Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1963, **29**, 311-322

A Comparative Study of Clinical Reaction observed after Application of Several Smallpox Vaccines in Primary Vaccination of Young Adults

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Four smallpox vaccines from different production laboratories were compared in primary vaccination of young adults. Morbidity rate, high fever rate and prolonged fever rate, as defined in this report, were used as gauges for pathogenic potency. Special batches were prepared, in addition, from two of these vaccines after three passages on calf skin. Two vaccines, prepared from the Elstree strain—a sheep lymph and its third calf-passage—were found to be of low pathogenicity. Two calf lymphs, prepared from the Copenhagen strain and the Bern strain respectively, caused clearly higher rates of illness and of height and duration of fever. Two calf lymphs from the Ecuador strain took up an intermediate position.

Vaccine potency, as assessed on the chorio-allantoic membrane of developing chick embryos, seems to be of no significance for the course of vaccinia disease caused by two pustules. Besides the pathogenicity of the vaccinia strain, ill-defined extraneous factors might be of importance for the degree of illness observed after primary smallpox vaccination.

INTRODUCTION

In order to assess the dose-effect relations for glycerolated smallpox vaccines, prepared by the Lister Institute of Preventive Medicine (England) and the Rijks Instituut voor de Volksgezondheid (National Institute of Public Health, Netherlands), 450 primary vaccinations were performed in an army unit. For young adult males, the infectivity per pock-forming unit (PFU) on the chorio-allantoic membrane (CAM) appeared to be equal for both vaccines (Polak et al., 1962). The rate of admission to sick quarters after successful primary vaccination with development of two pocks was, however, considerably higher for vaccine R, the Netherlands lymph, than for vaccine L, the English lymph. For that reason it was decided to extend the observations on that point and to include in a comparative study also vaccines of other origin, which were supposed to be of low pathogenic potency.

Furthermore, the significance of an apparently minor difference in production of vaccines R and L was tested, as well as the importance of vaccine potency, measured on the CAM of the developing chick embryo as PFU per ml.

When it emerged clearly that two vaccines of extraneous origin were less pathogenic for young adults than the local product (vaccine R), these vaccines were additionally introduced into this study after three calf-passages completed in the National Institute of Public Health, Utrecht.

MATERIAL AND METHODS

Vaccines (Table 1)

Vaccine R represents the lymph since 1956 in common use in the Netherlands. It is derived through one rabbit-passage and three calf-passages from vaccinia strain no. 9-521, received from the Statens Seruminstitut, Copenhagen.

Vaccines R-A and R-B, both prepared from the same pulp, belong to another batch of the production in the Netherlands. For R-A the routine scheme of preparation was followed, inasmuch as the calf pulp was treated with 1% phenol for 24 hours at 22°C, and glycerol was then added. For R-B, to imitate more closely a phase in production of vaccine L,

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storage with 1% phenol for 24 hours at 22°C was followed by storage for 12 days at 4°C; thereafter glycerol was added.

Vaccine L is a batch of sheep lymph from the routine production of the Lister Institute of Preventive Medicine, Elstree, provided by Dr C. Kaplan.

Vaccine L-R3 was prepared in the National Institute of Public Health, Utrecht, from L-vaccine through three calf-passages.

Vaccine M, a calf lymph from the Bayerische Landesimpfanstalt, Munich, is prepared with the Bern strain. We received one lot of this vaccine from Professor Herrlich. The Bern strain is recommended in the *Abhandlungen aus dem Bundesgesundheitsamt* (Germany, Federal Republic, 1959) for production of smallpox vaccine.

Vaccine E, a calf lymph, prepared in the Statens Seruminstitut, originates from a smallpox vaccine of the Instituto Nacional de Higiene "Leopoldo Izquieta Pérez", Guayaquil, Ecuador. At the Statens Seruminstitut the Ecuador vaccine, received in 1956, was given two consecutive series of cutaneous passages, the first consisting of three rabbit- and two calf-passages, the second of three rabbit- and three calf-passages. The freeze-dried Ecuador vaccine which was used in the WHO International Assay on Smallpox Vaccine [1] had been given only the first series of passages. The batch received from Dr E. Krag Andersen had been submitted to both series of passages. At the Statens Seruminstitut the Ecuador strain was found to be of low virulence for new-born mice and new-born rabbits, and it maintained this quality in spite of the two series of passages in rabbits and calves (E. Krag Andersen, personal communication).

Vaccine E-R3 was prepared in the National Institute of Public Health, Utrecht, from vaccine E through three calf-passages.

These batches were tested for potency (PFU) on the CAM of chick embryos several times (10 eggs per test). The median values found are indicated in Table 1. The vaccines were stored at −70°C in tubes containing 1-1.5 ml.

During the vaccination session, the lymphs were used in small bottles with code numbers on the labels.

Vaccinations

The vaccinations were performed, in three army units, with 21 trial groups (see Table 1). Two vaccinators, making two linear scratches of about 3 mm per inoculation on the deltoid region of the skin, were working simultaneously according to a programme fixed in advance. This programme fostered as far as possible an even distribution of the tested vaccines, as regards vaccinators and army sub-units. Each vaccinator used the contents of a bottle for a number of consecutive inoculations. This number varied in the programme from 4 to 11, depending on the total estimate of primary vaccinations for the session and the number of vaccines to be tested.

About one minute after vaccination, the vaccinee received, by intramuscular injection in the other arm, 2 ml of 16% vaccinia hyperimmune gamma-globulin for prevention of postvaccinal encephalitis (Nanning, 1962).

Clinical reaction

Measurement was made of the clinical reaction of those vaccinees showing a typical primary vaccinia with two pocks on or about the eighth day (in this report the vaccination day is taken as the first day). The frequency and the grade of observed clinical reactions are employed for assessing the pathogenic potency of a vaccine.

