Research letters

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 highly infectious with respiratory droplets suspected as the main route of transmission.[3] However, faeces were also suggested to have an important role in some outbreaks.[3] Therefore, viral shedding patterns in different body fluids and secretions are important, to know which are the most appropriate specimens for diagnosis and how to institute appropriate infection control measures.

In this prospective cross-sectional study, 2134 specimens were taken from 1041 patients between Feb 24 and July 24, 2003. All patients had clinical and epidemiological features consistent with a diagnosis of SARS as defined by WHO,[4] and were registered as probable SARS cases after thorough examination by the Department of Health, Hong Kong. Patients from 16 public hospitals throughout Hong Kong were examined, of which the largest contributor was Princess Margaret Hospital (367 patients, 35%). 467 (45%) patients were male, and 156 (15%) died. Age distribution was: less than 25 years, 180 patients (17%); 25–44 years, 454 (44%); 45–64 years, 258 (25%); more than 64 years, 149 (14%). 174 (17%) patients were health-care workers, and 250 (24%) were part of the Amoy Garden outbreak.[3]

Reverse-transcriptase PCR (RT-PCR) was undertaken with primer pair COR-1 (5′-CACCGTTTCTACAGGTTA GCTAACGA-3′) and COR-2 (5′-AAATGTTTACGCAG GTAAGCGTAAAA-3′). These primers were directed against the RNA polymerase of SARS coronavirus.[5] Identity of the 311 bp amplicon was confirmed by DNA sequencing or restriction enzyme analysis with *Alu*I, which yielded fragments of 129 bp, 112 bp, 51 bp, and 19 bp. Quantitative RT-PCR (RealArt HPA-Coronavirus LC RT-PCR kit, Artus, Hamburg, Germany) was done on 47 selected nasopharyngeal aspirates and 34 faecal specimens that were positive for SARS coronavirus by RT-PCR. Proportions of positive specimens were compared with χ^2 tests and Epi-Info, version 6.0.

Overall, 669 (31%) specimens were positive by RT-PCR (table 1). RT-PCR positive rates (expressed as percentages) differed significantly between different specimen types ($p<0{\cdot}0001$, in nasopharyngeal aspirates, upper respiratory tract specimens, and faeces). The positive rate was greatest in lower respiratory tract specimens, followed by

	Nasopharyngeal aspirates* (n=615)		Other upper respiratory tract*† (n=368)		Faeces* (n=366)		Serum (n=88)		Urine (n=119)		Lower respiratory tract specimens‡ (n=24)	
	Positive	N	Positive	N	Positive	N	Positive	N	Positive	N	Positive	N
Days after onset												
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 (16%)	43	1 (33%)	3	1 (100%)	1
6–8	87 (58%)	149	21 (39%)	54	23 (47%)	49	4 (33%)	12	0	5	11 (92%)	12
9–11	37 (60%)	62	6 (32%)	19	26 (70%)	37	1 (25%)	4	1 (25%)	5	4 (100%)	4
12–14	13 (42%)	31	4 (33%)	12	15 (68%)	22	0	3	0	8	2 (67%)	3
15–17	9 (39%)	23	4 (25%)	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	0	2	1 (100%)	1
>23	1 (10%)	10	8 (5%)	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 (76%)	29

n=Number of patients. N=total number of specimens in period. *$p<0{\cdot}0001$ 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 bronchioalveolar 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		Faeces	
	Number of specimens	GMT	Number of specimens	GMT
Days after onset				
0–2	8	7·7	0	..
3–5	10	9·7	4	76·0
6–8	10	15·3	4	3338·1
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	2271·5
21–23	0	..	5	133·0
>23	0	..	6	51·2
Total	47	13·8	34	676·1

Table 2: **Variation in geometric mean titre (GMT, copies per μL) for SARS coronavirus 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.[3,5] 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 workers were especially prone to infection, since most patients would be in hospital care at that time. This 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.

Contributors

W W L Lim and P K C Cheng were the lead investigators. W W L Lim initiated, supervised, and coordinated the study, and P K C Cheng designed, set up, and evaluated the PCR assay. D A Wong designed the RFLP assay and wrote the report. S M Ip, A T C Lo, and C S Lau undertook the PCR and helped evaluate the assay. L K L Tong and E Y H Yeung organised and analysed the data.

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 0–13%), compared with methylation changes in eight moderate (17%, 8–31%, p=0·026) and 15 severe (30%, 18–45%, p=0·002) oligozoospermic patients. Our data suggest an association between abnormal genomic imprinting and hypospermatogenesis, and that spermatozoa from oligozoospermic patients carry a raised risk of transmitting imprinting errors.

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