WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

SE/WP/72.11
ENGLISH ONLY

INDEXED

INTER-COUNTRY SEMINAR ON
SURVEILLANCE IN SMALLPOX ERADICATION
New Delhi, 30 October - 2 November 1972

VIRUS EXCRETION IN THE THROAT, URINE AND CONJUNCTIVA OF SMALLPOX CASES

by

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* Supported principally by a Research Grant of the World Health Organization, Geneva

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In earlier days vaccination and revaccination were thought to be enough for the prevention and control of smallpox in any area; special efforts to define the mechanism of the spread of infection were not thought to be essential. But since the World Health Organization began a global programme of smallpox eradication, the number of countries recording smallpox has progressively declined and the prospect of complete eradication of the disease in the near future is fairly bright. This situation has demanded critical study of the various factors responsible for the maintenance and spread of the infection. As a result, many new concepts have emerged. The recent finding of monkeypox virus infecting man (Report 1972), the development of the idea that surveillance is more effective than large-scale vaccination for the control of smallpox (Report 1972), etc., are only a few of many such revelations. The present studies to determine the duration and extent of virus excretion in the throat, urine and conjunctiva from smallpox cases on different days of illness was undertaken as it was thought that the findings might have an important bearing on the epidemiology of the disease.

Materials and methods

All patients in the series were hospitalized at the Infectious Disease Hospital (IDH), Calcutta, and were virologically positive, variola virus having been isolated from the blood of haemorrhagic cases and from vesicular or pustular fluid of non-haemorrhagic cases. A careful history of illness and of past vaccination was obtained. The presence of a vaccination scar was taken as evidence of primary vaccination. No attempt was made to determine the time of primary vaccination or history of revaccination as they were felt to be unreliable. Patients with no vaccination scar were considered to be unvaccinated. Day of illness was calculated from the date of onset of prodromal fever.

To denote the severity of the disease the cases were divided broadly into "haemorrhagic", "confluent" and "discrete" categories; (Sarkar and Mitra, 1967).

HAEMORRHAGIC - patients with early haemorrhage into the skin and mucous membranes. These patients were extremely toxic and the mortality was very high.

CONFLUENT - patients with such plentiful pocks that no healthy skin was visible between the lesions on the face or limbs. Toxicity and mortality were lower than in the haemorrhagic group.

DISCRETE - patients with less plentiful lesions. Healthy skin could be observed between the lesions on the face and limbs. These patients were not toxic and mortality was almost nil.

Roughly, the "fulminating", "malignant confluent" and "malignant semi-confluent" cases of Dixon (1964) correspond to the haemorrhagic cases of the present series, his "benign confluent" and "benign semi-confluent" cases to our confluent group and his "discrete", "mild" and "abortive" cases to a discrete group.

Collection of specimens was started on the day of admission or on the next day. Before swabbing the throat and conjunctiva, the cotton swabs were soaked in Hanks basal salt solution (BSS) containing 0.5 per cent bovine albumin and antibiotics (penicillin and streptomycin). Samples of midstream urine were collected aseptically from male patients only. No specimen was collected from patients having smallpox lesions about the urethal opening.

All specimens were immediately placed in iced containers and brought to the laboratory. The swabs were dipped and squeezed in one millilitre of Hanks BSS and the fluid was preserved at -20°C until inoculation. Egg inoculation and pock counts were made in the standard manner as described previously (Sarkar and Mitra, loc. cit.).

Results

VIRUS IN THE THROAT

Included in the study were 8 "haemorrhagic", 13 "confluent" and 11 "discrete" cases. Throat swabs were positive in all the cases. The titre of virus (pock forming units or pfu per ml of swab-washed fluid) on different days, as well as other data regarding the cases are shown in Table 1. It may be seen that (a) the haemorrhagic and confluent cases had higher titres of virus in their throats than the discrete cases; (b) the confluent cases had more prolonged periods of virus excretion than the discrete cases; (c) amongst the confluent group, the fatal cases had virus in their throats until the time of death; (d) all haemorrhagic cases were unvaccinated and the virus titres were high in all; (e) in the confluent and discrete cases there was no apparent relationship between the virus concentration and vaccination status, age or sex; and (f) the virus titre was highest in all three groups of cases on the third and fourth day and then the concentration gradually diminished until the seventh to ninth day in the discrete cases and the eighth to thirteenth day in the non-fatal confluent cases.

VIRUS IN THE URINE

Of 39 patients studied, 21 (5 haemorrhagic, 18 confluent and 16 discrete) showed virus in their urine, while urine samples of 18 cases (9 confluent and 9 discrete), all collected daily during the same period of illness as that of the virus positive cases, were negative. The titre of virus in the urine of 21 positive cases on different days of illness along with other data are shown in Table 2. It may be seen in Table 2 that (a) the haemorrhagic and confluent cases had higher titres of virus in the urine than the discrete cases, (b) the confluent cases had more prolonged periods of viruria than the discrete cases, and (c) previous vaccination, age and sex of the patients do not seem to have any effect on the virus excretion.