Morbidity rate

The proportion of bed-patients to successful vaccinations represents the morbidity rate. In this connexion, a difference of policy between unit A and units B and C is of importance.

In unit A, a vaccinee who felt ill reported to sick quarters and, if the body temperature amounted to 38.0°C or higher, was admitted. When he had no fever, he was admitted only if there were special reasons.

In units B and C the vaccinees themselves decided whether or not to become bed-patients, but their discharge was determined by the medical officer. This policy would, of course, entail a higher "morbidity rate". It was decided to accept a vaccinee who stayed in bed by day as a bed-patient if his temperature surpassed 37.9°C during his illness.

The usual medical care was given to all bed-patients, and their temperature was taken daily, generally twice. The highest temperature observed on each day was used for further calculations.

Soldiers are not allowed to leave their garrison for 18 days after primary vaccination; there is hence little reason for a vaccinee who feels ill to withdraw from admission to sick quarters.

[1] Krag, P., Weis Bentzon, M. & Olesen Larsen, S. (1962) *Draft report on the WHO International Assay on Smallpox Vaccine* (Unpublished document WHO/BS/546). See also the article on p. 299 of this issue.

TABLE 1

SUMMARY OF APPLIED VACCINES, TRIAL GROUPS AND OBSERVED MORBIDITY RATES

Characteristics of vaccines														Army unit	Date of vaccination	Trial group number
Vaccinia strain	Copenhagen						Elstree				Bern	Ecuador				
Notation in text	R			R-A		R-B	L			L-R3	M	E	E-R3			
Vaccine producer	R.I.V.			R.I.V.		R.I.V.	L.I.			R.I.V.	B.L.	S.S.I.	R.I.V.			
Batch mark	59134			6032 A		6032 B	3768			3rd calf-passage of 3768	2059	3/59	3rd calf-passage of 3/59			
Log potency (PFU/ml)	8.5			9.0		8.8	9.0			8.7	7.7	8.5	9.4 [a]			
Dilution	1 : 1	1 : 10	1 : 40 and higher	1 : 1	1 : 10	1 : 1	1 : 1	1 : 10	1 : 100 and higher	1 : 1	1 : 1	1 : 1	1 : 1 [a]			
Number of bed-patients / Number of successful primary vaccinations with two pocks	4/10	5/10	3/5	—	—	—	4/10	2/8	2/10	—	—	—	—	A	27. 6.60	1
	—	—	11/15	—	—	—	—	—	6/15	—	—	—	—	A	22. 8.60	2
	—	—	8/14	—	—	—	—	—	2/10	—	—	—	—	A	17.10.60	3
	—	—	5/13	—	—	—	—	—	–/12	—	—	—	—	A	9. 1.61	4
	12/33	—	—	—	—	—	2/31	1/24	—	—	6/22	—	—	A	17. 2.61	5
	8/13	—	—	—	—	—	5/11	2/9	—	—	6/10	—	—	C	6. 3.61	6
	10/12	—	—	—	—	—	4/11	2/7	—	—	7/8	—	—	B	14. 3.61	7
	10/24	—	—	—	—	—	4/34	—	—	—	12/27	4/30	—	A	14. 4.61	8
	—	—	—	6/17	6/18	10/18	—	—	—	—	—	—	—	B	23. 6.61	9
	7/26	—	—	—	—	—	2/22	—	—	—	8/26	8/45	—	A	28. 6.61	10
	15/28	—	—	—	—	—	5/31	—	—	2/27	—	10/32	—	A	21. 8.61	11
	—	—	—	11/12	10/12	11/13	2/10	—	—	—	—	—	—	B	11. 9.61	12
	—	—	—	5/8	4/10	—	—	—	—	—	—	—	—	C	5.10.61	13
	—	—	—	7/19	4/16	5/20	—	—	—	—	—	—	—	B	5.10.61	14
	10/23	—	—	—	—	—	—	—	—	9/43	—	—	13/34	A	16.10.61	15
	10/28	—	—	—	—	—	—	—	—	16/31	—	—	—	B	28.11.61	16
	—	—	—	—	—	—	—	—	—	6/51	—	—	17/49	C	30.11.61	17
	17/40	—	—	—	—	—	—	—	—	5/41	—	—	13/41	A	5.12.61	18
	19/30	—	—	—	—	—	11/30	—	—	9/33	—	—	—	A	5. 2.62	19
	17/42	—	—	—	—	—	—	—	—	6/43	—	—	—	C	6. 2.62	20
	—	—	—	—	—	—	9/31	—	—	13/27	—	—	—	B	2. 4.62	21

R.I.V. — Rijks Instituut voor de Volksgezondheid, Utrecht.
L.I. — The Lister Institute of Preventive Medicine, Elstree.
B.L. — Bayerische Landesimpfanstalt, Munich.
S.S.I. — Statens Seruminstitut, Copenhagen.
[a] Applied in dilution 1 : 5 (log potency 8.7) in trial groups 17 and 18.

Grade of illness

(*a*) High fever rate: the rate of the number of bed-patients with observed fever surpassing 38.9°C to the total number of bed-patients.

(*b*) Prolonged fever rate: the rate of the number of bed-patients having a fever of more than 37.9°C for three or more days to the total number of bed-patients.

These criteria for grade of illness are clearly interdependent.

Cumulative 90% temperature curve

For each day that 10% or more of the individuals successfully vaccinated with the same vaccine are bed-patients, the cumulative 90% temperature point can be assessed. This point indicates, as accurately as the data allow, the temperature surpassed by 10% of the successfully vaccinated population. As the temperature data of all non-bed or ambulant patients are unknown, assessments of points below 38.0°C cannot be made. It must be admitted, moreover, that a temperature of 38.0°C or more may have occurred unobserved in an ambulant patient. Missing observations will be more numerous on a low level of fever than on a higher one. Nevertheless, construction of cumulative 90% curves for two vaccines, involved in a comparative study, gives in one glance an approximate idea of the composite effects of morbidity rate, fever height, fever duration, and interval between vaccination and fever.

