WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

WHO/SE/71.26

ENGLISH ONLY

INDEXED

BIBLIOTHÈQUE OMS
23 FEV. 1971
WHO LIBRARY

A STUDY OF INAPPARENT INFECTION IN SMALLPOX[a]

by

Gordon G. Heiner, Nusrat Fatima, Richard W. Daniel, June L. Cole, Ronald L. Anthony and Fred R. McCrumb, jr

ABSTRACT

Heiner, G. G. (Institute of International Medicine, University of Maryland School of Medicine, Baltimore, Md. 21201), Nusrat Fatima, R.W. Daniel, J.L. Cole, R.L. Anthony and F.R. McCrumb, jr. A study of inapparent infection in smallpox.

A retrospective serologic study was conducted in villages and households in which recent smallpox outbreaks had occurred but in which vaccination by public health authorities had not been carried out. Sera were collected from 143 household and compound contacts of smallpox cases in 20 villages and from 62 controls in five of the same villages; all had been vaccinated in the past. Serum antibody was assayed by the techniques of complement-fixation, haemagglutination-inhibition, passive haemagglutination, neutralization and immunodiffusion. Among the contacts, 54.5 per cent. showed elevated complement-fixing antibody levels, compared to 6.2 per cent. of controls. Similar proportions were found employing the other serologic tests. Analysis of the 56 households from which the study subjects were drawn showed a clinically-overt secondary attack rate of 14 per cent. and a 27.3 per cent. incidence of serologic response suggestive of inapparent infection. Since 35 per cent. of the contacts were not tested serologically, the true incidence of inapparent infection may have been considerably higher.

Keywords: antibodies; communicable diseases; epidemiology; immunity; smallpox; virus diseases.

Smallpox has long been considered an essentially overt disease, a view based primarily on epidemiologic evidence. Cases of smallpox are easily detectable and distinguishable from other forms of vesicular disease, chains of infection usually can be traced readily, and there are seldom breaks in the chains which would suggest the occurrence of inapparent infection.

On the other hand, it is unlikely that the pattern of smallpox should be radically different from other viral diseases in which the ratio of subclinical to clinical cases is often very high. The clinical spectrum of smallpox itself is broad, ranging from fulminating and fatal haemorrhagic disease to mild cases without permanent scarring.

---

[a] From the Pakistan Medical Research Center (University of Maryland, International Center for Medical Research and Training), Lahore, West Pakistan and the Division of Virus Diseases, Institute of International Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201.

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

Variola sine eruptione, consisting of fever without rash, is an unusual but recognized phenomenon, which occasionally has been proven virologically.[1-4] Recently cases have been detected in which conjunctivitis was the only manifestation of illness, the etiology of which has been confirmed by isolation of virus from conjunctival swabs.[5,6]

Cases of subclinical infection have been demonstrated by serologic means in variola minor, both in unvaccinated and vaccinated subjects.[7,8] Several recent studies have also reported instances in which serologic evidence of infection was detected in persons who had been exposed to variola major but had no clinical illness. With few exceptions, these cases have occurred in previously vaccinated persons.[2,6,9]

The present study was undertaken in an effort to identify serologically cases of inapparent infection occurring in recent smallpox outbreaks and to evaluate their frequency and public health significance.

METHODS

A major problem in conducting serologic studies in smallpox-endemic areas is that concurrent vaccination programmes complicate the interpretation of results. When a village outbreak of smallpox is detected by public health authorities, a vaccination campaign normally is carried out especially among intimate contacts of known cases. However, small village outbreaks occasionally are missed by the authorities and a vaccination programme is not instituted. Even when an outbreak is detected and vaccination is carried out, some households containing smallpox cases or some individual household contacts of cases may be missed. Such missed villages, households or individuals, if discovered within a few weeks of the onset of the outbreak, lend themselves to the serologic study of inapparent infection without the influence of concomitant vaccination.

The basic plan of this study was to collect sera from close contacts of smallpox cases who had not recently been vaccinated, and to examine these sera for evidence of subclinical infection by comparing their antibody patterns with those of an appropriate control group from the same villages.

Study area

The field study was conducted in 1968-69 in 20 rural villages in Lahore and Bahawalnagar Districts of West Pakistan, all within a radius of 250 miles of the city of Lahore. Size of the villages ranged from a few households to approximately 2000 inhabitants. Outbreaks were detected by following epidemiologic leads from other outbreaks reported by the public health authorities.

Contacts

Any individual was included in the study who was a household or compound contact of a recent smallpox case, had no history of previous smallpox and had not been vaccinated within a period of at least nine months prior to the study. Only those households were included in which one or more recent cases were diagnosed by the investigators as smallpox and epidemiologically related to at least one other similarly diagnosed case. Contacts were included only if their first exposure to the index case was within 150 days of the date of entry into the study. First exposure was considered to be the first day of rush of the index case for all contacts living with the case at the onset of illness.

A contact was defined as a person sleeping regularly in the same household or compound as an index case during the period of his illness. The typical house in a Punjabi village is a mud-brick dwelling of one or two small rooms, usually without windows. A compound consists of two or more houses grouped around a common walled court-yard, which usually are occupied by relatives. It is customary for a case to be kept inside during the acute phase of illness, but after a few days his bed often will be placed in the courtyard during the day. Essentially no isolation measures are taken.

Cases and contacts were identified, enumerated and interviewed on the first visit to a study village. A questionnaire was completed for each study household, and a single venous blood specimen was collected from each available contact. The questionnaire included demographic data on each contact, vaccination scar status, and a record of vaccination history, previous smallpox and pattern of exposure to the case. Contacts who were absent or who refused bleeding were omitted from the study, although questionnaires were also completed on these persons. Follow-up visits were made to the village as required for completion of data collection, and a final visit was made to each household no less than 42 days (two maximum incubation periods) after the collection of blood specimens, to insure that subsequently overt cases had not been included in the study during the prodromal period.

Controls

A control group was comprised of residents of certain of the same villages who were not household or compound contacts of cases. Five such villages were selected; within each village, smallpox-free compounds were chosen at random and all available inhabitants were entered into the study as controls. A venous blood specimen was collected from each control and a questionnaire was completed. Persons with a history of smallpox or of vaccination within the previous nine months were excluded. As in the case of contacts, persons who were absent or refused bleeding were omitted from the study. A final visit was also made to each control household no less than 42 days after the collection of blood specimens, to insure that no cases had appeared subsequently in the household.