VIRUS IN THE CONJUNCTIVA

Of 16 cases studied, 12 had conjunctivitis. No virus was isolated from the four cases who had no conjunctivitis. Table 3 shows the results of examination of swabs from 12 cases, along with other data about them. From Table 3, it can be seen that (a) haemorrhagic and confluent cases had higher titres of virus, (b) confluent cases had longer periods of virus excretion than the discrete cases, and (c) previous vaccination, age and sex do not seem to be related to virus excretion in the conjunctiva.

Discussion

VIRUS EXCRETION IN THE THROAT

That transmission of infection by droplet nuclei is the most important mode of spread of smallpox is now a recognized fact. It is quite natural to think that the degree of infectivity at any given time will depend on the titre of virus in the throat at that time. Table 1 shows that haemorrhagic and confluent cases having higher virus titres in their throat than the discrete cases are more infectious than the latter. But the haemorrhagic cases, dying earlier, have less chance of spreading the disease than the confluent ones. The epidemiological observation of Rao et al. (1968), that no first generation cases got infected after the 13th day of the disease of the index case, is supported by the virological findings in this work, as none of the cases, except the fatal confluent ones, excreted the virus in the throat beyond the 13th day. However, Rao's observation that the vaccinated cases transmit infection less frequently than the unvaccinated patients is not supported by the findings of the present study. Age and sex of the patients seemed to have no influence on the virus titres in their throats. It is interesting to note that the five confluent cases which were fatal had positive throat swabs almost to the time of their death.

VIRUS EXCRETION IN THE URINE

The duration and degree of viruria have been found to be more in haemorrhagic and confluent cases than in the discrete (Table 2). Confluent cases excreted the virus for a more prolonged period than the discrete ones. The titre of the virus in the urine was highest on the 5th to 6th day of the disease and then deminished gradually. That haemorrhagic cases would excrete virus in their urine as long as they lived was expected because of the long persistence of the state of viraemia (Mitra et al. 1966), but how the non-haemorrhagic cases could continue to excrete virus for long periods cannot be explained. Also, why 18 of 39 cases did not excrete detectable amounts of virus in the urine, although they were comparable with the virus positive cases, cannot be explained. The duration or degree of viruria seemed to be unrelated to the vaccination status, age or sex of the patients.

It may be mentioned here that recently the colleagues of Marennikova[1] have also found virus in the urine of patients convalescing from smallpox.

VIRUS EXCRETION IN THE CONJUNCTIVA

The conjunctival swabs from all 12 patients showing clinical conjunctivitis were positive for virus and the trend of virus titres and the duration of virus excretion in the conjunctiva in general, follows what was found in the throat swabs and urine - that is, a higher virus titre in the haemorrhagic and confluent cases than in the discrete and a more prolonged period of virus excretion in the confluent than in the discrete. As the titre of virus in some was fairly high, it is reasonable to think that the bedding, etc., of the patients could be contaminated from this source. Absence of virus in the conjunctiva of four cases without conjunctivitis indicates that the presence of virus in that part of the body elicits inflammatory change. Kempe et al. (1969) isolated virus from 60 patients who developed conjunctivitis early in the disease and not from 24 who developed the inflammatory process during convalescence.

Conclusions

Smallpox patients excrete virus in the throat and urine for varying periods. Clinically more severe cases (haemorrhagic and confluent) excrete more virus than less severe (discrete) cases and the period of excretion is more in the former two than in the latter. Virus is present in the conjunctiva of those smallpox cases who show conjunctivitis. The duration and titre of virus excretion in the throat, urine, or conjunctiva seem to be unrelated to vaccination status. This does not mean that previous vaccination does not reduce the severity of the disease, but it suggests that if a vaccinated person does get a severe form of smallpox, excretion of virus in his throat, urine and conjunctiva will be like an unvaccinated person developing a similar illness.

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TABLE 1

VIRUS TITRE (pfu/ml) IN THE THROAT SWABS OF SMALLPOX CASES ON DIFFERENT DAYS OF ILLNESS

Serial No.	Type of case	Age	Sex	Primary vaccination	DAYS															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	H	32	M	-			10^5	10^5	10^5	E										
2	H	42	M	-			10^6	10^5		10^2E										
3	H	8	M	-			10^5	10^5	10^4	E										
4	H	27	M	-			10^3	10^3	10^3	10^2	E									
5	H	19	M	-			10^5	10^4	10^4	E										
6	H	31	F	-		10^5	10^5	10^4	10^4	E										
7	H	11	M	-		10^4	10^4	10^3	E											
8	H	17	M	-			10^3	10^3	10^3	E										
9	C	28	M	+				10^5	10^5	10^4	10^3			0	0					
10	C	12	M	-				10^5			10^3	10^3		10^2	10^1		0			
11	C	37	M	-				10^4	10^4		10^2	10^2	10^2	10^2			10^2		0	
12	C	57	M	+				10^5		10^4	10^3		10^3		10^2	10^2		10^1	E	
13	C	21	M	-				10^4	10^4		10^3	10^2			10^2	10^2	10^2	E		
14	C	17	M	+				10^5		10^4	10^4	10^4	10^3		10^3	10^2		10^2	E	
15	C	19	M	+			10^5	10^5		10^4	10^4	10^3	10^2	10^2	10^2			10^2	E	
16	C	9	M	-			10^5			10^4		10^3	10^2			10^2	10^2	10^1E		
17	C	11	M	-				10^4	10^3	10^3	10^3		10^2	10^1			0	0		
18	C	24	F	+					10^3		10^3	10^2	10^2		0	0	0			
19	C	27	F	+					10^5	10^4		10^4	10^3	10^3	10^2		10^2		0	
20	C	17	F	-					10^4	10^3	10^3	10^2		10^1	0	0	0			