All illnesses and fevers were ascribed to vaccinia infection unless—in very rare cases—there was clear-cut evidence that another cause for fever was present which could be distinguished in the temperature chart from vaccinia fever.

Comparison of vaccines in trial groups

It was supposed that differences in medical policy regarding acceptance as a bed-patient, in training conditions, in weather conditions and in eventual occurrence of other pathogenic organisms might impair the comparability of clinical reactions. Therefore, it was decided beforehand to compare the vaccines only as far as they were applied on the same days in the same trial groups. Moreover, it was decided to test the main vaccines (R, L, L-R3, M, E and E-R3) in army unit A in three sessions at least. This intention was not completely fulfilled for vaccine E-R3. After two observations in unit A (trial groups 15 and 18) and one in unit C (trial group 17), information on pathogenic potency, in conjunction with the data on vaccine E, was deemed sufficient.

The vaccinators and the personnel giving medical care to the vaccinees were unable to identify any vaccine, as a system of changing code numbers was used.

This programme was planned in January 1961, as the results in the trial groups 1-4 [1] became available, indicating a difference in pathogenic potency between vaccines R and L as used for assessment of dose-effect relations.

Except in trial groups 10 and 15, the vaccination programmes were meant to ensure approximately equal numbers of vaccinations for each vaccine tested in a trial group. As the number of vaccinees could be only roughly estimated at the beginning of each session, and as some vaccinations could not be accounted for, due to lack of pock development, appearance of one pock, or an accelerated reaction, slight differences in the denominator values on the same line in Table 1 were inevitable. In the direct comparisons between vaccines and between strains (see Tables 3-13, 17-19, pages 316-318/321) the results per vaccine in relevant trial groups were combined by mere addition, although the weight of a particular trial group was not identical for each vaccine.

Significancies of differences were tested in 2×2 tables with correction for continuity. The exact value of P has been computed if an expected frequency was below 5.

Double-tail probability values are indicated in the Tables as follows:

$P > 0.05$	*
$0.05 \geqslant P > 0.01$	**
$0.01 \geqslant P > 0.001$	†
$0.001 \geqslant P$	††

RESULTS

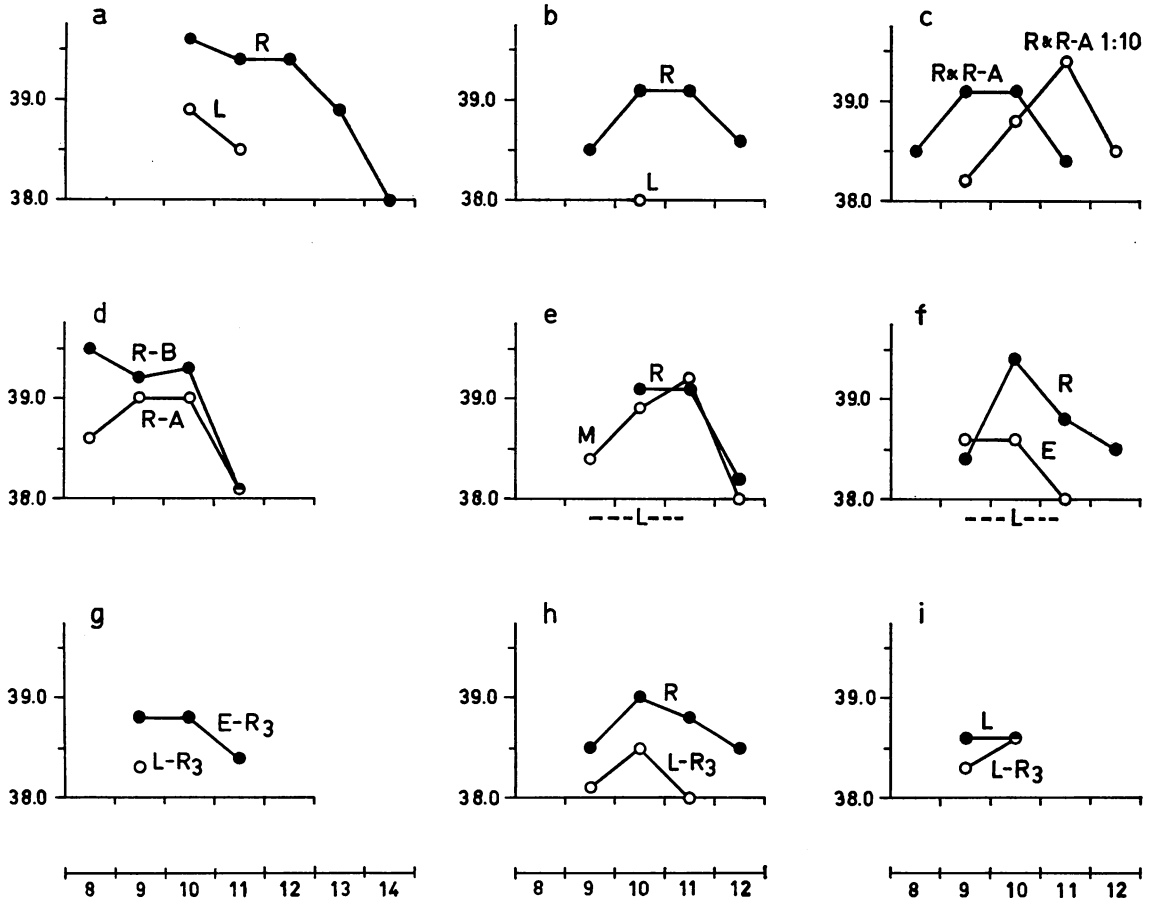
It is reasonable to assume, and this appeared to be confirmed by our data, that the number of primary pocks affects the outcome of the individual clinical reaction. Therefore, comparison between vaccines has been confined to vaccinees who showed two primary pustulae on the day of reading, generally the eighth day.

