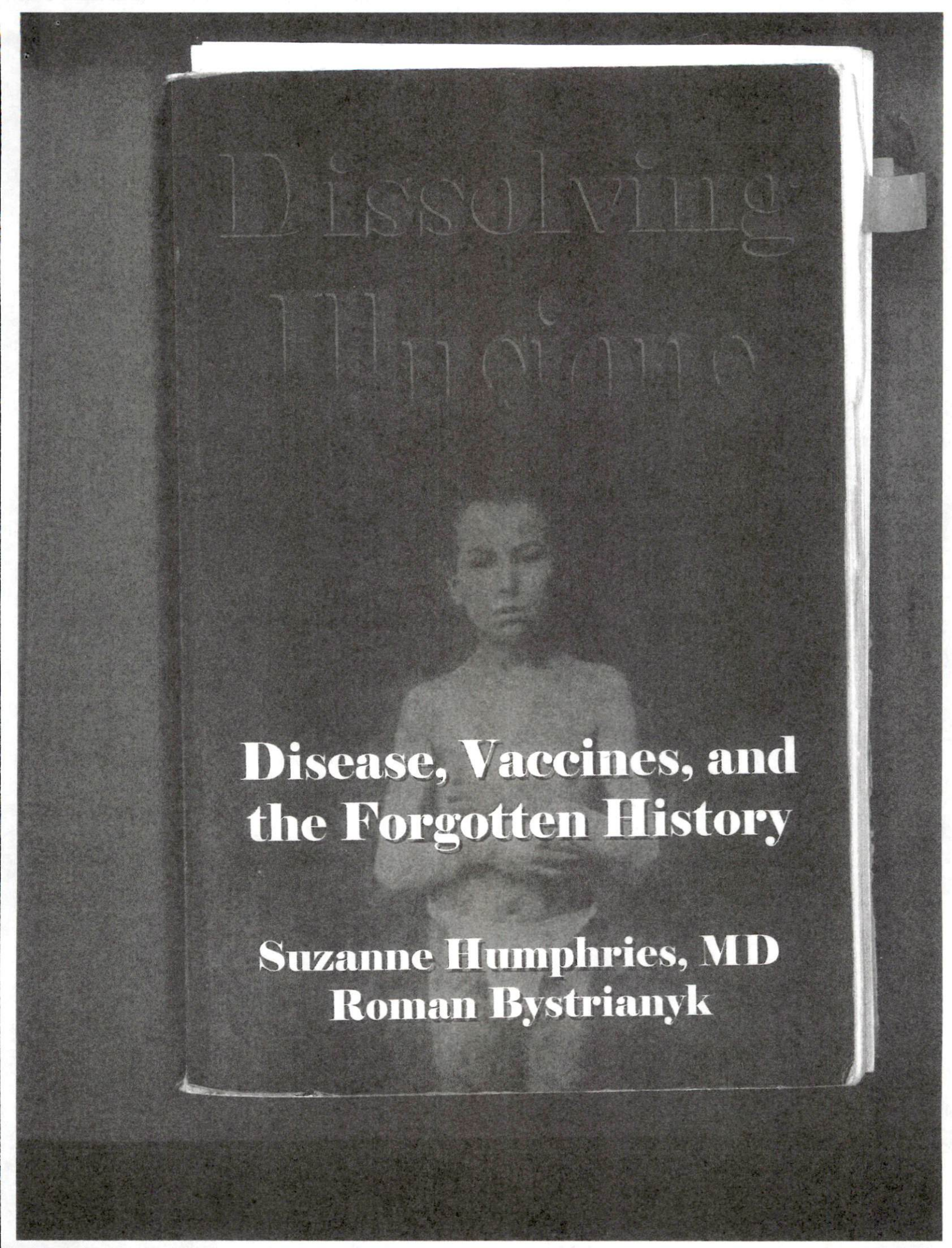
Dissolving Illusions
Disease, Vaccines, and the Forgotten History
Suzanne Humphries, MD
Roman Bystrianyk

Not too long ago, lethal infections were feared in the Western world. Since that time, many countries have undergone a transformation from disease cesspools to much safer, healthier habitats. Starting in the mid-1800s, there was a steady drop in deaths from all infectious diseases, decreasing to relatively minor levels by the early 1900s.

The history of that transformation involves famine, poverty, filth, lost cures, eugenicist doctrine, individual freedoms versus state might, protests and arrests over vaccine refusal, and much more.

Today, we are told that medical interventions increased our lifespan and single-handedly prevented masses of deaths. But is this really true?

Dissolving Illusions details facts and figures from long-overlooked medical journals, books, newspapers, and other sources. Using myth-shattering graphs, this book shows that vaccines, antibiotics, and other medical interventions are not responsible for the increase in lifespan and the decline in mortality from infectious diseases. If the medical profession could systematically misinterpret and ignore key historical information, the question must be asked, "What else is ignored and misinterpreted today?"

Perhaps the best reason to know our history is so that the worst parts are never repeated.

Roman Bystrianyk

Dr. Suzanne Humphries is a medical doctor, Internist, and Board Certified Nephrologist. She is currently in private practice and continues to dispel the mythology surrounding vaccination.

Roman Bystrianyk has been researching the history of diseases and vaccines since 1996. He has an extensive background in health and nutrition as well as a B.S. in engineering and M.S. in computer science.

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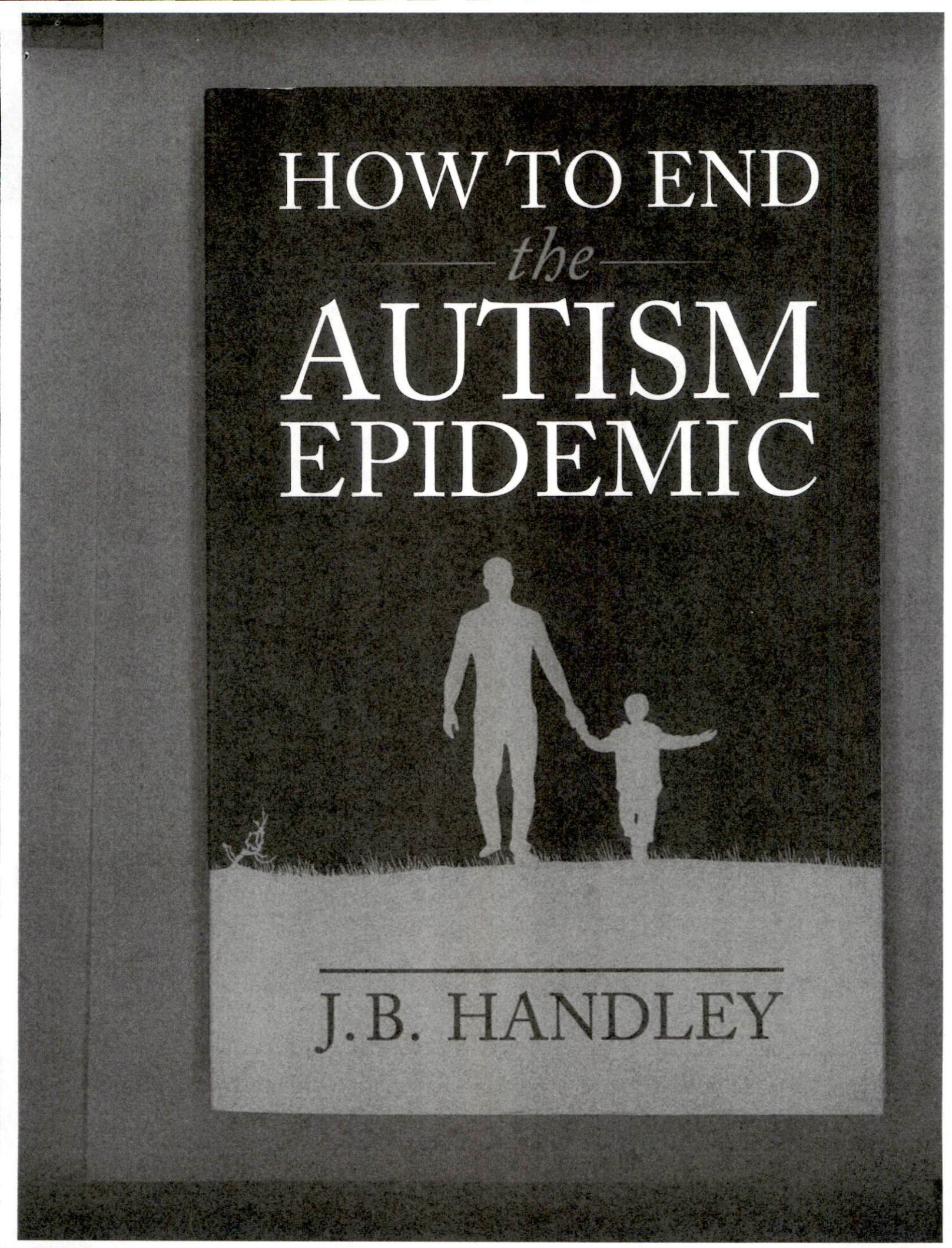
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the
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"This book is inspired, powerful, the unadulterated truth, and a must read.... Parents and future parents: Read this book now, and say No to business as usual and the status quo. If your pediatrician has not yet done his or her own research and is just parroting the 'vaccines are safe and effective' marketing phrase, it is time for you to get a new pediatrician." —**Paul Thomas**, MD, coauthor of *The Vaccine-Friendly Plan* and *The Addiction Spectrum*

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Infant mortality rates regressed against number of vaccine doses routinely given: Is there a biochemical or synergistic toxicity?

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Abstract

The infant mortality rate (IMR) is one of the most important indicators of the socio-economic well-being and public health conditions of a country. The US childhood immunization schedule specifies 26 vaccine doses for infants aged less than 1 year—the most in the world—yet 33 nations have lower IMRs. Using linear regression, the immunization schedules of these 34 nations were examined and a correlation coefficient of $r = 0.70$ ($p < 0.0001$) was found between IMRs and the number of vaccine doses routinely given to infants. Nations were also grouped into five different vaccine dose ranges: 12–14, 15–17, 18–20, 21–23, and 24–26. The mean IMRs of all nations within each group were then calculated. Linear regression analysis of unweighted mean IMRs showed a high statistically significant correlation between increasing number of vaccine doses and increasing infant mortality rates, with $r = 0.992$ ($p = 0.0009$). Using the Tukey-Kramer test, statistically significant differences in mean IMRs were found between nations giving 12–14 vaccine doses and those giving 21–23, and 24–26 doses. A closer inspection of correlations between vaccine doses, biochemical or synergistic toxicity, and IMRs is essential.

Keywords: infant mortality rates, sudden infant death, SIDS, immunization schedules, childhood vaccines, drug toxicology, synergistic effects, linear regression model

Introduction

The infant mortality rate (IMR) is one of the most important measures of child health and overall development in countries. Clean water, increased nutritional measures, better sanitation, and easy access to health care contribute the most to improving infant mortality rates in unclean, undernourished, and impoverished regions of the world.[1–3] In developing nations, IMRs are high

because these basic necessities for infant survival are lacking or unevenly distributed. Infectious and communicable diseases are more common in developing countries as well, though sound sanitary practices and proper nutrition would do much to prevent them.[1]

The World Health Organization (WHO) attributes 7 out of 10 childhood deaths in developing countries to five main causes: pneumonia, diarrhea, measles, malaria, and malnutrition—the latter greatly affecting all the others.[1] Malnutrition has been associated with a decrease in immune function. An impaired immune function often leads to an increased susceptibility to infection.[2] It is well established that infections, no matter how mild, have adverse effects on nutritional status. Conversely, almost any nutritional deficiency will diminish resistance to disease.[3]

Despite the United States spending more per capita on health care than any other country,[4] 33 nations have better IMRs. Some countries have IMRs that are less than half the US rate: Singapore, Sweden, and Japan are below 2.80. According to the Centers for Disease Control and Prevention (CDC), "The relative position of the United States in comparison to countries with the lowest infant mortality rates appears to be worsening."[5]

There are many factors that affect the IMR of any given country. For example, premature births in the United States have increased by more than 20% between 1990 and 2006. Preterm babies have a higher risk of complications that could lead to death within the first year of life.[6] However, this does not fully explain why the United States has seen little improvement in its IMR since 2000.[7]

Nations differ in their immunization requirements for infants aged less than 1 year. In 2009, five of the 34 nations with the best IMRs required 12 vaccine doses, the least amount, while the United States required 26 vaccine doses, the most of any nation. To explore the correlation between vaccine doses that nations routinely give to their infants and their infant mortality rates, a linear regression analysis was performed.

Methods and design

Infant mortality

The infant mortality rate is expressed as the number of infant deaths per 1000 live births. According to the US Central Intelligence Agency (CIA), which keeps accurate, up-to-date infant mortality statistics throughout the world, in 2009 there were 33 nations with better infant mortality rates than the United States (Table 1).[8] The US infant mortality rate of 6.22 infant deaths per 1000 live births ranked 34th.

Table 1.

2009 Infant mortality rates, top 34 nations[8]

Rank	Country	IMR
1	Singapore	2.31
2	Sweden	2.75
3	Japan	2.79
4	Iceland	3.23
5	France	3.33
6	Finland	3.47
7	Norway	3.58
8	Malta	3.75
9	Andorra	3.76
10	Czech Republic	3.79
11	Germany	3.99
12	Switzerland	4.18
13	Spain	4.21
14	Israel	4.22
15	Liechtenstein	4.25

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CIA. Country comparison: infant mortality rate (2009). *The World Factbook.* www.cia.gov (Data last updated 13 April 2010).[8]

Immunization schedules and vaccine doses

A literature review was conducted to determine the immunization schedules for the United States and all 33 nations with better IMRs than the United States.[9,10] The total number of vaccine doses specified for infants aged less than 1 year was then determined for each country (Table 2). A vaccine dose is an exact amount of medicine or drug to be administered. The number of doses a child receives should not be confused with the number of 'vaccines' or 'injections' given. For example, DTaP is given as a single injection but contains three separate vaccines (for diphtheria, tetanus, and pertussis) totaling three vaccine doses.

Table 2.

