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POTENCY CONTROL OF LIVE, ATTENUATED VACCINES AGAINST MEASLES USED IN CHILDREN VACCINATIONS IN THE STATE OF SÃO PAULO, BRAZIL (1976-1980)

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SUMMARY

Through the inoculation of serial dilutions of reconstituted live attenuated vaccines against measles in cultures of Vero cells, 161 different lots of vaccines imported from England, France and Belgium for the vaccination of infants of the State of São Paulo, Brazil, were titrated for potency. In the conditions of the test, titer failures of 20.0, 11.5 and 25.0% were observed among the tested vaccine lots imported from England, France and Belgium, respectively. None of the 72 lots imported from France as bulk and freeze-dried in Brazil has been impugnated due to lack of titer.

INTRODUCTION

Measles infection, a highly infectious disease in childhood can be prevented by vaccination which represents the most effective control measure available.

In Brazil, from 1968 to 1979, live attenuated vaccines for vaccination against measles were imported from France, U.S.A., England and Belgium. Since 1979, vaccines imported from France as bulk and submitted to the final steps of lyophilization in Rio de Janeiro, are being employed in the vaccination programs.

Since 1976 the potency of the measles vaccines used in children vaccinations in the State of São Paulo, is surveyed in routine basis at the Instituto Butantan. The main purpose is to prevent the administration of vaccines with titers under 1,000 $TCID_{50}$ per dose, considered as the minimum viral concentration capable of triggering the development of specific immunity against measles, according to international regulations [1].

MATERIAL AND METHODS

Vaccines

161 Lots of live attenuated vaccines against measles imported from Belgium (28 lots), England (35 lots), and France (26 lots) were titrated, as well as 72 lots imported from France in the form of bulk and freeze-dried in Rio de Janeiro (Brazil). Vials were stored at -20°C and protected against light until reconstitution, prior to the test.

Titration

Three vials containing 1-10 doses each were reconstituted, pooled, serially diluted (10^{-3} to 10^{-5}) in Eagle's medium [2] and kept in ice-bath, protected from light. Confluent cultures of Vero cells growing in Eagle's (0.17% $NaHCO_3$) with 10% calf serum and 25 mcg/ml Neomycin had their supernatants decanted and each of 8 tubes were inoculated with 0.5 ml of each virus dilution. After 60 min at 36.5°C for virus adsorption, the inoculated tubes received 1.5 ml of maintenance Eagle (0.22% $NaHCO_3$) plus 2% calf serum, 25 μg Neomycin per ml, and were held at 36.5°C for 7 days.

The cytopathic effect observed microscopically was recorded at the 4th, 6th and 7th day, when the titer was calculated by the REED & MÜENCH method [3]. Titrations were performed in duplicate and in parallel to a measles reference virus (titer $10^{3.5} \pm 0.5$ $TCID_{50}$/ml). The

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titer was expressed in tissue culture infective doses 50% ($TCID_{50}$). Neutralization of the maintenance medium was carried out on the 3th post-inoculation day by the addition of up to 0.1 ml of 2.5% $NaHCO_3$ solution per tube, and when necessary the medium was changed on the 5th day.

RESULTS

Data obtained in the titrations performed with vaccines imported from England, Belgium and France are shown in Fig. 1. In the conditions of the test a few lots of each origin failed to comply with the international minimum requirement titer of 1,000 $TCID_{50}$ per dose and thus, were discarded. It was noted that the highest titer presented by vaccines coming from the three different manufacturers was 5,000 $TCID_{50}$ per 0.5 ml dose.

Table I shows the percentages of titer failures found for the total number of vaccine lots titrated. From the 26 lots imported from France 11.5% had titers below the minimum allowed, while vaccines from England and Belgium presented titer failures of 20.0 and 25.0% respectivelly. Titers varying from 1,000 to 5,000 $TCID_{50}$ per dose were recorded, while among the 72 lots whose final steps of manufacturing were performed in Brazil no titer failure was observed. In the same group titers varied from 1,500 to 16,000 $TCID_{50}$ per dose.

TABLE I

Titer failures observed within the 161 measles vaccine lots of different origin

Vaccine Manufacturer	Lots titrated	Lots impugnated
England	35	7 (20.0%)
France	26	3 (11.5%)
Belgium	28	7 (25.0%)
France — (Brazil)	72	0 (0.0%)

DISCUSSION

The checking of potency titer of vaccines against measles is carried out as a routine procedure by the manufacturer to fulfill specific requirements for the release of the product.