Villages used for controls were selected principally for logistic reasons. Since resistance developed rapidly to the collection of blood specimens, it proved essential to complete this operation in each village in a single day. Within a control village, the specific random selection scheme was defined on the basis of village size and character. In two of the villages, every fifth house was visited; in the other three villages, the sequence of blocks to be used was determined by the drawing of straws and all houses in the selected blocks were then visited. In either case, no persons were accepted who fell outside the random selection scheme.

Since the control group was drawn from the same villages as the contacts, in all of which there had been recent cases of smallpox, it was recognized that some of the controls might have experienced inapparent infection during the current outbreaks. Therefore, two supplementary control groups were established. These control groups were intended to provide additional standards for the evaluation of serologic results and to verify the suitability of apparently unaffected village residents as the principal control group (Group 1). The first of the supplementary groups, designated Group 2, consisted of 37 adult Pakistani staff employees of the Pakistan Medical Research Center. Most had had annual smallpox vaccination for several years, and all had been revaccinated at the Center nine months before the collection of blood specimens. The other supplementary group, Group 3, consisted of 40 adult male inmate volunteers from the Maryland House of Correction at Jessup, Maryland. Twenty-four had been primarily vaccinated only in childhood, 16 had been revaccinated and four gave an uncertain history. None had been vaccinated within the previous five years.

Serologic tests

All serum specimens were stored at -20°C and shipped to Baltimore, where serologic tests were performed at the University of Maryland School of Medicine. International Standard Anti-Smallpox Serum was used as a positive control in each test.

Complement-fixation (CF) and haemagglutination-inhibition (HI) tests were performed by a micro-adaption of the procedures outlined by Kempe and St. Vincent.[10] CF titres were reported as the reciprocal of the highest dilution of serum which fixed two exact units of complement in the presence of four units of soluble antigen derived from variola-infected cell culture.[11] HI titres were recorded as the reciprocal of the highest dilution of serum contained in 0.025 ml capable of inhibiting the agglutinating activity of 0.025 ml of variola haemagglutinin[12] containing two HA units.

Passive haemagglutination (PHA) tests were performed using human "O" erythrocytes sensitized with variola-specific soluble antigens.[13] PHA titres were recorded as the reciprocal of the highest dilution of serum capable of agglutinating 0.025 ml of sensitized erythrocytes.

Virus neutralization tests were performed with vaccinia virus, using a plaque reduction technique.[14] Titres of virus neutralizing (NT) antibody were recorded as the reciprocal of that dilution of serum effecting a 50 per cent. reduction in the number of plaques observed in a virus control.

Virus-specific precipitating antibody was detected by the performance of micro-immunodiffusion (ID) tests. High molecular weight soluble antigens of variola virus served as the source of precipitinogens.[11]

RESULTS

During the two-year study period, 146 contacts residing in 56 households of 20 villages were entered into the study. The vast majority (143) of the contacts had scars providing evidence of vaccination at some time in the past; this reflected the fact that most unvaccinated contacts had developed overt disease and were thus not available for the study. It was therefore decided to limit the study group, for purposes of analysis, to these 143 vaccinated contacts. Three contacts who had no vaccination scars and no history of vaccination or of previous smallpox will be referred to as "Special cases" and discussed separately.

The interval between first exposure of the contact to the household or compound index case and collection of the blood specimen varied from six to 134 days, but in only one case was the interval less than two weeks. Specimens were obtained from 26.6 per cent. of contacts (38/143) within 30 days, from 35.7 per cent. (51/143) within 31-60 days and from 37.8 per cent. (54/143) later than 60 days after first exposure.

The principal control group (Group 1) consisted of 62 subjects, drawn from 26 households in five villages. Since the study group was limited to vaccinated contacts, unvaccinated persons were also excluded from the control group.

The composition of the study and control groups by age, sex and vaccination history is shown in Tables 1 and 2. The control group had a slightly greater proportion of females, relatively more persons under 10 years of age and fewer of 40 and older. However, the differences in age and sex distribution were not significant at the five per cent. level by the chi-square test, which was used throughout this analysis.

The control group showed a lower proportion of persons who had had primary vaccination only, but the difference was not significant. The controls also had a higher proportion of persons who reported recent vaccination; this difference was significant, whether measured as vaccination within the past three years ($p = .004$) or within the past 10 years ($p = .014$). Both differences, however, suggested that the controls were somewhat better vaccinated, which would tend to result in higher antibody titres in the control group. Since this could only bias the study against the finding of higher antibody levels among contacts, no adjustments were made for the differences.

Serologic findings

Results of serum antibody determinations among contacts and controls are summarized in Table 3 and in Figure 1. In each test, the geometric mean antibody titre observed in contacts was higher than that of controls, the distribution of titres of contacts was distinctly shifted and many individual titres were far above the levels of normal residual antibody.

In order to define antibody elevations and to determine the number of contacts who showed such elevations, standard values of residual antibody levels were established on the basis of findings in the principal control group. In each test a maximum titre was designated which would include the vast majority of controls and thereby define the upper limits of normal. The maximums established were a CF titre of 1:20, HI of 1:8, PHA of 1:160 and NT of 1:320. Thus elevated antibody levels, suggestive of recent infection, were defined as a CF titre of 1:40, HI of 1:16, PHA of 1:320 and NT of 1:640 or greater.

In the CF test, 54.5 per cent. of contacts (78/143) showed a titre of 1:40 or greater, compared to 6.5 per cent. of controls (4/62); the difference was highly significant ($p < .001$). Although a CF titre of 1:20 appears most appropriate as the upper limit of normal, the difference between contacts and controls in proportions of elevated titres remains significant if the upper limit is set at $<$ 1:10, 1:10, 1:40 or 1:80. Similar proportions were found in the other serologic tests, and in each test the difference between contacts and controls was highly significant ($p < .0001$).

It can be seen in Figure 1 that the distribution of CF and HI antibody titres was bimodal for contacts but unimodal for the controls. This indicates that there were two distinct populations among the contacts, one with low residual CF and HI titres similar to those of controls and another with elevated titres suggestive of recent infection. Bimodality cannot be detected in the distribution of PHA and neutralization titres, although there is a definite broadening of the distribution curves of titres of contacts as compared with those of controls. These differences among the tests are consistent with known differences in the persistence of detectable antibody after vaccination. Complement-fixing antibody titres return relatively quickly to low or undetectable levels. HI titres also decline but somewhat more slowly, whereas PHA and neutralization titres may remain at moderately high levels for many years (15-18). Thus residual and elevated titres are easily distinguishable in the CF and to some extent in the HI test, while in the PHA and neutralization tests, there is some overlap between high residual titres and those elevated as a result of recent antigenic stimulation.