Summary of International Immunization Schedules: vaccines recommended/required prior to one year of age in 34 nations

	BCG		
Andorra[a]	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), MenC (2)	23	
Austria	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), Rota (2)	23	
Ireland	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (2), MenC (2), BCG	23	
Greece	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), MenC (2)	23	
Monaco[a]	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), HepA, BCG	23	
Netherlands	DTaP (4), Polio (4), Hib (4), Pneumo (4)	24	5 (24–26)
Canada	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), MenC (2), Flu	24	
Australia	DTaP (3), Polio (3), Hib (3), HepB (4), Pneumo (3), Rota (2)	24	
United States	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), Rota (3), Flu (2)	26	

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[a] These four nations were excluded from the analysis because they had fewer than five infant deaths.

[b] DTaP is administered as a single shot but contains three separate vaccines (for diphtheria, tetanus, and pertussis). Thus, DTaP given three times in infancy is equivalent to nine vaccine doses. Immunization schedules are for 2008–2009.[9,10]

Nations organized into data pairs

The 34 nations were organized into data pairs consisting of total number of vaccine doses specified for their infants and IMRs. Consistent with biostatistical conventions, four nations—Andorra, Liechenstein, Monaco, and San Marino—were excluded from the dataset because they each had fewer than five infant deaths, producing extremely wide confidence intervals and IMR instability. The remaining 30 (88%) of the data pairs were then available for analysis.

Nations organized into groups

Nations were placed into the following five groups based on the number of vaccine doses they routinely give their infants: 12–14, 15–17, 18–20, 21–23, and 24–26 vaccine doses. The unweighted IMR means of all nations as a function of the number of vaccine doses were analyzed using linear regression. The Pearson correlation coefficient (r) and coefficient of determination (r^2) were calculated using GraphPad Prism, version 5.03 (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Additionally, the F statistic and corresponding p values were computed to test if the best fit slope was statistically significantly non-zero. The Tukey-Kramer test was used to determine whether or not the mean IMR differences between the groups were statistically significant. Following the one-way ANOVA (analysis of variance) results from the Tukey-Kramer test, a post test for the overall linear trend was performed.

Results

Nations organized into data pairs

A scatter plot of each of the 30 nation's IMR versus vaccine doses yielded a linear relationship with a correlation coefficient of 0.70 (95% CI, 0.46–0.85) and $p < 0.0001$ providing evidence of a positive correlation: IMR and vaccine doses tend to increase together. The F statistic applied to the slope [0.148 (95% CI, 0.090–0.206)] is significantly non-zero, with $F = 27.2$ ($p < 0.0001$; Figure 1).

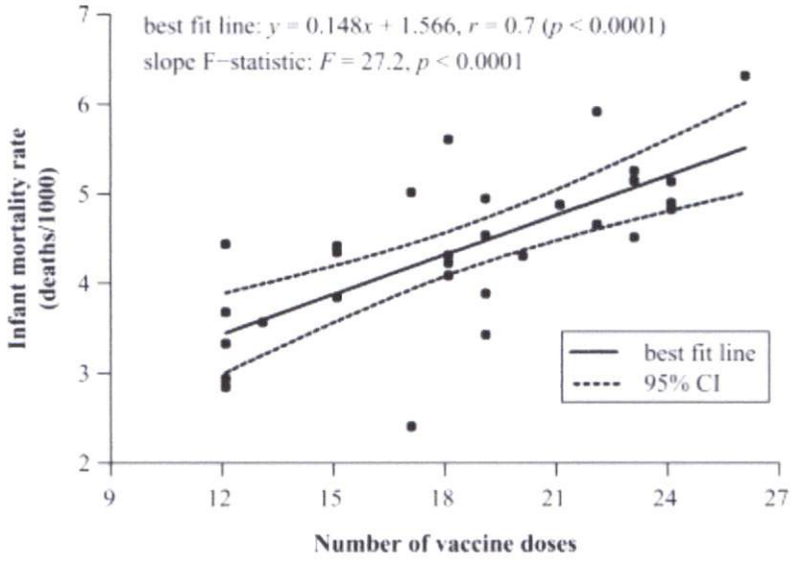


Figure 1.

2009 Infant mortality rates and number of vaccine doses for 30 nations.

Nations organized into groups

The unweighted mean IMR of each category was computed by simply summing the IMRs of each nation comprising a group and dividing by the number of nations in that group. The IMRs were as follows: 3.36 (95% CI, 2.74–3.98) for nations specifying 12–14 doses (mean 13 doses); 3.89 (95% CI, 2.68–5.12) for 15–17 doses (mean 16 doses); 4.28 (95% CI, 3.80–4.76) for 18–20 doses (mean 19 doses); 4.97 (95% CI, 4.44–5.49) for 21–23 doses (mean 22 doses); 5.19 (95% CI, 4.06–6.31) for 24-26 doses (mean 25 doses; Figure 2). Linear regression analysis yielded an equation of the best fit line, $y = 0.157x + 1.34$ with $r = 0.992$ ($p = 0.0009$) and $r^2 = 0.983$. Thus, 98.3% of the variation in mean IMRs is explained by the linear model. Again, the F statistic yielded a significantly non-zero slope, with $F = 173.9$ ($p = 0.0009$).

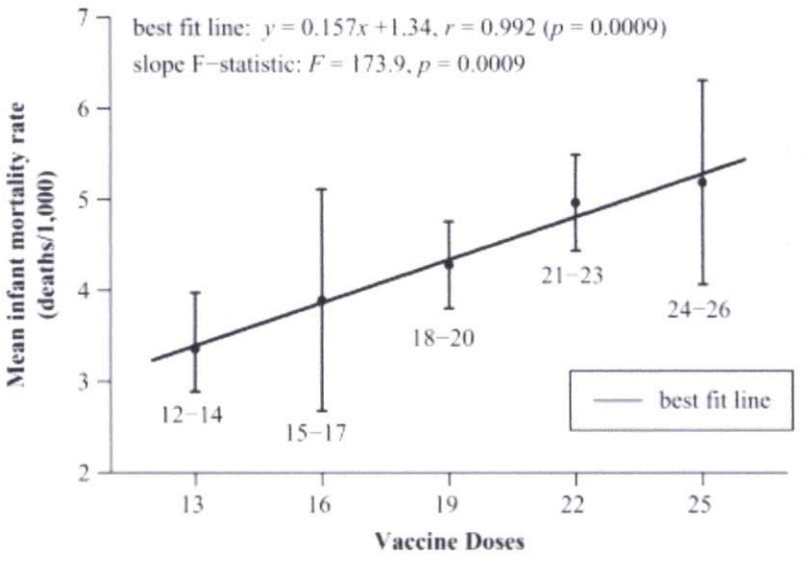


Figure 2.

2009 Mean infant mortality rates and mean number of vaccine doses (five categories).

The one-way ANOVA using the Tukey-Kramer test yielded $F = 650$ with $p = 0.001$, indicating the five mean IMRs corresponding to the five defined dose categories are significantly different ($r^2 = 0.510$). Tukey's multiple comparison test found statistical significance in the differences between the mean IMRs of those nations giving 12–14 vaccine doses and (a) those giving 21–23 doses (1.61, 95% CI, 0.457–2.75) and (b) those giving 24–26 doses (1.83, 95% CI, 0.542–3.11).

Discussion

Basic necessities for infant survival

It is instructive to note that many developing nations require their infants to receive multiple vaccine doses and have national vaccine coverage rates (a percentage of the target population that has been vaccinated) of 90% or better, yet their IMRs are poor. For example, Gambia requires its infants to

receive 22 vaccine doses during infancy and has a 91%–97% national vaccine coverage rate, yet its IMR is 68.8. Mongolia requires 22 vaccine doses during infancy, has a 95%–98% coverage rate, and an IMR of 39.9.[8,9] These examples appear to confirm that IMRs will remain high in nations that cannot provide clean water, proper nutrition, improved sanitation, and better access to health care. *As developing nations improve in all of these areas a critical threshold will eventually be reached where further reductions of the infant mortality rate will be difficult to achieve because most of the susceptible infants that could have been saved from these causes would have been saved.* Further reductions of the IMR must then be achieved in areas outside of these domains. As developing nations ascend to higher socio-economic living standards, a closer inspection of all factors contributing to infant deaths must be made.

Crossing the socio-economic threshold

It appears that at a certain stage in nations' movement up the socio-economic scale—after the basic necessities for infant survival (proper nutrition, sanitation, clean water, and access to health care) have been met—a counter-intuitive relationship occurs between the number of vaccines given to infants and infant mortality rates: nations with higher (worse) infant mortality rates give their infants, on average, more vaccine doses. This positive correlation, derived from the data and demonstrated in Figures 1 and 2, elicits an important inquiry: are some infant deaths associated with over-vaccination?

A closer inspection of infant deaths

Many nations adhere to an agreed upon International Classification of Diseases (ICD) for grouping infant deaths into 130 categories.[11–13] Among the 34 nations analyzed, those that require the most vaccines tend to have the worst IMRs. Thus, we must ask important questions: is it possible that some nations are requiring too many vaccines for their infants and the additional vaccines are a toxic burden on their health? Are some deaths that are listed within the 130 infant mortality death categories really deaths that are associated with over-vaccination? Are some vaccine-related deaths hidden within the death tables?

Sudden infant death syndrome (SIDS)

Prior to contemporary vaccination programs, 'Crib death' was so infrequent that it was not mentioned in infant mortality statistics. In the United States, national immunization campaigns were initiated in the 1960s when several new vaccines were introduced and actively recommended. For the first time in history, most US infants were required to receive several doses of DPT, polio, measles, mumps, and rubella vaccines.[14] Shortly thereafter, in 1969, medical certifiers presented a new medical term—sudden infant death syndrome.[15,16] In 1973, the National Center for Health Statistics added a new cause-of-death category—for SIDS—to the ICD. SIDS is defined as the sudden and unexpected death of an infant which remains unexplained after a thorough investigation. Although there are no specific symptoms associated with SIDS, an autopsy often reveals congestion and edema of the lungs and inflammatory changes in the respiratory system.[17] By 1980, SIDS had become the leading cause of postneonatal mortality (deaths of infants from 28 days to one year old) in the United States.[18]

In 1992, to address the unacceptable SIDS rate, the American Academy of Pediatrics initiated a 'Back to Sleep' campaign, convincing parents to place their infants supine, rather than prone, during sleep. From 1992 to 2001, the postneonatal SIDS rate dropped by an average annual rate of 8.6%. However, other causes of sudden unexpected infant death (SUID) increased. For example, the postneonatal mortality rate from 'suffocation in bed' (ICD-9 code E913.0) increased during this same period at an average annual rate of 11.2%. The postneonatal mortality rate from 'suffocation-other' (ICD-9 code E913.1-E913.9), 'unknown and unspecified causes' (ICD-9 code 799.9), and due to 'intent unknown' in the External Causes of Injury section (ICD-9 code E980-E989), all increased during this period as well.[18] (In Australia, Mitchell et al. observed that when the SIDS rate decreased, deaths attributed to asphyxia increased.[19] Overpeck et al. and others, reported similar observations.)[20,21]

A closer inspection of the more recent period from 1999 to 2001 reveals that the US postneonatal SIDS rate continued to decline, but *there was no significant change in the total postneonatal mortality rate.* During this period, the number of deaths attributed to 'suffocation in bed' and 'unknown causes,' increased significantly. According to Malloy and MacDorman, "If death-certifier preference has shifted such that previously classified SIDS deaths are now classified as 'suffocation,' the inclusion of these suffocation deaths and unknown or unspecified deaths with SIDS deaths then accounts for about 90 percent of the decline in the SIDS rate observed between 1999 and 2001 and results in a non-significant decline in SIDS"[18] (Figure 3).

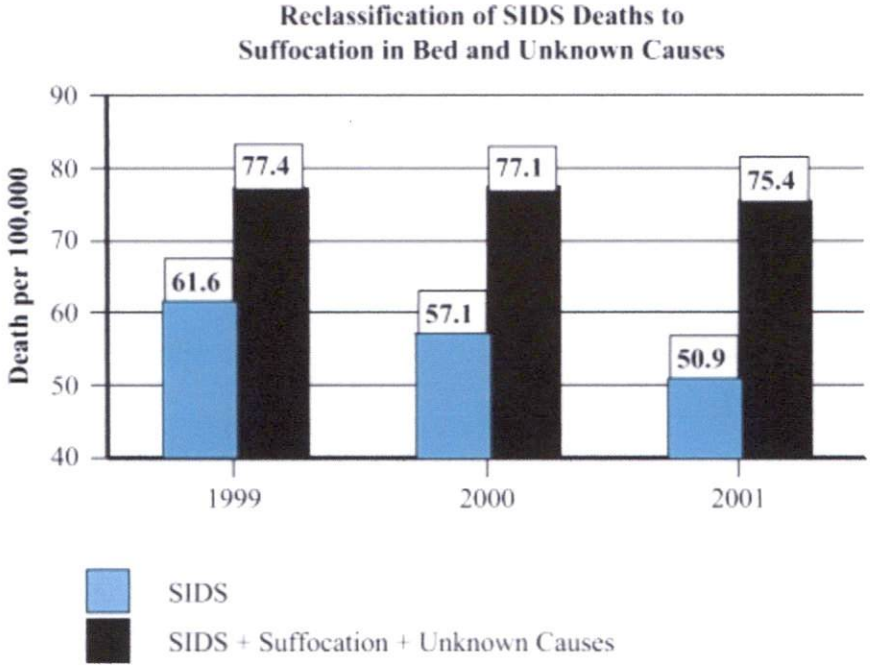
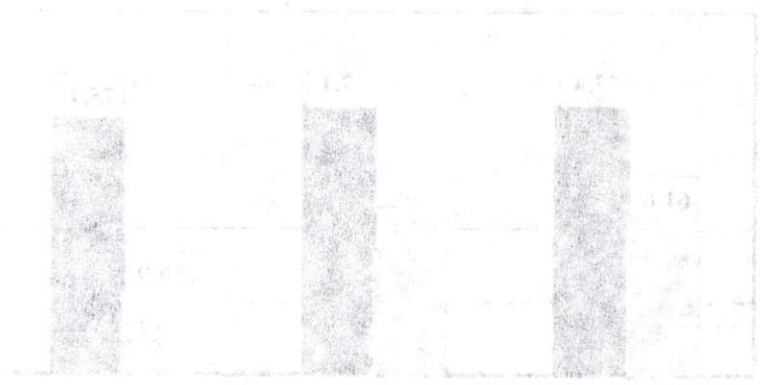


Figure 3.

