

SEROLOGICAL STUDIES ON AN OUTBREAK  
OF SMALLPOX IN THE STATE OF  
BAHIA — BRAZIL IN 1969<sup>1</sup>

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(With 6 figures)

**SUMMARY:** Four weeks after Containment Vaccination undertaken against the largest outbreak of smallpox occurred in Brazil in 1969, that of the municipality of Utinga, Bahia, 99 samples of serum were collected from the local population.

These samples were classified in four groups:

- a) — Individuals with a history of variola prior to the beginning of present outbreak in town (15 sera); "Previous smallpox group";
- b) — Individuals with primary vaccination, with no record of variola, at the time of containment measures (15 sera), "Primary vaccinated group";
- c) — Individuals with no previous record of variola revaccinated with "take" at the time of containment (15 sera), "Revaccinated group";
- d) — Individuals who contracted variola in present outbreak (54 sera) these were subdivided in four sub-groups, according to dates on which cases occurred, "Variola in outbreak group".

Serological study of samples was done by tests of hemagglutination inhibition, neutralization, and complement fixation.

It was observed that HI titers were significantly lower in cases of previous smallpox than in other groups. Although they were slightly higher on revaccinated individuals than on primary vaccinated group and than in the group of variola in outbreak, this difference was not significant. Those same antibodies were present in all cases of variola in outbreak, and it was found that titers decreased in direct proportion to time elapsed from occurrence of cases.

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Neutralizing antibodies proved to be significantly higher on the revaccinated group than on variola in outbreak group, and higher on these than on primary vaccinated and on the previous smallpox groups. In cases from the variola in outbreak it was verified that neutralizing antibodies remained stable, although with great variation in titers.

Tests of complement fixation could not be undertaken on all samples, because many of them proved to have anticomplementarity. However, it was found that complement fixing antibodies diminished rapidly, becoming negative for earlier infections. Finally, the authors suggest that there would be some evidence that HI titers are lower in variola minor under Brazilian conditions than in variola major.

THE smallpox eradication campaign ("Campanha de Erradicação da Variola" — CEV), was set up in Brazil in 1966. One of the first projects to be planned was the installation of Epidemiological Surveillance Units in the different States of Brazil. Reference Laboratories should be set up in order that these units might operate. By 1969 one Laboratory was in operation in the former National School of Public Health now Instituto Pres. Castello Branco (FIOCRUZ) in Rio de Janeiro. In the same year, following an agreement between SESP Foundation and CEV, four Epidemiological Units were set up in States where the mass vaccination was just being put into effect.

One of the interesting field observations made was that the hemagglutination-inhibiting (HI) antibody response of typical cases of variola in evolution or recently recovered never showed titers over 1/160 and most of them ranged from 1/20 to 1/40. There were noted also some typical cases of chickenpox who had been vaccinated against variola several years previously, showing also titers, turning sometimes difficult differential diagnosis, important to follow the chain of cases in an epidemic, as reported by one of the authors, **Azeredo Costa** (1).

At the beginning of August 1969, the Epidemiological Unit operating in the State of Bahia investigated an outbreak of smallpox in the municipality of Utinga. From one case reported it was possible to trace another 506 cases, which made this the largest epidemic recorded in Brazil in that year. Of 246 cases reported in the urban area, only one patient died (0.4%). One case was confirmed through isolation of the virus in scabs, and the others through clinical and epidemiological data. This outbreak will be described in another paper by **Azeredo Costa & Morris** (2).

This epidemic offered a good opportunity for further investigations into the serology of variola under Brazilian conditions (where there is not known circulating variola major virus), as all cases under study would be in almost the same ecological situation and just on strain of virus would have affected them all. A similar situation was described by **McCarthy & Downie** in 1953 (11).

In addition, serological studies could be also carried out amongst those people successfully vaccinated as a containment measure during the outbreak in the town, which could provide interesting comparisons.



## MATERIAL AND METHODS

On September 3, 1969, the surveillance Unit arrived at Utinga to check the efficiency of vaccination which had been carried out one month previously to contain the outbreak in the town.

At this time 99 samples of serum were taken from individuals noting name, age, sex, vaccination scars, and record of smallpox, and these were divided into four groups as follows:

- a) those with a convincing story of smallpox before the outbreak under study (15 sera) — “previous smallpox group” (PS);
- b) those who had been primary vaccinated with take during the containment operation on month earlier (15 sera) — “primary vaccinated group” (PV);
- c) those who had been revaccinated with take during the containment operation one month earlier (15 sera) — “revaccinated group” (RV); and
- d) those who contracted smallpox during the outbreak under study (54 sera) — “variola in the outbreak group” (VO).

PS group consists of one individual with infection less than ten years earlier, eight individuals infected between 10 and 20 years before and six individuals over 20 years before.

None of them had vaccination scar and three out of them were living during the outbreak in dwellings where smallpox affected one person at least.

In RV group seven subjects had their primary vaccination during previous ten years, and eight earlier than this. None of them had smallpox and three out of them were dwelling with persons affected by smallpox during the outbreak.

In PV group no individual either had smallpox or were dwelling with persons affected by smallpox during the outbreak.

Of 54 patients in the VO group 50 had never been vaccinated with take, one had a scar produced by vaccination in 1963, and three were vaccinated in the incuba-

tion period of the disease and subsequently suffered vaccinal and variolic infections simultaneously, developing variolic rash at three and seven days after the vaccination.

