Smallpox: Disease and Vaccine Resurfacing Concerns

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ABSTRACT

The potential threat of bioterrorist activities has caused concerns about smallpox disease and vaccine to resurface. The last naturally occurring case of smallpox was in 1949 in the United States and throughout the world in Somalia in 1977. In 1980, the World Health Organization (WHO) announced the eradication of smallpox. At that time, the WHO indicated that all countries should discontinue vaccination. There are two clinical forms of smallpox: variola major and variola minor. Variola major is the more prevalent and more virulent form of the disease. Smallpox is marked by a high rate of infectivity, pathogenicity, and virulence. There are six stages in the spectrum of the smallpox disease, beginning with an incubation period and ending with the resolving of scabs. The smallpox victims are contagious from the onset of the rash until the last scab falls off. Man is the only known host of smallpox. Smallpox can be transmitted from an infected individual to susceptible people by extended periods of face-to-face contact, direct contact, or touching of contaminated bodily fluids or objects, but it is rarely transmitted through air in an enclosed environment. The inoculation of individuals with smallpox matter, variolation, can be traced back to the early 18th century. Reports indicate that variolation decreased the rate of mortality of smallpox infections. In 1796, Jenner performed some of the first vaccination experiments, inoculating people with cowpox (vaccinia virus). During the global eradication of smallpox in the 1970s, two vaccination strategies were employed: mass vaccination and ring vaccination. All vaccination programs are based on herd immunity. That is, not everyone in the population has to be vaccinated to protect the entire population. The critical proportion of the population which must be vaccinated to bring about eradication of the disease is a function of the transmission rate. The vaccinia virus vaccine has a vaccine efficacy of around 95%. There are side-effects associated with the vaccine, although most are moderate. However, there are also serious, but not life-threatening complications, and life-threatening reactions which require medical attention. Vaccination was generally performed on young people, and therefore there is little information on the rate of adverse events in the adult population. Alternatives or supplements to the vaccine such as vaccinia immune globulin (VIG) and cidofovir are being developed. VIG in conjunction with the vaccine has been shown to be important in reducing the risk for the disease and adverse events. However, its currently limited supply prevents the prophylactic use of VIG. Thus, other alternatives must be considered.

HISTORY AND EPIDEMIOLOGY

Concerns over smallpox have resurfaced with the potential threat of bioterrorist activities. The use of smallpox as a weapon would not be novel. British forces in North America distributed blankets used by smallpox victims to Indians during the French and Indian War (1754 - 1767). This method of transmission resulted in smallpox epidemics that wiped out more than half the infected Indian populations.[4]

The last case of naturally occurring smallpox was 1949 in the United States and throughout the world in Somalia in 1977. In 1980, a World Health Organization (WHO) committee indicated that all laboratories eliminate their supplies of smallpox or send them to one of the WHO laboratories, the Institute of Virus Preparations in Moscow, Russia or the Center for Disease Control and Prevention (CDC) in Atlanta, Georgia. All countries were said to have followed suit.[4] That same year, the WHO committee decided that all supplies of smallpox should be destroyed by June of 1999. A 1996 World Health Assembly agreed to the destruction. However, in 1998 the Institute of Medicine (IOM) and the WHO committee determined that certain research questions could be answered if the smallpox supplies were not destroyed.[4] The former deputy director of the Soviet Union's civilian bioweapons program, Ken Alibek, reported that in 1980 the Soviet government was producing several tons of smallpox each year for use in bombs and intercontinental ballistic missiles. Virulent and contagious recombinant strains continue to be produced. Diminishing monetary assistance for laboratories increases fears that knowledge and equipment may fall into unfriendly hands.[4]

There are two clinical forms of smallpox, variola major and variola minor (alastrim). Variola major, the more common form, prevailed through the 19th century. Variola major is

virulent and marked by an extensive rash and high fever. There are four types of variola major: ordinary, modified, hemorrhagic, and malignant.[3, 4] The ordinary type predominates, accounting for 90 percent or more of the cases. The modified type occurs in individuals that have been vaccinated and is typically mild.[3] The flat, hemorrhagic type, affecting both sexes and all ages, is atypical, extremely virulent, and generally fatal. Death typically occurs five or six days after the rash appears.[3, 4] The malignant type is also typically fatal with survivors developing lesions that heal without scabbing, or in severe cases, losing large portions of their skin. It is thought that a portion of the hemorrhagic and malignant cases may have occurred in individuals with suppressed immune systems, however, this is not certain since smallpox was eradicated prior to the appearance of HIV and methods to assess cell-mediated immunity.[4] Variola minor, not as prevalent nor nearly as virulent as variola major, appeared at the beginning of the 20th century in South Africa and subsequently in Florida from which it spread throughout the United States and eventually into Europe and Latin America.[3, 4]

The smallest number of infectious particles required to initiate a smallpox infection is unknown, but it is thought to be just a few virions. The natural route of infection is intranasal or peroral.[4] For contact-transmitted diseases such as smallpox, a measure of infectivity is the secondary attack rate (SAR). The SAR is the frequency at which susceptible people contract the disease following exposure to an infected person.[17]

An SAR[I] for smallpox of 25 to 40 percent was reported by Breman and Henderson.[9] The pathogenicity of smallpox, the potential to bring about disease, is measured as the portion of those infected that develop the disease. No exact measure was found in the literature, although it is high

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[I] An equation for the SAR is provided by Fox, Hall, and Elveback:[17]
SAR = {the number of contacts contracting the disease within the maximum incubation period / total number of susceptible contacts} × 100.

and probably near 100 percent.[17]

When death is the basis of virulence, the case-fatality rate (CFR)[II] is used as a measure of virulence.[17] The CFR for variola major has been reported as approximately 30 percent, as observed during epidemics in Asia, and could be greater among unvaccinated populations.[3, 4, 8] Gani and Leach presented CFR for variola major of around 15 percent for epidemics in the United States and England during the 1700's.[7] CFR associated with variola minor are around one percent or less.[3, 4]

Table 1 shows the spectrum the of smallpox disease, indicating infectious stages. During the first week of the disease, the virus titers in saliva are the greatest.[4] However, the disease is seldom transmitted prior to the onset of rash, being most contagious 7 to 10 days thereafter and diminishing after the appearance of scabs.[3, 4, 5] Death, probably due to toxemia, typically occurs within the second week of the disease.[4] The pitted scarring, that remains after the scabs resolve, covers large portions of the body, in particular the face, and some survivors may be blind.[3] Large quantities of the viable virus are contained within the fibrin matrix of the scabs. A Dutch scientist isolated the virus from a 13-year-old scab. It is doubtful that the scabs are infectious to humans; all cases of smallpox have been traced to contact with a previously infected person.[4]

Man is the only known host of smallpox. No other invertebrate or vertebrate host of smallpox has been identified. Smallpox is typically transmitted from the reservoir (man) to susceptible people by extended periods of face-to-face contact, direct contact, and touching infected bodily fluids or objects. Viable smallpox virus is believed to endure in the laundry from infected individuals over prolonged periods of time.[3, 4]

[II] An equation for CFR for smallpox is presented by Fox, Hall, and Elveback:[17]
CFR = {the number of deaths attributed to smallpox in a defined time period / the number cases existing during the defined time period} × 100