Serologic results observed in the three control groups are presented in Table 4. Findings in the two supplementary groups were consistent with those of the principal control group. All were distinctly lower than the contact group in ranges of titres and geometric mean titres in each of the tests. On the other hand, there were clearly detectable differences among the three groups, reflecting differences in their vaccination status. Group 3, the American volunteer group, was the least well vaccinated, Group 2 was by far the best vaccinated, while Group 1, the principal control group, occupied an intermediate position. In all three groups, the vast majority of subjects showed low or negative CF and HI antibody levels, providing additional evidence of the relatively rapid decline of these antibodies

after vaccination, regardless of the degree of previous immunization. However, there were clear differences in the ranges and means of titres in the PHA test. Geometric mean titres were $< 1:10$ for the least well vaccinated Group 3, 1:41.6 for the intermediate Group 1 and 1:112 for Group 2, the best and most recently vaccinated. A similar pattern was seen in the results of the neutralization test.

The results of immunodiffusion tests in the contact group and in the three control groups are presented in Table 5. Precipitins were found in the sera of 62.9 per cent. of contacts (90/143), compared to 13.6 per cent. (8/59) in Group 1, the principal control group. This represents a highly significant difference ($p < .0001$). However, positive results were also obtained in sera of persons in Group 2 who had not been exposed to smallpox, and must indicate residual precipitins induced by previous immunization. It thus seems probable that some of the positive findings in the contact group also result from vaccine-induced antibody.

Epidemiologic factors

Within the contact group alone, the distribution of antibody titres was analysed in relation to epidemiologic factors (Table 6). In this analysis, only the CF results were used, since these provided the most clear-cut separation between persons with residual and recently elevated titres.

There was a distinct variation of titres with age of the contacts. Two-thirds (67.4 per cent.) of children under 15 showed elevated CF antibody levels, as compared to 49 per cent. of contacts of 15 or older, a significant difference ($p = .042$). The overall difference in antibody levels by sex was not significant. However, the percentage of females with elevated titres was somewhat greater (58.3 per cent.) than that of males (50.7 per cent.), and was significantly greater when contacts of 15 or older were examined alone (38.3 per cent. of males and 58.5 per cent. of females, $p = .044$).

Antibody levels also varied markedly with the relationship of contacts to the index case. Two-thirds (65.5 per cent.) of mothers but only one-third of fathers of child cases had elevated titres, while 78.6 per cent. of child siblings of cases showed such titres. The differences between fathers and mothers and between fathers and child siblings both were significant ($p = .036$ and .002 respectively).

There was a definite association between vaccination history and prevalence of elevated CF antibody. Elevated titres were found in more than two-thirds (67.9 per cent.) of contacts who had had primary vaccination alone, but in only 45.3 per cent. of those who also had been revaccinated; the difference was highly significant ($p = .009$). A similar difference was noted when adults (age 15 or above) and children (less than 15) were examined separately.

On the other hand, there was no clear association between the interval since last vaccination and prevalence of elevated titres. Persons vaccinated within the last 10 years showed a slightly lower proportion of elevated titres (50.5 per cent.) than those last vaccinated more than 10 years before (60.9 per cent.), but the difference was not significant. Even when persons with primary vaccination only and persons who had also had revaccination were examined separately, antibody levels appeared unrelated to the interval since vaccination.

CF titres showed a clear-cut association with the residence status and pattern of exposure of contacts. Elevated titres were found in nearly two-thirds of household contacts, but in less than one-third of persons who lived in the same compounds as cases but had no illness in their own households; the difference was highly significant ($p = .0007$). Persons who had constant contact with cases, i.e. slept in the same house or compound and also remained there during the day, showed a significantly higher proportion of elevated titres than contacts who were absent during the day ($p = .009$). Differences in titres of contacts in relation to numbers of overt cases in the house or compound were not statistically significant; however,

persons living in compounds with more than one overt case showed a slightly higher proportion of elevated titres than those in compounds with one case only, and the trend was more noticeable when household contacts were examined alone.

Interval since exposure

Intervals between first exposure of the contacts and collection of blood specimens varied from six to 134 days. As the period after exposure increased, there was a slightly greater tendency for antibody levels to be elevated; 44.7 per cent. of sera collected within 30 days, 52.9 per cent. of those collected within 31-60 days and 63 per cent. of those collected later than 60 days showed elevated CF titres. However, these differences were not significant.

Subjects with previous history of smallpox

Serum specimens were collected by error from nine contacts and nine controls who were found in subsequent interviews to have had a history of previous smallpox. These were excluded from the study; however, only two of the contacts and three of the controls had detectable facial scars, and it therefore seems probable that some of the others gave an erroneous history. The serologic findings are presented in Table 7. Ranges and means of titres of contacts were distinctly higher than those of controls. No control showed an elevated titre in any test (except for a single borderline HI titre of 1:16 in one subject) and none was positive in the immunodiffusion test. Three of the contacts showed elevated CF titres (1:80, 1:80 and 1:640), positive immunodiffusion and mixed results in the other tests; none of the three had facial scars but two were adults who had no vaccination scars, which would tend to support the history of smallpox. Thus there was suggestive but inconclusive evidence that inapparent smallpox infection also occurred in persons who previously had experienced overt disease.

Special cases

As mentioned above, three contacts were entered into the study who did not have vaccination scars. All were adults, and all gave a negative history of vaccination and previous smallpox. The serologic determinations for these three contacts are presented in Table 8. Subject 1 showed a low residual neutralization titre but no evidence of recent infection, subject 2 showed strong evidence of infection by our criteria in all tests, and the findings for subject 3 were mixed.

The extent of infection

Contacts included in this study were drawn from 56 separate households in 38 compounds. In order to estimate the extent of infection, both overt and inapparent, in households into which smallpox had been introduced, an analysis was made of all the residents of these households, whether or not they were included in the serologic study. The study households contained a total of 300 contacts, of whom 270 were examined. A history of smallpox was reported by 11.9 per cent. of persons examined, 82.2 per cent. were vaccinated or had a history of smallpox and 16.7 per cent. were unvaccinated or probably unvaccinated. These proportions correspond closely to findings in other rural studies in this area.[19-20]

The distribution of overt secondary cases and of contacts with elevated titres is presented in Table 9. Fourteen per cent. of the 300 contacts developed overt smallpox. Fifty-one per cent. of contacts were tested serologically. Using the criteria established for the CF test, 27.3 per cent. of the contacts showed elevated titres suggestive of infection, while 23.7 per cent. had titres within the normal range. It should be noted that 35 per cent. of contacts did not have overt disease but were not tested serologically, and presumably some of these also experienced inapparent infection. Thus at least 41.3 per cent. of all contacts developed overt smallpox or had evidence of inapparent infection. Inapparent infection was at least twice as frequent as overt disease.