Grouping were not made strictly by random, but rather in accordance with receptivity found in individuals during the performance of this work. Size of sample was limited by the material we had at hand for collection. Allocation of individuals into groups was thus by “quota-sampling”. Age distribution resulting of this process is shown in Table 1. Serum samples were sent to the Reference Laboratory in Rio de Janeiro as soon as possible. The Laboratory was not informed of any data on any person or group. The hemmagglutination inhibition test was made in accordance with technique described by KEMPE & St. VINCENT (10).

Complement fixation test was done according described methods (15).

Neutralization test was carried out essentially as described by CUTCHINS & cols. (3) using the LLC-MK<sub>2</sub> cell strain. However, the serum virus mixtures, after the incubation period were inoculated directly into the cell culture tubes with 1 ml of Eagle basal medium. The plaques were counted by staining with 0.2 ml of 0.13% crystal violet added after incubation of racks at 35-36°C for 40-44 hours. As antigen was used a vaccinia virus (freeze-dried egg vaccine) obtained from Instituto Oswaldo Cruz and adapted to the LLC-MK<sub>2</sub> cell strain.

## Results

### 1 — Hemagglutination inhibition test:

On Table 2 and Figure 1 is shown results obtained and geometric mean titers of each group, which was lowest in PS group, followed, by VO, PV, and RV, in this order. However, statistically, PS was the only group different from the others (at 5% level).

TABLE I

AGE DISTRIBUTION OF THE UTINGA SAMPLE SUBMITTED TO  
SEROLOGICAL EXAMINATION — BAHIA, 1969

Age Group (Years)	Previous Smallpox	Primary Vaccinated	Revacci- nated	Variola in outbreak	Total
0 — 4	—	1	—	1	2
5 — 14	1	10	5	38	54
15 — 49	12	4	10	14	40
50 +	2	—	—	1	3
<b>Total</b>	15	15	15	54	99

FIGURE 1

HEMAGGLUTINATION INHIBITION TEST WITH VACCINIA ANTIGEN :  
GEOMETRIC MEAN TITERS OF THE GROUPS OF THE UTINGA  
SAMPLE - BAHIA , 1969 .

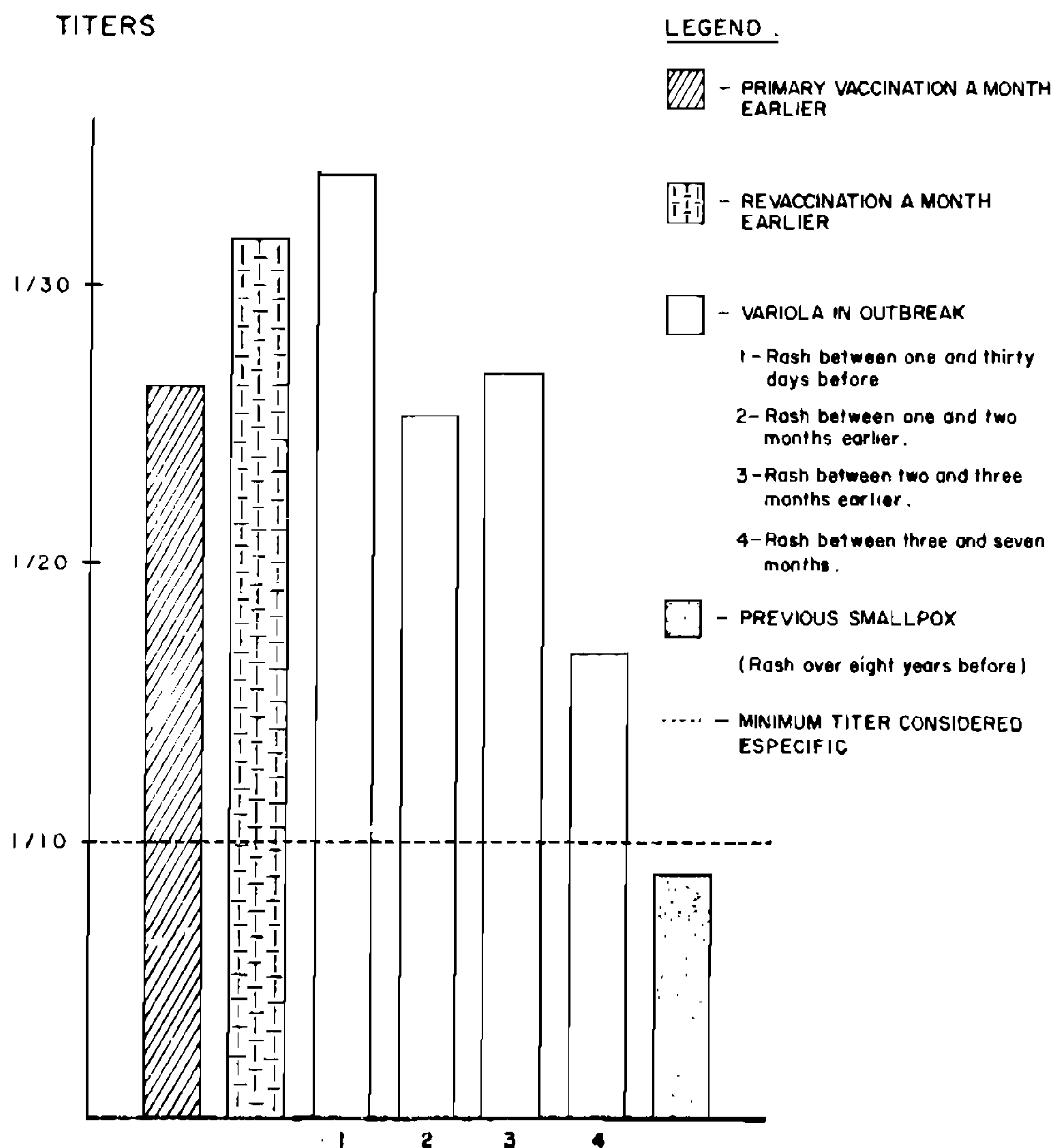


FIGURE 2

HEMAGGLUTINATION INHIBITION TEST RESULTS AND GEOMETRIC MEAN TITERS AGAINST VACCINIA ANTIGEN AMONG PATIENTS WHO HAD HAD VARIOLIC RASH SINCE MAY 13 - UTINGA SAMPLE BAHIA, 1969.

