

## Clinical and subclinical variola minor in a ward outbreak

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### INTRODUCTION

Missing links in the chain of variola transmission are usually ascribed to unrecognized instances of clinically manifest infection or, less frequently, to instances of subclinical infection. Published virological studies of variola epidemics do not include a systematic search for subclinical infections, and in only two reports are isolated instances recorded. Verlinde & van Tongeren (1952) reported isolation of variola virus from the throat of a healthy contact of a patient with overt variola, a finding questioned by Dixon (1962) because of the long interval after contact. Rodrigues-da-Silva, Rabello & Angulo (1963) found significant complement-fixation titres in six asymptomatic contacts of housemates or schoolmates with overt variola minor. The present paper describes an outbreak of variola minor occurring in a hospital ward and, particularly, a serological survey aimed at disclosing subclinical variola.

### MATERIALS AND METHODS

#### *The locale and population*

The outbreak occurred in the 4th General Medical Infirmary of the Santa Casa de Misericórdia, a charity hospital of the city of São Paulo, in 1959. The infirmary occupied the right wing of a floor, in one of the six buildings composing the hospital. All beds were located in a single, large ward and the infirmary also comprised a first-aid room, a dining room, a waiting room for visitors and two sets of water closet and bathroom (Fig. 1). Corridors and stairways connected the infirmary with the rest of the floor and with other floors from the building.

On admission of the introducer of infection, there were thirty-four patients, all adult males, in the ward. Two deaths, one discharge and five admissions occurred during the outbreak. The patient occupying bed twenty-one on admission of the introducer died on 29 July 1959, this bed being soon occupied by one of the admissions. These two patients will be referred to as 'patient 21A' and 'patient 21B' respectively. The same nomenclature is applied to the patients successively occupying bed 33, the first of which, 'patient 33A', was discharged on 11 August, the second, 'patient 33B', being admitted on 12 August. The third admission occupied bed 23 which was empty on admission of the introducer. The fourth admission occupied bed 36 for a short interval (see below) while the fifth admission did not occupy a bed but slept on a mattress placed on the floor every night and removed the next morning. This mattress is represented as bed 0 in Fig. 1, the

patient being referred to correspondingly in the text. The patient from bed 17, who was suffering from Chagas disease with heart involvement, died on 2 August.

Three physicians and five male nurses took care of the patients and there was an orderly for the infirmary. While several patients were bedridden, others moved around through the infirmary and sporadically visited other infirmaries from the same or other buildings. Seven patients were suffering from allergic dermatitis, seven patients exhibited leishmaniasis, four patients had leg ulcers, three patients showed blastomycosis and other patients were suffering from liver cirrhosis, nephrosis, nephritis, Chagas disease, verminosis, etc. Only a male nurse had previously suffered from variola in the study population group.

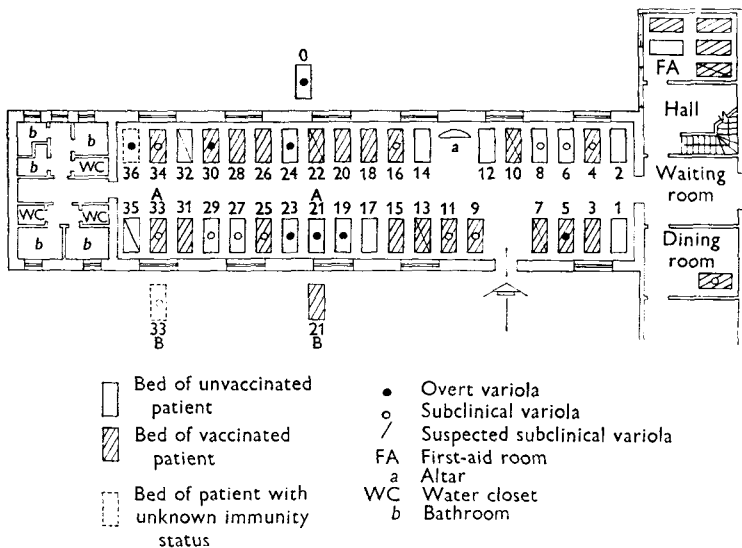


Fig. 1. Spatial relations among individual infections.