Secondary attack rates varied markedly with vaccination status. The overt attack rate is unvaccinated or probably unvaccinated contacts was 68.8 per cent., but only 3.2 per cent. in vaccinated contacts. Among the unvaccinated, no contacts were found with serologic evidence of inapparent infection, with the exception of one and possibly two of the three unvaccinated adults discussed above (listed in Table 9 as "Special cases"). Among the vaccinated, the incidence of elevated titres suggestive of inapparent infection was 41.1 per cent.; however, since nearly one-fourth of the vaccinated contacts were not tested serologically, the true incidence of inapparent infection may have been considerably higher.

DISCUSSION

Technical considerations

This study was based principally on the comparison of contacts of smallpox cases with an appropriate control group from the same population. At the same time, we have attempted with this and two supplementary control groups to establish standards of residual antibody titres. In this laboratory, the upper limits of normal appear to be represented by a complement-fixation titre of 1:20, haemagglutination-inhibition of 1:8, passive haemagglutination of 1:160 and neutralization of 1:320. The CF and NT titres correspond well with the generally accepted limits of normal;[16,21,22] HI titres employing variola-derived haemagglutinin are lower in this laboratory; and the PHA test is a newly-developed procedure not yet tested elsewhere. However, it is apparent from findings in our control groups that in each test some sera show titres well above the normal range and that an elevated titre alone cannot be considered diagnostic of infection. Downie and associates, on the basis of studies in Madras, define a CF titre of 1:20 or greater, HI of 1:80 or greater, neutralization of 1:500 or greater and a positive immunodiffusion test as indicative of recent infection if found in a single serum specimen.[22]

The time of appearance and decline of CF antibody is of obvious importance, since much of our analysis has been based on this assay. In a serial study of the response to revaccination, we found that in approximately 50 per cent. of cases, CF antibody first appeared or showed a rise in titre by day seven and that most became positive or demonstrated a rise by day 14 after revaccination.[18] In the present study, only one specimen was obtained less than two weeks after first exposure of the contact; this was at six days, and showed a CF titre of 1:320 and correspondingly elevated titres in other tests. This may indicate that the response is more rapid when the booster stimulus occurs via the respiratory tract rather than by revaccination.

In studies of sera of smallpox patients, we have found that mean CF titres begin to decline after day 30 of illness in previously unvaccinated cases and after day 20 in previously vaccinated cases.[23] As noted above, no significant differences were found in the present study between proportions of elevated titres in sera of contacts collected within 30 days, within 31-60 days and later than 60 days. However, it may be that titres of some of the later sera had declined by the time of collection, and that the overall proportion of elevated titres was in fact higher.

Other investigators have reported that persons who have had recent smallpox infection can be distinguished from vaccinees by the demonstration of precipitating antibody by the technique of immuno-diffusion.[9,22,24,25] Rao found that sera of smallpox patients, collected at least 10 days after onset of illness, invariably gave a positive result with variola antigen derived from pooled vesicular fluid and usually with vaccinia antigen. Post-vaccination sera sometimes gave a positive result with vaccinia antigen but never with variola.[9]

In our laboratory, however, precipitins have also been demonstrated in the sera of vaccinated persons not exposed to smallpox. A purified antigen derived from variola-infected cell culture was employed.[11] In a study of the serologic response to revaccination, post-vaccination sera became positive in 19 of 25 subjects tested, precipitins being detectable

first on day 10 after vaccination.[18] Furthermore, a considerable number of persons in two of the control groups of the present study demonstrated precipitins.

It is thus clear that precipitins may be formed after vaccination, and may persist for an as yet undetermined period, but their detection appears to depend upon the concentration of antigen and consequent sensitivity of the test. In Rao's study, the use of pooled vesicular fluid apparently provided an antigen of relatively low potency and thus allowed a quantitative distinction to be made between the reactions of smallpox cases, including subclinical cases, and those of vaccinees. However, since the distinction is quantitative, such a test may also yield false positives, especially in view of the great variability in antigenic content of different specimens and pools of vesicular fluid. In the present study, in which a more concentrated antigen was used, no distinction could be made in individual cases between residual post-vaccination precipitins and those due to a recent variola stimulus. However, as with the other serologic tests, a significantly higher proportion of positive results was found among contacts than among controls.

We cannot be certain that some of the contacts in this study who showed elevated antibody titres had not had mild symptoms prior to our first visit, and thus should be classified as cases of variola sine eruptione rather than inapparent infection. Many of the households were visited while cases were still convalescent, but few contacts were seen during the first two or three weeks of illness of the index case. It seems unlikely, however, that many of the elevated titres can be accounted for by such cases, since other studies have shown the incidence of variola sine eruptione to be very low. In prospective studies conducted during the same period among contacts of active smallpox cases, we encountered only one serologically proven case, one possible and three doubtful cases of variola sine eruptione among nearly 200 contacts.[20]

Dekking and associates[5] and Kempe et al.[6] have reported a high incidence of conjunctivitis among mothers and other family attendants sleeping with smallpox patients in the infectious disease hospital in Madras. This also may have occurred in some of the contacts in this study, but it seems equally unlikely to have accounted for a large number of the elevated titres found. These authors expressed the view that the cases noted in Madras appear to have represented infection, possibly auto-inoculation, through the conjunctiva, rather than infection "naturally" acquired via the respiratory tract. This may account for its high incidence among persons living in a smallpox ward and handling contaminated linen and clothing.

Infectivity and rates of infection

It would be of great interest to know what proportion of smallpox contacts actually become "infected" with virus, since this has a direct bearing on evaluation of the natural infectivity of smallpox. Some of the present data suggest that a substantial number of additional contacts may in fact have received a potentially infective virus dose but because of their immune status, neutralized the virus before it could multiply and provide a stimulus to further antibody production. There is no reason to suppose that there was a difference in the exposure of contacts who had had primary vaccination only and those who had also had revaccination, since the two groups are similar in age and sex distribution. However, 67.9 per cent. of those with primary vaccination and only 45.3 per cent. of those with revaccination showed elevated CF titres, a highly significant difference. Presumably some of the latter group were in fact exposed to virus but failed to experience an anamnestic response because of rapid neutralization of the infecting dose. It is well known that vaccine of higher titre is required for successful revaccination than for primary vaccination,[24] and there probably is an analogous difference in dosage required for a booster by natural infection, varying with the degree of immunity of the subject. Evidence of a similar variation has recently been reported in measles. When neutralizing antibody was measured in serial serum specimens of 39 mothers of children with measles, only those mothers with low residual levels showed a detectable rise in titre.[26]