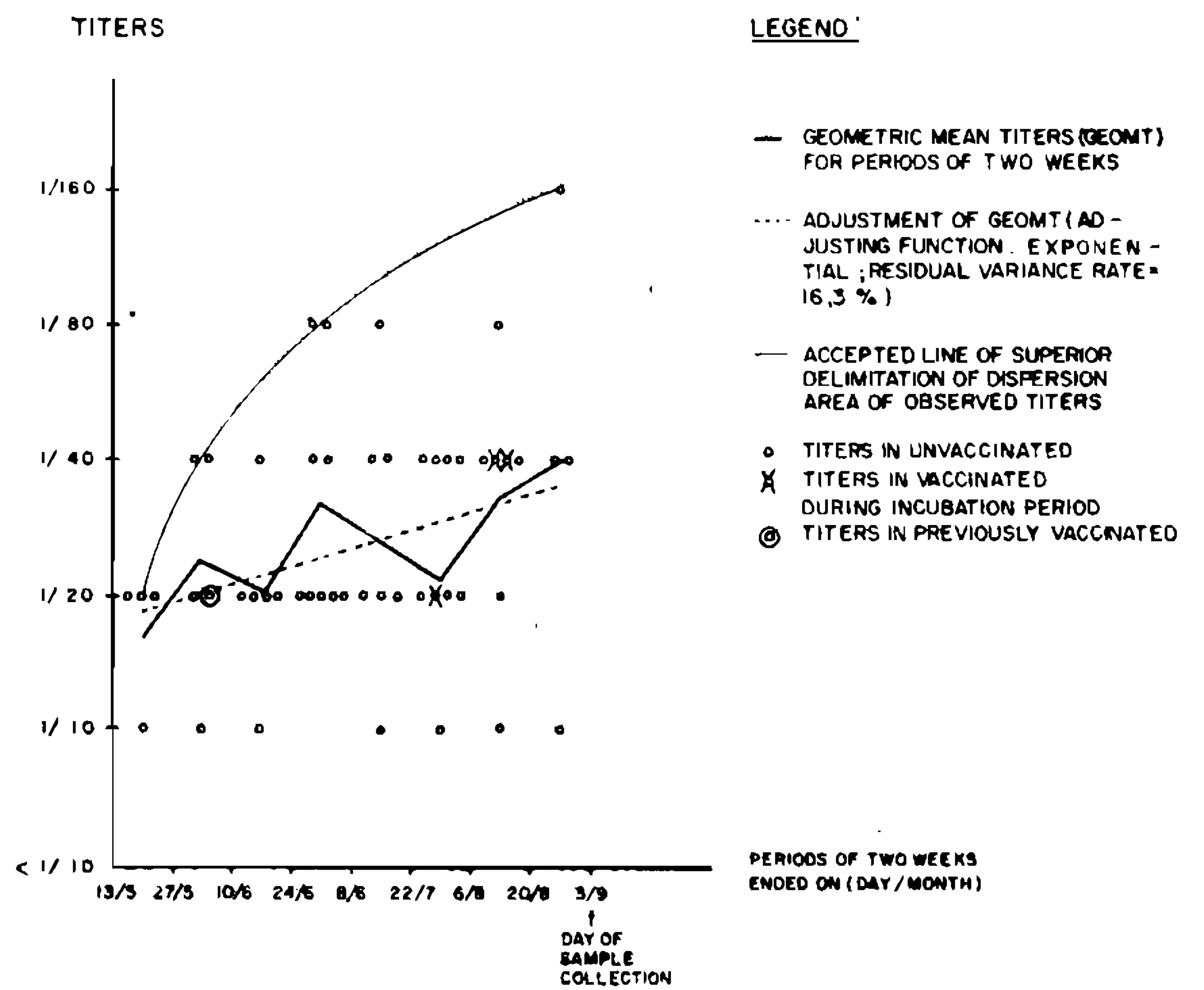
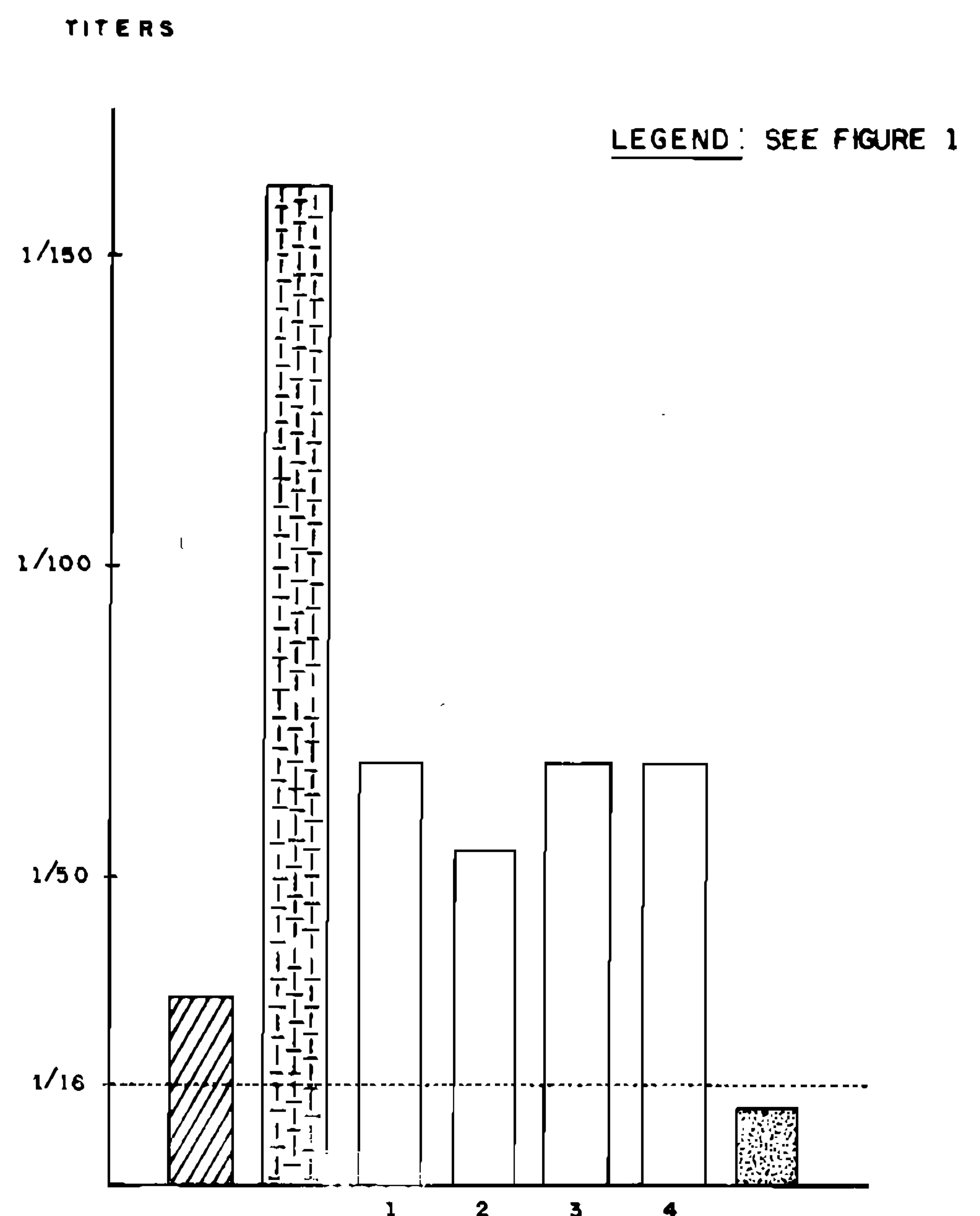