Reclassification of sudden infant death syndrome (SIDS) deaths to suffocation in bed and unknown causes. The postneonatal SIDS rate appears to have declined from 61.6 deaths (per 100,000 live births) in 1999 to 50.9 in 2001. However, during this period there was a significant increase in postneonatal deaths attributed to suffocation in bed and due to unknown causes. When these sudden unexpected infant deaths (SUIDs) are combined with SIDS deaths, the total SIDS rate remains relatively stable, resulting in a non-significant decline.

Is there evidence linking SIDS to vaccines?

Although some studies were unable to find correlations between SIDS and vaccines,[22–24] there is some evidence that a subset of infants may be more susceptible to SIDS shortly after being vaccinated. For example, Torch found that two-thirds of babies who had died from SIDS had been vaccinated against DPT (diphtheria–pertussis–tetanus toxoid) prior to death. Of these, 6.5% died within 12 hours of vaccination; 13% within 24 hours; 26% within 3 days; and 37%, 61%, and 70% within 1, 2, and 3 weeks, respectively. Torch also found that unvaccinated babies who died of SIDS did so most often in the fall or winter while vaccinated babies died most often at 2 and 4 months—the same ages when initial doses of DPT were given to infants. He concluded that DPT "may be a generally unrecognized major cause of sudden infant and early childhood death, and that the risks of immunization may outweigh its potential benefits. A need for re-evaluation and possible modification of current vaccination procedures is indicated by this study."[25] Walker et al. found "the SIDS mortality rate in the period zero to three days following DPT to be 7.3 times that in the period beginning 30 days after immunization."[26] Fine and Chen reported that babies died at a rate nearly eight times greater than normal within 3 days after getting a DPT vaccination.[27]



Ottaviani et al. documented the case of a 3-month-old infant who died suddenly and unexpectedly shortly after being given six vaccines in a single shot: "Examination of the brainstem on serial sections revealed bilateral hypoplasia of the arcuate nucleus. The cardiac conduction system presented persistent fetal dispersion and resorptive degeneration. This case offers a unique insight into the possible role of hexavalent vaccine in triggering a lethal outcome in a vulnerable baby." Without a full necropsy study in the case of sudden, unexpected infant death, at least some cases linked to vaccination are likely to go undetected.[28]

Reclassified infant deaths

It appears as though some infant deaths attributed to SIDS may be vaccine related, perhaps associated with biochemical or synergistic toxicity due to over-vaccination. Some infants' deaths categorized as 'suffocation' or due to 'unknown and unspecified causes' may also be cases of SIDS reclassified within the ICD. Some of these infant deaths may be vaccine related as well. This trend toward reclassifying ICD data is a great concern of the CDC "because inaccurate or inconsistent cause-of-death determination and reporting hamper the ability to monitor national trends, ascertain risk factors, and design and evaluate programs to prevent these deaths."[29] If some infant deaths are vaccine related and concealed within the various ICD categories for SUIDs, is it possible that other vaccine-related infant deaths have also been reclassified?

Of the 34 nations that have crossed the socio-economic threshold and are able to provide the basic necessities for infant survival—clean water, nutrition, sanitation, and health care—several require their infants to receive a relatively high number of vaccine doses and have relatively high infant mortality rates. These nations should take a closer look at their infant death tables to determine if some fatalities are possibly related to vaccines though reclassified as other causes. Of course, all SUID categories should be re-inspected. Other ICD categories may be related to vaccines as well. For example, a new live-virus orally administered vaccine against rotavirus-induced diarrhea—Rotarix®—was licensed by the European Medicine Agency in 2006 and approved by the US Food and Drug Administration (FDA) in 2008. However, in a clinical study that evaluated the safety of the Rotarix vaccine, *vaccinated babies died at a higher rate than non-vaccinated babies*—mainly due to a statistically significant increase in pneumonia-related fatalities.[30] (One biologically plausible explanation is that natural rotavirus infection might have a protective effect against respiratory infection.)[31] Although these fatalities appear to be vaccine related and raise a nation's infant mortality rate, medical certifiers are likely to misclassify these deaths as pneumonia.

Several additional ICD categories are possible candidates for incorrect infant death classifications: unspecified viral diseases, diseases of the blood, septicemia, diseases of the nervous system, anoxic brain damage, other diseases of the nervous system, diseases of the respiratory system, influenza, and unspecified diseases of the respiratory system. All of these selected causes may be repositories of vaccine-related infant deaths reclassified as common fatalities. All nations—rich and poor, industrialized and developing—have an obligation to determine whether their immunization schedules are achieving their desired goals. Progress on reducing infant mortality rates should include monitoring vaccine schedules and medical certification practices to ascertain whether vaccine-related infant deaths are being reclassified as ordinary mortality in the ICD.

How many infants can be saved with an improved IMR?

Slight improvements in IMRs can make a substantial difference. In 2009, there were approximately 4.5 million live births and 28,000 infant deaths in the United States, resulting in an infant mortality rate of 6.22/1000. If health authorities can find a way to reduce the rate by 1/1000 (16%), the United States would rise in international rank from 34th to 31st and about 4500 infants would be saved.

Limitations of study and potential confounding factors

This analysis did not adjust for vaccine composition, national vaccine coverage rates, variations in the infant mortality rates among minority races, preterm births, differences in how some nations report live births, or the potential for ecological bias. A few comments about each of these factors are included below.

Vaccine composition

This analysis calculated the total number of vaccine doses received by children but did not differentiate between the substances, or quantities of those substances, in each dose. Common vaccine substances include antigens (attenuated viruses, bacteria, toxoids), preservatives (thimerosal, benzethonium chloride, 2-phenoxyethanol, phenol), adjuvants (aluminum salts), additives (ammonium sulfate, glycerin, sodium borate, polysorbate 80, hydrochloric acid, sodium hydroxide, potassium chloride), stabilizers (fetal bovine serum, monosodium glutamate, human serum albumin, porcine gelatin), antibiotics (neomycin, streptomycin, polymyxin B), and inactivating chemicals (formalin, glutaraldehyde, polyoxyethylene). For the purposes of this study, all vaccine doses were equally weighted.

Vaccine coverage rates

No adjustment was made for national vaccine coverage rates—a percentage of the target population that received the recommended vaccines. However, most of the nations in this study had coverage rates in the 90%–99% range for the most commonly recommended vaccines—DTaP, polio, hepatitis B, and Hib (when these vaccines were included in the schedule). Therefore, this factor is unlikely to have impacted the analyses.[9]

Minority races

It has been argued that the US IMR is poor in comparison to many other nations because African–American infants are at greater risk of dying relative to White infants, perhaps due to genetic factors or disparities in living standards. However, in 2006 the US IMR for infants of all races was 6.69 and the IMR for White infants was 5.56.[13] In 2009, this improved rate would have moved the United States up by just one rank internationally, from 34th place to 33rd place.[8] In addition, the IMRs for Hispanics of Mexican descent and Asian–Americans in the United States are significantly lower than the IMR for Whites.[6] Thus, diverse IMRs among different races in the Unites States exert only a modest influence over the United States' international infant mortality rank.

Preterm births

Preterm birth rates in the United States have steadily increased since the early 1980s. (This rise has been tied to a greater reliance on caesarian deliveries, induced labor, and more births to older mothers.) Preterm babies are more likely than full-term babies to die within the first year of life. About 12.4% of US births are preterm. In Europe, the prevalence rate of premature birth ranges from 5.5% in Ireland to 11.4% in Austria. Preventing preterm births is essential to lower infant mortality rates. However, it is important to note that some nations such as Ireland and Greece, which have very low preterm birth rates (5.5% and 6%, respectively) compared to the United States, require their infants to receive a relatively high number of vaccine doses (23) and have correspondingly high IMRs. Therefore, reducing preterm birth rates is only part of the solution to reduce IMRs.[6,32]

Differences in reporting live births

Infant mortality rates in most countries are reported using WHO standards, which do not include any reference to the duration of pregnancy or weight of the infant, but do define a 'live birth' as a baby born with any signs of life for any length of time.[12] However, four nations in the dataset—France, the Czech Republic, the Netherlands, and Ireland—do not report live births entirely consistent with WHO standards. These countries add an additional requirement that live babies must also be at least 22 weeks of gestation or weigh at least 500 grams. If babies do not meet this requirement and die shortly after birth, they are reported as stillbirths. This inconsistency in reporting live births artificially lowers the IMRs of these nations.[32,33] According to the CDC, "There are some differences among countries in the reporting of very small infants who may die soon after birth. However, it appears unlikely that differences in reporting are the primary explanation for the United States' relatively low international ranking."[32] Nevertheless, when the IMRs of France, the Czech Republic, the Netherlands, and Ireland were adjusted for known underreporting of live births and the 30 data pairs retested for significance, the correlation coefficient improved from 0.70 to 0.74 (95% CI, 0.52–0.87).

Ecological bias

Ecological bias occurs when relationships among individuals are inferred from similar relationships observed among groups (or nations). Although most of the nations in this study had 90%–99% of their infants fully vaccinated, without additional data we do not know whether it is the vaccinated or unvaccinated infants who are dying in infancy at higher rates. However, respiratory disturbances have been documented in close proximity to infant vaccinations, and lethal changes in the brainstem of a recently vaccinated baby have been observed. Since some infants may be more susceptible to SIDS shortly after being vaccinated, and babies vaccinated against diarrhea died from pneumonia at a statistically higher rate than non-vaccinated babies, there is plausible biologic and causal evidence that the observed correlation between IMRs and the number of vaccine doses routinely given to infants should not be dismissed as ecological bias.

Conclusion

The US childhood immunization schedule requires 26 vaccine doses for infants aged less than 1 year, the most in the world, yet 33 nations have better IMRs. Using linear regression, the immunization schedules of these 34 nations were examined and a correlation coefficient of 0.70 ($p < 0.0001$) was found between IMRs and the number of vaccine doses routinely given to infants. When nations were

grouped into five different vaccine dose ranges (12–14, 15–17, 18–20, 21–23, and 24–26), 98.3% of the total variance in IMR was explained by the unweighted linear regression model. These findings demonstrate a counter-intuitive relationship: *nations that require more vaccine doses tend to have higher infant mortality rates*.

Efforts to reduce the relatively high US IMR have been elusive. Finding ways to lower preterm birth rates should be a high priority. However, preventing premature births is just a partial solution to reduce infant deaths. A closer inspection of correlations between vaccine doses, biochemical or synergistic toxicity, and IMRs, is essential. All nations—rich and poor, advanced and developing—have an obligation to determine whether their immunization schedules are achieving their desired goals.

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Thank you Madam Chair and committee
Hello my name is Brittany Olig
I am against HB19-1312.
My son was born a healthy 8 lb. 15 oz with an Apgar score of 9, he received a vitamin K shot at birth and now my family and I have to deal with consequences of our decision for the rest of lives! My son suffered one of the worst side effects this injection has to offer.

My son has suffered from hundreds of seizures a day. He lives in a prison, and my heart is broken daily. I have often wondered if my son will live.

I had two choices, do a corpus colostomy or try medical marijuana. I opted for natural medicine, and so our family moved here to build our lives, and find healing in the beautiful state of Colorado. We chose Colorado instead of California, strictly because of vaccine laws. While HB1312 is different from the California vaccine laws, it is dangerously similar. My family moved here for medical freedom, we are medical refugees.

My son is almost 6, and will live always live in our home, with 100 % full time care. We utilize the personal and religious belief exemption to avoid any further injections, and with the benefits of medical marijuana, he is now very healthy.

I did not know that vitamin K is a Blackbox shot, and neomycin sulfate side effects in this shot can lead to epilepsy and brain damage, which my son has been diagnosed with, along with autism, he is completely nonverbal!

None of my son's experiences qualifies him for a medical exemption according to HB19-1312 pg 8 lines 9-15. This law will cause a hardship for my family and any future laws that tighten restrictions on exemption rights will cause further injury to my son, un-needed pain and suffering, and will require us to move again to find medical freedom.

The Federal vaccine schedule directs doctors to give infants and children a whopping 69 doses of vaccines by the age of six. What are the effects of multiple vaccine ingredients on the human body? We are just beginning to learn about these injuries, because there have not been any double blind placebo tests in the United States in the last 30 years. Under these circumstances, we have the right to deny any vaccine we see fit.

Please do not proceed with HB19-1312, for the sake of Coloradan's children, for the sake of my son. Our healthcare decisions are a private matter, between parents and their healthcare providers. I work hard to give the best care to my son, please don't make it harder on me.

Polysorbate 80: A Risky Vaccine Ingredient

by Rishma Parpia
Published January 7, 2016 | Vaccination, Ingredients

STORY HIGHLIGHTS

- Polysorbate 80, a surfactant, is an ingredient used in several vaccines.
- There are serious safety concerns regarding its use as a vaccine ingredient.
- There is glaring lack of research evaluating the risks to human health of including Polysorbate 80 in vaccines.

The recent public conversation about the safety of vaccine ingredients has centered primarily around mercury (thimerosal), aluminum and formaldehyde. However, there are other concerning ingredients in vaccines that are not talked about as often, one of which is Polysorbate 80—also known as Tween 80 and polyoxyethylene-sorbitan-20 mono-oleate.[1]

What is Polysorbate 80?