H - Haemorrhagic
C - Confluent
0 - Absence of virus

D - Discrete
E - Expired

Table continued on next page

TABLE 1 (continued)

Serial No.	Type of case	Age	Sex	Primary vaccination	DAYS															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
21	D	24	M	+				10^3	10^3	10^2		0	0							
22	D	11	M	+				10^3	10^3	10^2	0	0								
23	D	31	M	+				10^4		10^3	10^3	10^2	10^2		0	0	0			
24	D	12	M	+				10^3		10^2	10^1	0	0							
25	D	47	M	-						10^1	10^1	0								
26	D	35	M	+						10^3	10^2		0	0						
27	D	18	M	+						10^2	10^2		10^1	0						
28	D	62	F	+					10^3	10^3	10^2	0	0							
29	D	37	F	+				10^4	10^3	10^2	10^2	10^2	0	0						
30	D	19	M	-					10^3	10^3	10^2	10^1	0							
31	D	10	M	-				10^3	10^3	10^2	10^2	10^1	0	0						
32	D	12	F	+					10^4	10^3	10^3	10^2	10^2	10^1		0				

H - Haemorrhagic
C - Confluent
O - Absence of virus

D - Discrete
E - Expired

TABLE 2

VIRUS TITRE (pfu/ml) IN THE URINE OF SMALLPOX CASES ON DIFFERENT DAYS OF ILLNESS

Sl. No.	Type of cases	Age	Sex	Primary vaccination	DAYS																			
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	H	32	M	-			10^5	10^5	10^3	E														
2	H	42	M	-				10^3		10^2E														
3	H	8	M	-			10^4	10^4	10^3	E														
4	H	27	M	-			10^3	10^3	10^2	10^2	E													
5	H	19	M	-			10^5	10^4	10^3	E														
6	C	28	M	+					10^3		10^3	10^2	10^2	10^2		10^2			10^2				10^1	0
7	C	12	M	-			10^4		10^4	10^3		10^2	10^2				10^2		10^2		10^2	0	0	
8	C	37	M	-				10^4	10^3		10^2		10^2		10^2		10^2		10^2	0	0			
9	C	57	M	+				10^3	10^3		10^2		10^2		10^2		10^2		E					
10	C	21	M	-					10^4	10^4		10^2		10^2	10^2		10^1	E						
11	C	17	M	+					10^3	10^3	10^2		10^2		10^1		10^1		E					
12	C	19	M	+						10^4		10^2		10^1		10^1			E					
13	C	9	M	-					10^4	10^3	10^3			10^2		10^2			E					
14	C	11	M	-					10^3	10^2	10^1	10^1		0	0									
15	D	24	M	+				10^2	0		0													
16	D	11	M	+				10^2	10^2	0	0													
17	D	31	M	+					10^2	0		0												
18	D	12	M	-						10^2		10^1	10^1		0									
19	D	47	M	+						10^2		0	0											
20	D	35	M	+						10^2	0		0											
21	D	18	M	+					10^2	10^2	0	0												

H - Haemorrhagic C - Confluent D - Discrete E - Expired O - Absence of virus

TABLE 3

VIRUS TITRE (pfu/ml) IN THE CONJUNCTIVAL SWAB OF SMALLPOX ON DIFFERENT DAYS OF ILLNESS

Sl. No.	Type of cases	Age	Sex	Primary vaccination	DAYS														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	H	32	M	-			10^5		10^3	E									
2	H	42	M	-				10^4	10^2	10^2E									
3	H	8	M	-			10^5	10^3	10^2	E									
4	C	28	M	+					10^5		10^4		10^3	10^2		0	0		
5	C	12	F	-						10^4		10^3	10^3		0	0	0		
6	C	37	M	-					10^5		10^4		10^3		10^1	0	0		
7	C	57	F	+						10^4	10^4		10^3	0	0	0	0		E
8	C	21	M	-					10^5	10^5	10^4		10^3	0	0	0	0	E	
9	C	17	M	+						10^4		0	0	0	0	0	0		E
10	D	24	M	+				10^4		0	0		0	0		0			
11	D	11	M	+					10^3	10^2	0		0		0				
12	D	31	M	+						10^3	10^2	0	0		0				

H - Haemorrhagic
C - Confluent
0 - Absence of virus
D - Discrete
E - Expired