FIGURE 3

NEUTRALIZATION TEST WITH VACCINIA ANTIGEN : GEOMETRIC MEAN TITERS OF THE GROUPS OF THE UTINGA SAMPLE - BAHIA , 1969



In case of VO group the farther we went in time the lower titers became. This phenomenon was studied separately in Figure 2, where the retrospective observation was limited to May 13 because before this date we did not have at least three cases for each period of two weeks. The exponential proved to be the best adjusting function, and laboratorial tests were all positive for the 16 weeks record.

2 — Neutralization test:

Some sera could not be test for they proved to be toxic to cells.

Remainning sera are tabulated in Table 3, and presented graphically in Figure 3. The highest geometric mean

TABLE II

HEMAGGLUTINATION INHIBITION TEST: SEROLOGICAL TITERS AGAINST  
VACCINIA ANTIGEN OBSERVED IN THE UTINGA SAMPLE — BAHIA, 1969

Titers	Previous smallpox	Primary vaccinated	Revacci- nated	Variola in Outbreak				
				Total	Feb-May	June	July	Aug-Sept
< 1/10	7(1)	—	—	—	—	—	—	—
1/10	5(2)	2	3(2)	10	5	1	2	2
1/20	2	7	3(1)	22(ab)	4(a)	7	9	2(b)
1/40	1	4	5	17(bb)	2	2	6	7(bb)
1/80	—	2	4	4	—	2	1	1
1/160	—	—	—	1	—	—	—	1
Total	15(3)	15	15(3)	54	11	12	18	13
Geometric mean titer	1/8,71 ( < 1/10)	1/26,39	1/31,75	1/25,20	1/16,55	1/26,70	1/25,20	1/34,09

(n) — Number of individuals with close contact with smallpox patients.

a — Smallpox in individual previously vaccinated.

b — Represents one individual presenting vaccinia and smallpox infections simultaneously.



titer was that for RV, next highest that of VO, then PV, and, finally, PS group. The test showed that all four groups differed significantly from one another and that this difference was maintained even when the VO group was shown through recent cases (July to September).

On the other hand, there were a clear stability of geometric mean titers according to the several months in which cases occurred (VO group). Figure 4 shows the development of the phenomenon up to 16 weeks from the beginning of variolic rash. The best adjusting function was a 2nd degree parabola. It should be noted that a great variation in results was

FIGURE 4

NEUTRALIZATION TEST: RESULTS AND GEOMETRIC MEAN TITERS AGAINST VACCINIA ANTIGEN AMONG PATIENTS WHO HAD HAD VARIOLIC RASH SINCE MAY 13 - UTINGA SAMPLE - BAHIA - 1969.