### *Method of investigating the outbreak*

When the occurrence of variola was recognized (see below), a case-finding survey was conducted in retrospect and all inmates and staff members were examined and questioned about occurrence of skin or constitutional manifestations attributable to variolous rashes or *variola sine eruptione* during the interval after admission of the introducer. Demographic characteristics were also recorded and more clinical examinations and questioning were later made at variable intervals in all patients and staff members. Cases of overt variola were identified through the classical clinical criteria, length of interval between illness onset in the infecting and infected cases, antibody titrations and, in three cases, by virus isolation from skin pocks. The previous immunity status was determined through inspection of the usual vaccination sites and careful questioning. Lack of co-operation made it impossible, in a few instances, to obtain some data.

An environmental survey was made, aimed at disclosing the spatial relations among cases and contacts and, particularly, the personal associations which might have influenced spread of infection. Since the population of a ward offers peculiar

facilities for disclosing subclinical infections, a serological survey was also conducted. Patients and staff members were bled on 3, 12 and 28 August that is, 28, 37 and 53 days after introduction of infection in the ward. The earliest bleeding was made on the day preceding a mass vaccination of the ward population. Two patients were not bled and several donors were bled only once or twice because of death, discharge, admission during the outbreak or temporary absence. No blood could be obtained from the attending physicians because of their reluctance.

Blood obtained by venipuncture was kept overnight at 4° C.; the serum was then separated and stored at -25° C. Before testing, sera were inactivated at 56° C. for 30 min. Complement-fixation (CF) and haemagglutination-inhibition (HI) tests were conducted on each serum following the techniques of McCarthy & Downie (1953). The Instituto Butantan strain of vaccinia virus was employed in the preparation of antigens and hyperimmune rabbit serum. In some tests, serum from a donor with virologically confirmed, clinically typical generalized vaccinia was employed as a positive control, while, in other tests, hyperimmune rabbit serum was used. All sera from a given donor were simultaneously tested by one of the tests. The lowest serum dilution employed in the CF was 1/5, while in the HI it was 1/10. Titres are expressed in terms of serum dilution before addition of the CF antigen or the haemagglutinin.

From each of three patients with a presumptive variolous eruption, a specimen was collected for virus isolation. The specimen consisted of liquid content from several pocks aspirated with a capillary pipette as well as of pieces excised with forceps from the roof of these or other pocks. This material was placed in a tube containing 0.5 ml of broth and then stored at -25° C. until testing, when the solid material was ground and penicillin and streptomycin were added to the specimen for preventing bacterial growth. Embryonated hen eggs were inoculated on the chorioallantois by the usual technique. Besides serial passaging on the chorioallantois, the strains were inoculated on rabbit skin by scarification. One of the strains was subjected to a neutralization test according to McCarthy & Downie (1953) using serum from a rabbit hyperimmunized with the Instituto Butantan strain of vaccinia virus, as well as to the test devised by Helbert (1957) for differentiation between variola minor strains and variola major strains after previous titration on the chorioallantois. Each egg from a series of ten was inoculated with 10,000 pock-forming doses, while each egg from another series of ten was inoculated with 100,000 pock-forming doses and embryo mortality was determined at daily intervals until the 7th day after inoculation.

## RESULTS

### *Course of the outbreak*

On 6 July 1959, an 18-year-old man was admitted to the ward because of generalized leishmaniasis and occupied bed 24 (Fig. 1). He also had a pock eruption the identity of which was not suspected mainly because attention was focused on mutilating lesions of leishmaniasis frankly predominating in the clinical picture. The pock eruption started 5 days before admission, after a pre-

eruptive illness lasting 2 days. The patient had never been vaccinated against variola and had not suffered from this disease. Suspicion of variola arose 23 days after his admission, when a pathologist found umbilicated pustules on the face and extremities of patient 21 A who had just died. By the time variola virus was isolated and identified in the material collected from the cadaver pustules, most

Table 1. *Characteristics of cases of overt variola*

Bed no.	Age	Immunity status	Laboratory findings	Date of illness onset (1959)	Behaviour during the outbreak
24	18	No scar	High CF titre. Rather high HI titre	29 June	Introducer of infection
30	51	37-year-old scar	Very high CF titre	18 July	Secondary case (first generation)
19	25	No scar	Fairly high HI titre	18 July	Secondary case (first generation)
5	15	10-year-old scar	Very high CF titre. High HI titre	19 July	Secondary case (first generation)
21-A	68	No scar	Virus isolation	20 July	Secondary case (first generation)
23	28	No scar	Virus isolation. Eightfold HI titre increase	1 August	Secondary case (second generation)
36	?	?	—	2 August	Secondary case (second generation)
0	?	No scar	Virus isolation. Fourfold HI titre increase	22 August	Secondary case (third generation)

Scar means vaccination scar; no patient had previously suffered from variola.