From the earlier study on dose-effect relation with diluted vaccines, 132 usable observations were available after 450 vaccinations in trial groups 1-4. Trial groups 5-21 provided 1333 observations from

[1] In the report on dose-effect relation (Polak et al., 1962) the present trial group 1 was used for a pilot study.

FIG. 1

CUMULATIVE 90% TEMPERATURE CHARTS FOR COMPARISON OF VACCINES



Abscissa: days (first day is of vaccination)
Ordinate: body temperature (°C)

Trial groups, relating to each chart:

a: 1-4
b: 5-8, 10, 11, 19
c: 1, 9, 12-14
d: 9, 12, 14
e: 5-8, 10
f: 8, 10, 11
g: 15, 17, 18
h: 11, 15, 16, 18-20
i: 11, 19, 21

1418 vaccinations with full-potency vaccines (see Table 2). It emerges, from the figures in Table 2, that selection procedures for primary vaccination were not on the same level of efficiency in the three units; some differences of effect in primary vaccination are also noticeable. For the present purpose, these factors have no real consequence.

Morbidity rates are summarized in Table 1 and further particulars may be found in Tables 3-12. In Fig. 1 a series of cumulative 90% temperature curves are shown.

First comparison between R and L (Table 3, Fig. 1, a)

The morbidity rate is clearly higher for R; as to height and duration of fever, the differences, although favourable for L, are not significant. These observations were derived from the study with, mainly, diluted vaccines to determine dose-effect relations.

Second comparison between R and L (Table 4, Fig. 1, b)

The use of undiluted vaccines demonstrates the same difference between R and L. In each of 7 trial

TABLE 2
RESULTS OF VACCINATION IN ARMY UNITS A, B AND C

Vaccination results	A Trial groups		B	C
	1-4	5, 8, 10, 11, 15, 18, 19		
Primary vaccinia, 2 pocks	132	777	310	246
Primary vaccinia, 1 pock	93	1	3	9
Negative	222	—	—	2
Non-primary vaccinia	3	20	22	20
Lost for observation	—	7	—	1

groups morbidity was lowest for L (see Table 1, page 313).

Comparison between undiluted and diluted vaccines (Tables 5, 6, Fig. 1, c)

Once inoculation has caused development of two primary pocks, the CAM potency of vaccine as applied would seem to have no influence on the outcome of the clinical reaction. One might suppose, however, that a delay in pock development could engender less illness after the use of diluted vaccine because immunity had already attained a higher level.

Our study afforded several opportunities for comparing portions of different potency on CAM, not all on a level of practical utility (see Table 5).

It seems that the use of a low-potency vaccine *per se* will not give a lower morbidity rate. It is not justifiable to exclude any possible benefit, but certainly the difference as shown between R and L seems much more convincing, while the vaccine with a low illness rate shows about three times higher potency on CAM.

A closer examination of observations with vaccines R and R-A, as given in Table 6, shows some delay as regards the first day of illness for the 1:10 dilution, but for the clinical reaction there is no real difference.

TABLE 3
COMPARISON BETWEEN VACCINES R AND L, MAINLY APPLIED IN DILUTION

	R		L
Morbidity rate	54 % (36/67)	†	25 % (16/65)
High fever rate	72 % (26/36)	*	50 % (8/16)
Prolonged fever rate	56 % (20/36)	*	25 % (4/16)

Trial groups 1-4.

TABLE 4
COMPARISON BETWEEN VACCINES R AND L, NOT DILUTED

	R		L
Morbidity rate	49 % (81/166)	††	19 % (33/170)
High fever rate	51 % (41/81)	*	39 % (13/33)
Prolonged fever rate	32 % (26/81)	**	12 % (4/33)

Trial groups 5-8, 10-11, 19.

R-A and R-B compared (Table 7, Fig. 1, d)

The data do not show differences of any significance. A minor deviation from the routine production method was of no importance for the pathogenic potency.

M compared with R and L (Table 8, Fig. 1, e)

R and M may be considered to be of about equal pathogenic potency, and the morbidity rate for M appears to be significantly higher than for L. This holds for all five relevant trial groups in Table 1. Vaccine M shows, moreover, a higher prolonged fever rate than L.

E compared with R and L (Table 9, Fig. 1, f)

E is of lower pathogenic potency than R, but its position to L is not clear. The morbidity rates

TABLE 5
COMPARISON BETWEEN UNDILUTED AND DILUTED VACCINES

Trial groups	Vaccine	Dilution	Morbidity rate	
1, 9, 12-14	R & R-A	1 : 1	50 % (33/66)	*
		1 : 10	44 % (29/66)	
2-4	R	1 : 40	61 % (17/28)	*
		1 : 200 and higher	50 % (7/14)	
1, 5-7	L	1 : 1	24 % (15/63)	*
		1 : 10	15 % (7/48)	
2-4	L	1 : 100	13 % (3/23)	*
		1 : 500 and higher	36 % (5/14)	

TABLE 6

COMPARISON BETWEEN UNDILUTED AND 1 : 10 DILUTED VACCINE (R & R-A)

	R & R-A 1 : 1		R & R-A 1 : 10
Morbidity rate	50 % (33/66)	*	44 % (29/66)
High fever rate	48 % (16/33)	*	55 % (16/29)
Prolonged fever rate	27 % (9/33)	*	41 % (12/29)
First day of illness			
until 9th day	19		7
from 9th day	14		22

Trial groups 1, 9, 12-14.