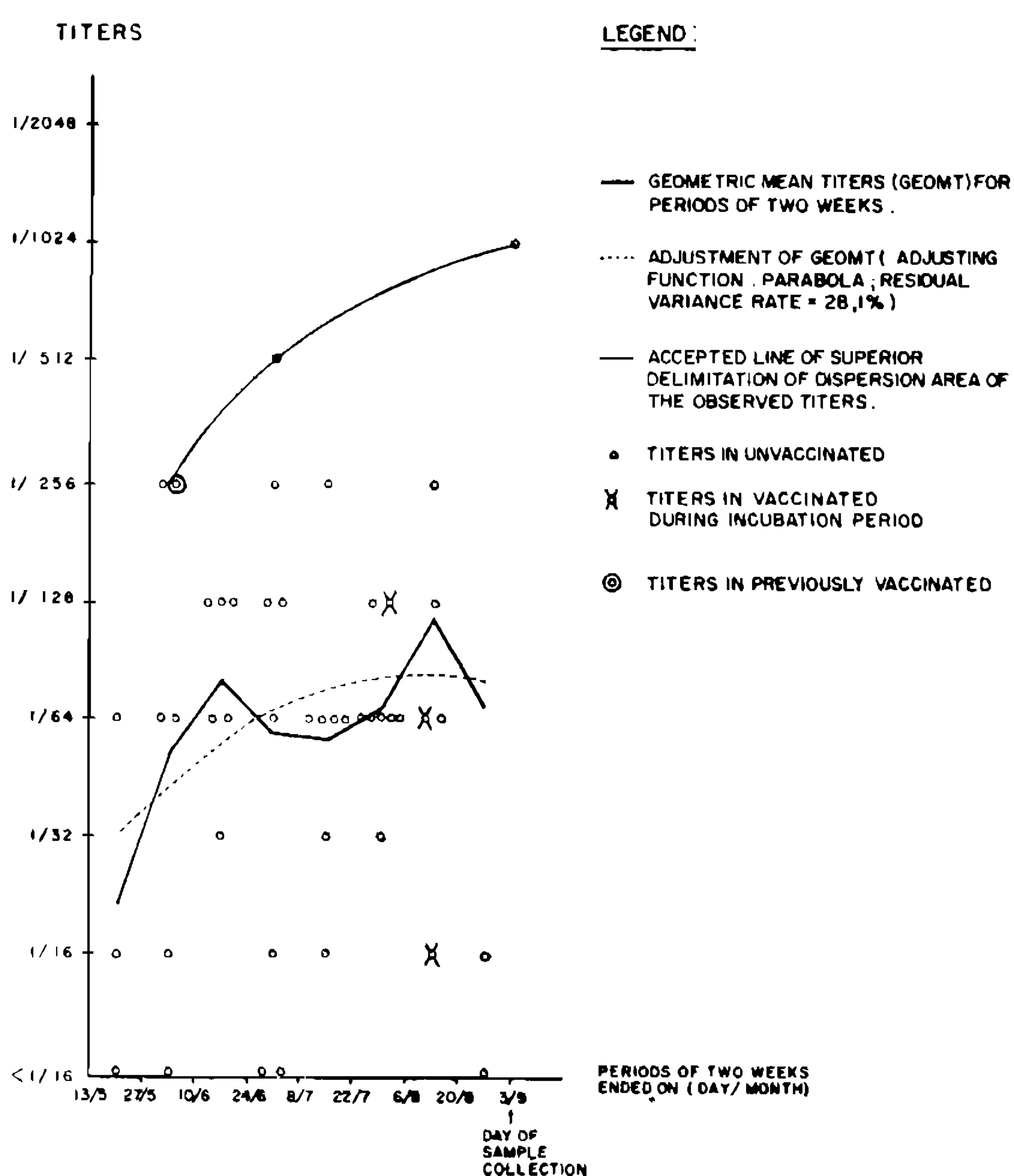
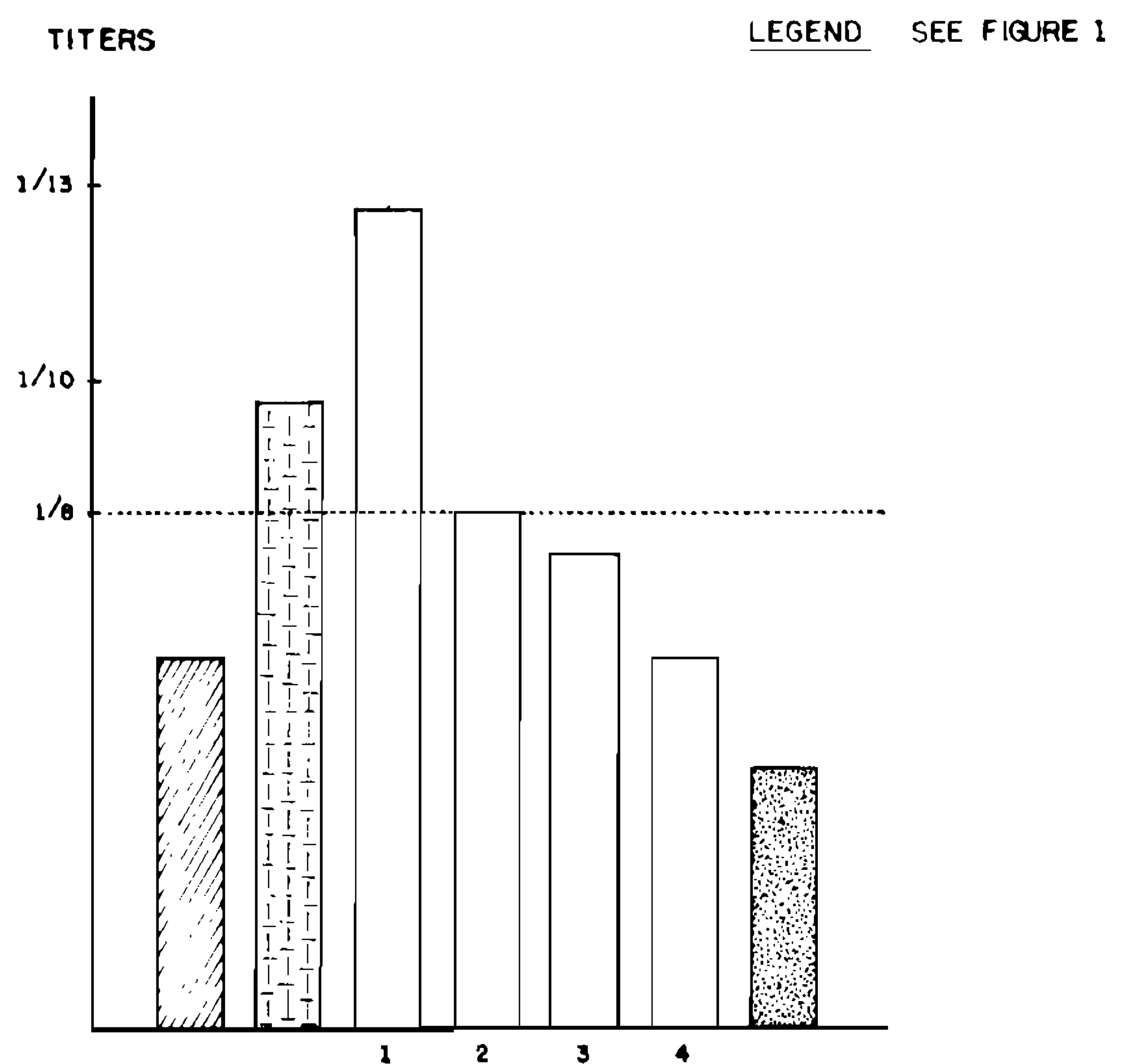