Among unvaccinated contacts in this study, the overt secondary attack rate was 68.8 per cent. This is somewhat higher but in the same order of magnitude as rates found in previous studies in this and other endemic areas.[19,20,27-30] It is our impression from epidemiologic studies conducted in West Pakistan that the lower rates reported may be somewhat misleading. On closer examination, many of the unvaccinated who escape infection are found to be infants presumably protected by maternal antibody or persons vaccinated soon after exposure, and the number of true susceptibles who escape infection appears to be extremely low.[19,20]

It remains uncertain whether or not inapparent infection may occur in the unvaccinated. Subclinical cases of variola minor have been demonstrated serologically,[7,8] but this is in fact a different disease in which the entire spectrum of illness is much milder. Verlinde and van Tongeren isolated virus from one apparently unvaccinated subject who had been in contact with a case of variola major and who showed no signs of illness.[1] Rao has found evidence of subclinical infection in three unvaccinated contacts by the immuno-diffusion test;[9] but as indicated above, it would appear that this test cannot alone be considered diagnostic.

In the present study, of the three unvaccinated contacts from whom sera were obtained, one had positive and one possible evidence of inapparent infection. However, it was the opinion of the investigators that the initial immune status of all three was questionable. All were adults and all were generally poor respondents who were vague on most of their history. The possibility must be considered that they had been successfully vaccinated in childhood without detectable scarring or that they had had very mild smallpox which was misdiagnosed or forgotten. Previous studies in West Pakistan have shown that as many as a third of diagnosed smallpox cases may show no detectable pocks a year after their illness.[31]

Among vaccinated contacts in this study, the overt secondary attack rate was 3.2 per cent. This too is similar to findings in other studies.[19,20,27] The secondary attack rate of inapparent infection appears to be at least 41 per cent., on the basis of the observed incidence of elevated antibody titres. Since nearly one-fourth of the vaccinated contacts in the study households were not tested, the rate can be presumed to be somewhat higher. Furthermore, as discussed above, a considerable number of additional vaccinated contacts can be presumed to have been infected but to have neutralized the virus dose without a serologic response.

The protective effect of vaccination against both overt and inapparent infection was clearly demonstrated. The overt attack rates in unvaccinated and vaccinated contacts were 68.8 and 3.2 per cent., respectively, giving a vaccination-protection ratio of 95.4 per cent. The efficacy of revaccination was shown by the significantly lower incidence of elevated titres in those contacts who had been revaccinated. The study did not show any clear advantage of recent vaccination; however, the data on time of revaccination are inherently the least reliable of the study, both because of inaccuracies in history and because of erratic revaccination take rates. Throughout the period of this study, only liquid vaccine was being used by the local public health authorities.

Epidemiologic questions

The close association of exposure factors with infection is one of the most striking findings of this study. In the first place, the difference between contacts and controls was unexpectedly clear-cut. In the village setting, many controls obviously had some exposure due to the nature of village life and the complete lack of isolation of cases. Yet this casual type of exposure apparently was not enough for any significant amount of infection to occur. Only four of the controls showed elevated CF titres. Three of these showed mixed results in the other tests, and the CF elevations (1:40, 1:40 and 1:80) may represent normal variations in residual titres; similar variations occurred in the two supplementary control groups. Only one control showed clear evidence of inapparent infection in all tests (CF 1:320, HI 1:64, PHA 1:1280, NT 1:5120 and ID positive).

This was a 17-year-old girl who was a "control" by the random selection scheme but who sat during each of our visits on the low compound wall which separated her by only a few feet from her neighbour's three children who had overt smallpox.

This sharp difference between household and compound contacts and outsiders is consistent with the belief that smallpox is spread principally by direct droplet transmission from the respiratory tract of the patient. Within a compound, it might be expected that most of the residents would soon be exposed to this type of infection. Outside the compound, however, there would be little possibility of transmission by this means.

Within a household or compound, the overall rate of infection was high, but differences in incidence of inapparent infection could still be correlated closely with differences in degrees of exposure. Household contacts showed a higher rate of infection than compound contacts; persons with constant contact had a higher rate than those absent during the day; and houses with more than one overt case showed more inapparent infection than those with a single overt case. Adult females and children showed a higher incidence of elevated titres than did the adult males, including the fathers of cases; this too presumably reflected differences in exposure, since females and young children rarely left the compounds whereas adult males were working in the fields throughout the day.

The importance of the degree of infectivity of the index case cannot be fully evaluated from our data. Other studies have shown that the infectivity of the index case, in terms of resulting secondary cases, increases as severity of illness increases.[27,28,32] We have found that the pattern of infection is quite spotty, that in some houses no overt or subclinical secondary cases occurred, in some there were many secondary cases and in some there were many subclinical cases only. Since the patterns of exposure and of vaccination status did not vary greatly from household to household, it would seem probable that variations in infectivity of the index case might at least in part explain the differences.

It may be concluded that smallpox is much more infective than has been generally believed, but that transmission usually requires an exposure close enough to permit direct droplet spread from patient to contact. When smallpox is introduced into a village household, it appears that the vast majority of contacts will become infected. Most of the unvaccinated will develop overt disease; some infants may escape due to the protection of maternal antibody; and a very few may escape effective exposure. A few of the vaccinated contacts will show overt disease; half or more will develop inapparent infection which results in a demonstrable rise in antibody titres; and presumably some of the better vaccinated will become infected but will neutralize the virus without showing a serologic effect. This general pattern may of course vary considerably in individual households; and may be influenced by the infectivity of the case, vaccination status of the contacts and specific patterns of exposure.

An important question remaining is the effect of the phenomenon of inapparent infection on the development and maintenance of immunity in an endemic area. It is obvious from the findings of this study that in affected households, inapparent infection plays a major role in the maintenance of a high level of immunity among the residents of those households. In villages which suffer major outbreaks, affecting many households, it may serve to raise significantly the immunity level of the villages concerned. Thus it must be considered, as is overt smallpox, a factor in evaluating the immune status of endemic communities. On the other hand, it is unlikely that inapparent infection is of great importance in the maintenance of immunity in larger regions, for the simple reason that even in an endemic area, the overall incidence of smallpox is very low in terms of numbers of communities affected. In comparison to vaccination, even overt smallpox probably plays a relatively small role in most areas today in maintaining hard immunity; and since inapparent infection occurs only where there is overt disease, the same may be said for that.