TABLE 7

COMPARISON BETWEEN VACCINES WITHOUT (R-A) AND WITH (R-B) EXTENDED PHENOL ACTION (12 DAYS AT 4° C)

	R-A		R-B
Morbidity rate	50 % (24/48)	*	51 % (26/51)
High fever rate	46 % (11/24)	*	69 % (18/26)
Prolonged fever rate	25 % (6/24)	*	27 % (7/26)

Trial groups 9, 12, 14.

TABLE 8

COMPARISON BETWEEN VACCINE M AND VACCINES R AND L

	R		M		L
Morbidity rate	44 % (47/108)	*	42 % (39/93)	††	16 % (17/109)
High fever rate	47 % (22/47)	*	59 % (23/39)	*	29 % (5/17)
Prolonged fever rate	15 % (7/47)	*	33 % (13/39)	**	6 % (1/17)

Trial groups 5-8, 10.

for E and L do not differ much, but the course of fever looks more favourable for L.

L-R3 and E-R3 compared (Table 10, Fig. 1, g)

For both these third calf-passages of L and E, the difference is now more convincing, L-R3 showing the lowest rates. The difference in morbidity rate

TABLE 9

COMPARISON BETWEEN VACCINE E AND VACCINES R AND L

	R		E		L
Morbidity rate	41 % (32/78)	†	21 % (22/107)	*	13 % (11/87)
High fever rate	53 % (17/32)	*	64 % (14/22)	*	27 % (3/11)
Prolonged fever rate	34 % (11/32)	*	64 % (14/22)	††	0 % (0/11)

Trial groups 8, 10, 11.

TABLE 10

COMPARISON BETWEEN VACCINES L-R3 AND E-R3

	L-R3		E-R3
Morbidity rate	15 % (20/135)	††	35 % (43/124)
High fever rate	30 % (6/20)	*	44 % (19/43)
Prolonged fever rate	15 % (3/20)	*	35 % (15/43)

Trial groups 15, 17, 18.

TABLE 11

COMPARISON BETWEEN VACCINES R AND L-R3

	R		L-R3
Morbidity rate	46 % (88/191	††	22 % (47/128)
High fever rate	53 % (47/88)	**	30 % (14/47)
Prolonged fever rate	48 % (42/88)	†	23 % (11/47)

Trial groups 11, 15, 16, 18-20.

is significant, while again the course of fever is more favourable for the Elstree strain.

R and L-R3 compared (Table 11, Fig. 1, h)

The results are clearly favourable for L-R3, notwithstanding one exception observed in trial group 16 (Table 1). There the morbidity was highest for L-R3, but the fever data were better for L-R3 than for R.

L and L-R3 compared (Table 12, Fig. 1, i)

There is a good agreement between the data for these closely related vaccines.

TABLE 12

COMPARISON BETWEEN VACCINES L AND L-R3

	L		L-R3
Morbidity rate	27 % (25/92)	*	28 % (24/87)
High fever rate	40 % (10/25)	*	38 % (9/24)
Prolonged fever rate	16 % (4/25)	*	21 % (5/24)

Trial groups 11, 19, 21.

The index of pathogenicity

It seems from our data that, in general, a significantly higher morbidity rate goes parallel with a fever of higher degree and longer duration.

In an attempt to summarize the over-all effect of pathogenic potency in one figure, the number of observed days of fever above 38.7°C was related to the number of successful vaccinations per vaccine, dilutions included. This lower limit was somewhat arbitrarily chosen so as to include most cases of serious health impairment but avoid the low fever region where completeness of the data is more doubtful. For vaccine R, R-A and R-B together, 276 such fever days were counted after 529 vaccinations (52 per 100).

In order to compute a comparative index of pathogenic potency of each vaccine, vaccines R, R-A and R-B together were taken as the standard, and the average number of fever days above 38.7°C per vaccination for any vaccine was expressed in terms of the average for the standard, given an index

TABLE 13

THE INDEX OF PATHOGENICITY

Vaccine	Fever days >38.7°C / vaccinations	Fever days >38.7°C / vaccinations with vaccines R [a, b]	Index of pathogenicity [c]
R [a]	276/529	276/529	100
M	34/93	42/108	94
E	24/107	30/78	58
E-R3	22/75	34/63	54
L	42/285	170/270	23
L-R3	32/218	99/191	28

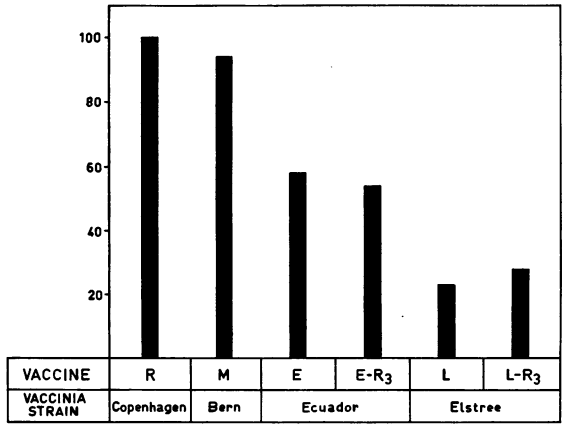
[a] R-A and R-B inclusive.

[b] From same trial groups as second column.

[c] Index of pathogenicity = $\frac{\text{rate of second column}}{\text{rate of third column}} \times 100$.

FIG. 2

INDEX OF PATHOGENICITY FOR VARIOUS VACCINES



Number of fever days (>38.7°C) in 100 vaccinations ; R = 100

value of 100. The index value for each vaccine was derived from simultaneous observations in relevant trial groups, as indicated in Table 13 and Fig. 2.