Admission of the introducer was made on 6 July 1959, when he was in the 8th day of illness. The date of illness onset in the patient from bed 36 is approximate.

? the pertinent information is missing.

pocks on the introducer (bed 24) were in the crust stage. Six secondary cases had already appeared and these and the introducer were soon identified as cases of variola. The introducer had been moving around through the infirmary and had even gone to another building to be photographed.

Visits of friends and relatives were forbidden and a mass vaccination of the ward population was conducted on 4 August. The patients with generalized allergic dermatitis were spared and twenty-three patients and one staff member were vaccinated, but this was only successful in the patients from beds 7, 11 and 12. No current or terminal disinfection of the ward was carried out nor were patients with variola placed in an isolation room.

A case occurred in a Japanese who did not belong to the infirmary but who was allowed to sleep on bed 36 for two nights on about 19 and 20 July, returning to his

original infirmary to leave for an unknown address soon after the appearance of the variolous eruption. The appearance of this eruption was reported to the authors by the staff of the other infirmary, in which no other case appeared. A total of eight cases of overt variola was recorded and some characteristics of these cases appear in Table 1. The seven cases secondary to that of the introducer were seemingly grouped in three generations and the last case started on 22 August. No case of overt variola occurred among staff members or visitors nor in the adjoining infirmary or elsewhere in the hospital.

Clinical findings were typical of variola minor and did not include any unusual characteristics. All cases showed a benign eruption consisting of discrete pocks, after intense systemic manifestations lasting the usual time. The only fatal outcome could not reasonably be attributed to variola, since the eruption in this case did not include haemorrhages or other manifestations of malignant character and was not even confluent. Death might be attributed to pneumonia complicating the generalized cysticercosis with right hemiplegia from which this 68-year-old patient 21 A was suffering. No post-mortem examination could be made because of refusal of relatives.

#### *Identification of the outbreak*

All three specimens of pock material yielded variola virus. The virus was identified by: (a) the macroscopic morphology of chorioallantoic lesions on isolation and the maintenance of this morphology in three serial passages; (b) histological examination of inoculated membranes; (c) frequent embryo survival and absence of membrane haemorrhages; (d) absence of definite lesions on rabbit skin inoculated through scarification; (e) significant neutralization (more than 50%) of the virus from the patient occupying bed 23 by antiserum prepared in the rabbit; (f) Results typical of variola minor strains in Helbert's test conducted on the same strain.

All cases of clinically manifest infection were identified by typical clinical and epidemiological findings and, particularly, by aetiological (laboratory) data from all but one case (Table 1). The pock eruption in the introducer (patient from bed 24) was identified by: (a) A 1/160 CF titre and a 1/160 HI titre in the absence of previous variola or successful vaccination; (b) a fourfold decrease of the CF titre and a twofold decrease of the HI titre within 9 days after the first bleeding; (c) a typical pre-eruptive illness followed by an eruption of morphology, distribution and course typical of variola minor; (d) occurrence of four laboratory-confirmed cases of variolous eruption within 2 days and all appearing 12–14 days after the admission of the introducer (Table 1).

One case from each of the three generations of secondary cases was identified by virus isolation from pocks (Table 1). In the patients from beds 23 and 0 a significant (fourfold or higher) titre increase of the HI antibody was also found. A secondary case occurring in the patient from bed 19 was identified through a 1/320 HI titre falling to 1/40 within 9 days, in the absence of previous variola and successful vaccination. The two secondary cases occurring in the patients from beds 5 and 30 had very high CF titres of 1/640 and 1/320 falling within

25 days to 1/160 and  $< 1/5$  respectively. This rapid fall adds to the significance of the early titres. Only the case occurring in the patient from bed 36 was not identified through virological data for reasons already mentioned.