Another major question that remains unanswered is whether or not an individual with inapparent infection can himself be a source of infection. As a general rule, this would appear unlikely. As mentioned above, it has been demonstrated that the infectivity of a case varies directly with its severity, which probably reflects the duration and intensity of virus shedding. On the other hand, previous studies have confirmed the presence of virus and thus the potential infectivity of the mildest clinical forms of the disease. Several investigators have isolated virus from cases of variola sine eruptione;[1-4] and positive isolates have been obtained from throat washings of contacts with pharyngitis and fever but no rash[4] and from the conjunctivae of contacts with conjunctivitis who were otherwise asymptomatic.[5,6] Virus multiplication presumably occurs in inapparent infection as in variola sine eruptione, in order to produce the observed antigenic stimulus, and a transient period of virus shedding may also take place. The high incidence of subclinical cases in smallpox outbreaks has now been clearly demonstrated. Even if only a small percentage of such cases were infective, this would be of obvious epidemiologic significance

TABLE 1. DISTRIBUTION OF CONTACTS AND VILLAGE CONTROLS BY AGE AND SEX

Age (years)	Contacts				Controls			
	Males	Females	Both sexes	% of total	Males	Females	Both sexes	% of total
<10	13	10	23	16.1	10	9	19	30.6
10-14	11	9	20	14.0	4	6	10	16.1
15-19	4	7	11	7.7	3	3	6	9.7
20-29	10	13	23	16.1	1	5	6	9.7
30-39	11	14	25	17.5	5	7	12	19.4
≥40	22	19	41	28.7	4	5	9	14.5
All ages	71	72	143	100.0	27	35	62	100.0
% of total	49.7	50.3	100.0		43.5	56.5	100.0	

TABLE 2. DISTRIBUTION OF CONTACTS AND VILLAGE CONTROLS BY VACCINATION HISTORY

Vaccination history	Contacts		Controls	
	No.	% of total	No.	% of total
Primary only	53	37.1	18	29.0
Primary and revaccination	86	60.1	39	62.9
Unknown	4	2.8	5	8.1
Vaccinated within last 3 years	52	36.4	34	54.8
Vaccinated within last 10 years	93	65.0	48	77.4
Total vaccinated	143	100.0	62	100.0

TABLE 3

Variola antibody determinations in sera of contacts and village controls

CF			HI			PHA			NT		
Reciprocal of titer	No. of contacts	No. of controls	Reciprocal of titer	No. of contacts	No. of controls	Reciprocal of titer	No. of contacts	No. of controls	Reciprocal of titer	No. of contacts	No. of controls
<10	46	45	<4	45	36	<10	9	9	<10		1
10	8	6	4	16	11	10	6	4	10		
20	10	3	8	11	7	20	7	8	20	2	3
40	21	1	16	19	2	40	7	14	40	3	5
80	15	2	32	24	3	80	19	15	80	8	12
160	16		64	14	2	160	22	8	160	15	17
320	9	1	128	12		320	14	2	320	28	13
640	9		256	1		640	24	1	640	26	3
1,280	3					1,280	19	1	1,280	32	1
2,560	3					2,560	10		2,560	9	2
5,120	2					5,120	3		5,120	9	1
						10,240			≥10,240	8	
						≥20,480	3				
Not tested*	1	4	Not tested*	1	1	Not tested*			Not tested*	3	4
Total	143	62	Total	143	62	Total	143	62	Total	143	62
Geometric mean	38.6	<10	Geometric mean	10.5	<4	Geometric mean	225.6	41.6	Geometric mean	659.2	157.6
No. sera ≥ 1:40	78	4	No. sera ≥ 1:16	70	7	No. sera ≥ 1:320	73	4	No. sera ≥ 1:640	84	7
%sera ≥ 1:40	54.5	6.5	% sera ≥ 1:16	49.0	11.3	% sera ≥ 1:320	51.0	6.5	% sera ≥ 1:640	58.7	11.3
International Standard Anti-Smallpox Serum: 1:160			International Standard Anti-Smallpox Serum: 1:128			International Standard Anti-Smallpox Serum: 1:160			International Standard Anti-Smallpox Serum: 1:10,240		

*Includes specimens of insufficient volume or exhibiting anti-complementary activity

TABLE 4

Variola antibody determinations in sera of three control groups

CF				HI				PHA				NT			
	No. of controls				No. of controls				No. of controls				No. of controls		
Reciprocal of titer	Group 1	Group 2	Group 3	Reciprocal of titer	Group 1	Group 2	Group 3	Reciprocal of titer	Group 1	Group 2	Group 3	Reciprocal of titer	Group 1	Group 2	Group 3
<10	45	24	26	<4	36	14	11	<10	9		24	<10	1		1
10	6	8	6	4	11	11	8	10	4		9	10			
20	3	2	3	8	7	7	12	20	8		3	20	3		4
40	1	2	1	16	2	5	6	40	14	8	3	40	5		1
80	2	1	2	32	3		3	80	15	11	1	80	12	3	11
160			1	64	2			160	8	12		160	17	6	8
320	1							320	2	4		320	13	13	9
								640	1	2		640	3	7	3
								1,280	1			1,280	1	6	3
												2,560	2	2	
												5,120	1		
Not tested*	4		1	Not tested*	1			Not tested*				Not tested*	4		
Total	62	37	40	Total	62	37	40	Total	62	37	40	Total	62	37	40
Geometric mean	<10	<10	<10	Geometric mean	<4	4.2	5.8	Geometric mean	41.6	112.0	<10	Geometric mean	157.6	406.4	144.0
No. sera ≥ 1:40	4	3	4	No. sera ≥ 1:16	7	5	9	No. sera ≥ 1:320	4	6	0	No. sera ≥ 1:640	7	15	6
% sera ≥ 1:40	6.5	8.1	10.0	% sera ≥ 1:16	11.3	13.5	22.5	% sera ≥ 1:320	6.5	16.2	0.0	% sera ≥ 1:640	11.3	40.5	15.0
International Standard Anti-Smallpox Serum: 1:160				International Standard Anti-Smallpox Serum: 1:128				International Standard Anti-Smallpox Serum: 1:160				International Standard Anti-Smallpox Serum: 1:10,240			

*Includes specimens of insufficient volume or exhibiting anti-complementary activity

TABLE 5

Results of immunodiffusion test in sera of contacts and controls

	Total no. of sera	No. of sera positive	% of sera positive
Contacts	143	90	62.9
Controls			
Group 1	59	8	13.6
Group 2	37	5	13.5
Group 3	40	0	0.0

TABLE 6

Proportions of subgroups of contacts showing elevated CF antibody titers

	No. of contacts	No. with CF titer $\geq$ 1:40	% with CF titer $\geq$ 1:40
Children (< 15 years)	43	29	67.4
Adults ($\geq$ 15 years)	100	49	49.0
Child siblings of cases*	28	22	78.6
Mothers of cases**	26	17	65.4
Fathers of cases**	18	6	33.3
Other contacts	71	33	46.5
Primary vaccination only	53	36	67.9
Primary and revaccination	86	39	45.3
Living in same house as case	111	69	62.2
Living in same compound as case	32	9	28.1
Constant exposure to case +	87	55	63.2
Daily exposure to case +	56	23	41.1
Total contacts	143	78	54.5
Controls	62	4	6.5

* Siblings < 15 years.
** Fathers and mothers of child cases (< 15 years).
\+ Exposure was defined as constant when the contact slept in the same house or compound as the index case and also remained there during the day, and as daily when the contact slept in the same house or compound but was absent during the day.