Spectrum of Smallpox Disease [3]			
	Duration	Contagious	Symptoms
Incubation Period	7 to 17 days (12 to 14 days on average)	No	None
Prodromal Phase	2 to 4 days	Rarely	Fever, malaise, head and body aches, and vomiting
Early Rash	Approximately 4 days	Yes, highly	Rash appears as small red spots on the tongue and in the mouth which develop into sores, erupting and distributing large quantities of the virus into the mouth and throat. A rash then appears on the skin, starting at the face and extending to the arms, legs, hands and feet within 24 hours. Fever may subside. On day three, the rash evolves into raised bumps. On day four, the bumps, containing a depression, fill up with fluid. Fever may return and persist until the scabs develop.
Pustular Rash	Approximately 5 days	Yes	Bumps evolve into pustules.
Pustules and Scabs	Approximately 5 days	Yes	Pustules develop a crust that evolve into scabs. The majority of lesions will have scabbed over two weeks after the onset of the rash.
Resolving Scabs	Approximately 6 days	Yes, until the last scab has fallen off.	Scabs fall off, leaving pitted scars. The majority of scabs will have fallen off three weeks after the onset of the rash.

Table 1

Within two days, under laboratory settings, 90 percent of aerosolized smallpox virus becomes inactive, and this percentage increases upon exposure to ultraviolet light.[3, 4] Aerosolized vaccinia virus has an extended period of survival under conditions of low temperature and humidity. At low temperatures (10°C - 11°C) and humidity (20%), approximately one-third of the aerosolized vaccinia virus dies within 24 hours. However, at high temperatures (31°C - 33°C) and humidity (80%), almost all of the aerosolized vaccinia virus becomes inactive within 6 hours. These findings are thought to be consistent with what would be observed for the variola (smallpox) virus.[4]

It is unusual for smallpox to be transmitted through the air in a confined environment.[3] Although, higher rates of transmission have been observed in hospitals during smallpox outbreaks.[5] The transmission rate, R_o, is defined by Gani and Leach as the "average number of secondary cases infected by each primary case in a population composed entirely of susceptible individuals." They also define another transmission rate, R, which is analogous to R_o, but it doesn't take into account the percentage of susceptible individuals. Since the literature has varying values of R_o, from 1.5 to more than 20, Gani and Leach set out to derive a consistent value using epidemic modeling. They found estimated values for R_o between 3.5 and 6 in pre twentieth century Europe with insignificant herd immunity and in 30 outbreaks in 20th century Europe taking into account vaccination levels and hospital associated infections.[7] In 1972, a smallpox outbreak occurred in Kosovo which went undetected until the second generation after which time the contacts were quarantined and vaccinated along with 95 percent of the population. The estimated R value was 5 at the onset of the outbreak and dropped to below 1 upon quarantine and vaccination. Assuming that half of the population had been previously vaccinated, R_o was estimated to be 10. For the 30 European outbreaks between 1958 and 1973, R was

similarly estimated at 5.5 and an estimated R_0 of 11, taking into account that 50 percent of the population had been previously vaccinated. Half of the cases in the Kosovo outbreak and most of the European outbreaks were due to transmission in a hospital setting. By excluding these hospital-acquired cases, an estimated R_0 of 5.5 was assigned to community-acquired illnesses.[7]

The estimated values of R_0 were in agreement with those described by Dietz and Heesterbeck on an analysis by Bernoulli of a smallpox outbreak in Paris with an estimated R_0 between 4 and 5.[III] Gani and Leach indicate that during the 1700's to 1900's R_0 was estimated to be between 3.5 and 6, although larger values of R_0, 10 to 11, did occur in 18th century London as a consequence of crowding and poor socio-economic conditions and in more recent times in hospital settings prior to taking appropriate infection controls. They believe that if smallpox should appear again similar estimates of R_0 would apply, with larger values occurring until the disease is accurately identified and appropriate controls put in place.[7]

VACCINATION (PRIMARY PREVENTION)

History

Descriptions of the inoculation of humans with smallpox matter can be traced back to the early 18th century. The *Philosophical Transactions of the Royal Society of London* published in 1714 contains papers written by two Italians, Timoni and Pylarini, who discussed a practice, performed by their parents and grandparents, of variolation, inoculating people with smallpox matter.[1, 2] James Jurin (1722) and Zabdiel Boylston (1726) conducted studies on the mortality of individuals inoculated with smallpox, revealing that mortality was around 1 in 50 for inoculated

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[III] In Bernoulli's analysis the transmission rate was estimated as $R_0 = \lambda L$ or $R_0 = \lambda L + 1$ where λ is the estimated force of infection = 0.125 and L is the life expectancy = 32 years.

individuals and around 1 in 6 for those naturally infected. Inoculation techniques had improved by 1765, decreasing mortality to below 1 in 500.[1] The practice of infecting people with fluid from smallpox lesions occurred at least 82 years before vaccination experiments were conducted by Jenner.[1,2] In 1796, Jenner took material from cowpox lesions on a milkmaid's hand and placed it into an incision on a boy's arm; the boy was subsequently challenged with the smallpox virus and failed to contract the disease.[2,3]

Working as a physician in the Hospital for the Maintenance and Education of Deserted Children in London in 1767, William Watson conducted some of the first "clinical trials". Since the opening of the Hospital in 1739, the children had been inoculated with matter from smallpox lesions. At the time, it was also common to administer the inoculum with a laxative and mercury. Watson didn't believe that the mercury was beneficial and therefore set out to perform experiments to test his hypothesis. He conducted three trials on children of similar ages and of both sexes. In his first experiment, three groups of children were inoculated with material from an early lesion and assigned either a treatment of mercury plus a laxative, a laxative, or no medication. In a second experiment, three groups of children were inoculated with material from a mature pock and assigned a treatment of mercury, a laxative, or no medication. In a third experiment, a single group of children was inoculated with material from a late lesion and received no medication. The number of pocks on each child was counted, since the number of pocks was indicative of the prognosis of the disease. Based on the mean number of pocks that formed within each group, Watson concluded that mercury did not improve the outcome and that material from early and mature lesions produced better results than that from late lesions. The number of pocks counted on 74 children was 2353, which was less than the number that typically formed on the arm of an individual naturally infected with smallpox. Also, the pocks counted on five of the 74

children accounted for more than half the total number of pocks counted. The other 69 children had a mean pock count of 17. Arthur W. Boylston's paper[1] discussing Watson's experiments included an analysis of Watson's findings using the Kruskal-Wallis test for comparing independent groups. From the Kruskal-Wallis test[IV], Boylston determined that there was not sufficient evidence ($p>0.05$) to conclude that the treatments differ or that the sources of the inoculum differ.

In 1971, recommendations for routine vaccination in the United States were no longer in place and were discontinued by 1972. Prior to 1972, vaccination was recommended for children at one year of age and required before entry into school in most states. Before entering most countries, individuals had to have been vaccinated within the previous 3 years. In 1982, Wyeth Laboratories ceased production of the vaccinia vaccine for civilian use, and international travelers were no longer required to have proof of vaccination.[4] In 1980, the World Health Organization (WHO) announced the eradication of smallpox and advised all countries to discontinue vaccination.[3, 4] Vaccination of all military personnel continued until 1990.