Polysorbate 80 is a surfactant commonly used in the pharmaceutical, cosmetic and food industry.[1] Surfactants are compounds that reduce the surface tension and increase the solubility between two liquids that would normally be unable to dissolve together, e.g. oil in water.[2 3] Because of their ability to lower surface tension between liquids, surfactants are known for their spreading and wetting properties. Therefore, surfactants act as detergents, emulsifiers, wetting agents, foaming agents and dispersants.[1 2]

To prevent separation of liquids, Polysorbate 80 is present in several formulas such as skin care products, shampoos, makeup, ice-cream, processed salad dressings and sauces.[3 4] Within the pharmaceutical industry, polysorbate 80 is used to improve and maintain consistency of gel capsules; assist in keeping medication suspended in liquids; in preparation of intravenous fluids; as an excipient in tablets, and in the manufacture of vaccines.[1 4]

Polysorbate 80 in Vaccines: Not Well Tested

The U.S. Centers for Disease Control and Prevention (CDC) Vaccine Excipient and Media Summary lists 11 vaccines that contain polysorbate 80:

- DTaP (Infanrix);
- DTaP—IPV (Kinrix);
- DTap-HepB-IPV (Pediarix);
- DTaP-IPV-Hib (Pentacel);
- Gardasil
- Influenza (Agriflu);
- Influenza (Fluarix);
- Meningococcal (MenB-Trumenba);
- Pneumococcal (PCV13—Prevnar13);
- Rotavirus (RotaTeq);
- Tdap (Boostrix)[5]

According to the Material Safety and Data Sheet (MSDS) on ScienceLab.com, Polysorbate 80 was tested for inhalation and ingestion and demonstrated to be slightly hazardous in case of skin contact.[6] The MSDS does not address the effects of polysorbate through injection. Nevertheless, in the same toxicology section under special remarks on chronic and toxic effects on human, it states that Polysorbate 80:

> *May cause adverse reproductive effects based on animal test data. No human data found. May cause cancer based on animal test data. No human data found. May affect genetic material (mutagenic). Ingestion of very large doses may cause abdominal spasms and diarrhea. Animal studies have shown it to cause cardiac changes, changes in behavior (altered sleep time) and weight loss (upon repeated or prolonged ingestion). However, no similar human data has been reported.*[6]

The fact that Polysorbate 80 "may cause cancer based on animal test data" and may be mutagenic alone should be enough to require vaccine manufacturers and the Food and Drug Administration to provide credible scientific evidence that it is safe to include Polysorbate 80 in vaccines given to humans.

Polysorbate 80: Damage to the Brain?

In vaccines, Polysorbate 80 acts as an *emulsifier* to disperse all the other ingredients evenly within the liquid. Pediatrician Lawrence Palevsky, MD warns of the potential danger of using Polysorbate 80 as a vaccine ingredient.[8] He notes,

> *Polysorbate 80 is used in pharmacology to assist in the delivery of certain drugs or chemotherapeutic agents across the blood-brain-barrier.*[8]

This raises serious concerns of using Polysorbate 80 in combination with other reactive vaccine ingredients, which have the potential to damage the brain.[8]

The blood-brain-barrier is a barrier that separates the brain from the circulatory system and protects the central nervous system from harmful chemicals and other toxins. The blood brain barrier is particularly weak and more easily penetrated during infancy and in old age. Consequently, the concern with using Polysorbate 80 in vaccines is that it may permit the entry of other toxic ingredients, such as heavy metals, into the brain.[2]

Dr. Palevsky asks some crucial questions regarding whether Polysorbate 80 is having negative effects on and facilitating toxic insults to the brain via vaccination.

> *What viral, bacterial, yeast, heavy metal or other vaccine containing ingredient needs to pass into the brains of our children? Do they belong in the brain? Is that part of the needed immune response to protect our children from disease? Do vaccine materials pass across the blood-brain barrier with the help of Polysorbate-80? If so, are there complications from being in the brains of our children?*[8]

Furthermore, once injected into the body, polysorbate 80 can rapidly break down into sorbitol and ethylene oxide. Sorbitol has the ability to increase the risk of diabetes, cell death, mitochondrial failure and DNA fragmentation.[2]

Polysorbate 80: Damage to Uterus?

Research has demonstrated that Polysorbate 80 can lead to infertility in rats. A study published in the *Journal of Food and Chemical Toxicology*...

> *discovered that Tween80 accelerated the rats' maturation, prolonged the estrous cycle, decreased the weight of the uterus and ovaries, and caused damage to the lining of the uterus indicative of chronic estrogenic stimulation. The rats' ovaries were also damaged, with degenerative follicles and no corpora lutea (a mass of progesterone-secreting endocrine tissue that forms immediately after ovulation). Such severe deformities to the ovary can lead to infertility.*[10]

The question that needs to be answered is whether the demonstrated toxic effects of Polysorbate 80 in animals, including damage to the uterus and ovaries leading to infertility, applies to humans as well.

Polysorbate 80: Damage to Immune Function?

There are also concerns with the use of *detergents* in vaccines. Our body has something called the Membrane Attack Complex (MAC)—one of the immune system's toughest weapons.[2] When the body identifies a pathogen, MAC proteins kill the cells of pathogens by tunneling through their surface membranes thus causing them to leak or explode.

Although there are similarities between MAC proteins and detergents in terms of their "attacking" function, detergents are not regulated in body in the same manner as MAC proteins.[2] A protein known as CD59 regulates MAC; its role is to restrain other MAC proteins from attaching to our cells thus preventing them from rupturing.[2]

Due to the lack to regulating proteins in detergents, they attack cells randomly and have the potential to attack our own cells ignoring immune system alerts to cease attacking.[2]

Several studies illustrate that lack of CD59 protection can lead to damaged neuromuscular transmission junctions, rheumatoid arthritis, kidney disease, stroke or fatal cerebral hemorrhage. Considering that regulatory CD59 protein is non-existent in injected detergents, all of the mentioned medical conditions are to be expected when using detergents in vaccines since they have no regard for other cells that would otherwise be protected by CD59 or other regulating proteins.[2]

Verdict on Polysorbate 80

It is obvious that there is a glaring lack of basic science research into the toxic effects of the vaccine ingredient, Polysorbate 80, on human health. Some argue that ingredients like Polysorbate 80 in vaccines are safe and not dangerous simply because they are present in vaccines in miniscule amounts.

However, the fact remains that the federal vaccine schedule directs doctors to give infants and children a whopping 49 doses of vaccines by the age of six.[11] What are the cumulative effects of multiple vaccine ingredients on the human body? Shouldn't we have the right to ask for evidence of short and long-term safety if we are going to inject something into our bodies or our child's body?

References:

ALUMINUM: WHAT DOES THE SCIENCE SAY?

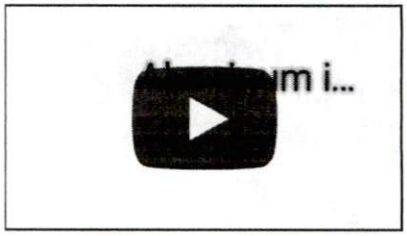
It seems these days, the push to be vaccinated is everywhere, for children, for pets, for seniors, for everyone. But most people, including most doctors and nurses, have no idea what's actually in a vaccine. And pharmaceutical companies like it that way...

One common vaccine ingredient is the known neurotoxin Aluminum. It's a metal, that has no purpose in the human body, and is added to most vaccines to help stimulate an immune response to the disease component in the vaccine (i.e. the virus or bacteria). Because vaccines are injected, they bypass the body's natural immune reaction pathway, and therefore must include an additive that is known to be toxic to stimulate an antibody response.

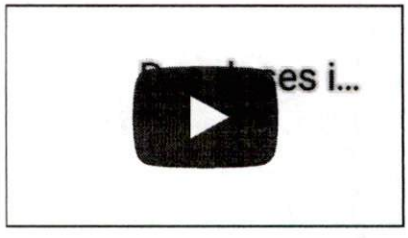
Aluminum in vaccines is linked to food allergies...

Of course, this can create problems, including a chain reaction of autoimmune traumas that create the basis for the common autoimmune health disorders and illnesses we now see in skyrocketing numbers. Because aluminum is such a powerful toxin, it actually causes the body to have an autoimmune reaction to all components within the needle, including to the animal and food-based proteins in the vaccines, which includes dairy, yeast, latex, soy, and nut. This sets the stage for food allergies, including and also to any fragments of the person's skin, muscle, and blood that may have entered from damage by the needle.

Dr. Chris Shaw, a Professor of Neuroscience at the University of British Columbia, explains how aluminum in vaccines is far more dangerous than ingested aluminum and how vaccines can cause brain damage



GROUNDBREAKING STUDY: Professor Christopher Exeley, Professor at Keele University (UK) and a world-leading expert on aluminum, has found elevated aluminum levels in those with autism



"The aluminum content of brain tissue in autism was consistently high."

Aluminum has been linked to autoimmune issues...

Thus it has a harmful effect on the central nervous system and can activate an immune response. This is thought to be a factor in the epidemic of autoimmune issues, such as asthma, allergies, eczema and more. Aluminum in vaccines is also thought to be a factor in neurological problems because it accumulates in the brain over time.

Ever wonder about the huge rise in Alzheimer's and dementia-related conditions, especially as early-onset cases skyrocket? They are neurological conditions linked to environmental toxins in our environment — aka a load of chemicals, we subject ourselves to every day. Synthetic chemicals that are in our food, our body care products, our water, our air...and our vaccines.

Aluminum has been proven to cause brain damage...

Aluminum is the main toxic additive put into vaccines to elicit an immune response. It's been shown to cause brain damage in animal studies, in even tiny doses, and it accumulates in our brains so further chemical exposure does even MORE damage. A recent Daily Mail article published by Professor Chris Exley reveals that aluminum in jabs may cause sufferers to have TEN TIMES more of the metal in their brain compared to those who opted out.

> "The metal accumulates in cells that maintain a constant internal environment and autism sufferers may have genetic changes that cause them to hold aluminum", according to world reknowned expert in aluminum, Prof. Christopher Exley from Keele University (UK)."

Christopher Exeley calls out the pharmaceutical industry for hiding dangers

Aluminum is also linked to breast cancer, neurological issues, autoimmune issues like lupus and IBS, sudden infant death and autism and more.

Every time we are vaccinated, our health is damaged to some degree, whether or not it is noticeable right away. Because vaccines are injected into muscle tissue, the aluminum takes days to weeks to leach out into the circulatory system and accumulate in amounts high enough to cause inflammation and the resulting issues (neurological damage, autoimmune issues, etc).

How this damage manifests depends on a combination of previous toxic load in the body and a person's genetic vulnerabilities. Common chronic issues include memory issues, Alzheimer's, autism, speech delays, lupus, IBD, autoimmune issues, allergies, skin issues like eczema, cancer...or even organ failure and sudden death.

Injection vs Ingestion...is there a difference?

Injected aluminum = around 95% absorbed into body tissues
Ingested aluminum = around 95% excreted through the body's detox pathway

We all can handle different levels of synthetic chemicals — determined by our genetics — before inflammation takes hold and cause a health issue, most often chronic. You NEVER know when you will be pushed past the tipping point of what your body can handle and develop a neurological or autoimmune issue, cancer, heart disease or even death.

"The advent of the aluminum age and the consequence of the omnipresence of aluminum, not only in the environment but also throughout the human body, is that we are all subject to chronic aluminum intoxication. Every minute of each day we expend energy coping with the presence of biologically reactive aluminum in our bodies. The higher the body burden of aluminum the more likely that this coping mechanism will manifest itself as disease.

All exposure adds to the toxic mix, but when it's injected, the aluminum bypasses the body's natural detox pathway. Around 95% of injected toxins, including aluminum, pass from muscle tissue and go straight into the circulatory system (aka bloodstream). From there, the aluminum and other toxins can reach vital organs like the brain, heart, kidney, liver, and others. One study shows that most aluminum remains in the body for months, possibly years, after the injection. This can cause chronic health issues as the toxins raise inflammation levels in the body.

When aluminum is ingested (via food, water or air), only about 5% is absorbed into the body, whereas most if filtered through the body's natural detox pathway. So the toxic chemicals in vaccines are FAR MORE POTENT than those that come into the body in other ways. The toxic ingredients in vaccines include aluminum, but also mercury, barium, monosodium glutamate (MSG) formaldehyde (known to cause cancer in humans).

More info

More than a thousand scientific studies show the chronic health issues that arise from vaccinations, including neurological issues, autoimmune issues, inflammatory diseases, even death. Click here to see these studies.

Did diseases decline because of vaccines? Not according to history... Click here for more.

In the brain, aluminum will contribute toward neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and multiple sclerosis. There is now a clear requirement for therapy or treatment, which could lower the body burden of aluminum, and particularly the aluminum content of the brain."

Click here to read the full article

A must-watch video on how aluminum remains embedded in the muscle tissue after vaccinees

Additional information packet:

The first two pages of this packet of documents contain the current Personal and Religious Belief exemption form directly from the CDPHE website. I have highlighted the portions that I disagree with. Really, the whole first paragraph is entirely unnecessary.

The next two pages are an email I received from Smoky Hill High School about a pertussis outbreak. Notice the highlight line that says, "Children and adults may get sick with whooping cough even if fully vaccinated." That means the vaccine isn't really working, then.

The last page is the current vaccination rate at Smoky Hill High School for the DTap and Tdap vaccines – both of which purport to protect recipients from pertussis. They have a 99.66% compliance rate for DTap, and a 96.59% compliance rate for Tdap. If 95% coverage will cause "herd immunity" then there should have been no outbreak at the high school. This means the vaccine isn't working.

-Tricia Roush (tricia.roush@gmail.com) – contact me if you have any further questions.

Immunization

Non-Medical Exemption Form (Religious and Personal Belief)

Vaccines are one of the greatest public health achievements of the past century and save an estimated 3 million children's lives every year. The Colorado Department of Public Health and Environment strongly supports vaccination as one of the easiest and most effective tools in preventing diseases that can cause serious illness and even death. For nearly all children, the benefits of preventing disease with a vaccine far outweigh the risks. Declining to follow the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP) immunization schedule for number, space and timing of doses, may endanger an unvaccinated child's health and others who come into contact with him/her. Some vaccine-preventable diseases are common in other countries and unvaccinated children could easily get one of these diseases while traveling or from a traveler.

Colorado law C.R.S. § 25-4-902 requires all students attending any school in the state of Colorado to be vaccinated against certain vaccine-preventable diseases as established by Colorado Board of Health rule 6 CCR 1009-2, unless an exemption is filed. This law applies to students attending public, private and parochial kindergarten, elementary and secondary schools through 12th grade, colleges or universities, and child care facilities licensed by the Colorado Department of Human Services including child care centers, school-age child care centers, preschools, day camps, resident camps, day treatment centers, family child care homes, foster care homes, and Head Start programs. Prior to kindergarten, a non-medical exemption must be filed each time a student is due for vaccines according to the schedule developed by the ACIP.[1,2] From kindergarten through 12th grade, a non-medical exemption must be filed every year during the student's school enrollment/registration process.[1] Students with a recorded immunization exemption may be kept out of a child care facility or school during a disease outbreak; the length of time will vary depending on the type of the disease and the circumstances of the outbreak.