FIGURE 5

COMPLEMENT FIXATION TEST WITH VACCINIA ANTIGEN: GEOMETRIC MEAN TITERS OF THE GROUPS OF THE UTINGA SAMPLE - BAHIA, 1969



found, but up to the last period of observation the curve of geometric mean titers stayed within the criterion of positivity.

### 3 — Complement fixation test:

As can be noted from totals in groups of Table 4 many samples of serum were not titrated due to anti-complementary shown.

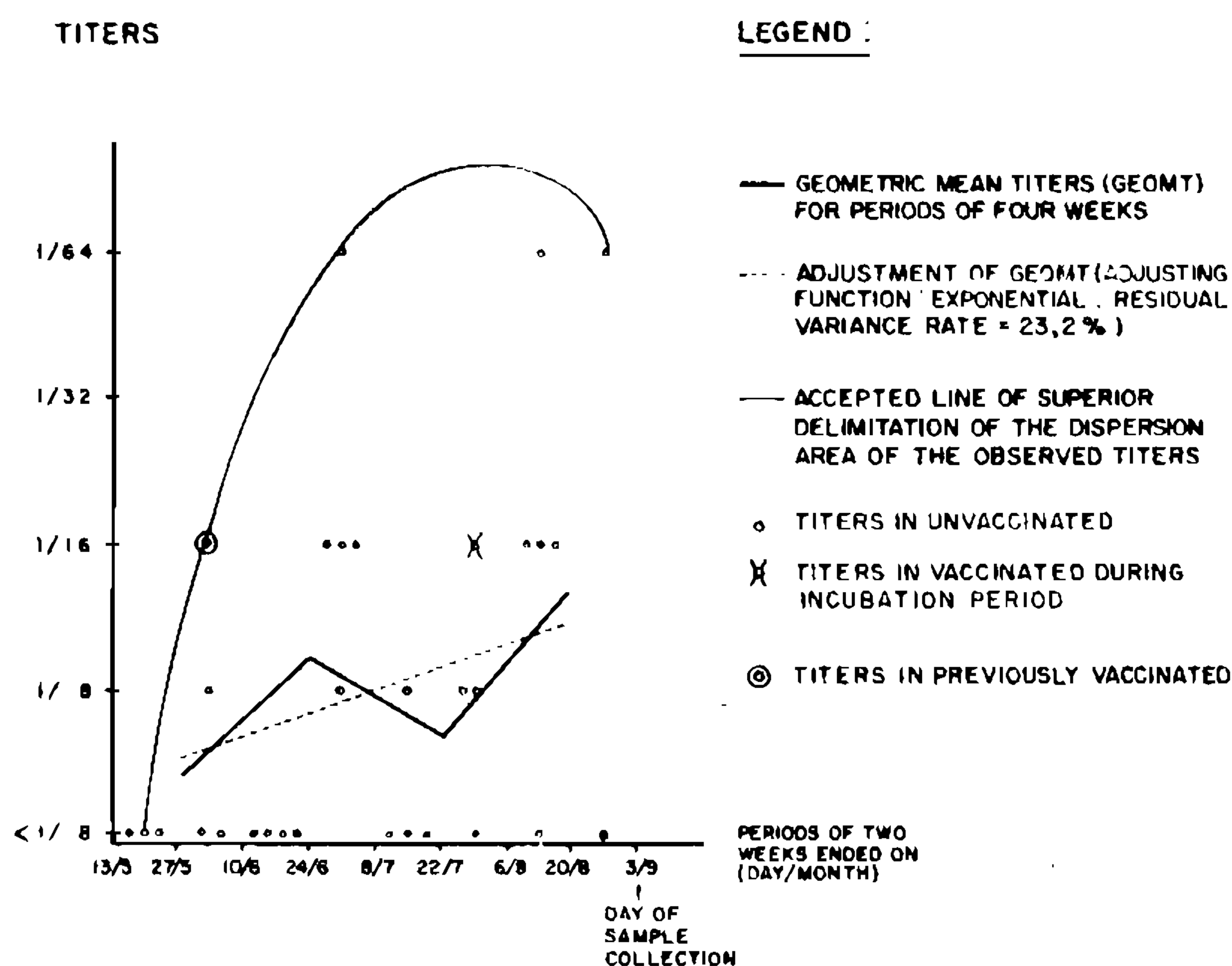
The geometric mean titer was higher among RV than in other groups, which, excepting cases of VO, showed it below the limit of positivity (Table 4 and Figure 5). The PS group did not provide any positive sample and the VO group exhibited a diffe-