Strategies

Until recently, only scientists and medical personnel working with vaccinia virus, recombinant vaccinia virus, and other nonvariola Orthopoxviruses were vaccinated.[3, 5] On December 13, 2002, President George W. Bush introduced a smallpox vaccination policy which included the mandatory vaccination of 500,000 military personnel and voluntary vaccination of a similar number of health care workers and other critical personnel that would be the first to respond to a clandestine release of smallpox.[3, 10] The vaccine is also being offered to civilian and

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[IV]For a discussion of the Kruskal-Wallis test, see Douglas C. Montgomery. Design and Analysis of Experiments (New York: John Wiley & Sons, Inc., 2001) 116. The p-value calculations of Boylston were replicated using Minitab and can be found in the appendix along with the original data.

US personnel who are or may be located in high threat areas, but it is not currently available to the general public.[3] The individuals within the aforementioned groups would not be vaccinated if they or any close contact possessed a condition that is contraindicated for vaccination.

Two different strategies were used in the global eradication of smallpox in the 1970's: mass vaccination and ring vaccination. Both of these strategies are now being considered by the Center for Disease Control and Prevention (CDC) for implementation in a response to the use of smallpox as a bioweapon.[3] Mass vaccination involves vaccinating a large number of people who frequently have not come in contact with the smallpox virus. Ring vaccination, also referred to as surveillance-containment, involves isolating and vaccinating those who have been exposed to the smallpox infection and their contacts. This in effect creates a ring of vaccination around the infected person and prevents further transmission of the disease.[3, 12] This method has been likened to the fire-ring method used by fire fighters.

It has been argued by Henderson and others that mass vaccination alone would not have been enough to eradicate smallpox and that ring vaccination was the method that eventually led to its eradication. Mass vaccination leaves some people unprotected, allowing for infection and spread of the disease.[3, 4, 11, 12] Recently, however, the findings of Henderson and others have come under scrutiny by Kaplan who argues that ring vaccination "made a marginal contribution at best". Kaplan indicates that a graph presented by Henderson and others that shows a decrease in the number of cases following ring vaccination is misleading due to plotting the data on a logarithmic scale and the use of data on the expected number of cases from a year of low vaccination. When Kaplan graphed the number of smallpox cases and the percent unvaccinated over time on an arithmetic scale, he determined that these two variables decreased in concordance over time, and there was not great change upon the introduction of ring vaccination.[11]

Vaccination programs are based on "herd immunity", meaning that not everyone in the population has to be vaccinated to protect the entire population. The level of vaccination in a population needed to confer immunity on everyone depends on the infectivity of the disease, the susceptibility of the population, and environmental factors.[13] In a "homogeneously mixed" population, a critical proportion of the population must be successfully immunized to bring about the eradication of the disease. In Anderson[14], this critical value is given by $p_c = 1 - 1/R_o$. The R_o values given by Gani and Leach (3.5 to 6) would give values of p_c from 71 to 83 percent which are in line with values of 70 to 80 percent which were found to be effective in the eradication of smallpox in industrialized countries.[7] However, in India where there were large population densities and poor socio-economic conditions, vaccination levels greater than 90 percent did not allow for eradication of smallpox. The eventual eradication is attributed to the introduction of ring vaccination.[7, 11] Gani and Leach have estimated that the level of "herd immunity" is now 18 percent in industrialized countries. This estimate is based on the current UK population, assuming that 50 percent of the population was immunized as an infant up until 1972, 60 percent of these individuals are still alive, and of these 50 percent still have immunity to smallpox.[7]

If faced with the necessity of eradicating smallpox as a result of reintroduction under a cataclysmic event, vaccination would currently be the only option. The only vaccine currently licensed for use in the United States is Dryvax manufactured by Wyeth Laboratories in Lancaster, Pennsylvania. Dryvax is produced from lyophilized calf lymph containing the live vaccinia virus.[3, 4, 8] The vaccines used for measles, mumps, rubella, and chickenpox are also live virus vaccines. The vaccinia virus is a member of the Orthopoxvirus genus along with cowpox, monkeypox, and smallpox.[3] These viruses are all capable of infecting humans; as recently as 1997, cases of human

monkeypox were reported in the Democratic Republic of the Congo.[9] However, smallpox is the only one that has a propensity to be transmitted from human to human.[3, 9]

Efficacy

The smallpox vaccine is administered, usually in the upper arm, using a bifurcated (two-prong) needle which contains a drop of vaccine. The needle is used to prick the skin 15 times in a few seconds within an area ~5 mm in diameter, drawing a few drops of blood and causing soreness. The undiluted vaccine, containing approximately 10^8 pock-forming units per milliliter (pfu/ml), is reconstituted using a diluent of 0.25 percent phenol, 50 percent glycerin, and 0.005 percent brilliant green.[3, 4, 8] A recent clinical trial has shown diluted vaccine ($10^{7.2}$ pfu/ml and $10^{7.0}$ pfu/ml) to be as effective as the undiluted vaccine[V] in providing immunity as judged by a successful vaccination.[15] A successful vaccination, referred to as a "take", in a primary vaccinee is marked by the formation of a red, itchy bump at the site of vaccination within 3 to 4 days. The bump develops into a puss-filled blister with drainage by week one, by week two the blister begins to dry up and form a scab, and the scab falls off during week three, leaving a slight scar.[3, 8] The reaction may be more pronounced in a primary vaccinee than in a secondary vaccinee with partial immunity, who might display faster maturation and recovery. The appearance of a pus-filled lesion or a region of fluid build-up or hardening around a lesion within 6 to 8 days signifies a successful reaction for an individual being revaccinated. Erythema (reddening of the skin) reaching a maximum within 48 hours is indicative of an accelerated reaction to the vaccine and requires revaccination.[3, 4, 5]

[V]The diluted vaccines were said to be as effective as the undiluted vaccine if the difference in the success rates was five percent or less. They calculated a 95 percent upper confidence limit (1-sided) for the difference in the success rates.

A definitive measure of efficacy of the vaccinia vaccine has never been made under a controlled trial.[5] The vaccine "take" rate has been used as a measure of vaccine efficacy[VI]. The level of antibody response and the period over which they remain protective is a function of the extent of viral replication. Vaccine efficacy for the vaccinia vaccine has been reported as 95 percent based on historical events.[3, 8] A vaccine efficacy of 97.5 percent for uninfected individuals and 30 percent for infected and latent individuals were used by Gani and Leach in calculating a R_o for Kosovo following a smallpox outbreak, taking into account subsequent quarantine and intervention.[7] A recent clinical trial using the vaccinia virus vaccine (undiluted, $10^{8.1}$ pfu/ml) found a success rate for primary vaccinees of 103 out of 106 or 97.2 percent (95% CI: 92.0–99.4 percent)[VII] for subjects 18 to 32 years old.[15]

The smallpox vaccine does not confer lifelong immunity, and the level of protection exhibited by individuals that were vaccinated more than 30 years ago is uncertain.[4] However, epidemiologic studies suggest that the level of immunity remains high for three to five years with diminishing but significant levels of protection lasting for 10 or more years following primary

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[VI] Vaccine efficacy (VE) is defined as the proportionate reduction in risk of disease in the vaccinated population over the unvaccinated population. In a vaccine trial setting, vaccine efficacy can be calculated using the equation: $VE = [(\beta+\delta)-\beta]/(\beta+\delta) = 1-(1/\psi)$ where $(\beta+\delta)$ is the disease rate without vaccination, β is the disease rate with vaccination, δ is the excess risk, and ψ is the relative risk or risk ratio.