Please complete all required fields below; incomplete forms will not be accepted. *All fields are required unless noted optional.*

Type of Non-Medical Exemption Claimed: ☐ Personal Belief ☐ Religious

Student Information:

Last Name:	First Name:	(optional) Middle Name:
Gender: ☐ Female ☐ Male	Date of Birth:	
Street #:	Street Name:	Street Type (e.g. Ave.):
Unit #:	P.O. Box:	
City:	State:	Zip Code:
Email Address:		County:
Phone Number:		☐ Home ☐ Cell

Parent/Guardian Completing This Form: ☐ Check if an emancipated student or student over 18 years old

Last Name:	First Name:	(optional) Middle Name:
Relationship to student: ☐ Mother ☐ Father ☐ Guardian		
Street #:	Street Name:	Street Type (e.g. Ave.):
Unit #:	P.O. Box:	
City:	State:	Zip Code:
Email Address:		County:
Phone Number:		☐ Home ☐ Cell

School/Licensed Child Care Facility Information:

School Name/Licensed Child Care Facility:		
School District:		☐ Check if Not Applicable
Address:		
City:	State:	Zip Code:
Phone Number:		Grade of Student:

[1] Colorado Board of Health rule 6 CCR 1009-2: https://www.sos.state.co.us/CCR/GenerateRulePdf.do?ruleVersionId=7698&fileName=6%20CCR%201009-2.

[2] 2018 Recommended Immunizations from Birth through 6 Years Old: www.cdc.gov/vaccines/parents/downloads/parent-ver-sch-0-6yrs.pdf. Based on this schedule, a non-medical exemption would be submitted at 2 months, 4 months, 6 months, 12 months and 18 months of age.

Vaccine Preventable Disease Information

The information provided below is to ensure parents/guardians/students are informed about the risks of not vaccinating.

Diphtheria, tetanus, pertussis (DTaP, Tdap) - Unvaccinated children may be at increased risk of developing diphtheria, tetanus and/or pertussis if exposed to these diseases. Serious symptoms and effects of diphtheria include heart failure, paralysis, breathing problems, coma, and death. Serious symptoms and effects of tetanus include "locking" of the jaw, difficulty swallowing and breathing, seizures, painful tightening of muscles in the head and neck, and death. Serious symptoms and effects of pertussis (whooping cough) include severe coughing fits that can cause vomiting and exhaustion, pneumonia, seizures, brain damage, and death. For more information: http://www.cdc.gov/vaccines/hcp/vis/vis-statements/dtap.pdf and http://www.cdc.gov/vaccines/hcp/vis/vis-statements/tdap.pdf

Haemophilus influenzae type b (Hib) - Unvaccinated children may be at increased risk of developing invasive Hib disease if exposed to this disease. Serious symptoms and effects include bacterial meningitis, pneumonia, severe swelling in the throat, brain damage, deafness, infections of the blood, joints, bones, and covering of the heart, and death. For more information: http://www.cdc.gov/vaccines/hcp/vis/vis-statements/hib.pdf

Hepatitis B - Unvaccinated children may be at increased risk of developing hepatitis B if exposed to this disease. Serious symptoms and effects include jaundice, life-long liver problems such as liver damage, scarring, liver cancer, and death. For more information: http://www.cdc.gov/vaccines/hcp/vis/vis-statements/hep-b.pdf

Inactivated poliovirus (IPV) - Unvaccinated children may be at increased risk of developing polio if exposed to this disease. Serious symptoms and effects include paralysis of muscles that control breathing, meningitis, permanent disability, and death. For more information: http://www.cdc.gov/vaccines/hcp/vis/vis-statements/ipv.pdf

Measles, mumps, rubella (MMR) - Unvaccinated children may be at increased risk of developing measles, mumps, and/or rubella if exposed to these diseases. Serious symptoms and effects of measles include pneumonia, seizures, brain damage, and death. Serious symptoms and effects of mumps include meningitis, painful swelling of the testicles or ovaries, sterility, deafness, and death. Serious symptoms and effects of rubella include rash, arthritis, and muscle or joint pain. If a pregnant woman gets rubella, she could have a miscarriage or her baby could be born with serious birth defects such as deafness, heart problems, and mental retardation. For more information: http://www.cdc.gov/vaccines/hcp/vis/vis-statements/mmr.pdf

Pneumococcal conjugate (PCV13) - Unvaccinated children may be at increased risk of developing pneumococcal disease if exposed to this disease. Serious symptoms and effects include pneumonia, lung infections, blood infections, meningitis and death. For more information: http://www.cdc.gov/vaccines/hcp/vis/vis-statements/pcv13.pdf.

Varicella (chickenpox) - Unvaccinated children may be at increased risk of developing varicella if exposed to this disease. Serious symptoms and effects include severe skin infections, pneumonia, brain damage, and death. For more information: http://www.cdc.gov/vaccines/hcp/vis/vis-statements/varicella.pdf

Required Vaccines for School Entry - Place an "X" next to each vaccine you are declining.

	Diphtheria, tetanus, pertussis (DTaP)		Inactivated poliovirus (IPV)
	Tetanus, diptheria, pertussis (Tdap)		Measles, mumps, rubella (MMR)
	Haemophilus influenzae type b (Hib)		Pneumococcal conjugate (PCV13)
	Hepatitis B		Varicella (chickenpox)

Statement of Exemption

I am the parent/guardian of the above-named student or am the student himself/herself (emancipated or over 18 years of age) and am declining the vaccine(s) indicated above due to a religious or personal belief that is opposed to vaccines. The information I have provided on this form is complete and accurate.

- I may change my mind at any time and accept vaccination(s) for my child/myself in the future.
- I can review evidence-based vaccine information at www.colorado.gov/cdphe/immunization-education, or www.ImmunizeforGood.com for additional information on the benefits and risks of vaccines and the diseases they prevent.
- I can contact the Colorado Immunization Information System (CIIS) at www.ColoradoIIS.com or my health care provider to locate my child's/my immunization record.[3]

I acknowledge that I have read this document in its entirety.

Parent/Guardian/Student (emancipated or over 18 yrs old) signature: _____ Date: _____

(Optional) I authorize my/my student's school to share my/my student's immunization records with state/local public health agencies and the Colorado Immunization Information System, the state's secure, confidential immunization registry.

Parent/Guardian/Student (emancipated or over 18 yrs old) signature: _____ Date: _____

[3] Under Colorado law, you have the option to exclude your child's/your information from CIIS at any time. To opt out of CIIS, go to www.colorado.gov/cdphe/ciis-opt-out-procedures. Please be advised you will be responsible for maintaining your child's/your immunization records to ensure school compliance.

SMOKY HILL HIGH SCHOOL <noreply@cherrycreekschools.org> 2/25/2019 11:37 PM

SHH: Important notice

To tricia.roush@comcast.net

A message from SMOKY HILL HIGH SCHOOL

Tri-County
HEALTH DEPARTMENT

IMPORTANT NOTICE TO PARENTS

February 25, 2019

Tri-County Health Department has been notified of multiple cases of pertussis, also known as whooping cough, at Smoky Hill High School. This letter is to notify you that your child may have been exposed to whooping cough and to provide recommendations for actions that you should take.

What you need to know:

- Whooping cough is an illness that is spread when someone sick with whooping cough sneezes or coughs and another person breathes in the germs.
- Symptoms of whooping cough may begin 4-21 days after exposure.
- Whooping cough begins with a cough that becomes worse over time. It can cause vomiting and/or coughing fits, and sometimes difficulty breathing.
- A whooping sound may or may not follow the coughing fits.
- Whooping cough is easily spread among family members and close contact with other people.
- Children and adults may get sick with whooping cough even if fully vaccinated. However, the symptoms may be less severe if all vaccines have been given.
- Whooping cough may be very severe in infants. Sometimes babies develop a more severe illness and must be hospitalized.

How you can avoid getting sick or getting others sick:

- Always cover your cough with your shoulder or elbow (not your hands).
- Wash your hands often.
- Stay home when you are sick!
- Vaccinate yourself and your children with the whooping cough vaccine (DTaP or Tdap). You can get the vaccine from your doctor or at the Tri-County Health Department Immunization Clinic (303-451-0123).

People ill with whooping cough are infectious until after they take the correct antibiotic for five days or until they have coughed for 3 weeks. Ofen times they may cough longer than 3 weeks, but they are no longe intectious to others.

In summary, we recommend the following:

- If your child has symptoms of whooping cough, call your doctor to have your child examined and to receive appropriate antibiotics.
- If diagnosed with whooping cough, your child cannot return to school until after taking five full days of antibiotics or after coughing for 21 days.
- If your child does not have symptoms, no treatment is needed at this time.
- Make sure all of your children are fully immunized.

If you have any questions please contact Kaitlin Harame, Communicable Disease Epidemiologist, at 720-200-1530. For more information, visit CDC's website: https://www.cdc.gov/pertussis/.

Undo

Site Name	District	County	Facility Type	Survey Status	Enrollment	Vaccine
Smoky Hill High School	Cherry Creek 5	Arapahoe	Public School	Completed	2084	DTaP
						Tdap

Fully Immunized	In Process	Medical Exemption	Religious Exemption	Personal Exemption	Incomplete Record	No Record
99.66%	0.00%	0.00%	0.00%	0.00%	0.00%	0.34%
96.59%	0.00%	0.19%	0.05%	2.11%	0.72%	0.34%

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HB19-1312 Should Not Amend Current Exemption Process

Testimony of Kelli Martin, Castle Rock, CO

House Health & Insurance Committee Hearing
Colorado General Assembly
April 15, 2019

My name is Kelli Martin, and my family has lived in Castle Rock 13 years. I'm here today because my daughter Tessa began having seizures just a few hours after receiving DTaP and PCV on her first birthday. She had up to 10 episodes a day, and as the tetanus in DTaP caused her right leg to twist inward, she went from walking confidently to falling every few steps. These symptoms continued for about 6 months, and it was the most frightened I've ever been.

Tessa's pediatrician and neurologist determined her body couldn't metabolize the toxins and decided she shouldn't be vaccinated again. But this bill would revoke her medical exemption because the onset of her symptoms doesn't meet ACIP standards.

Legislators say, well, just use a personal exemption, but I have three problems with that: First, the state-required form I would have to sign would likely say I am negligent to not vaccinate my child -- my child who was injured by vaccines. Second, the handling of this form by the state violates FERPA laws. And third, many legislators hope to remove personal exemptions, too -- so what then? This bill simply creates an untenable situation.

I understand the goal of the bill's sponsors is to create healthier children, and I appreciate their passion for that; but, many children are healthier *without* vaccines, because vaccines simply aren't safe for *all* children. This bill shouldn't disrupt the current exemption process: medical exemptions should be decided by doctors, not ACIP guidelines; and personal exemptions should remain personal, not be restricted by a FERPA-violating form that implies we're negligent or lazy.
Please vote NO on this bill.

HB19-1312 Testimony

Good Afternoon, my name is Nicole Beck and I live in Parker, and I oppose HB1312.

I was raised a Democrat and was a Democrat my entire adult life, until 2013.

It was at this time that SB 277, which sought to remove both personal and religious exemptions was introduced by Senator Pan, and heavily supported by the Democratic majority in the House as well as the Senate, much like we see with HB1312.

This bill was shocking for many reasons, in particular because a bill the previous year, AB2109, which stated you needed a doctors signature to submit an exemption was not heavily opposed, and Pan assured everyone that he would never restrict or remove exemptions because he highly valued Parental Rights. He Lied. Next came SB277, which was heavily opposed. I myself took an overnight bus with other concerned families to Sacramento to oppose this bill, thousands of us lined the halls of the capital, where we again saw him lie, lie about aborted fetal cells in vaccines, again when he said water was the most dangerous ingredient in a vaccine, and again, and again. Pan swore over and over during SB277 that he would never touch medical exemptions, that they were at the doctors discretion, that they would be safe.

Now he has introduced SB276, limiting medical exemptions exactly like this bill HB1312.
This bill, like Pans restricts medical exemptions so severely, that we may as well not have them at all.

Representative Mullica is pulling a backwards Pan here. I guarantee it.

Limiting Medical Exemptions to ACIP guidelines would restrict them so severely that a patients doctor would be taken out of the equation entirely. Family history, prior vaccine reactions and epigenetics would cease to matter. Adverse events after vaccination including but not limited to: Grand Mal Seizures, Guillain-Barre Syndrome, Diabetes, Respiratory Arrest, Transverse Myelitis, Encephalitis, Vision Loss, Vaccine Related Death of a Sibling, Organ Failure, Sepsis, Heart Failure and Cerebral Atrophy, would also cease to matter.

Please don't California Colorado.
Thank you,
Nicole Beck (Parents United 4 Kids)
(949) 356-8577
baggo72@yahoo.com

SOUND CHOICE
PHARMACEUTICAL
INSTITUTE

OPEN LETTER TO LEGISLATORS REGARDING FETAL CELL DNA IN VACCINES

April 8, 2019

My name is Dr. Theresa Deisher. I am Founder and Lead Scientist at Sound Choice Pharmaceutical Institute, whose mission is to educate the public about vaccine safety, as well as to pressure manufacturers to provide better and safer vaccines for the public. I obtained my doctorate from Stanford University in Molecular and Cellular Physiology in 1990 and completed my post-doctoral work at the University of Washington. My career has been spent in the commercial biotechnology industry, and I have done work from basic biological and drug discovery through clinical development.

I am writing regarding unrefuted scientific facts about fetal DNA contaminants in the Measles-Mumps-Rubella vaccine, which must be made known to lawmakers and the public.

Merck's MMR II vaccine (as well as the chickenpox, Pentacel, and all Hep-A containing vaccines) is manufactured using human fetal cell lines and is heavily contaminated with human fetal DNA from the production process. Levels in our children can reach up to 5 ng/ml after vaccination, depending on the age, weight and blood volume of the child. That level is known to activate Toll-like receptor 9 (TLR9), which can cause autoimmune attacks.

