoospermic patients. In the mesodermal specific transcript gene (MEST), bisulphite genomic sequencing showed that maternal imprinting was correctly erased in all 123 patients. However, methylation of the H19 gene did not change in any of 27 normozoospermic individuals (0%, 95% CI 0–33%), compared with methylation changes in eight moderate (17%, p<0.026) and 15 severe (30%, 18–45%, p=0.002) oligozoospermic patients. Our data suggest an association between abnormal genomic imprinting and hypospermogenesis, and that spermatozoa from oligozoospermic patients carry a raised risk of transmitting imprinting errors.

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