[VII] Frey et al. used the exact binomial method (Clopper-Pearson method) to calculate the 95% confidence limits.

$$p_U = \left[1 + \frac{(n-y)}{(y+1)\cdot F_{1-\frac{\alpha}{2},2(y+1),2(n-y)}}\right]^{-1} \qquad p_L = \left[1 + \frac{(n-y+1)}{y\cdot F_{1-\frac{\alpha}{2},2y,2(n-y+1)}}\right]^{-1}$$

$$\left[1 + \frac{(106-103)}{104\cdot qF(0.975,208,6)}\right]^{-1} = 0.994 \qquad \left[1 + \frac{(106-103)+1}{103\cdot \frac{1}{qF(0.975,8,206)}}\right]^{-1} = 0.92$$

greater than 102°F. Fever occurs with less frequency among adults.[5] Although relatively rare, serious and potentially life-threatening reactions can occur. Table 2a[VIII] and Table 2b contain the adverse event rates and number of reported cases, respectively, for serious, but not life-threatening reactions (inadvertent inoculation, generalized vaccinia, and erythema multiforme) and for life-threatening reactions (postvaccinial encephalitis, vaccinia necrosum, and eczema vaccinatum) from a ten-state survey (1968) and from reports by the CDC on vaccinations conducted this year.[3, 16]

Approximately one out of every million primary vaccinees has a moderate adverse reaction, including erythema multiforme, inadvertent inoculation, and generalized vaccinia.[3, 16] Erythema multiforme (Steven-Johnson syndrome) is a rash that develops at the site of vaccination due to a toxic or allergic reaction. Other rashes including urticarial, maculopapular, and blotchy erythematous may occur ten days after vaccination and may be mistaken for generalized vaccinia. These rashes are generally self-limited with clearing in two to four days.[3, 4, 8] Generalized vaccinia results from dissemination of the vaccinia virus through the blood to other regions of the body. This complication generally occurs in primary vaccinees who are otherwise healthy and resolves with little or no treatment except in the case of a toxic reaction or suppressed immune system.[3, 4]

Since the smallpox vaccine contains the live vaccinia virus, it is possible for a vaccine recipient to inadvertently inoculate others or themselves at different locations on the body. This complication, which accounts for approximately half of all the adverse events in primary vaccinees and revaccinees, can be minimized by washing one's hands after touching the site or anything which may come in contact with the vaccination site. The CDC web site contains information

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[VIII] The calculations of the upper one-sided 95% confidence limits using the Clopper-Pearson method can be found in the appendix.

Adverse Event Rates from a Ten-State Survey, Cases per Million Vaccinees (Upper One-sided 95% Confidence Limit)

Age (yrs) and Status	Vaccinia necrosum	Post-vaccinial encephalitis	Eczema vaccinatum	Generalized Vaccinia	Accidental Implantation	Erythema multiforme	Other	Total
Primary Vaccinations								
<1		42.3	14.1	394.4	507	436.6	154.9	1549.3
1 to 4	3.2	9.5	44.2	233.4	577.3	157.7	236.6	1261.8
5 to 19		8.7 (27.5)	34.9	139.7 (187.7)	371.2	87.3	214 (271.5)	855.9
>20			30.3	212.1 (398.4)	606.1	30.3	636.4 (916.1)	1515.2
Total	1.5 (7.3)	12.3 (22.2)	38.5 (53.7)	241.5 (275.7)	529.2 (578.6)	164.6 (193.3)	266.2 (301.9)	1253.8 (1328)
Revaccination								
<1					109.1	72.7	18.2	200
1 to 4			2	9.9	47.7	2	23.9	85.5
5 to 19	6.8	4.5	4.5	9.1	25	9.1	54.5	113.6
>20	3 (7.8)	2 (6.3)	3 (7.8)	9 (15.7)	42.1 (54.4)	10 (17)	39.1 (51.0)	108.2
Total								
Grand Total	2.4 (5.6)	6.1 (10.3)	24.9 (32.3)	101.3 (115.2)	251.8 (273.1)	71.6 (83.4)	129.2 (144.8)	587.4 (619.4)

Rates for Vaccination of US Civilian Healthcare Workers* and US Troops and Medical Personnel** (Cases per Million Vaccinees)

Age (yrs) and Status	Post-vaccinial encephalitis**	Generalized Vaccinia*	Other*
Primary Vaccinations			
5 to 19	20 (63.0)		
>20		136.0 (644.9)	3128 (4428)

Table 2a.

Reported Complications of Smallpox Vaccination from a Ten-State Survey

Age (yrs) and Status	Estimated Number of Vaccinations	Vaccinia necrosum	Post-vaccinial encephalitis	Eczema vaccinatum	Generalized Vaccinia	Accidental Implantation	Erythema multiforme	Other	Total
Primary Vaccinations									
<1	71,000	0	3	1	28	36	31	11	110
1 to 4	317,000	1	3	14	74	183	50	75	400
5 to 19	229,000	0	2	8	32	85	20	49	196
>20	33,000	0	0	1	7	20	1	21	50
Total	650,000	1	8	25	157	344	107	173	815
Revaccination									
<1	0	0	0	0	0	0	0	0	0
1 to 4	55,000	0	0	0	0	6	4	1	11
5 to 19	503,000	0	0	1	5	24	1	12	43
>20	440,000	3	2	2	4	11	4	24	50
Total	998,000	3	2	3	9	42	10	39	108
Grand Total	1,648,000	4	10	41	167	415	118	213	968

Reported Complications from Vaccination of US Civilian Healthcare Workers and US Troops and Medical Personnel (Cases per Million Vaccinees)

Status	Number of Vaccinations	Post-vaccinial encephalitis**	Generalized Vaccinia*	Other*
Primary Vaccinations				
US Troops and Medical Personnel	100,000	2		
US Civilian Healthcare Workers	7354		1	23

Table 2b.

about how to care for the vaccination site along with other procedures to be followed to reduce inadvertent inoculation of vaccinees or their contacts.[3] The vaccination site remains infectious until the scab falls. The highest levels of viral shedding occur 3 to 14 days after vaccination, determined by cultures obtained from the vaccination site, and continue until the scab falls off.[5, 8] Based on the data from the 1968 ten-state survey, for every one million vaccinees, 27 infections result from inadvertent inoculation of a contact, 44% are children less than five years of age, and 60% were generally healthy individuals.[5, 16] The majority of these cases healed without treatment.[4, 5]

For every million primary vaccinees, 14 to 52 will experience a severe adverse reaction including eczema vaccinatum, progressive vaccinia, and postvaccinial encephalitis, necessitating medical attention. These severe adverse events typically occur in individuals that have health problems such as indicated as a contradiction for vaccination (e.g., suppressed immune system, eczema, and atopic dermatitis).[3, 16] Eczema vaccinatum is a serious rash that occurs in primary vaccinees or contacts who have or once had eczema or atopic dermatitis. Contact related cases account for about 30 percent of all cases and may be more virulent as a result of inoculating more than a single site.[3, 4, 5, 16] The case-fatality rate is 10 percent or less overall, but children less than 2 years of age experience a higher rate of 30 to 40 percent.[8] Although this complication can be fatal or severe, it is generally minor and self-limited. The most severe cases generally occur in primary vaccinees.[5]