To illustrate the autoimmune capability of very small amounts of fetal DNA, consider this: labor is triggered by fetal DNA from the baby that builds up in the mother's bloodstream, triggering a massive immune rejection of the baby. This is labor.

It works like this: fetal DNA fragments[i] from a baby with about 300 base pairs in length are found in a pregnant mother's serum. When they reach between 0.46– 5.08 ng/mL in serum, they trigger labor via the TLR9 mechanism[ii]. The corresponding blood levels are 0.22 ng/ml and 3.12 ng/ml. The fetal DNA levels in a child after being injected with fetal-manufactured vaccines reach the same level that triggers autoimmune rejection of baby by mother.

Anyone who says that the fetal DNA contaminating our vaccines is harmless either does not know anything about immunity and Toll- like receptors or they are not telling the truth.

If fetal DNA can trigger labor (a naturally desired autoimmune reaction), then those same levels in vaccines can trigger autoimmunity in a child. Fragmented fetal DNA contained in vaccines is of similar size, ~215 base pairs.[iii]

This is direct biological evidence that fetal DNA contaminants in vaccines are not in low innocuous amounts. They are a very strong proinflammatory trigger.

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www.soundchoice.org

Administration of fragments of human fetal (primitive) non-self DNA to a child could generate an immune response that would also cross-react with the child's own DNA, since the contaminating DNA could have sections of overlap very similar to the child's own DNA.

Children with autistic disorder have antibodies against human DNA in their circulation that non-autistic children do not have. These antibodies may be involved in autoimmune attacks in autistic children.[iv]

Duke University demonstrated in a recently conducted study that significant improvements in behavior were observed when children with autism spectrum disorder were treated with their own banked autologous cord blood[v]. This treatment clearly shows that most children with autism are not born with it since genetic diseases like Down syndrome or muscular fibrosis cannot be treated with autologous stem cells. Therefore, an environmental trigger, or triggers, introduced to the world around 1980 when autism first began to rise, must be identified and eliminated or reduced in the environment.

- Strong change-point correlation exists between rising autism rates and the US vaccine manufacturing switch from animal-derived cell lines for rubella vaccine to human aborted cell lines in the late 70s[vi].
- The earliest change point for Autistic Disorder (AD) birth year was identified for 1981 for California and U.S. data, preceded by a switch in the manufacturing process:
    - In January 1979, the FDA approved the manufacturing switch for the rubella virus from animal based (high passage virus, HPV-77, grown e.g. in duck embryo cells) to the human fetal cell line WI-38 using the RA27/3 virus strain[vii]. Both the newly approved monovalent rubella vaccine and a trivalent mumps, measles and rubella vaccine utilize the WI-38 fetal cell line for manufacturing of the rubella vaccine portion.
- Prior to 1980, autism spectrum disorder was a very rare, almost unknown disease. According to the figures of the CDC, the rate of autism in 2014 was 1 in 59 children , a very steep increase since just 2000, when it was 1 in 150. CDC: "The total costs per year for children with ASD in the United States were estimated to be between $11.5 billion – $60.9 billion (2011 US dollars)[viii]."
- Recently, duplications and de novo deletions have been recognized in up to 10% of simplex autism spectrum disorders, corroborating environmental triggers on the genetics of autism spectrum disorders[ix].
- The rubella portion of the MMR vaccine contains human derived fetal DNA contaminants of about 175 ngs, more than 10x over the recommended WHO threshold of 10 ng per vaccine dose[x].
- No other drug on the market would receive FDA approval without thorough toxicity profiling (FDA follows international ICH guidelines) -> this was never conducted by the pharmaceutical industry for the DNA contamination in the MMR vaccine.
- Vaccines produced with human fetal cell lines contain cell debris and contaminating residual human DNA, which cannot be fully eliminated during the downstream purification process of the virus[xi]. Moreover, DNA is not only characterized by its sequence (ATCG), but also by its epigenetic modification (e.g. DNA methylation pattern etc.). This decoration is highly species specific, which is why non-human DNA will be eliminated, while this is not necessarily the case with fetal human DNA.

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Injecting our children with human fetal DNA contaminants bears the risk of causing two well-established pathologies:

1) Insertional mutagenesis: fetal human DNA incorporates into the child's DNA causing mutations. Gene therapy using small fragment homologous recombination has demonstrated that as low as 1.9 ng/ml of DNA fragments results in insertion into the genome of stem cells in 100% of mice injected[xii]. The levels of human fetal DNA fragments in our children after vaccination with MMR, Varivax (chickenpox) or Hepatitis A containing vaccines reach levels beyond 1.9 ng/ml.
2) Autoimmune disease: fetal human DNA triggers a child's immune system to attack his/her own body.

An additional concern: *retrovirus contamination.*

Human endogenous retrovirus K (HERVK) is a contaminant in the measles/mumps/rubella vaccine[xiii].

- HERVK can be reactivated in humans[xiv]. It codes for a protein (integrase) specialized in integrating DNA into the human genome.
- Several autoimmune diseases have been associated with HERVK activity[xv].
- It is also in the same family of retroviruses as the MMLV virus used in a gene therapy trial, in which inappropriate gene insertion (insertional mutagenesis) led to subsequent additional somatic mutations and cancer in 4 of 9 young boys[xvi].
- It is therefore possible that the HERVK gene fragment present in the MMR vaccine is active, codes for the integrase or the envelope protein, and thus has the potential to induce gene insertion, fostering insertional mutagenesis and autoimmunity.

The presence of both the high level contaminating fetal DNA as well as the HERVK contamination in the MMR vaccine is an unstudied risk with huge implications and dangers for individual and public health.

Solution: Pressure manufacturers to switch back to animal cell line derived rubella vaccines as was successfully done in Japan:

- Based on Takahashi strains of live attenuated rubella virus, produced on rabbit kidney cells. A single dose of this vaccine has been recently proven to retain immunity for at least 10 years when rubella was under regional control[xvii].
- Split MMR vaccine into three individually offered options as done in Japan.

The MMR vaccine manufacturing process needs to be changed to address and eliminate the above risks for the public.

Thank you for your consideration. I will be happy to address any questions you may have concerning the above.

Sincerely,

Theresa A. Deisher, Ph.D.

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END NOTES

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Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model

Jason M. Warfel, Lindsey I. Zimmerman, and Tod J. Merkel

SIGNIFICANCE

Pertussis has reemerged as an important public health concern since current acellular pertussis vaccines (aP) replaced older whole-cell vaccines (wP). In this study, we show nonhuman primates vaccinated with aP were protected from severe symptoms but not infection and readily transmitted *Bordetella pertussis* to contacts. Vaccination with wP and previous infection induced a more rapid clearance compared with naïve and aP-vaccinated animals. While all groups possessed robust antibody responses, key differences in T-cell memory suggest that aP vaccination induces a suboptimal immune response that is unable to prevent infection. These data provide a plausible explanation for pertussis resurgence and suggest that attaining herd immunity will require the development of improved vaccination strategies that prevent *B. pertussis* colonization and transmission.

Keywords: whooping cough, T-cell memory, animal models, adaptive immunity, IL-17

ABSTRACT

Pertussis is a highly contagious respiratory illness caused by the bacterial pathogen *Bordetella pertussis*. Pertussis rates in the United States have been rising and reached a 50-y high of 42,000 cases in 2012. Although pertussis resurgence is not completely understood, we hypothesize that current acellular pertussis (aP) vaccines fail to prevent colonization and transmission. To test our hypothesis, infant baboons were vaccinated at 2, 4, and 6 mo of age with aP or whole-cell pertussis (wP) vaccines and challenged with *B. pertussis* at 7 mo. Infection was followed by quantifying colonization in nasopharyngeal washes and monitoring leukocytosis and symptoms. Baboons vaccinated with aP were protected from severe pertussis-associated symptoms but not from colonization, did not clear the infection faster than naïve animals, and readily transmitted *B. pertussis* to unvaccinated contacts. Vaccination with wP induced a more rapid clearance compared with naïve and aP-vaccinated animals. By comparison, previously infected animals were not colonized upon secondary infection. Although all vaccinated and previously infected animals had robust serum antibody responses, we found key differences in T-cell immunity. Previously infected animals and wP-vaccinated animals possess strong *B. pertussis*-specific T helper 17 (Th17) memory and Th1 memory, whereas aP vaccination induced a Th1/Th2 response instead. The observation that aP, which induces an immune response mismatched to that induced by natural infection, fails to prevent colonization or transmission provides a plausible explanation for the resurgence of pertussis and suggests that optimal control of pertussis will require the development of improved vaccines.

Pertussis is a highly contagious, acute respiratory illness caused by the bacterial pathogen *Bordetella pertussis* (1, 2). Infection results in a wide spectrum of clinical manifestations ranging from mild respiratory symptoms to a severe cough illness accompanied by marked leukocytosis and the hallmark inspiratory whoop and posttussive emesis (3). Because acellular pertussis vaccines replaced whole-cell vaccines in the 1990s, pertussis has reemerged at a startling rate in the United States despite nationwide vaccine coverage in excess of 95% (4). With a 50-y high of 42,000 reported cases in the United States in 2012, pertussis is the most common of the vaccine-preventable diseases (5). This resurgence is mirrored throughout the industrial world despite similar high rates of vaccination (6–9). Two common hypotheses for the resurgence have been proposed: *i*) current acellular pertussis vaccines (aP) vaccines are less effective than the whole-cell pertussis (wP) vaccines they replaced and *ii*) aP-induced immunity wanes more quickly than anticipated (10–13). However, pertussis resurgence is not completely understood (14, 15).

Hampering our ability to counteract this resurgence is the fact that pertussis pathogenesis and immunity to natural infection have not been well studied in humans because typical pertussis is sporadic given high rates of vaccination in developed countries. Human challenge studies have been proposed but never conducted due to a variety of logistical and ethical problems including the

potential for severe disease, the lack of an effective therapeutic for established disease, and the highly contagious nature of pertussis. Although a variety of small-animal models have been used to study pertussis, none of them adequately reproduce the human disease (16). To address this gap, we recently developed a nonhuman primate model of pertussis using baboons (*Papio anubis*) and found the disease is very similar to severe clinical pertussis. Upon challenge, baboons experience 2 wk of heavy respiratory colonization and leukocytosis peaking between 30,000–80,000 cells/mL, similar to the range in pertussis-infected infants (1, 17). In addition, baboons experience a paroxysmal cough illness characterized by repeated fits of 5–10 coughs. The coughing fits last on average >2 wk in the baboon, although this is less than some severely infected children, where the cough can last up to 12 wk (1, 17). We also characterized airborne transmission of *B. pertussis* from infected to naïve animals, which is the route of transmission postulated to occur between humans (18). Because this is the only model of pertussis to reproduce the cough illness and transmission of the human disease, we believe it provides the unique opportunity to test our hypothesis that aP vaccines fail to prevent *B. pertussis* colonization, thus enabling transmission among vaccinated individuals.

Using this model we have confirmed that, as in humans, aP vaccines provide excellent protection against severe disease in baboons. However, aP vaccines do not prevent colonization following direct challenge or infection by transmission. In addition, aP-vaccinated animals are capable of transmitting disease to naïve contacts. By comparison, wP-vaccinated animals cleared infection significantly more quickly than aP-vaccinated or naïve animals. We also found that aP vaccination induces T helper 2 (Th2) and T helper 1 (Th1) immune memory responses, whereas infection and—to a lesser extent—wP vaccination induce Th17 and Th1 memory. Our results suggest that in addition to the potential contribution of reduced efficacy and waning immunity of aP, the inability of aP to prevent colonization and transmission provides a plausible explanation for pertussis resurgence.

RESULTS

Acellular Pertussis Vaccines Protect Against Disease but Fail to Prevent Infection. Several observational studies recently concluded that children primed with aP vaccine are at greater risk for pertussis diagnosis compared with wP-primed children (19–22). Although these data suggest aP vaccine is less effective than wP vaccine at preventing colonization, the rate of undiagnosed *B. pertussis* carriage in vaccinated individuals is unknown. To assess the ability of each vaccine to prevent colonization and clinical pertussis symptoms, baboons were vaccinated according to the US schedule at 2, 4, and 6 mo of age with human doses of combination diphtheria, tetanus, and pertussis vaccines containing aP or inactivated wP (Table 1 provides a list of the components of each vaccine). At 7 mo of age, vaccinated, naïve, and previously infected (convalescent) animals were challenged with D420, a *B. pertussis* clinical isolate that causes severe infection in humans and baboons (17). Naïve animals were heavily colonized with peak levels between 10^7–10^8 cfu/mL in nasopharyngeal washes (Fig. 1*A*). After 2 wk, colonization gradually decreased, and the infection cleared after 30 d. Consistent with our previous finding, none of the convalescent animals were colonized (17). Compared with naïve animals, aP-vaccinated animals had slightly reduced colonization for the first 10 d but remained consistently colonized before clearing after 35 d. In wP-vaccinated animals the initial colonization was similar to aP-vaccinated animals but the infection cleared after 18 d, significantly faster than naïve and aP-vaccinated animals (Fig. 1*B*).

Table 1.

Components of aP and wP vaccines used in this study

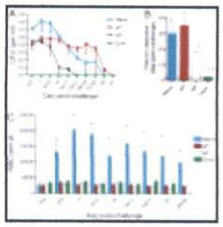

Fig. 1.

The effect of vaccination or convalescence on colonization and leukocytosis. Naïve animals, aP-vaccinated animals, wP-vaccinated animals, and previously infected [convalescent conv.)] animals were directly challenged with *B. pertussis* (n = 3–4 ...

To assess the efficacy of the vaccines in preventing the symptoms of severe pertussis, peripheral blood was drawn serially, and complete blood counts were performed to monitor leukocytosis, a significant marker of morbidity in pertussis-infected infants (23). Compared with preinfection levels, naïve animals had a significant increase in circulating white blood cells at each time point, peaking at over 40,000 cells per μL, an eightfold increase over preinfection levels (Fig. 1*C*). In contrast to the colonization data, aP vaccination, wP vaccination, and convalescence all prevented leukocytosis (Fig. 1*C*). In addition, wP-vaccinated, aP-vaccinated, and convalescent animals did not cough and showed no reduction of activity, loss of appetite, or other outward signs of disease.