Table 2. *Serological evidence of subclinical variola*

Patient's bed no.	Complement fixation	Haemagglutination inhibition	Immunity status
8	$< 1/5$ $< 1/5$ $< 1/5$	$< 1/10$ $< 1/10$ 1/20	No scar
33-B	N.D. $< 1/5$ $< 1/5$	N.D. $< 1/10$ 1/160	Unrecorded
Ward orderly	1/40 1/160 1/20	1/160 1/640 1/160	6-year-old scar
6	1/40 1/40 1/20	1/160 1/160 1/80	No scar
29	1/10 1/10 1/5	$< 1/10$ $< 1/10$ $< 1/10$	No scar
34	1/320 1/640 N.D.	1/160 1/80 N.D.	32-year-old scar
16	1/320 1/160 1/40	1/320 1/640 1/640	39-year-old scar
4	1/160 1/80 N.D.	1/640 1/640 N.D.	30-year-old scar
11	1/80 1/40 1/20	N.D. 1/20 1/20	20-year-old scar
25	1/40 1/40 N.D.	1/80 1/80 N.D.	26-year-old scar
9	1/80 1/80 N.D.	1/20 1/10 N.D.	15-year-old scar
27	$< 1/5$ $< 1/5$ N.D.	1/160 1/80 N.D.	No scar
33-A	$< 1/5$ N.D. N.D.	1/320 N.D. N.D.	35-year-old scar

Patient 33A was bled only on 3 August, while patient 33B was bled on 12 and 28 August. Each of the patients from beds 4, 9, 25, 27 and 34 were bled only on 3 and 12 August. The remaining patients were bled on 3, 12 and 28 August.

N.D. Not done because no serum was available.

Scar. Vaccination scar. No patient had previously suffered from variola.



*Subclinical variola*

Table 2 presents serological evidence of current infection in donors without clinical manifestations of variola according to the following criteria: (a) a fourfold or greater increase of either CF or HI titre; (b) a 1/10 or higher CF titre in a donor without previous variola whose last successful vaccination occurred 15 or more years before or had not occurred at all; and, (c) a fairly high (1/160 or higher) HI titre in a donor without previous variola and whose last successful vaccination had occurred 35 years before or had not occurred at all. In some published reports, titres are expressed in terms of the final serum dilution, after addition of the antigen, and this makes them higher than the corresponding titres presented here. It should be emphasized that, in this outbreak, the first and second mass bleedings were made after onset of six of the seven secondary cases while the third bleeding was made 6 days after onset of the last case.

A fourfold or greater rise of titre was observed in two patients and one staff member (Table 2). The patient from bed 8 had no vaccination scar or previous variola and showed a fourfold increase of the HI titre. Patient 33B, whose immunity status was not found in the records, was admitted on 12 August and bled for the first time on the same day. This serum was negative in both CF and HI tests, while the serum collected on 28 August exhibited a 1/160 HI titre and negative CF titre. The ward orderly, who took care of patients' meals and clothing, showed a four-fold increase of both CF and HI titres. These titres fell rapidly during the next 16 days. This woman had been successfully vaccinated for the first and only time 6 years before the outbreak. Moreover, the CF was negative in all other (five) staff members bled and the same applies to the HI, except for a 1/80 titre found in the only serum obtained from a male nurse.

Eight patients did not show a rise of titre but showed significant (1/10 or higher) CF titres (Table 2). The patient from bed 29 twice showed a 1/10 CF titre which declined to 1/5 in the third serum, while the patient from bed 6 showed higher CF and HI titres in his three sera and these also declined in the third serum. These two patients had had no previous variola or successful vaccination. The remaining six patients had not suffered from variola but had successful vaccination 15–39 years before and this would not justify the CF titres observed. In each of these six patients, the CF titre was significant in all sera and most titres were high and usually declined in the last serum, a finding also made in patients with overt variola. Moreover significant, at times high, HI titres supported the CF titres observed in these patients.

The patient from bed 27 had not suffered from variola or successful vaccination and showed a rather high HI titre in the first serum, with a lower titre in the second serum (Table 2). Patient 33A showed a fairly high HI titre which cannot be ascribed to a previous successful vaccination occurring 35 years before, especially since negative or low HI titres were found in several patients with more recent successful vaccination.

Inconclusive serological evidence of subclinical variola was found in some persons not included in Table 2. The patients from beds 10 and 32 both showed HI titres