Progressive vaccinia, also referred to as vaccinia necrosum or vaccinia gangrenosa, is an infection of the skin that advances to the point of necrosis (tissue destruction), often leading to death especially in individuals with a suppressed immune system. This complication occurs in both primary vaccinees and revaccinees with a case-fatality rate of 75 to 100 percent.[3, 4, 5, 8]

Progressive vaccinia along with generalized vaccinia, eczema vaccinia and postvaccinial encephalitis are rare complications that occur with greater frequency in infants and in primary vaccinees who are ten or more times likely to experience these adverse events than a revaccinee. Progressive vaccinia and postvaccinial encephalitis seldom occur in contact related cases.[5] Postvaccinial encephalitis is an inflammation of the brain with symptoms typically appearing 8 to 15 days after vaccination. There is no treatment for postvaccinial encephalitis; fatality and permanent neurological residua occurs in 15 to 25 percent and 25 percent of cases, respectively.[3, 4, 5]

Seven to nine deaths occurred each year during the United States vaccination program, with infants experiencing a greater risk, especially for postvaccinial encephalitis. About 1 or 2 primary vaccinees per million vaccinated and 0.25 revaccinees per million vaccinated will die of complications of the vaccinee, primarily from postvaccinial encephalitis and progressive vaccinia.[3, 5] The statistics used by the CDC on vaccine complications are derived from the 1968 ten-state and national surveys. The CDC points out that at present the rates may be higher due to an increase in the population of individuals with suppressed immune systems, eczema, or atopic dermatitis. However, the gravity of these adverse events may be lower today as a result of improvements in medicine.[3] Higher rates may be seen in primary vaccinees than in revaccinees and in countries using more virulent strains. Previously, primary vaccinees were generally children or adolescents. Thus, little is known about the rate of adverse events in the adult population.[8, 16]

Evolving Alternatives

Vaccinia immune globulin (VIG) and cidofovir are two potential therapies for the complications of the smallpox vaccine. VIG is an isotonic sterile solution of the immunoglobulin

fraction of plasma obtained from the sera of hyperimmunized army recruits vaccinated with the vaccinia virus vaccine.[3, 5, 8] VIG, procured by the Red Cross, is now the property of the Department of Defense (DOD). The CDC contains a small supply for the most serious cases.[8] VIG has been found to be beneficial in the treatment of severe eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and periocular implantation as a result of inadvertent inoculation.[4, 8] Individuals with vaccinial keratitis, however, should not receive VIG due to the potential for increased corneal scarring. VIG is also not therapeutic for postvaccinial encephalitis or smallpox.[5] However, a double-blind, randomized, controlled trial conducted on Dutch military recruits indicated that VIG delivered together with the smallpox vaccine reduced the number of cases of postvaccinial encephalitis. More than 106,000 recruits randomly received either the smallpox vaccine plus VIG or the smallpox vaccine plus a placebo. Thirteen cases of postvaccinial encephalitis developed in the group receiving the placebo, whereas three cases occurred in the VIG group ($p<0.05$, from previous author). The limited availability of VIG, however, does not permit the administration of VIG as a preventive measure.[5, 8]

Most of the data on the effectiveness of VIG in treating the adverse events of the smallpox vaccine are derived from case series, anecdotal evidence, and unblinded controlled studies. A case series study found VIG decreased the case-fatality rate and severity of serious smallpox vaccine complications.[8] A soldier with AIDS who developed progressive vaccinia after being vaccinated benefitted from VIG and ribavirin therapy. Administration of VIG at this time is deemed experimental and falls under investigational new drug (IND) protocol.[3, 4] Individuals for whom vaccination is not recommended can receive VIG (0.3 ml/kg of body weight) together with the smallpox vaccine if they have come in contact with a smallpox victim or are otherwise at risk for exposure to smallpox.[4] A dose of 0.6 ml/kg of body weight used to treat serious

complications of the smallpox vaccine is delivered intramuscularly in two stages over a 24 to 36 hour period and can be administered again in 2 to 3 days if there is no change in the conditions. This large dose can result in trauma and nerve damage.[4, 8] Complications in the method of delivery along with production indicate the need for a new therapy. Antivirals and monoclonal antibodies might provide an alterative to VIG.[4, 5, 8]

Cidofovir, a viral DNA synthesis inhibitor, may serve as an alternative or supplement to vaccination.[4, 6] If delivered within two days of exposure, cidofovir may provide protection against smallpox.[4] Cidofovir has been shown to be effective against poxvirus infections in animal models and humans as shown in Table 3.[6]

	Infection	**Route of Infection**	**Drug Delivery**
Mouse Model	vaccinia	intravenous	subcutaneous
		intranasal	intraperitoneal
	cowpox	intranasal	subcutaneous
		aerosolized	peroral
Monkey Model	monkeypox	aerosolized	
Humans (Patients)	molluscum contagiosum		intravenous
			local
	orf		topical

Table 3.

In addition to the poxviruses indicated in Table 3, variola and camelpox have also been shown to be sensitive to cidofovir.[6] Cidofovir protected cultured cells from infection with the variola virus.[4] In a susceptibility study of poxviruses to cidofovir, variola virus had the lowest concentration required for 50 percent inhibition (IC_{50} = 1.5 µg/ml).[6]

To date, cidofovir is licensed only for use in the treatment of AIDS patients with cytomegalovirus (CMV) retinitis and by intravenous delivery. The approved method of administration along with the potential for serious renal toxicity limit the utility of cidofovir.[4, 6] However, based on the results from animal models, researchers believe that other delivery routes (topic, intranasal, peroral) could be useful and made available if needed.[6]

Alternatives to the currently licensed vaccinia virus vaccine are also needed since the method of production, scarified calves, is no longer allowed due to the limited, but unavoidable, presence of bacteria and other undesirable agents. The antigenic and allergenic properties of animal proteins can induce sensitization and allergic reactions.[4, 8] Thus, new vaccinia virus vaccines will need to be created using tissue cell cultures. This requires finding a suitable cell substrate. A candidate cell substrate might be one already approved for use in the production of a live-virus vaccine, including primary cell substrates (e.g., embryonated chicken-egg), diploid cell strains (e.g., MRC-5 or WI-38 used to develop the licensed live-virus vaccines for varicella and rubella), or continuous cell lines (e.g., Vero used in the production of licensed inactivated polio vaccine which may be appropriate for the smallpox vaccine).[4, 8] Due to the current complications associated with vaccinia virus vaccine, it may be desirable to use a more attenuated strain such as Lc16m8 or the modified vaccinia Ankara. Both of these attenuated strains have been developed and tested in more than 100,000 people. Lc16m8, derived from the Lister strain and produced in rabbit kidney cell cultures, was created and tested by Japan in 1975. The modified vaccinia Ankara, derived from the Ankara vaccinia strain, was tested in Turkey and Germany.[4, 8] In order to be licensed for use, these new vaccines will need to go through clinical trials. The discussion by Rosenthal on what should be accomplished at each phase of the clinical trials is briefly outlined below.[8]