rence in titers in accordance to the time elapsed, which was better represented by an exponential curve (Figure 6). It is worth to note that after the tenth week only two samples showed positive results: one on the titer limit and the other represented by a case previously vaccinated.

Finally, no significant difference was found between vaccinated individuals (PV plus RV group) and recent cases of smallpox (July to September sub-group of VO).

FIGURE 6

COMPLEMENT FIXATION TEST: RESULTS AND GEOMETRIC MEAN TITERS AMONG PATIENTS WHO HAD HAD VARIOLIC RASH SINCE MAY 13 - UTINGA SAMPLE - BAHIA, 1969.



### Discussion

The several circulating antibodies which can be detected in infections by the pox group are not necessarily related, since they are produced by different antigenic fractions. However, since the characteristics of the ap-

pearance of such antibodies are already known, they can be used for diagnosis, evaluation of the immunizing capacity of a vaccine, and several related purposes.

The titration of such antibodies depend on factors inherent to each technique, each laboratory, and each technician Downie & cols. (7). Therefore, it is extremely important to establish and to describe accurately the objective criteria, the determinables ones used by different institutions.

Anyway, even if the possibility of comparing results found by different researchers is rendered more difficult, the possibility is still yielded by results obtained by each laboratory.

Preliminary considerations are needed as to the gathering of information that made possible the organization of groups:

Although criterion was very objective in regards to vaccination — the presence of a vaccination scar — this was not true in cases of previous smallpox. Since facial scars, or other, occur but infrequently in our field observations, a case history of the patient becomes essential. However, considering the characteristics of the disease and the information that everybody has on it, the percentage of error is negligible. Another difficulty is the exact dating of the beginning of the disease when it had occurred three or more months previously, especially in rural populations with low cultural level. The sorting of this information into monthly or, at least fortnightly periods, allows us to lower the margin of error.

It is important also to remind that some individuals of PS, PV and RV



groups might have been affected by sub-clinical infections during this outbreak, possibility pointed out by **Kempe & cols.** and **Salles Gomes & cols.** (9, 14). One attempt to evaluate this effect would be to look at the titers of individuals who had close contact with smallpox cases (living in the same dwelling). It seems, by the distribution of such cases (Table 2, 3, and 4), that all individuals came from the same population regarding titers, for each group and test.

After those preliminary remarks we are ready to discuss our findings.

Generally speaking, antibodies found in different groups are as was to be expected **Downie & Kempe** (4), **Downie & McCarthy** (5), **Downie & cols.** (6), **Downie & cols.** (7), **McCarthy & cols.** (12) and **Noble & cols.** (13).

Among those receiving primary vaccination and revaccination, no individuals had HI titers below 1/10; although some had neutralizing antibodies below 1/16 and CF antibodies of less than 1/8.

The geometric mean titer of antibody among vaccines in one group were significantly higher than those found by **Downie & cols.** (7), but similar to those detected sixteen days after vaccination by **McCarthy & cols.** (12) except for HI titers among primary vaccinated individuals whose titers higher than ones and even than their on revaccinated individuals.

The group that had smallpox some years earlier (PS group) had also similar antibody titer levels to those published by **Downie & McCarthy** (5).

This is would appear that the techniques used in our laboratory are not less sensitive than those used

elsewhere, but it is important to notice that those series are not necessarily comparable as regarding to many others attributes of individuals.

Some differences between this and other studies, however, were found in regard to antibody titer levels in recent cases of smallpox (VO group).

Comparisons are recognizedly difficult as most previous papers are concerned with patients who experienced variola major and/or had many previously vaccinated subjects. Most comparable are individuals studied by **McCarthy and Downie** (11), seventeen samples of serum taken from "alastrim" patients (mean time from onset: thirteen days), and by **Downie & cols.** (6) thirteen samples taken from unvaccinated non-haemorrhagic variola major patients (mean time from onset: thirteen days). Our eleven samples were obtained from Utinga patients with a mean time from onset of disease of sixteen days and as in the other studies, also by **Noble & cols.** (13), were obtained in the first four weeks of disease.

The geometric mean neutralizing and CF antibody titers in our study were higher than in the other two series, but those differences were not significant at the 5% level. Some of our cases lacked CF antibodies, an observation also made by **Herrlich & cols.** (8), by **Salles-Gomes** (personal communication) and also by **Noble & cols.** (13) in variola minor patients.