TABLE 7

Variola antibody determinations in sera of contacts and village controls who had a history of previous smallpox

	CF	HI	PHA	NT	ID
Contacts					
No. of subjects	9	9	9	9	9
Geometric mean titer	27.0	5.4	63.6	172.8	-
No. with elevated titers*	3	2	1	1	6
% with elevated titers*	33.3	22.2	11.1	11.1	67.0
Controls					
No. of subjects	9	9	9	9	9
Geometric mean titer	<10	<4	17.1	25.2	-
No. with elevated titers*	0	1	0	0	0
% with elevated titers*	0.0	11.1	0.0	0.0	0.0

*CF $\geq$ 1:40, HI $\geq$ 1:16, PHA $\geq$ 1:320, NT $\geq$ 1:640, ID positive

TABLE 8

Variola antibody determinations in sera of contacts without vaccination scars

Subject no.	Age & Sex	Interval, first exposure to specimen collection	Reciprocal of antibody titers CF	HI	PHA	NT	ID test
1	35 F	44 days	<10	<4	<10	160	0
2	50 F	35 days	160	128	640	640	+
3	50 M	79 days	10	64	160	1280	+

TABLE 9

Distribution of total contacts, overt secondary cases and contacts tested serologically in 54 households studied*

Vaccination status of contacts	Total no. of contacts	Distribution of contacts							
		Numbers				Per cent			
		Overt secondary cases	Contacts with inapparent infection ‡	Contacts without inapparent infection	Contacts not tested	Overt secondary cases	Contacts with inapparent infection ‡	Contacts without inapparent infection	Contacts not tested
Unvaccinated	41	29	-	-	12	70.7	-	-	29.3
Probably unvaccinated**	4	4	-	-	-	100.0	-	-	-
Special cases +	3	-	1	2	-	-	33.3	66.7	-
Unvaccinated or probably unvaccinated	48	33	1	2	12	68.8	2.1	4.2	25.0
Vaccinated ++	190	6	78	64	42	3.2	41.1	33.7	22.1
History of smallpox	32	-	3	5	24	-	9.4	15.6	75.0
Vaccinated or having a history of smallpox	222	6	81	69	66	2.7	36.5	31.1	29.7
Not examined	30	3	-	-	27	10.0	-	-	90.0
Total	300	42	82	71	105	14.0	27.3	23.7	35.0

* Two households for which data were incomplete were excluded.

** Died before examination, or had confluent lesions preventing determination of vaccination scar status.

\+ 3 adults with no detectable vaccination scars and with a negative history of vaccination and of smallpox.

++ Not including persons with a history of smallpox.

‡ Contacts with a CF titer $\geq$ 1:40

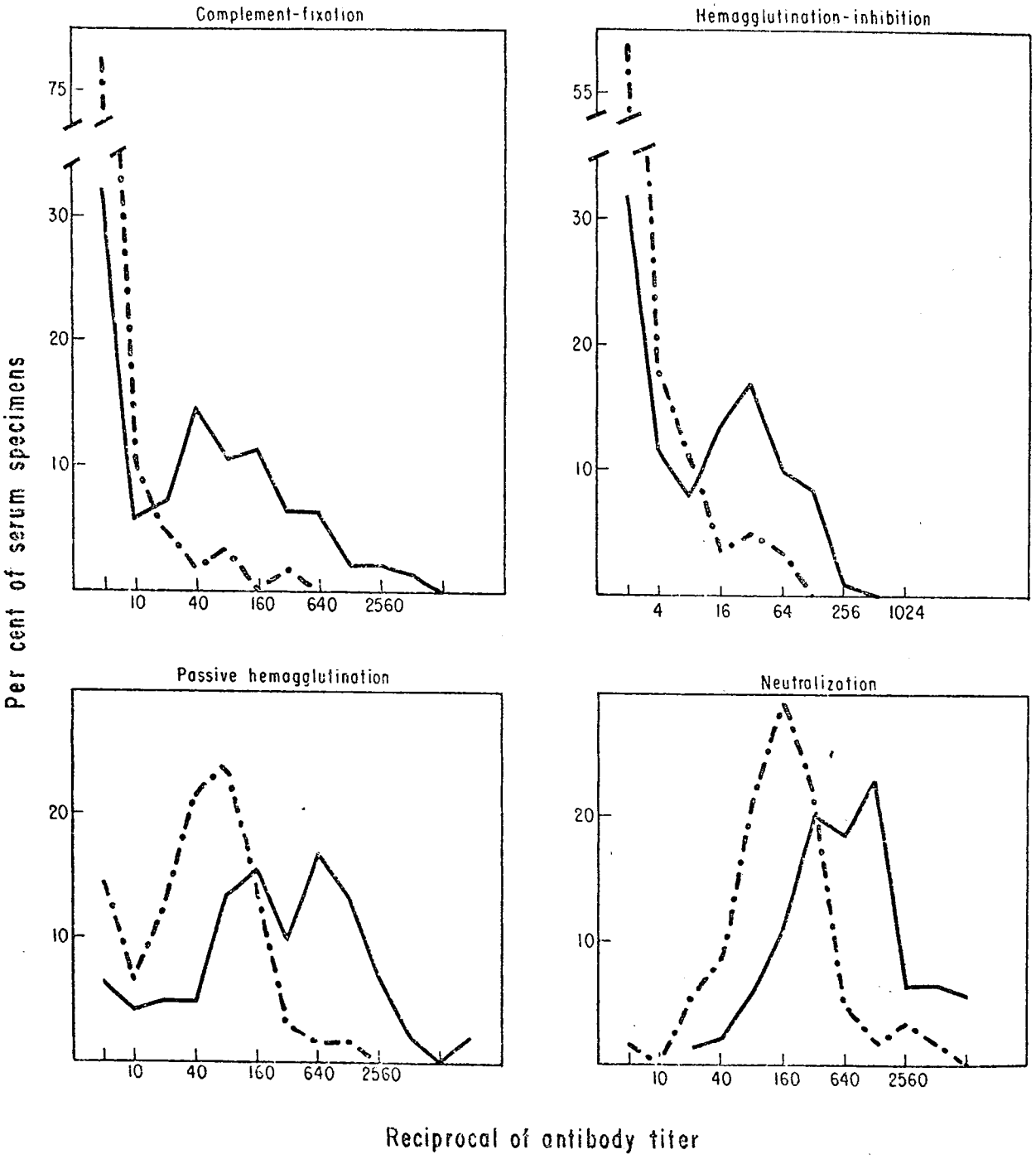

FIG. 1 DISTRIBUTION OF VARIOLA ANTIBODY DETERMINATIONS IN SERA OF CONTACTS AND VILLAGE CONTROLS. (———— CONTACTS;—•—•—CONTROLS)