1. Phase 1
    i. Small Enrollment of 10 to 20 Healthy Adults
    ii. Safety and Immunogenicity Measurements
        (1) Local signs and symptoms, including lesion size and frequency
        (2) Frequency and severity of systemic signs and symptoms
        (3) Serum chemistries
        (4) Hematologic testing
    iii. Containment and Survival of the Virus
    iv. Shedding and the Possibility of Inadvertent Inoculation

2. Phase 2
    i. Larger Enrollment - Size Determined by the Power Needed to Detect Differences in "Take" Rate, Immune Response, and Safety Between the New Vaccine and the Licensed Dryvax Vaccine
    ii. Additional Safety and Immunogenicity Measurements
    iii. Determine the Desirable Vaccine Formulation and Dosage

3. Phase 3
    i. Unable to Perform Large-Scale Clinical Efficacy Trials Due to the Absence of a Population at Risk for Smallpox
    ii. Efficacy Measurements
        (1) Vaccine "take" rate
        (2) Neutralizing antibody response
    iii. Large-Scale Trial to Assess Rare Complications

Due to the absence of a natural population at risk for smallpox, the Food and Drug Administration (FDA) is faced with a difficult task of evaluating the effectiveness of new drugs and vaccines. The FDA has therefore set forth guidelines for new drugs and vaccines, including proof of effectiveness from animal studies and well-controlled clinical trials on the safety and immunogenicity of vaccines in humans.[8]

TREATMENT (SECONDARY/TERTIARY PREVENTION)

Currently, there is no treatment for the smallpox disease. Intravenous fluids, drugs to minimize fever and pain, and antibiotics for secondary infections, although not common, are the only therapies presently available to smallpox victims.[3, 4] Although VIG has been shown to

decrease the risk of disease, its limited supply prevents prophylactic use. A VIG relative efficacy of 70%[IX] was obtained from a randomized trial conducted in Madras, India on 705 family contacts of 208 smallpox victims ($p<0.05$, from previous author). Of the 705 contacts, 326 were administered VIG plus the smallpox vaccine and 379 received the smallpox vaccine alone. Five of the VIG recipients became infected with smallpox, while 21 of those receiving only the smallpox vaccine contracted smallpox.[8] The only licensed protection against the smallpox virus is vaccination with the Dryvax vaccine.

CONCLUSIONS

The enormity of the reintroduction of smallpox exists not only in the infectivity, virulence, and pathogenicity of the disease, but also in the complications of the prevention. The potential for adverse events following vaccination with the vaccinia virus vaccine were highlighted recently. The heart attack deaths reemphasize how little is known about the vaccination of the adult population. This also points to the need for supplements such as VIG to minimize the risk for adverse events as well as the disease. The limited availability of VIG which prohibits its use as a prophylactic along with the cumbersome method of administration indicate the necessity of other alternatives.

[IX] VIG Efficacy = $(\varphi_1 - \varphi_2)/ \varphi_1$, where φ_1 is the true risk for those not receiving VIG and φ_2 is the true risk for those receiving VIG. VIG Efficacy = $(0.0554 - 0.0153)/0.0554 = 0.72$, where $\varphi_1 = 21/379 = 0.0554$, $\varphi_2 = 5/326 = 0.0153$. (See footnote VI for same definition in alternative notation.)

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APPENDICES

APPENDIX A

CONFIDENCE LIMITS

$$p_U = \left[1 + \frac{(n-y)}{(y+1)\cdot F_{\frac{\alpha}{2},2(y+1),2(n-y)}}\right]^{-1}$$

$$\left[1 + \frac{(650000-1)}{2\cdot qF(0.95,4,1.3\cdot 10^6)}\right]^{-1} = 7.298\cdot 10^{-6}$$

$$2\cdot(650000-1) = 1.3\cdot 10^6$$

$$\frac{1}{650000}\cdot 1000000 = 1.538$$

$$\frac{1}{650000} = 1.538\cdot 10^{-6}$$

$$\left[1 + \frac{(650000-8)}{9\cdot qF(0.95,18,1.3\cdot 10^6)}\right]^{-1} = 2.221\cdot 10^{-5}$$

$$2\cdot(650000-8) = 1.3\cdot 10^6$$

$$\frac{8}{650000}\cdot 1000000 = 12.308$$

$$\frac{8}{650000} = 1.231\cdot 10^{-5}$$

$$\left[1 + \frac{(650000-25)}{26\cdot qF(0.95,52,1.3\cdot 10^6)}\right]^{-1} = 5.372\cdot 10^{-5}$$

$$2\cdot(650000-25) = 1.3\cdot 10^6$$

$$\frac{25}{650000}\cdot 1000000 = 38.462$$

$$\frac{25}{650000} = 3.846\cdot 10^{-5}$$

$$\left[1 + \frac{(650000-157)}{158\cdot qF(0.95,316,1.3\cdot 10^6)}\right]^{-1} = 2.757\cdot 10^{-4}$$

$$2\cdot(650000-157) = 1.3\cdot 10^6$$

$$\frac{157}{650000}\cdot 1000000 = 241.538$$

$$2\cdot 158 = 316$$

$$\frac{157}{650000} = 2.415\cdot 10^{-4}$$

$$\left[1+\frac{(650000-344)}{345\cdot qF\left(0.95,690,1.299\cdot 10^{6}\right)}\right]^{-1}=5.786\cdot 10^{-4}$$

$$2\cdot 345=690$$

$$2\cdot(650000-344)=1.299\cdot 10^{6}$$

$$\frac{344}{650000}\cdot 1000000=529.231$$

$$\frac{344}{650000}=5.292\cdot 10^{-4}$$

$$\left[1+\frac{(650000-107)}{108\cdot qF\left(0.95,216,1.3\cdot 10^{6}\right)}\right]^{-1}=1.933\cdot 10^{-4}$$

$$2\cdot 108=216$$

$$2\cdot(650000-107)=1.3\cdot 10^{6}$$

$$\frac{107}{650000}\cdot 1000000=164.615$$

$$\frac{107}{650000}=1.646\cdot 10^{-4}$$

$$\left[1+\frac{(650000-173)}{174\cdot qF\left(0.95,348,1.3\cdot 10^{6}\right)}\right]^{-1}=3.019\cdot 10^{-4}$$

$$2\cdot 174=348$$

$$2\cdot(650000-173)=1.3\cdot 10^{6}$$

$$\frac{173}{650000}\cdot 1000000=266.154$$

$$\frac{173}{650000}=2.662\cdot 10^{-4}$$

$$\left[1+\frac{(650000-815)}{816\cdot qF\left(0.95,1632,1.298\cdot 10^{6}\right)}\right]^{-1}=1.328\cdot 10^{-3}$$

$$2\cdot 816=1.632\cdot 10^{3}$$

$$2\cdot(650000-815)=1.298\cdot 10^{6}$$

$$\frac{815}{650000}\cdot 1000000=1.254\cdot 10^{3}$$

$$\frac{815}{650000}=1.254\cdot 10^{-3}$$

$$p_U = \left[1 + \frac{(n-y)}{(y+1)\cdot F_{\frac{\alpha}{2},2(y+1),2(n-y)}}\right]^{-1}$$

$$\left[1 + \frac{(998000-3)}{4\cdot qF(0.95,8,1.996\cdot 10^6)}\right]^{-1} = 7.769\cdot 10^{-6}$$