of  $< 1/10$  on 3 August and 12 August and both showed a rise to  $1/10$  on 28 August. The two negative results form a control for the titratable but low titre of the third serum from each patient, but no four-fold increase was observed in either of these two patients, one of whom (patient from bed 32) had had no previous variola or successful vaccination. The patient from bed 13 showed a fairly high ( $1/320$ ) HI titre on 3 August which fell to  $1/80$  by 12 August. This rapid decline within 9 days and the fairly high titre in the early serum strongly suggest that current infection had occurred in this patient who had been revaccinated 8 years before. The patient from bed 22, who occupied a bed adjacent to that of the introducer of infection, and who had not suffered from variola, showed a  $1/80$  HI titre in all three sera. This finding can hardly be attributed to previous successful vaccination 16 years earlier. The patient from bed 35 had no vaccination scar nor previous variola and showed a  $1/5$  CF titre in the early serum. This is significant according to Downie & McCarthy (1958), although it is not so according to the last criterion of Downie (1959). The  $1/5$  CF titre became negative in the serum collected 9 days later from this patient, and the HI titre also declined from  $1/20$  in the first serum to  $1/10$  in the second serum. A male nurse showed a  $1/80$  HI titre in the only serum obtained from him and he had had variola and successful vaccination more than 20 years before, while three other male nurses successfully vaccinated 6, 6 and 7 years before, respectively, showed negative HI and CF titres in all their sera.

In addition to this inconclusive evidence of subclinical variola, there was a significant increase of the CF titre in the sera from the patient occupying bed 7 and this increase might perhaps be attributed to subclinical variola instead of to a successful vaccination on the day (4 August) following the first bleeding because: (a) this patient occupied a bed adjacent to bed 5, of a patient with overt variola in the first generation of secondary cases; (b) vaccination did not provoke CF antibody response in the remaining two patients successfully vaccinated on 4 August; (c) vaccination does not provoke CF antibody response (Sindo & Nisimura, 1940; Herrlich, Mayr & Munz, 1956); and, (d) significant titre increases were observed in the ward orderly (Table 2) who was not vaccinated during the outbreak nor showed clinical manifestations of variola.

#### DISCUSSION

The identity of the disease was clearly established by clinical and epidemiological findings and, particularly, by virus isolation or antibody titrations in seven of the eight persons with overt variola. Instances of subclinical variola were deduced from serological evidence according to three criteria, the first of which, a significant titre increase, is the classical criterion and has general acceptance, while the remaining criteria are based upon known peculiarities of the persistence of variolous and vaccinal CF and HI antibodies. With an unconfirmed exception, reports of CF antibody response either show the persistence of this antibody for only a few months after successful vaccination or variola (Andres *et al.* 1958; McCarthy, Downie & Bradley, 1958; Downie & McCarthy, 1958), or the non-



appearance of CF antibody after successful vaccination (Sindo & Nisimura, 1940; Herrlich, Mayr & Munz, 1956; McCarthy *et al.* 1958) or after variola minor (de Jongh, 1956; Herrlich, Mayr & Mahnel, 1959). The HI antibody does not always appear after successful vaccination (McCarthy *et al.* 1958) but, when present, it persists longer than the CF antibody, although after 1 year its titre is low or negative (Herrlich *et al.* 1956; McCarthy *et al.* 1958). The latter finding has also been made in patients who had overt variola (Downie & McCarthy, 1958; Herrlich, Mayr & Mahnel, 1959). Unpublished work in this laboratory disclosed negative CF in a rather large proportion of patients suffering from variola minor or successful vaccination, as well as a rapid and marked decline of the CF titre and a definite decline of the HI titre within a few months after variola minor, generalized vaccinia or successful vaccination.

It is remarkable that at least four cases of subclinical variola occurred in fully susceptible individuals, that is, in persons without previous variola or successful vaccination. Moreover, three of these patients had not even suffered from varicella, a disease which might be confused with variola minor, and one of these three showed a significant antibody titre increase. In addition, the fully susceptible patients from beds 32 and 35 showed very suggestive though inconclusive evidence of subclinical variola.

Regarding the finding of subclinical variola in this outbreak, it should be remembered that recent work has established the actual occurrence of subclinical infections by the viruses of mumps, equine encephalomyelites, Japanese encephalitis, poliomyelitis, Coxsackie, etc. Moreover, virological and epidemiological studies disclosed that subclinical infections are the explanation for the apparently low infectiousness of mumps in spite of infection by mumps virus being highly communicable, affecting most persons in a manner essentially similar to that of measles virus (Meyer, 1962).

#### SUMMARY

A variola minor outbreak in a 36-bed hospital-ward comprised seven cases of overt variola after the first case. Clinical and epidemiological findings were typical, seven of the eight cases of overt variola being confirmed by virus isolation or antibody titrations. In addition, thirteen definite and seven possible instances of subclinical variola were deduced from complement-fixation or haemagglutination-inhibition tests. A discussion is made of the validity of serological criteria of variolous and vaccinal infections.

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