The geometric mean titers of HI antibody, however, were lower than in the other two series. The difference was not significant at the 5% level from the serie reported by **McCarthy & Downie**, "alastrim" group (11), but

TABLE III  
NEUTRALIZATION TEST: SEROLOGICAL TITERS AGAINST VACCINIA  
ANTIGEN OBSERVED IN THE UTINGA SAMPLE — BAHIA, 1969

Titers	Previous smallpox	Primary vaccinated	Revacci- nated	Variola in Outbreak				
				Total	Feb-May	June	July	Aug-Sept
<1/16	8(2)	3	1	5	1	1	2	1
1/16	3(1)	4	—	6(b)	2	1	1	2(b)
1/32	2	1	3	4	1	1	2	—
1/64	1	2	1	18(b)	3	4	7	4(b)
1/128	—	2	—	8(b)	—	4	2	2(b)
1/256	—	1	2(1)	6(a)	3(a)	—	2	1
1/512	—	—	2	2	1	1	—	—
1/1024	—	—	3(1)	1	—	—	—	1
<b>Total</b>	<b>14(3)</b>	<b>13</b>	<b>12(2)</b>	<b>50</b>	<b>11</b>	<b>12</b>	<b>16</b>	<b>11</b>
<b>Geometric mean titer</b>	<b>1/13,13 ( &lt; 1/16)</b>	<b>1/30,34</b>	<b>1/161,20</b>	<b>1/65,76</b>	<b>1/68,16</b>	<b>1/67,81</b>	<b>1/53,82</b>	<b>1/68,16</b>

(n) — Number of individuals with close contact with smallpox patients.

a — Smallpox in individual previously vaccinated.

b — Represents one individual presenting vaccinia and smallpox infections simultaneously.

TABLE IV

COMPLEMENT FIXATION TEST: SEROLOGICAL TITERS AGAINST VACCINIA ANTIGEN OBSERVED IN THE UTINGA SAMPLE — BAHIA, 1969

Titers	Previous smallpox	Primary vaccinated	Revaccinated	Variola in Outbreak				
				Total	Feb-May	June	July	Aug-Sept
<1/8	11(3)	4	3	15	5	5	3	2
1/8	—	1	4(1)	5	2	1	2	—
1/16	—	1	3(1)	8(ab)	1(a)	1	3	3(b)
1/32	—	—	—	—	—	—	—	—
1/64	—	—	1(1)	2	—	1	—	1
<b>Total</b>	<b>11(3)</b>	<b>6</b>	<b>11(3)</b>	<b>30</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>6</b>
<b>Geometric mean titer</b>	<b>1/4</b> ( < 1/8)	<b>1/5,66</b> ( < 1/8)	<b>1/9,66</b>	<b>1/7,82</b> ( < 1/8)	<b>1/5,66</b> ( < 1/8)	<b>1/7,34</b> ( < 1/8)	<b>1/8</b>	<b>1/12,7</b>

(n) — Number of individuals with close contact with smallpox patients.

a — Smallpox in individual previously vaccinated.

b — Represents one case presenting vaccinia and smallpox infections simultaneously.



significantly different from the titers reported by Downie & cols. (6) unvaccinated non-haemorrhagic variola major group ( $P < 0.001$ ).

Although such analyses would not resist to a careful criticism it could support previous field observations that HI titers found in smallpox cases in Brazil should not to be expected in the levels found in variola major as usually reported in literature.

### Sumário

Quatro semanas após a Vacinação de Contenção, realizada com a finalidade de controlar o maior surto de varíola ocorrido em 1969, no Brasil, no município de Utinga na Bahia, 99 amostras de sangue foram coletadas da população local.

Estas amostras foram classificadas em quatro grupos:

a) — Indivíduos com uma história de varíola anterior ao início do surto de 1969 (15 soros); "Grupo varíola prévia";

b) — Indivíduos com vacinação primária, sem história de varíola por ocasião da vacinação de contenção (15 soros), "Grupo primo-vacinados";

c) — Indivíduos sem história de varíola prévia, revacinados com "pega" por ocasião da vacinação de contenção (15 soros), "Grupo revacinado";

d) — Indivíduos que contraíram varíola no surto de 1969 (54 soros), os quais, foram subdivididos em quatro subgrupos, de acordo com as datas

em que ocorreram os casos "Grupo varíola no surto".

Os estudos sorológicos foram realizados através testes de inibição da hemaglutinação, neutralização e fixação do complemento.

Observou-se que os títulos de anticorpos inibidores foram significativamente mais baixos em casos de varíola prévia de que nos outros grupos. Embora estes anticorpos se apresentassem ligeiramente mais elevados em indivíduos revacinados do que nos grupos de vacinação primária e com varíola no surto, esta diferença não foi significativa. Estes mesmos anticorpos estavam presentes em todos os casos de varíola no surto e foi possível mostrar que os títulos decresciam em relação direta ao tempo decorrido após a doença clínica.

Anticorpos neutralizantes mostraram ser significativamente mais elevados no grupo revacinado do que no grupo com varíola no surto e mais elevados neste último grupo do que nos grupos primo-vacinados e com varíola prévia.

As provas de fixação do complemento não puderam ser realizadas em todas as amostras, porque muitas delas mostraram atividade anticomplementar. No entanto, foi possível demonstrar que os anticorpos fixadores do complemento diminuem rapidamente, tornando-se negativos nos casos de infecções antigas. Finalmente, os autores mostram que os títulos inibidores da hemaglutinação são mais baixos na varíola minor, em condições brasileiras do que na varíola major.

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