Acellular Vaccines Fail to Prevent Infection Following Natural Transmission. To assess the ability of vaccination to prevent pertussis infection by transmission, two aP-vaccinated animals and one unvaccinated animal were cohoused with a directly challenged, unvaccinated animal. Similar to our previous findings (18), all animals became colonized 7–10 d after cohousing with the infected animal (Fig. 2). The peak levels and kinetics of colonization were indistinguishable between the naïve and aP-vaccinated animals.

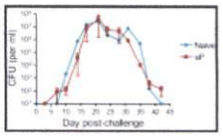

Fig. 2.

aP does not protect against colonization following natural transmission. A naïve animal was directly challenged. After 24 h, a naïve animal and two aP-vaccinated animals were placed in the same cage as the directly challenged animal and ...

Acellular-Vaccinated Animals Are Capable of Transmitting *B. pertussis* to Naïve Contacts. Because aP fails to prevent colonization we hypothesized that aP-vaccinated animals can transmit *B. pertussis* infection to contacts. To test this hypothesis, two aP-vaccinated animals were challenged with *B. pertussis* and placed in separate cages. After 24 h, a naïve animal was added to each cage, and all animals were followed for colonization. Both of the naïve animals were infected by transmission from their aP-vaccinated cage mates (Fig. 3).

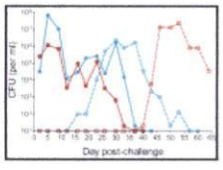

Fig. 3.

Infected aP vaccinees can transmit pertussis to naive contacts. Two animals vaccinated with aP were housed in separate cages, and each was directly challenged. Twenty four hours after challenge, an unchallenged naïve animal was placed ...

Vaccination and Previous Infection Induce Robust Antibody Responses. Sera collected before vaccination or primary infection and again at 1 wk before challenge were analyzed for IgG antibodies against heat-killed *B. pertussis* and the vaccine antigens pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae types 2 and 3 (FIM). We show that wP, aP, and natural infection induce high-antibody titers to all antigens, and the aP group generally possessed equivalent or greater prechallenge titers, suggesting that the differences in colonization between the groups do not correlate with levels of circulating antipertussis antibodies (Fig. 4). Following challenge, the titers for vaccinated animals were essentially unchanged, whereas boosting was observed for some antigens in convalescent animals (Fig. S1).

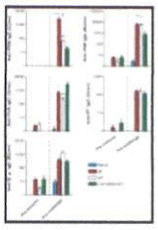

Fig. 4.

Vaccination and previous infection induce robust serum antibody responses. Antibody responses to the four vaccine antigens—PRN, FIM, FHA, and PT—and to heat-killed *B. pertussis* (*B. p.*) were measured by ELISA. Preimmune sera were collected ...

T-Cell Memory Response Elicited by Acellular Pertussis Vaccination Is Mismatched Compared with Natural Infection. Although a large number of clinical studies have characterized the antibody response to pertussis infection and vaccination, key deficiencies remain in our understanding of pertussis-induced helper T-cell immune responses in humans and primates. Importantly, no clinical studies have investigated whether the primary series of pertussis vaccines induce Th17 memory, a recently identified T cell that specializes in controlling extracellular bacterial infections at mucosal surfaces through stimulating neutrophil recruitment (24). To assess *B. pertussis*-specific T-cell memory responses in naïve, aP-vaccinated, wP-vaccinated, and convalescent animals, peripheral blood mononucleated cells (PBMCs) were collected 1 wk before infection. Total PBMC were incubated either with medium alone or with heat-killed *B. pertussis* as an ex vivo simulation of the memory responses recalled during the ensuing challenge. Following an overnight incubation, nonadherent PBMC, including T cells, were collected and separated using magnetic beads into the following fractions: CD4−, CD4+, CD95–CD4+, or left unseparated (total nonadherent cells). Memory helper T cells in primates are characterized by surface expression of CD4 and CD95 (25, 26). After further culture of all fractions, the supernatants were analyzed for secretion of IL-17, IFN-γ, and IL-5; cytokines that are characteristic of Th17, Th1, and Th2 cells, respectively. Very low background cytokine secretion was observed from nonstimulated cells isolated from naïve, vaccinated, or convalescent animals or from stimulated cells from naïve animals (Figs. S2 and S3). When stimulated with heat-killed *B. pertussis*, both total nonadherent cells and CD4+ cells from convalescent animals secreted high levels of IL-17, some IFN-γ, and no IL-5. When the CD95+ memory cells were depleted, the CD95–CD4+ cells did not secrete IL-17 or IFN-γ, consistent with induction of *B. pertussis*-specific Th17 and Th1 memory cells (Fig. 5). Stimulated total nonadherent cells and CD4+ cells from aP-vaccinated animals secreted significant IFN-γ, but the response was weaker than convalescent cells ($P = 0.01$), and there was no significant increase in IL-17 secretion. However, there was a significant IL-5 response, consistent with skewing toward Th2 and Th1 memory (Fig. 5). Total nonadherent cells and CD4+ cells from wP-vaccinated animals secreted similar IFN-γ compared with aP cells, but no IL-5. IL-17 secretion was between levels for naïve and convalescent cells, suggesting that T-cell memory induced by wP vaccination is similar to natural infection, but the Th17 and Th1 memory responses were weaker.

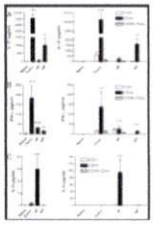

Fig. 5.

Helper T-cell responses induced by vaccination and infection. PBMC collected from naïve, aP-vaccinated, wP-vaccinated, and conv. animals 1 wk before infection were incubated overnight with either medium alone or medium containing heat-killed ...

DISCUSSION

The introduction of whole-cell vaccines consisting of inactivated *Bordetella pertussis* organisms in the United States in the 1940s caused a precipitous decrease in pertussis incidence (27). However, over the past 30 y, pertussis has resurged in the United States. The resurgence began during the wP vaccine era, but the pace has quickened since aP vaccines were recommended for all primary and booster doses (11). This correlation has led many to hypothesize that aP vaccines are less effective on a population scale than the wP vaccines they replaced (10, 12, 13). Consistent with this notion, several recent observational studies concluded that children primed with aP vaccine had a twofold to fivefold greater risk of pertussis diagnosis compared with wP-primed children (19–22).

Our results in nonhuman primates add to these findings by showing that animals vaccinated with wP cleared infection by a direct challenge twice as fast as animals vaccinated with aP. However, neither vaccine was able to prevent colonization as well as immunity from a previous infection.

Another hypothesis as to why pertussis is reemerging is that the duration of immunity in aP-vaccinated children is shorter than anticipated. Although some first-generation acellular vaccines had poor immunity and efficacy, double-blinded clinical trials and field-efficacy studies for the US-licensed acellular vaccines estimated the short-term efficacy to be excellent: ~85% after three doses and 98% after five doses (28–30). However, recent cohort and case-control studies concluded that 5 y following the fifth aP dose, children are fourfold to 15-fold more likely to acquire pertussis compared with within the first year, consistent with waning aP immunity (30–33).

We hypothesized an additional explanation for pertussis resurgence is that aP-vaccinated individuals can act as asymptomatic or mildly symptomatic carriers and contribute significantly to transmission in the population. Observational studies suggest that asymptomatic pertussis can occur in vaccinated children and adults based on PCR or serological data (34, 35). However, during the aP vaccine trials, participants were not screened for *B. pertussis* infection unless they presented with pertussis-like symptoms and at least 7–21 d cough (12). Therefore, no experimental data exist on whether vaccination prevents *B. pertussis* colonization or transmission in humans. In the present study we show that aP-vaccinated primates were heavily infected following direct challenge, and the time to clearance was not different compared with naïve animals. Similarly, there was no difference in the kinetics or peak level of colonization between aP-vaccinated and naïve animals that were infected by natural transmission. Importantly, we also show in two experiments that aP-vaccinated animals transmitted *B. pertussis* to naïve cage mates. Together these data form the key finding of this study: aP vaccines do not prevent infection or transmission of *Bordetella pertussis* even 1 mo after completing the primary vaccination series.

We show that wP, aP, and natural infection all induce high-antibody titers. The prechallenge titers in aP-vaccinated animals were generally equivalent or higher than those observed in convalescent and wP-vaccinated animals, suggesting that aP is immunogenic in baboons and that the inability to prevent infection was not due to low-antibody titers. Compared with the large number of clinical studies that have characterized the antibody response to pertussis infection and vaccination, very few have investigated pertussis-induced helper T-cell immune responses in humans. Taken as a whole, these limited data suggest that aP vaccination induces Th2 or mixed Th2/Th1 responses, whereas wP vaccination and natural infection induce a Th1 response (13). However, none of these studies tested for Th17 memory, a recently identified T cell that specializes in controlling extracellular bacterial infections at mucosal surfaces (24). Our data show that natural infection induced robust Th17 and Th1 immunity. Animals vaccinated with wP, which cleared infection faster than naïve and aP-vaccinated animals, showed similar but weaker T-cell responses. wP vaccination is generally believed to induce strong Th1 responses, but what we observed here was relatively weak. This observation might be explained by heterogeneity in the manufacturing of different wP vaccines. Future studies will compare the immune response induced by wP vaccines produced by three different manufacturers. In comparison with natural infection and wP, aP-induced immunity was mismatched, showing a Th2 response with a weaker Th1 response and no significant Th17 response.

Together, the cytokine and T-cell immunological data observed in baboons are generally consistent with those observed in mice (13). We previously showed that pertussis infection in baboons induces a mucosal immune response characterized by production of IL-17 and a variety of chemokines and cytokines associated with IL-17 signaling, including IL-6 and IL-8. This primary immune response correlated with long-lived Th17 and Th1 memory responses that lasted >2 y (36). Mice infected with *B. pertussis* also express mucosal IL-17, IL-6, and IL-8 homologs and induce Th17 and Th1 memory (37–40). Mice vaccinated with wP also develop Th17 and Th1 memory that results in partial protective immunity, similar to what we observed in the baboon model (41, 42). A recent report by Ross et al. (42) concluded that an aP containing PT, FHA, and PRN induces Th1, Th2, and Th17 immune responses in C57BL/6 mice (42). However, a previous study from the same group found Th1 and Th2 but no significant Th17 responses in C3H/HeJ and C3H/HeN mouse strains vaccinated with an aP containing PT and FHA (41). Nevertheless, data from two clinical studies recently showed negligible Th17 recall responses (~10 pg/mL) in PBMC isolated from aP-vaccinated 4-y-old children before and after booster, suggesting aP does not induce Th17 memory in humans (43, 44).

Taken as a whole, the data presented in this study suggest that antibodies induced by aP vaccination are sufficient for preventing severe pertussis symptoms but do not mitigate colonization. Inhibition of leukocytosis likely occurs through antibody-mediated neutralization of PT, a toxin which interferes with leukocyte extravasation by blocking chemokine receptor signaling (1). The mechanism by which aP prevents coughing despite heavy bacterial colonization is not known but deserves further attention. On the other hand, induction of Th17/Th1 memory responses correlated with the ability to clear infection: convalescent and wP-vaccinated animals possessed strong Th17 responses and Th1 responses and cleared infection more quickly than aP-vaccinated animals which lacked Th17 responses but possessed Th1/ Th2 memory. Although we have not definitively shown that Th17 cells are required for *B. pertussis* clearance, this correlation is consistent with the role these cells play in fighting extracellular bacterial infections at mucosal surfaces by inducing neutrophil chemotaxis. The current studies were not designed to look at immune cell recruitment to the respiratory tract, but additional experiments are underway to determine the role of neutrophils in the immune response to pertussis infection and vaccination in baboons. We are also investigating other possible mechanisms that could prevent mucosal colonization; for example, a possible role for IgA and IgD which are secreted in primate lower and upper respiratory tracts, respectively (45, 46).

The baboon model offers many advantages, chiefly the ability to investigate pertussis pathogenesis, transmission, and host immune responses to infection and vaccination in a primate species that is >96% genetically similar to humans (47). However, there are also several limitations associated with this model. There are far fewer animals available for research compared with smaller-animal models. In addition, there is a paucity of immunological reagents that are validated for baboons compared with mice and humans. Although antibodies against cell surface markers are generally cross-reactive, anti-cytokine antibodies tend to be much more species-specific. For this reason we have so far been unable to assess T-cell responses using intracellular cytokine staining and flow cytometry. This led us to develop the cell separation assay as an alternative method for phenotyping the memory T-cell responses induced by pertussis infection and vaccination (36). One limitation of our assay is that during the CD4+ cell purification, antigen-presenting cells such as macrophages and dendritic cells are removed after an overnight incubation. This likely explains the low IFN-γ secretion observed in all groups because antigen-presenting cells increase IFN-γ secretion by antigen-specific CD4+ T cells through a positive feedback loop (48). In line with this hypothesis, our previous data showed that restimulated whole PBMC from convalescent animals secreted much higher levels of IFN-γ. In addition, restimulation assays using human PBMC or murine splenocytes after infection or vaccination also show higher levels of secreted IFN-γ (42, 49). Together these observations suggest that although our assay is valuable for phenotyping T-cell memory, it likely underrepresents the magnitude of Th1 memory responses. We used heat-killed *B. pertussis* as an antigen for our restimulation assays because we believe this is the most relevant method for ex vivo simulation of T-cell memory recalled during infection. However, it is possible that this assay underdetects immune responses that would be observed had we used purified vaccine antigens. Another disadvantage of primate models is that it is not feasible to directly link an immune response to protection. Although protection from pertussis has been shown to be mediated by IFN-γ and, to a lesser extent, IL-17 signaling using knockout mouse strains lacking specific gene products (13), the relative protection afforded by Th17 or Th1 responses in vaccinated or convalescent baboons or humans is not known.