$$2\cdot(998000-3) = 1.996\cdot 10^6$$

$$\frac{3}{998000}\cdot 1000000 = 3.006$$

$$\frac{3}{998000} = 3.006\cdot 10^{-6}$$

$$\left[1 + \frac{(998000-2)}{3\cdot qF(0.95,6,1.996\cdot 10^6)}\right]^{-1} = 6.308\cdot 10^{-6}$$

$$2\cdot(998000-2) = 1.996\cdot 10^6$$

$$\frac{2}{998000}\cdot 1000000 = 2.004$$

$$\frac{2}{998000} = 2.004\cdot 10^{-6}$$

$$\left[1 + \frac{(998000-3)}{4\cdot qF(0.95,8,1.996\cdot 10^6)}\right]^{-1} = 7.769\cdot 10^{-6}$$

$$2\cdot(998000-3) = 1.996\cdot 10^6$$

$$\frac{3}{998000}\cdot 1000000 = 3.006$$

$$\frac{3}{998000} = 3.006\cdot 10^{-6}$$

$$\left[1 + \frac{(998000-9)}{10\cdot qF(0.95,20,1.996\cdot 10^6)}\right]^{-1} = 1.574\cdot 10^{-5}$$

$$2\cdot(998000-9) = 1.996\cdot 10^6$$

$$\frac{9}{998000}\cdot 1000000 = 9.018$$

$$\frac{9}{998000} = 9.018\cdot 10^{-6}$$

$$\left[1+\frac{(998000-42)}{43\cdot qF\left(0.95,86,1.996\cdot 10^{6}\right)}\right]^{-1}=5.443\cdot 10^{-5}$$

$$2\cdot(998000-42)=1.996\cdot 10^{6}$$

$$\frac{42}{998000}\cdot 1000000=42.084$$

$$\frac{42}{998000}=4.208\cdot 10^{-5}$$

$$\left[1+\frac{(998000-10)}{11\cdot qF\left(0.95,22,1.996\cdot 10^{6}\right)}\right]^{-1}=1.7\cdot 10^{-5}$$

$$2\cdot(998000-10)=1.996\cdot 10^{6}$$

$$\frac{10}{998000}\cdot 1000000=10.02$$

$$\frac{10}{998000}=1.002\cdot 10^{-5}$$

$$\left[1+\frac{(998000-39)}{40\cdot qF\left(0.95,80,1.996\cdot 10^{6}\right)}\right]^{-1}=5.104\cdot 10^{-5}$$

$$2\cdot(998000-39)=1.996\cdot 10^{6}$$

$$\frac{39}{998000}\cdot 1000000=39.078$$

$$\frac{39}{998000}=3.908\cdot 10^{-5}$$

$$\left[1+\frac{(998000-108)}{109\cdot qF\left(0.95,218,1.996\cdot 10^{6}\right)}\right]^{-1}=1.27\cdot 10^{-4}$$

$$2\cdot(998000-108)=1.996\cdot 10^{6}$$

$$\frac{108}{998000}\cdot 1000000=108.216$$

$$\frac{108}{998000}=1.082\cdot 10^{-4}$$

$$p_U = \left[1 + \frac{(n-y)}{(y+1)\cdot F_{\frac{\alpha}{2},2(y+1),2(n-y)}}\right]^{-1}$$

$$\left[1 + \frac{(1648000-4)}{5\cdot qF(0.95,10,3.296\cdot 10^6)}\right]^{-1} = 5.554\cdot 10^{-6}$$

$$2\cdot(1648000-4) = 3.296\cdot 10^6$$

$$\frac{4}{1648000}\cdot 1000000 = 2.427$$

$$\frac{4}{1648000} = 2.427\cdot 10^{-6}$$

$$\left[1 + \frac{(1648000-10)}{11\cdot qF(0.95,22,3.296\cdot 10^6)}\right]^{-1} = 1.029\cdot 10^{-5}$$

$$2\cdot(1648000-10) = 3.296\cdot 10^6$$

$$\frac{10}{1648000}\cdot 1000000 = 6.068$$

$$\frac{10}{1648000} = 6.068\cdot 10^{-6}$$

$$\left[1 + \frac{(1648000-41)}{42\cdot qF(0.95,84,3.296\cdot 10^6)}\right]^{-1} = 3.228\cdot 10^{-5}$$

$$2\cdot(1648000-41) = 3.296\cdot 10^6$$

$$\frac{41}{1648000}\cdot 1000000 = 24.879$$

$$\frac{41}{1648000} = 2.488\cdot 10^{-5}$$

$$\left[1 + \frac{(1648000-167)}{168\cdot qF(0.95,336,3.296\cdot 10^6)}\right]^{-1} = 1.152\cdot 10^{-4}$$

$$2\cdot(1648000-167) = 3.296\cdot 10^6$$

$$\frac{167}{1648000}\cdot 1000000 = 101.335$$

$$2\cdot 168 = 336$$

$$\frac{167}{1648000} = 1.013\cdot 10^{-4}$$

$$\left[1+\frac{(1648000-415)}{416\cdot qF\left(0.95,832,3.295\cdot 10^{6}\right)}\right]^{-1}=2.731\cdot 10^{-4}$$

$2\cdot(1648000-415)=3.295\cdot 10^{6}$

$2\cdot 416=832$

$\frac{415}{1648000}\cdot 1000000=251.82$

$\frac{415}{1648000}=2.518\cdot 10^{-4}$

$$\left[1+\frac{(1648000-118)}{119\cdot qF\left(0.95,238,3.296\cdot 10^{6}\right)}\right]^{-1}=8.343\cdot 10^{-5}$$

$2\cdot(1648000-118)=3.296\cdot 10^{6}$

$2\cdot 119=238$

$\frac{118}{1648000}\cdot 1000000=71.602$

$\frac{118}{1648000}=7.16\cdot 10^{-5}$

$$\left[1+\frac{(1648000-213)}{214\cdot qF\left(0.95,428,3.296\cdot 10^{6}\right)}\right]^{-1}=1.448\cdot 10^{-4}$$

$2\cdot(1648000-213)=3.296\cdot 10^{6}$

$2\cdot 214=428$

$\frac{213}{1648000}\cdot 1000000=129.248$

$\frac{213}{1648000}=1.292\cdot 10^{-4}$

$$\left[1+\frac{(1648000-968)}{969\cdot qF\left(0.95,1938,3.294\cdot 10^{6}\right)}\right]^{-1}=6.194\cdot 10^{-4}$$