Immunization against measles in São Paulo was started in 1968 using imported vaccines. In some occasions the vaccine containers delivered by air freight in dry ice, were held at the customs longer than expected and thus, a criteria was established that all vaccines should be submitted to titration prior to administration to make sure they had not suffered any loss of titer. The storage of the freeze-dried vaccine is done at -20°C and unless the results of the potency tests comply with the international regulation which states that an individual human immunizing dose shall be no less than 1,000 $TCID_{50}$ in terms of the assigned titer of a reference measles virus [1], the vaccine lote is impugnated and discarded.

Although a limit for minimum titer exists, there is no indication as to the maximum level of $TCID_{50}$ (tissue culture infective dose 50%) allowed per human dose. Carrying out the titration of 161 lots of live, attenuated vaccines against measles administered to children from 1976 to 1978, it was noted that among vaccines imported from England, Belgium and France, while the highest titer recorded was 5,000 $TCID_{50}$ per dose, a percentage of titer failures occurred, and vaccines had to be discarded. The vaccine lots whose final steps of filling and freeze-drying were performed in Brazil showed no failures. Their titers varied from 1,500 to 16,000 $TCID_{50}$ per dose, higher than the titers presented by the other three vaccines studied, probably because the bulk was dispensed straight, without further dilution, and lyophilized.

The results obtained led to the conclusion that for the studied period of 1976-1980, the quality of live attenuated vaccines against measles administered to infants from the State of São Paulo, as far as potency titers were concerned, was in accord to the international requirement of minimal concentration per human dose, considering that lots showing insufficient number of viral particles per dose were discarded.

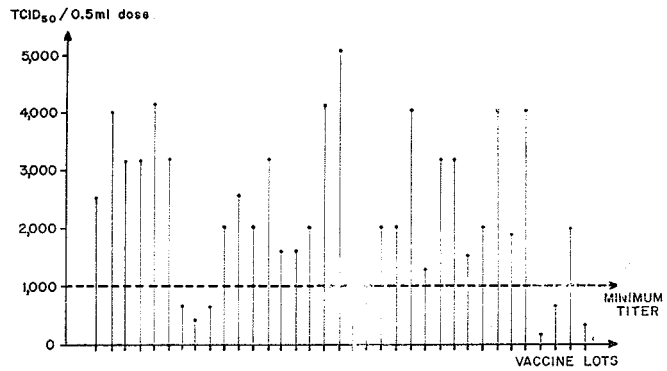
RESUMO

Controle de potência de vacinas vivas, atenuadas, contra o sarampo, utilizadas em vacinações da população infantil do Estado de São Paulo, Brasil (1976-1980)

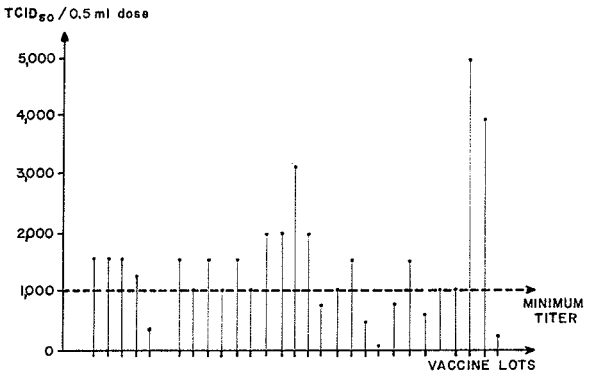
F I G U R E 1

POTENCY TITER OF VACCINES AGAINST MEASLES USED IN CHILDREN VACCINATION IN THE STATE OF SÃO PAULO, BRAZIL (1976-1978).

