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Developing the concept of beneficial non-specific effect of live vaccines with epidemiological studies

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Abstract

**Background and objectives** Epidemiological and immunological studies are increasingly reporting non-specific effects (NSEs) of vaccines; i.e. vaccines may affect the risk and severity of non-targeted infections. We reviewed how epidemiological studies developed the concept of beneficial NSEs of live vaccines.

**Sources** This is a personal narrative of how we came to pursue the concept of NSEs in studies of measles vaccine (MV) from the late 1970s. We also searched Pubmed for epidemiological studies of non-specific/nonspecific effects (NSEs) of the most common human vaccines.

**Content** When smallpox vaccine was introduced around 1800, BCG against tuberculosis in the 1920s and oral polio vaccine (OPV) in the 1960s, there were suggestions that these live attenuated vaccines reduced mortality more than expected. However, scientific follow-up was limited and the concept of beneficial NSEs did not become mainstream.

We observed beneficial NSEs after MV was introduced in low-income countries in the 1970s. Subsequent observational studies and randomised trials confirmed beneficial NSEs of smallpox vaccine, BCG and OPV. Recently, beneficial NSEs have been claimed for the non-live diphtheria-tetanus-pertussis and rabies vaccines. However, no non-live vaccine has yet been documented to produce beneficial NSEs.

**Implications** Observational and experimental research has shown beneficial NSEs of four live attenuated vaccines, smallpox vaccine, BCG, OPV and MV. With immunological evidence now supporting the epidemiological observations, it is urgent to take both the specific and NSEs into account in the planning of vaccination programs.
Introduction

Human vaccines were developed to prevent a specific disease, be it smallpox, tuberculosis, polio or measles infections. The practice for testing new vaccines has been disease-specific: does it induce protective antibodies or protective cellular immunity and does it provide clinical protection against the targeted infection. However, this perception of vaccines is crumbling as more and more epidemiological (1-3) and immunological studies (4-6) are finding beneficial or deleterious non-specific effects (NSEs) of vaccines (heterologous or off-target effects). In other words, a vaccine may have effects on infections not targeted by the vaccine.

Here, we review which human vaccines have been linked to beneficial NSEs and we focus particularly on how epidemiological studies have developed and justified the concept of beneficial NSEs of live vaccines. This is partly a personal story of how we came to use the concept of NSEs of measles vaccine (MV) stating from the late 1970s, but we have also reviewed the literature for possible NSEs of other vaccines (Figure 1).

Methods

Starting from 1979, in our attempt to understand the incredible high mortality in measles infection in West Africa and how it could be prevented, we came to pursue the concept of NSEs of MV. This led us to conduct a systematic investigation of NSEs of other routine vaccines, as outlined in the personal narrative below.

For the purpose of the present review, we furthermore searched Pubmed for epidemiological studies of non-specific/nonspecific effects (NSEs) of the most common human vaccines, including smallpox (vaccinia), Bacilli Calmette-Guérin (BCG) vaccine against tuberculosis, oral polio vaccine (OPV), measles vaccine (MV), rubella, mumps, pertussis, tetanus, diphtheria, varicella, yellow fever, RSV, rabies, meningitis, hepatitis B, pneumococcus, influenza and malaria (Supplementary Table 1). Abstracts were reviewed and the papers read if potentially relevant. Immunological studies, animal studies, case reports and papers where other vaccines were the main focus were not considered. For many vaccines there are no real epidemiological data on NSEs; other vaccines were only related to NSEs because the vaccine was co-administered with either MV or pertussis vaccine. We have therefore focused on smallpox vaccine, BCG, OPV, MV, and diphtheria-tetanus-
pertussis (DTP) where most of the research has been concentrated (see Supplementary Table 1).

The introduction of smallpox vaccine around 1800 and of BCG vaccine in 1921 happened long before the medical literature became searchable. However, it is known that already when these vaccine were first introduced, it was discussed whether they had beneficial NSEs (7-9).

Results

Non-specific effects of measles vaccine

Starting from 1979, studies of the mortality of measles-vaccinated and measles-unvaccinated individuals in Guinea-Bissau led to the suggestion that MV had beneficial NSEs.