Correlation of morbidity rates for two vaccinia strains

As mentioned earlier, it was assumed that comparison of two vaccines was not justified unless they were applied in the same trial group. The difference in admission policy between army units could affect the observed morbidity, and, within a unit, extraneous influences on the illness rates of trial groups seemed probable.

The connexion between morbidity rates for two vaccines is best studied in army unit A, which provided 11 of our 21 trial groups. Here, vaccines R, L and L-R3 were tested 11, 9 and 4 times respectively. Taking into consideration the data given in Table 12, we felt justified in combining the observations of L and L-R3 for characterization of vaccinia strain Elstree. The data collected for vaccine R, vaccinia strain Copenhagen, served as a counterpart.

From 11 pairs of morbidity percentages (see Fig. 3) the following regression equation was computed:

$$y = 30.8\% + 0.938x$$

y = morbidity percentage for strain Copenhagen

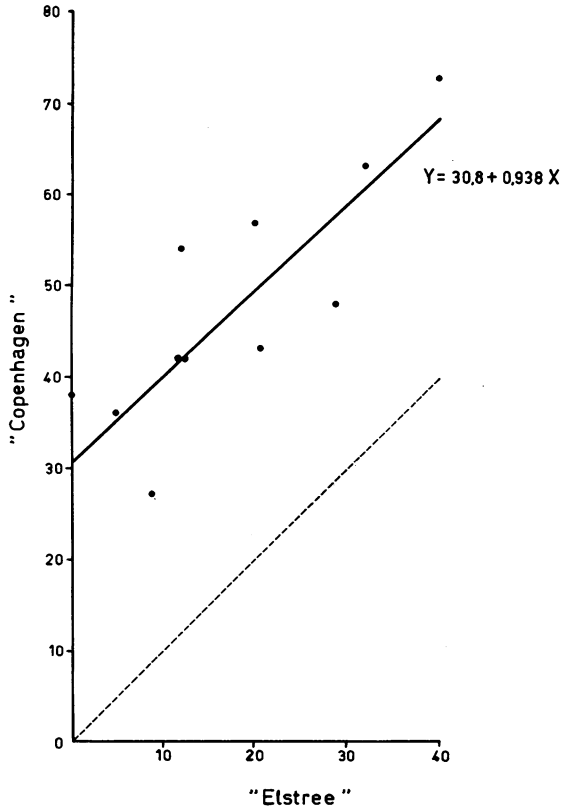
x = morbidity percentage for strain Elstree

The coefficient of correlation (Spearman) amounted to $+0.86$ ($P < 0.01$).

The vaccination procedure and the medical supervision of vaccinees in unit A have not changed

FIG. 3

MORBIDITY PERCENTAGES IN 11 TRIAL GROUPS OF UNIT A FOR TWO VACCINIA STRAINS



in the course of trial. The subjects of the separate trial groups represented different drafts, but there is no reason to suppose the existence of constitutional differences inherent to these drafts. Therefore, the positive correlation between morbidity percentages for two vaccinia strains is best attributed to extraneous influence. The nature of this influence is a matter of conjecture. No relationship with the incidence of respiratory diseases in recruits stationed in the same unit was discernible.[1] It is, of course, possible that the nature of military duties during incubation has its influence on the frequency and grade of vaccinia illness.

Analysis of variance

The method of analysis of variance has been applied to morbidity percentages obtained from the

[1] We are indebted to Dr R. Brouwer for making available data on respiratory diseases, collected for a special study, in unit A.

TABLE 14

TAIL PROBABILITIES (*P*-VALUES) FROM ANALYSES OF VARIANCE

	All trial groups (21)		Trial groups in unit A (11)	
	Between strains	Between trial groups	Between strains	Between trial groups (= between days)
Morbidity rate	<0.0001	0.0005	<0.0001 [a]	<0.0001
High fever rate	0.015	0.5	0.05	0.7
Prolonged fever rate	0.01	0.5	0.05	0.5

[a] Batches L and L-R3 and batches E and E-R3 separately.

rates of Table 1, after angular transformation (carried out by Dr E. F. Drion, Statistics Department, Central Organization for Applied Scientific Research in the Netherlands T.N.O.).[2] Table 14 summarizes the results for the complete trial, where trial groups vary in both date of vaccination and army unit, as well as for the separate results in army unit A, where besides strain variation only inter-day differences remain.

The choice of vaccinia strain is obviously of significance for the observed morbidity rate and the grade of illness. Between trial groups there are clear differences in illness frequencies, but high fever and prolonged fever rates appear rather homogeneous (*P*: 0.5-0.7). A high morbidity in some trial group thus is not attributable to a more liberal admission policy in the relevant period.

[2] A fixed-effect model was used of the form:

$$\underline{y}_{ij} = M + A_i + B_j + \underline{\epsilon}_{ij}$$

where $\underline{y}_{ij}$ is the angular transform of the percentage ill in the group, M is the general mean of the observations, A_i the effect of the i-th "date" of vaccination, B_j the influence of the j-strain and $\underline{\epsilon}_{ij}$ the random variation, which is supposed to be normally distributed for all combinations of i and j with zero mean and variance σ^2. The quantities A_i and B_j are subject to the restrictions $\Sigma h_i A_i = 0$ and $\Sigma k_j B_j = 0$ where h_i (and respectively k_j) is the number of observations on the i-th date (and for respectively j-th strain).

The values of A_i and B_j are estimated by means of the least square method without weighting for differences in the number of vaccinees in the groups; the variance σ^2 of $\underline{\epsilon}$ is estimated as $\sum_{ij} (\underline{y}_{ij} - M - A_i - B_j)^2$ divided by the number of degrees of freedom.