LIVE ATTENUATED VACCINE AGAINST MEASLES (SCHWARZ STRAIN)
ORIGIN: ENGLAND



LIVE ATTENUATED VACCINE AGAINST MEASLES (SCHWARZ STRAIN)
ORIGIN: BELGIUM



LIVE ATTENUATED VACCINE AGAINST MEASLES (SCHWARZ STRAIN)
ORIGIN: FRANCE

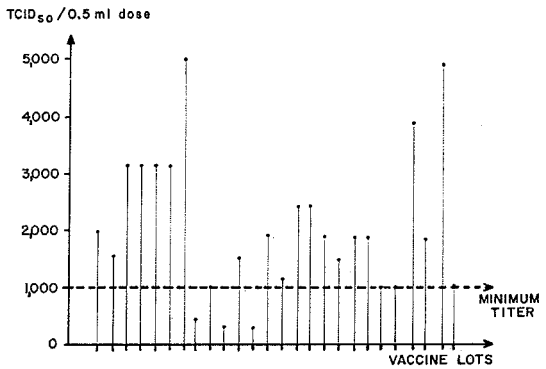
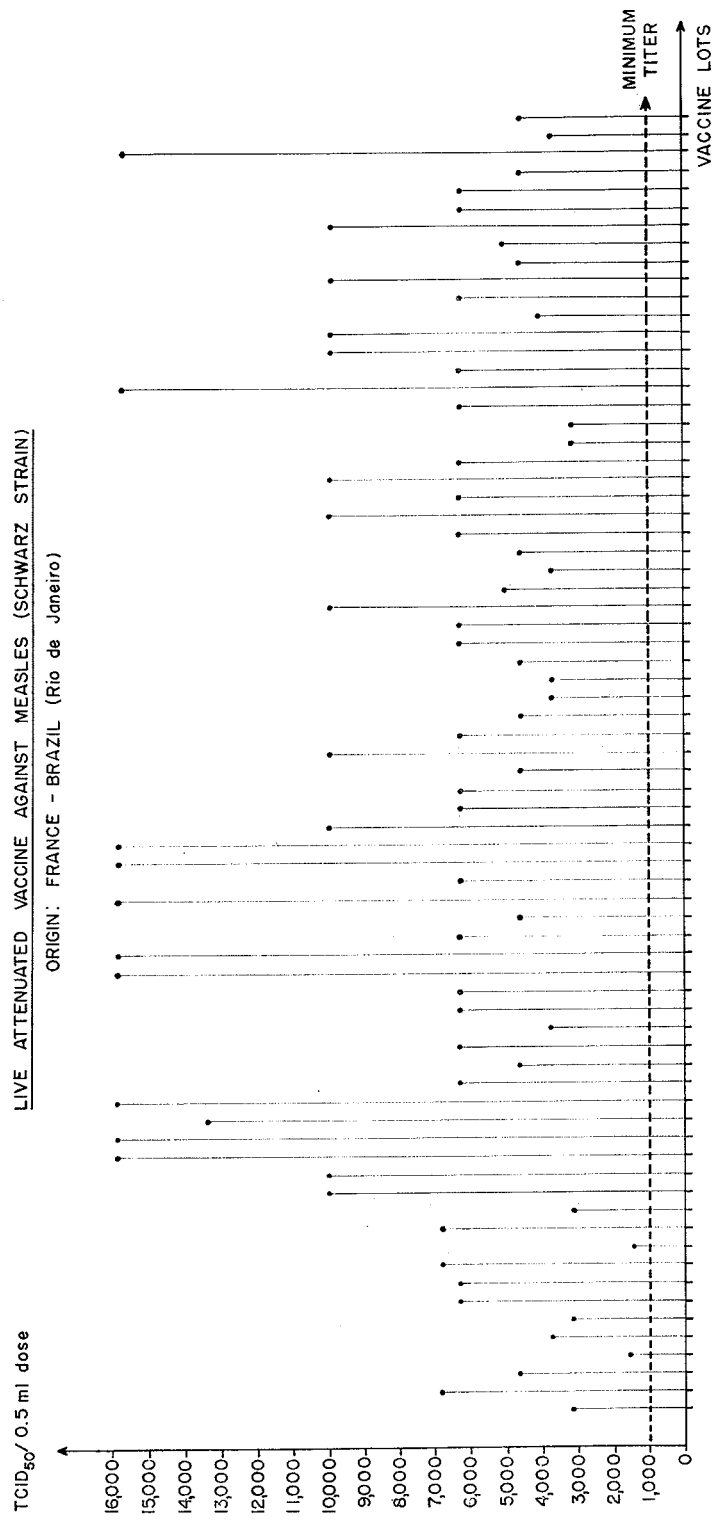


FIGURE 2

TITER OF VACCINES AGAINST MEASLES ADMINISTERED TO INFANTS IN THE STATE OF SÃO PAULO, BRAZIL (1979-1980).



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Pela inoculação de diluições seriadas da vacina viva, atenuada, contra o sarampo, em culturas de células Vero, foram titulados para controle de potência, 161 lotes de vacinas importadas da Inglaterra, França e Bélgica, destinadas à vacinação da população infantil do Estado de São Paulo. Nas condições do teste, 20,0, 11,5 e 25,0% dos lotes de vacina testadas, importadas da Inglaterra, França e Bélgica respectivamente, não apresentaram o título mínimo exigido; dentre os 72 lotes importados da França e liofilizados no Brasil, não ocorreram impugnações devidas a insuficiência de título.

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