REFERENCES

1. Verlinde, J. D. & van Tongeren, H. A. E. (1952) Isolation of smallpox virus from the nasopharynx of patients with variola sine eruptione, Antonie v. Leeuwenhoek, 18, 109-112

2. Bingel, K. F. & Kruse, F. (1959) Methoden und Ergebnisse der virologischen und serologischen Untersuchungen bei den Pockenerkrankungen in Heidelberg (Dezember 1958/Januar 1959), Medizinische, (1959) 20, 961-969

3. Marennikova, S. S., Gurvich, E. B. & Yumasheva, M. A. (1963) Laboratory diagnosis of smallpox and similar viral diseases by means of tissue vulture methods, Acta virol., 7, 124-129

4. Surowcowa-Swidzinska, A. et al. (1964) The clinical course of smallpox in the Wroclaw epidemic in 1963, Epid. Review, 18, 69-79

5. Dekking, F. et al. (1967) The weeping mother, an unusual source of variola virus, Arch. es. Virusforsch., 22, 215-218

6. Kempe, C. H. et al. (1969) Conjunctivitis and subclinical infection in smallpox, J. Hyg. (Lond.), 67, 631-636

7. Rodrigues-da-Silva, G., Ivo Rabello, S. & Angulo, J. J. (1963) Epidemic of variola minor in a suburb of São Paulo, Publ. Hlth. Rep. (Wash.), 78, 165-171

8. De Salles-Gomes, L. F. et al. (1965) Clinical and subclinical variola minor in a ward outbreak, J. Hyg. (Lond.), 63, 49-58

9. Rao, A. R. et al. (1970) Precipitation in gel test in diagnosis of smallpox, Indian J. med. Res., 58, 271-282

10. Kempe, C. H. & St. Vincent, L. (1964) Variola and vaccinia viruses. In: Diagnostic procedures for viral and rickettsial diseases, 3rd ed., Lennette, E. H. & Schmidt, N. J. (Eds.), American Public Health Association, New York, pp. 665-692

11. Anthony, R. L. et al., Studies of variola virus and immunity in smallpox. II. Recovery of virus-specific soluble antigens from infected Rhesus monkey kidney cells (Submitted for publication)

12. Anthony, R. L. et al. (1970) Studies of variola virus and immunity in smallpox. I. Variola virus haemagglutinins, J. infect. Dis., 121, 295-302

13. Anthony, R. L. et al., Studies of variola virus and immunity in smallpox. III. Measurement of antibodies to high molecular weight soluble antigens of variola virus using the bis-diazotized benzidine passive haemagglutination technique (Submitted for publication)

14. Cutchins, E., Warren, J. & Jones, W. P. (1960) The antibody response to smallpox vaccination as measured by a tissue culture plaque method, J. Immunol., 85,275-283

15. McCarthy, K. & Downie, A. W. (1958) The antibody response in man following infection with viruses of the pox group. II. Antibody response following vaccination, J. Hyg. (Lond.), 56, 466-478

16. Downie, A. W. (1965) Poxvirus group. In: Viral and Rickettsial Infections of Man, 4th ed., Horsfall, F. L. jr. & Tamm, I. (Eds), J. B. Lippincott Co., Philadelphia, pp. 932-964

17. Collier, W. A. (1951) Individual immunity against smallpox, Docum. Neerl. indones. Morb. trop., 3, 163-176

18. Anthony, R. L. et al., Studies of variola virus and immunity in smallpox. VI. Use of passive haemagglutination technique for measurement of the antibody response to vaccination (In preparation)

19. Mack, T. M. et al., Epidemiology of smallpox in West Pakistan. I. Acquired immunity and the distribution of smallpox by time, place and person (In preparation)

20. Pakistan Medical Research Center, unpublished data

21. Downie, A. W. & McCarthy, K. (1958) The antibody response in man following infection with viruses of the pox group. III. Antibody response in smallpox, J. Hyg., (Lond.), 56, 479-487

22. Downie, A. W. et al. (1969) Antibody response in non-haemorrhagic smallpox patients, J. Hyg. (Lond.), 67, 609-618

23. Anthony, R. L. et al., Studies of variola virus and immunity in smallpox. V. Use of passive haemagglutination technique for measurement of the antibody response in human variola infection (In preparation)

24. World Health Organization (1964) WHO Expert Committee on Smallpox, First Report, Wld Hth Org. techn. Rep. Ser., 283

25. Downie, A. W. et al. (1969) Antibody response following smallpox vaccination and revaccination, J. Hyg. (Lond.), 67, 603-608

26. Ueda, S., Toyoshima, K. & Kurose, T. (1969) Observation of booster infections of measles in mothers of children with measles, Biken's J. 12, 127-128

27. Rao, A. R. et al. (1968) Epidemiologic studies in smallpox. A study of intrafamilial transmission in a series of 254 infected families, Indian J. med. Res., 56, 1826-1854

28. Thomas, D. B. et al., Endemic smallpox in rural East Pakistan. Part 2: Intravillage transmission, communal susceptibility and infectiousness (In preparation)

29. Bauer, D. J. et al. (1969) Prophylaxis of smallpox with methisazone, Amer. J. Epid., 90, 130-145

30. Rao, A. R. et al. (1969) Chemoprophylaxis and chemotherapy in variola major. I. An assessment of CG 662 and Marboran in prophylaxis of contacts of variola major, Indian J. med. Res., 57, 477-483

31. Mack, T. M., Thomas, D. B. & Khan, M. M. (1970) Variola major in West Pakistan, J. infect. Dis., 122 (In press)

32. Mack, T. M., Thomas, D. B. & Khan, M. M., Epidemiology of smallpox in West Pakistan. II. Determinants of intravillage spread other than acquired immunity (In preparation)