The estimated values for the effect of each date or each strain are obtained by adding the estimated value of A_i (or B_j, as the case may be) to M and converting the angle to a percentage; these results have been called either the effect of the date corrected for inter-vaccine variation or the effect of strain corrected for inter-days variation, respectively.

TABLE 15

BEST ESTIMATES (PERCENTAGES) OF MORBIDITY, HIGH FEVER IN BED PATIENTS AND PROLONGED FEVER IN BED PATIENTS IN UNIT A, CORRECTED FOR INTER-DAYS' VARIATION

Vaccinia strain	Vaccine batch	Morbidity	High fever	Prolonged fever
Copenhagen	R	46	60	41
Bern	M	46	71	34
Ecuador	E	26	57	49
	E-R3	33		
Elstree	L	16	37	22
	L-R3	12		

Best estimates of morbidity per 100 vaccinations, high fever cases and prolonged fever cases per 100 bed-patients are shown, after correction for inter-days variation, in Table 15. The Copenhagen and Bern strains seem to possess a similar pathogenicity; the Elstree strain displays a much milder character. The position of the Ecuador strain is intermediate as regards morbidity, but the combined measures for grade of illness do not appear to be more favourable than those for Copenhagen and Bern.

Finally, the variation in morbidity that has been observed in 11 trial groups of army unit A is given in Table 16 after correction for vaccine variation.

TABLE 16

MORBIDITY PERCENTAGES FOR 11 TRIAL GROUPS IN UNIT A, CORRECTED FOR INTER-VACCINE VARIATION

Trial group	Vaccination date	Morbidity per 100
1	27. 6.1960	38
2	22. 8.1960	57
3	17. 10.1960	37
4	9. 1.1961	18
5	17. 2.1961	16
8	14. 4.1961	23
10	28. 6.1961	18
11	21. 8.1961	31
15	16. 10.1961	34
18	5. 12.1961	28
19	5. 2.1962	49

This series of values does not suggest a seasonal influence on illness rate.

DISCUSSION

This study on pathogenicity of vaccinia strains was started on a somewhat tentative basis. Analysis of admission and fever data obtained from the early trial groups (1-4) demonstrated a clear disparity between two smallpox lymphs, differing chiefly in vaccinia strain and choice of animal for virus propagation.

The ensuing study aimed to check the feasibility of these clinical data for estimating the comparative pathogenicity of various vaccines, to select a vaccine of low pathogenic potency, and to produce such a mild vaccine in the Netherlands Smallpox Vaccine Laboratory.

Rates of admission, high fever and prolonged fever, as defined in this report, were easily obtained and showed rather a high degree of consistency between trial groups. These rates reflect a general systematic quality of vaccinia infection—a fact of considerable practical importance. Evaluation of eventual differences in local reactions or complications is a much more cumbersome procedure and was not a particular aim of this trial. Our general impression was, however, that differences in degree of local reactions between vaccines were not observed, and that " complications " could all be accepted as coincidences unassociated with any particular vaccine.

The purpose of selecting a vaccine of low pathogenicity brought about the exclusion of vaccine M (Bern strain) after trial group 10, while the Ecuador strain, tested in two batches (vaccines E and E-R3), was not used after trial group 18. The most extensive observations were thus performed with a Netherlands routine production vaccine R (strain Copenhagen) and two lymphs (L and L-R3), prepared from the Elstree strain of the Lister Institute of Preventive Medicine.

The question now is, how far the pathogenic qualities tested might be attributes of particular batches. The bacterial counts were low and the usual tests failed to isolate pathogenic bacteria. A few microbes may have been introduced from a vaccine in or near the scratch, but it seems highly improbable that this could affect the clinical picture of vaccinia infection—the more so in this investigation, as the vaccines of high pathogenicity (R, R-A, R-B and M) gave the lowest bacterial counts (< 10 per ml).

TABLE 17

COMPARISON BETWEEN VACCINE R (BATCH 59134, COPENHAGEN STRAIN) AND VACCINES R-A AND R-B (BATCH 6032, COPENHAGEN STRAIN) IN DIFFERENT TRIAL GROUPS OF UNITS B AND C

	R		R-A & R-B
	Trial groups 6, 7, 16, 20		Trial groups 9, 12, 13, 14
Morbidity rate	47 % (45/95	*	48 % (79/163)
High fever rate	40 % (18/45)	*	53 % (42/79)
Prolonged fever rate	20 % (9/45)	*	28 % (22/79)

Our data show that the strain of vaccinia virus is the sole or principal factor in determining the pathogenicity of a vaccine, as measured by the criteria chosen in this trial. An Elstree strain sheep lymph (vaccine L) and its derivative through three calf-passages (vaccine L-R3) resulted in practically the same morbidity figures (see Table 12, page 318). In an indirect comparison, the pathogenicity of the Ecuador strain in calf lymph vaccine E was nearly equal to that of its derivative E-R3 (see Table 13, page 318). Although two batches (no. 59134 and no. 6032) prepared from a common seed virus of the Copenhagen strain were used, the respective observations should not, strictly, be compared, as these were not collected in the same trial groups, and even data for an indirect comparison by the intermediary of a common third vaccine are too meagre. The usual figures are, however, presented in Table 17, restricted to the observations in army units B and C, as batch no. 6032 was not used for unit A. Data for both batches are derived from four trial groups each, and are in rather good agreement. These observations are not unusual in the light of general experience of routine primary vaccination in the army with lymphs prepared from the Copenhagen strain.

Thus we notice good intra-strain agreement, in contrast with obvious inter-strain differences (see Tables 14, 15, pages 319, 320). Vaccines R, L and E have been prepared in different institutes, but R, L-R3 and E-R3 were all produced in one smallpox vaccine laboratory according to the same scheme of manufacture (Brans, 1959). Notwithstanding this, the interstrain differences in pathogenicity were maintained.