Currently, a major focus of public health agencies is the prevention of pertussis infection in young infants who have not completed their primary aP series and have considerable morbidity and mortality to pertussis infection (1). One recommendation to reduce transmission of pertussis to infants is by "cocooning," or vaccinating people who have contact with infants (11). Our data show that aP-vaccinated animals are infected and transmit pertussis to naïve contacts. Consistent with these findings, seroepidemiological studies have concluded that *B. pertussis* circulation is still high in countries with excellent aP uptake (27, 50), and a cross-sectional study showed that postpartum aP vaccination of mothers did not reduce pertussis illness in young infants (51). These data suggest that cocooning is unlikely to be an effective strategy to reduce the burden of pertussis in infants. However, it is important to note that our data in combination with human data show that vaccination with aP provides excellent protection from severe pertussis (52). Therefore, any short-term plan for addressing the resurgence of pertussis should include continued efforts to enhance aP immunization. However, to protect the most vulnerable members of the population and achieve optimal herd immunity, it will be necessary to develop a vaccination strategy that effectively blocks pertussis infection and transmission.

MATERIALS AND METHODS

Ethics Statement. All animal procedures were performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International in accordance with protocols approved by the Center for Biologics Evaluation and Research Animal Care and Use Committee and the principles outlined in the *Guide for the Care and Use of Laboratory Animals* by the Institute for Laboratory Animal Resources, National Research Council (53).

Bacterial Strains and Media. *B. pertussis* strain D420 was grown on Bordet–Gengou and Regan–Lowe plates prepared as described previously (17). Heat-killed *B. pertussis* was prepared by resuspending to an OD_{600} of 0.90 (5×10^9 cfu/mL) in PBS and heating at 65 °C for 30 min.

Vaccination, Infection, and Evaluation of Baboons. Baboons obtained from the Oklahoma Baboon Research Resource at the University of Oklahoma Health Sciences Center were inoculated with human doses of aP or wP administered intramuscularly at 2, 4, and 6 mo of age. For studies using aP, equal numbers of animals were vaccinated with Daptacel (Sanofi Pasteur Ltd.) and Infanrix (GlaxoSmithKline). For wP, animals were vaccinated with Triple Antigen (Serum Institute of India Ltd.), which meets the World Health Organization (WHO) recommendations for potency. Naïve animals were age-matched but not vaccinated. Previously infected animals were clear of *B. pertussis* infection for 1 to 2 mo before reinfection. Direct challenge and transmission studies were performed as described previously (17, 18). The inoculum for each direct challenge was between 10^9–10^{10} cfu as determined by measurement of optical density and confirmed by serial dilution and plating to determine the number of cfu per mL of inoculum. Baboons were evaluated twice weekly as described previously for enumeration of circulating white blood cells and serum separation (17). Nasopharyngeal washes were diluted and plated on Regan–Lowe plates to quantify bacterial cell counts.

Isolation of PBMC and Cell Separation. Baboons were anesthetized, and PBMC were isolated from peripheral blood as described previously (36) and cryopreserved in RPMI-1640 medium supplemented with 10% (vol/vol) DMSO and 12.5% (wt/vol) BSA using Mr. Frosty containers (Nalgene). After thawing, cells were washed twice and nonadherent cells were collected as described previously. For each growth condition, cells were incubated overnight with either medium alone or medium containing heat-killed *B. pertussis* (50 bacteria:1 PBMC). Nonadherent cells were collected, and 2×10^6 cells were left unseparated (total nonadherent cells). Using the method previously described, 4×10^6 cells were separated using anti-CD4 magnetic particles, and another 4×10^6 cells were depleted of CD95+ cells and then separated with anti-CD4 magnetic particles (36). The following fractions were collected: Total nonadherent, CD4−, CD4+, and CD95–CD4+. After incubation with or without heat-killed *B. pertussis*, cells were pelleted and supernatants were collected for IL-17A quantitation by ELISA (Aniara) and quantitation of IFN-γ and IL-5 using the Milliplex MAP nonhuman primate kit according to the manufacturer's instructions (Millipore). Data are presented as the cytokine concentration secreted by *B. pertussis*-stimulated cells minus the basal concentration secreted by cells incubated with medium alone.

Detection of Serum Antibodies to Pertussis Antigens. Nunc Maxisorp 96-well plates were coated overnight with 0.2 μg/mL PT, 0.5 μg/mL FHA, 2 μg/mL PRN, or 0.2 μg/mL FIM (List Biologicals) as described previously (17, 54). For whole-bacteria ELISA, plates were coated overnight at 37 °C with heat-killed *B. pertussis* prepared as described above. Serum IgG for each antigen was measured as described previously (17). Each plate contained a standard curve from the WHO international standard pertussis antiserum (National Institute for Biological Standards and Control) used to assign international units for PT, FHA, and PRN and relative units for FIM and heat-killed *B. pertussis* by comparison with the linear portion of the standard curve. Because Infanrix does not contain FIM, only Daptacel-vaccinated animals were included in the anti-FIM ELISA.

Statistics. All data are reported as mean ± SEM. Statistical analyses were performed by ANOVA with post hoc *t* test using JMP (version 9) software (SAS Institute, Inc.). Antibody and cytokine data were normalized by log transformation before analysis.

SUPPLEMENTARY MATERIAL

Supporting Information:

Click here to view.

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FOOTNOTES

The authors declare no conflict of interest.

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From the Cover

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MEDICINE

Baboon Study Reveals New Shortcoming of Pertussis Vaccine

The shortcomings of the whooping cough vaccine may help explain the disease's resurgence

By Tara Haelle on February 1, 2014

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Pertussis, better known as whooping cough, once sickened more than 100,000

Americans a year. The bacterial illness, which is particularly dangerous to infants, was brought under control in the 1940s with the introduction of pertussis vaccines. But in the past two decades pertussis has made an alarming comeback.

In 2012 the number of U.S. cases rose to 48,277—the most since 1955. The resurgence has led researchers to reexamine the workings of the current vaccine, which uses bits and pieces of the *Bordetella pertussis* bacterium to stimulate the production of antibodies. This so-called acellular pertussis (aP) vaccine is in the widely used DTaP and TdaP shots, which also protect against diphtheria and tetanus. An older formulation with whole, inactivated *B. pertussis* cells was phased out in the 1990s because of its side effects.

Recent studies have shown that immunity from the acellular vaccine wanes relatively quickly. In 2012, for instance, a *New England Journal of Medicine* study determined that children's odds of catching pertussis rose by 42 percent each year after receiving the final dose of DTaP, usually given between ages four and six, in the childhood vaccine series.

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Tod Merkel and his colleagues at the U.S. Food and Drug Administration suspected another weakness lurked in the acellular vaccine—that it might not block the spread of the disease. To test their hypothesis, Merkel's team members infected baboons with pertussis. Some of the animals had been vaccinated, and some had acquired natural immunity from a past bout of the illness. None of the vaccinated or naturally immune baboons fell ill, but the bacterium lingered for 35 days in the throats of the baboons that had received the acellular vaccine. Animals that had received the whole-cell vaccine cleared the infection nearly twice as fast.

During their infections, acellular-vaccinated baboons were able to pass the bacterium to unprotected animals, Merkel's team recently reported in the *Proceedings of the National Academy of Sciences USA*. The study, says Eric Harvill, a professor of microbiology and infectious disease at Pennsylvania State University, "explains a lot of the observations about the circulation of pertussis in highly vaccinated populations."

Finding out exactly how the different vaccines convey immunity might lead to a better pertussis shot, which Harvill, Merkel and their colleagues hope to develop over the next several years. "Clearly, the natural infection and whole-cell vaccine are stimulating some response besides the antibody response, and we're trying to find out what," Merkel says.

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ABOUT THE AUTHOR(S)

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(Modernization of Immunization Requirements to Improve Vaccination Rates)

James K. Todd, MD 10 April 2019

1. **Vaccines have been shown to be safe and highly effective in Colorado, saving tens of thousands of hospitalizations and hundreds of millions of dollars annually.[1] [2]**

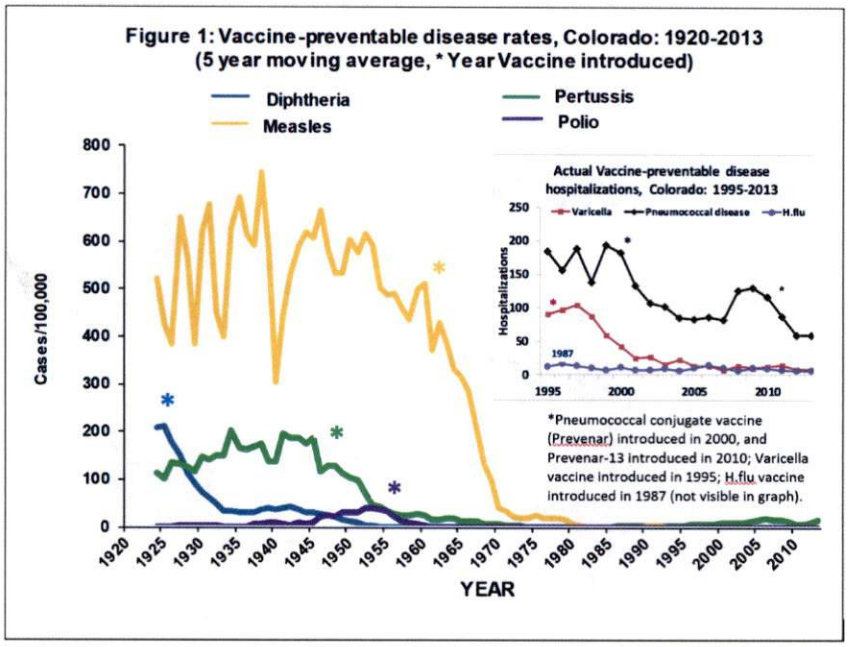


Table 2: Hospital cases and charges prevented among Colorado children due to vaccination, 2014

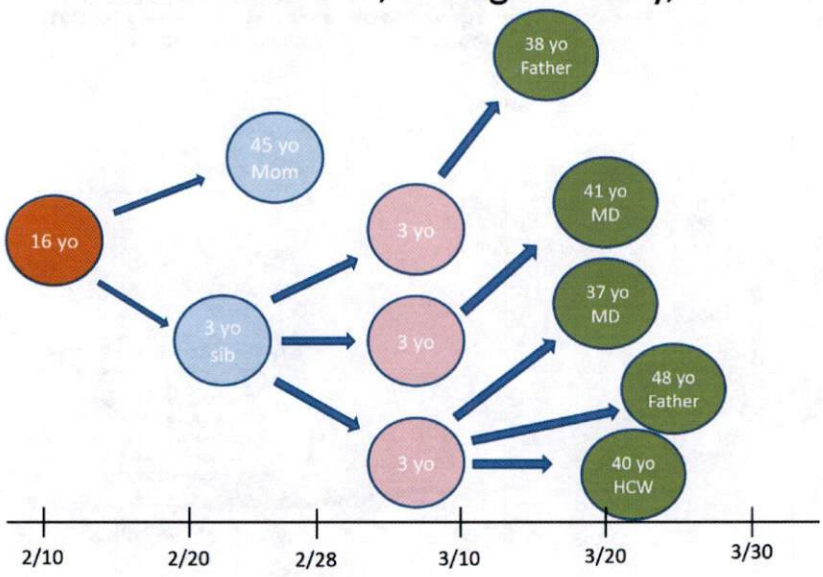
Disease	Index years[a]	Statewide pre-vaccination rate per 100,000[b]	Statewide rate per 100,000 2014[b]	Statewide reportable cases prevented: 2014[c]	Actual hospitalized cases: 2014[d]	Estimated hospitalized cases prevented: 2014[e]	Estimated hospital charges prevented: 2014[f]
Diphtheria	1920-1922	461	0.0	6,471	0	1,760	$75,359,651
H. influenzae	1984-1986	12.4	1.0	173	1	47	$2,051,743
Measles	1960-1962	784	0.0	11,004	2	2,993	$128,153,488
Mumps	1964-1966	408	0.0	5,736	0	1,560	$66,800,189
Pertussis	1945-1947	328	66.4	4,577	35	1,245	$68,223,045
Pneumococcal disease	1997-1999	14.8	2.6	159	48	148	$21,045,008
Polio	1952-1954	68	0.0	948	0	258	$11,040,083
Rubella	1966-1968	124	0.0	1,743	0	474	$20,301,335
Tetanus	1927-1929	1.1	0.0	15	0	4	$174,691
Varicella	1995-1997	8.7	22.6	113	9	114	$7,289,274
Total		**2,210**	**92.6**	**30,939**	**95**	**8,603**	**$400,438,507**

[1] https://www.childrenscolorado.org/globalassets/healthcare-professionals/vaccine-preventable-disease-2014.pdf

[2] https://www.childrenscolorado.org/globalassets/healthcare-professionals/vaccine-preventable-disease-2015.pdf

2. **Unvaccinated individuals put many others at risk at great cost to the community[3]**
    a. **Sick, unvaccinated individuals are highly contagious.**
    b. **Vaccinated individuals may still be at risk if heavily exposed.**
    c. **Immune compromised individuals are at special risk.**
    d. **Individual decisions impose public health costs on the community (tax payers).**

Measles Cluster 1, Orange County, 2014



Healthcare Workers with Measles Clinical and Epidemiologic Features, 2014

Age (y)	Measles Immunity Prior to Exposure	Exposure	Illness Onset	Fever	Cough	Coryza	Rash	Days Considered infectious while asymptomatic	Days working during active symptoms	Number of patients exposed
32	IgG+	3/3/2014	3/17/2014	Y	Y	N	3/18/14	3	0	0
36	IgG+	3/3/2014	3/14/2014	Y	N	N	3/18/14	0	4	850
41	2 MMR	3/7/2014	3/18/2014	Y	N	N	3/20/14	2	2	26
37	4 MMR IgG+	3/7/2014	3/16/2014	N	Y	N	3/20/14	0	4	72
40	Unknown vaccine history, IgG equivocal	3/7/2014	3/19/2014	Y	Y	Y	3/21/14	2	0	0

Cost of Washington's Measles Outbreak Tops $1 Million

The Seattle Times reports that a state health official expects that number to climb.

By **Megan Trimble**, Digital News Editor Feb. 21, 2019

[3] Matt Zahn, MD; Medical Director Epidemiology and Assessment, Orange County Health Care Agency