$2\cdot(1648000-968)=3.294\cdot 10^{6}$

$2\cdot 969=1.938\cdot 10^{3}$

$\frac{968}{1648000}\cdot 1000000=587.379$

$\frac{968}{1648000}=5.874\cdot 10^{-4}$

$$p_U = \left[1 + \frac{(n-y)}{(y+1)\cdot F_{\frac{\alpha}{2},2(y+1),2(n-y)}}\right]^{-1}$$

$$\left[1 + \frac{(229000-2)}{3\cdot qF(0.95,6,4.58\cdot 10^5)}\right]^{-1} = 2.749\cdot 10^{-5}$$

$$\left[1 + \frac{(229000-32)}{33\cdot qF(0.95,66,4.579\cdot 10^5)}\right]^{-1} = 1.877\cdot 10^{-4}$$

$$\left[1 + \frac{(33000-7)}{8\cdot qF(0.95,16,6.599\cdot 10^4)}\right]^{-1} = 3.984\cdot 10^{-4}$$

$$\left[1 + \frac{(229000-49)}{50\cdot qF(0.95,100,4.579\cdot 10^5)}\right]^{-1} = 2.715\cdot 10^{-4}$$

$$2\cdot(229000-2) = 4.58\cdot 10^5$$

$$\frac{2}{229000}\cdot 1000000 = 8.734$$

$$\frac{2}{229000} = 8.734\cdot 10^{-6}$$

$$2\cdot(229000-32) = 4.579\cdot 10^5$$

$$\frac{32}{229000}\cdot 1000000 = 139.738$$

$$\frac{32}{229000} = 1.397\cdot 10^{-4}$$

$$2\cdot(33000-7) = 6.599\cdot 10^4$$

$$\frac{7}{33000}\cdot 1000000 = 212.121$$

$$\frac{7}{33000} = 2.121\cdot 10^{-4}$$

$$2\cdot(229000-49) = 4.579\cdot 10^5$$

$$\frac{49}{229000}\cdot 1000000 = 213.974$$

$$\frac{49}{229000} = 2.14\cdot 10^{-4}$$

$$\left[1+\frac{(33000-21)}{22\cdot qF\left(0.95,44,1.996\cdot 10^{6}\right)}\right]^{-1}=9.161\cdot 10^{-4}$$

$$2\cdot(33000-21)=6.596\cdot 10^{4}$$

$$\frac{21}{33000}\cdot 1000000=636.364$$

$$\frac{21}{33000}=6.364\cdot 10^{-4}$$

$$p_U = \left[1 + \frac{(n-y)}{(y+1)\cdot F_{\frac{\alpha}{2},2(y+1),2(n-y)}}\right]^{-1}$$

$$\left[1 + \frac{(100000-2)}{3\cdot qF(0.95,6,2\cdot 10^5)}\right]^{-1} = 6.296\cdot 10^{-5}$$

$$\left[1 + \frac{(7354-1)}{2\cdot qF(0.95,4,1.471\cdot 10^4)}\right]^{-1} = 6.449\cdot 10^{-4}$$

$$\left[1 + \frac{(7354-23)}{24\cdot qF(0.95,48,1.446\cdot 10^4)}\right]^{-1} = 4.428\cdot 10^{-3}$$

$2\cdot(100000-2) = 2\cdot 10^5$

$\frac{2}{100000}\cdot 1000000 = 20$

$\frac{2}{100000} = 2\cdot 10^{-5}$

$2\cdot(7354-1) = 1.471\cdot 10^4$

$\frac{1}{7354}\cdot 1000000 = 135.98$

$\frac{1}{7354} = 1.36\cdot 10^{-4}$

$2\cdot(7354-23) = 1.466\cdot 10^4$

$\frac{23}{7354}\cdot 1000000 = 3.128\cdot 10^3$

$\frac{23}{7354} = 3.128\cdot 10^{-3}$

APPENDIX B

KRUSKAL-WALLIS TEST

Kruskal-Wallis Test

Kruskal-Wallis Test on No. of P

Pretreat	N	Median	Ave Rank	Z
Mercury	10	4.500	16.1	0.04
None	11	16.000	17.9	0.85
Senna	10	4.000	13.8	-0.91
Overall	31		16.0	

H = 1.02 DF = 2 P = 0.600
H = 1.04 DF = 2 P = 0.595 (adjusted for ties)

Kruskal-Wallis Test

Kruskal-Wallis Test on No. of P

Pretreat	N	Median	Ave Rank	Z
Mercury	8	21.00	12.8	0.39
None	7	15.00	7.2	-2.24
Senna	8	26.00	15.4	1.78
Overall	23		12.0	

H = 5.64 DF = 2 P = 0.060
H = 5.75 DF = 2 P = 0.056 (adjusted for ties)

Kruskal-Wallis Test

Kruskal-Wallis Test on No. of P

Source o	N	Median	Ave Rank	Z
Early	11	16.00	15.0	-1.61
Late	20	45.00	23.2	2.18
Mature	7	15.00	16.0	-0.92
Overall	38		19.5	

H = 4.78 DF = 2 P = 0.092
H = 4.92 DF = 2 P = 0.085 (adjusted for ties)

	C1	C2
	Pretreatment	No. of Pocks
1	Mercury	25
2	Mercury	13
3	Mercury	12
4	Mercury	6
5	Mercury	5
6	Mercury	4
7	Mercury	4
8	Mercury	3
9	Mercury	0
10	Mercury	0
11	Senna	30
12	Senna	5
13	Senna	5
14	Senna	5
15	Senna	4
16	Senna	4
17	Senna	2
18	Senna	2
19	Senna	0
20	Senna	0
21	None	200
22	None	17
23	None	16
24	None	16
25	None	16
26	None	16
27	None	3
28	None	2
29	None	2
30	None	0
31	None	0

	C1	C2
	Pretreatment	No. of Pocks
1	Mercury	440
2	Mercury	25
3	Mercury	21
4	Mercury	21
5	Mercury	21
6	Mercury	21
7	Mercury	20
8	Mercury	7
9	Senna	64
10	Senna	26
11	Senna	26
12	Senna	26
13	Senna	26
14	Senna	26
15	Senna	18
16	Senna	3
17	None	60
18	None	15
19	None	15
20	None	15
21	None	15
22	None	3
23	None	2

	C1	C2
	Source of Inoculum	No. of Pocks
1	Early	200
2	Early	17
3	Early	16
4	Early	16
5	Early	16
6	Early	16
7	Early	3
8	Early	2
9	Early	2
10	Early	0
11	Early	0
12	Mature	60
13	Mature	15
14	Mature	15
15	Mature	15
16	Mature	15
17	Mature	3
18	Mature	2
19	Late	250
20	Late	168
21	Late	93
22	Late	45
23	Late	45
24	Late	45
25	Late	45
26	Late	45
27	Late	45
28	Late	45
29	Late	45
30	Late	45
31	Late	45
32	Late	45
33	Late	4
34	Late	4
35	Late	4
36	Late	2
37	Late	0
38	Late	0

APPENDIX C

VIG EFFICACY

Prophylactic use of VIG

	Disease	No Disease	
Vaccine	21	358	379
Vaccine + VIG	5	321	326
	26	679	705

$\varphi 1$ is true risk for those not receiving VIG (21/379 = 0.0554)
$\varphi 2$ is true risk for those receiving VIG (5/326 = 0.0153)
VIG Efficacy = $(\varphi 1 - \varphi 2)/\varphi 1$ = (0.0554 - 0.0153)/0.0554 = 0.72

Prophylactic Use of VIG

	Encephalitis	No Encephalitis	
Vaccine + Placebo	13	52987	53000
Vaccine + VIG	3	52997	53000
	16	105984	106000

$\varphi 1$ is true risk for those not receiving VIG (13/53000 = 0.0002)
$\varphi 2$ is true risk for those receiving VIG (3/53000 = 0.0001)
VIG Efficacy = $(\varphi 1 - \varphi 2)/\varphi 1$ = (0.0002 - 0.0001)/0.0002 = 0.50