In a controlled trial to study the effect of 2 ml of 16% vaccinia hyperimmune gamma-globulin in primary vaccination, Nanning (1962) noticed a minor but significant decrease in the admission rate from 63.3% to 58.6%. His criteria for the admission rate were more liberal than ours for the morbidity rate. A similar mitigating effect may also have been present in our study, and might have been discriminative between various vaccinia strains. The hyperimmune gamma-globulin was, however, largely or completely homologous to the Copenhagen strain. If bias was present—a remote possibility—it favoured vaccine R, and our conclusions remain essentially unaffected.

TABLE 18

COMPARISON BETWEEN COPENHAGEN STRAIN (VACCINE R) AND ECUADOR STRAIN (VACCINES E AND E-R3)

	Copenhagen		Ecuador
Morbidity rate	42 % (59/141)	†	26 % (48/182)
High fever rate	58 % (34/59)	*	50 % (24/48)
Prolonged fever rate	44 % (26/59)	*	42 % (20/48)

Trial groups 8, 10, 11, 15, 18.

We found no evidence that vaccine potency, expressed as PFU/ml on the chick CAM is of any significance (see Tables 1, 5, 6, pages 313, 316, 317).

There may, perhaps, be a difference in the relative benign qualities of the Ecuador and Elstree strains. As far as our data go, the Ecuador strain demonstrates an advantage to the Copenhagen strain only as regards morbidity, whilst the Elstree strain gives also lower high fever and prolonged fever rates. This is shown in Tables 18 and 19, which differ from the other tables in presenting data not from separate batches but from strains, in one or two batches, applied in the same trial groups. The

TABLE 19

COMPARISON BETWEEN COPENHAGEN STRAIN (VACCINE R) AND ELSTREE STRAIN (VACCINES L AND L-R3)

	Copenhagen		Elstree
Morbidity rate	50 % (203/403)	††	21 % (101/476)
High fever rate	53 % (107/203)	**	37 % (37/101)
Prolonged fever rate	38 % (78/203)	††	18 % (18/101)

Trial groups 1-8, 10-12, 15, 16, 18-20.

Elstree strain emerged from our study as indubitably the mildest for primary vaccination of young adults, with an " index of pathogenicity " of about 25, the Copenhagen strain being fixed at 100 (see Table 13, page 318). This computation accounts for the number of days of fever above 38.7°C. As regards morbidity (admission with fever of 38.0°C or higher), if a vaccine of the Copenhagen strain causes an illness rate of 50%, a morbidity of about 20% may be expected with a vaccine of the Elstree strain.

ACKNOWLEDGEMENTS

Thanks are due to Dr E. Krag Andersen (Statens Seruminstitut, Copenhagen), Dr C. Kaplan (The Lister Institute of Preventive Medicine, Elstree) and Professor A. Herrlich (Bayerische Landesimpfanstalt, Munich) who supplied vaccines used in this study.

The authors are indebted to Dr E. F. Drion (Statistics Department of the Central Organization for Applied Scientific Research in the Netherlands T.N.O., The Hague), who gave general advice and carried out the analysis of variance, and to Lieutenant H. G. van Riet, who observed the clinical reactions in trial group 21.

RÉSUMÉ

Afin d'évaluer les divers vaccins antivarioliques, l'on a appliqué à 1968 jeunes soldats, répartis en 21 groupes, différents vaccins: essentiellement le vaccin L à base de lymphe de mouton, souche d'Elstree, préparé par l'Institut Lister en Angleterre et le vaccin R, lymphe de veau, souche de Copenhague, préparé par l'Institut national de Santé publique des Pays-Bas. En outre, l'on a utilisé du vaccin M (lymphe de veau, souche de Berne), du vaccin E (lymphe de veau, souche d'Equateur), du vaccin E-R3 (dérivé du vaccin E par trois passages sur le veau) et du vaccin L-R3 (dérivé du vaccin L par trois passages sur le veau). Enfin, deux sous-lots (RA et RB) et un autre lot préparé à partir de la souche de Copenhague, distincts l'un de l'autre par une petite différence dans le procédé de préparation, ont été testés.

La pathogénicité de chaque vaccin a été établie d'après l'indice de morbidité (pourcentage de sujets devant s'aliter avec une température égale ou supérieure à 38°C par rapport au nombre total de sujets présentant une réaction positive) l'indice d'élévation de la température et l'indice de durée de température (respectivement pourcentage des sujets présentant une fièvre égale ou supérieure à 39°C et des sujets présentant pendant plus de deux jours une élévation thermique par rapport au nombre total des sujets fébriles) après la formation de deux pustules primaires.

L'on a pu mettre en évidence des différences de pathogénicité entre les vaccins; la pathogénicité de lots différents de vaccins de même souche était identique. La souche d'Elstree (vaccins L et L-R3) est la plus bénigne, les souches de Copenhague (vaccins R, R-A et R-B) et de Berne (vaccin M) ont montré le plus haut degré de pathogénicité; la souche d'Equateur (vaccins E et E-R3) occupe une position moyenne.

Il doit y avoir, en plus de la souche elle-même, un second facteur exogène de morbidité puisque d'autres différences ont été notées entre groupes de la même unité.

En ce qui concerne la morbidité, celle du vaccin fabriqué à partir de la souche de Copenhague est d'environ 50%, tandis que celle de la souche d'Elstree est d'environ 20%.

L'activité du vaccin, exprimée par le nombre d'unités (par ml) formant des pustules sur la membrane chorio-allantoïdienne, n'a eu aucune influence sur la fréquence et la gravité des réactions vaccinales observées et étudiées dans ce travail.

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