In the 1970s, the predominant thinking was that malnutrition was the main determinant for severe measles infection, at the time the single largest killer of children in developing countries. Hence, many believed that measles vaccination would have limited effect on child survival, because frail children saved by MV would probably just die of other infections (10,11). This perspective was supported by a paper in Lancet comparing child mortality in two urban areas in Congo in which MV had been introduced at 7 months of age in one area but not in the other. The paper concluded “it may be useful to think twice before allocating already scarce resources to such a programme [measles vaccination]” (12).

In 1979-1980, after a measles epidemic in Guinea-Bissau, a Swedish funded nutritional project showed that nutritional status was not a determinant of the risk of dying of measles infection; there was essentially no difference in anthropometry between children, who died or survived measles infection (13,14). At the time, MV was not used in Guinea-Bissau. Due to the devastating measles case fatality rates of 21% and 34% for children under 5 years of age in urban and rural areas, MV campaigns were implemented in connection with nutritional surveys in 1979 and 1980 (14,15).

Since measles mortality was not related to nutritional status it raised the question of what would happen to overall mortality when children had received MV. A preliminary check of reported deaths after the MV campaign in the urban area suggested that nearly all deaths had occurred among children, who on the day of the campaign had not been home because they had travelled to visit family in the rural areas (15). The difference was stunning, 6-fold higher mortality for children
who were unvaccinated compared with those who had received MV during the
campaign (Table 1). A strong survival benefit of MV was also supported by the small
data set from a rural area (Table 1). In a later “natural experiment”, none of the
vaccinated child had seroconverted after MV during a one month period; they had
received an ineffective vaccine. Comparing the mortality of children who had
received effective MV versus ineffective MV showed a 3-fold reduction in mortality
after effective MV (Table 1). Maybe even more important, a re-examination of the
Congo-study showed that mortality from 7 to 36 months of age among the measles
vaccinated children was 53% (-9-80%) lower than among the unvaccinated children
from the control areas (Table 1) (1,20).

The observations were not based on individually randomised trials (RCTs) but it was
unlikely that selection biases could explain a difference in mortality of more than
50%. In Guinea-Bissau, there were no apparent differences in health or social
conditions between travelling and non-travelling children (15,21). In Congo, children
from two areas had been compared after the introduction of MV in one area; in the
year prior to the MV experiment there had been no mortality difference between
the two areas (12). Importantly, measles did not usually cause more than 10-15% of
all deaths; prevention of measles infection could thus not explain the reductions in
mortality of more than 50% (22).

These observations of strong non-specific reductions in mortality after MV raised
numerous issues. We briefly describe three key questions.

1) **Were the findings of non-specific benefits of MV consistent?**

To assure that the trends were reproducible, further studies of measles epidemics
and MV were initiated in Senegal, Kenya and Bangladesh (17,23,24). The
observation that MV had strong beneficial effects on overall child survival turned
out to be reproducible. In Guinea-Bissau, it had been possible to compare the
general mortality rate before and after the introduction of MV and this suggested a
reduction of more than 50% (15). Before-after studies from Senegal (N=2), Congo
and India found similar reductions in mortality (25). Surprisingly, the benefit from
MV was more important for females than for males in several studies and the
benefit was stronger when MV was given early in life (1).

To assess how much reduction was due to measles prevention and how much was
not explained by measles prevention, the vaccine efficacy against death (VED) was
estimated in studies including all deaths or excluding measles cases (Table 2). In the
larger studies, there was no difference in the VED estimates with and without censoring for measles infection. Hence, the specific protection against measles infection contributed little to the reduction in overall mortality after the introduction of MV (Table 2).

2) Could mortality be reduced further with early MV?

We examined whether further reductions in mortality could be obtained by using medium-titre measles vaccine (MTMV) or high-titre measles vaccine (HTMV) already from 4 months of age (3,26). In the 1970s, WHO had recommended MV from 9 months of age based on seroconversion studies (27), but studies in the early 1980s showed that HTMV could immunise already from 4 months of age, in the presence of maternal measles antibodies. Hence, RCTs with MTMV and HTMV were initiated in Guinea-Bissau, The Gambia, Senegal (26) and Sudan.

HTMV was clearly protective against measles infection in Guinea-Bissau and Senegal. However, the RCTs of HTMV were disastrous with nearly two-fold higher mortality for females who had received HTMV. The HTMV recipients had higher mortality than control children only after controls had received standard-titre MV at 9-10 months of age (3,26). Since HTMV was protective against measles infection this made little sense. It was speculated that this was an adverse event or that HTMV had come to close to the natural disease, inducing immunosuppression similar to measles infection (1). However, these speculations did not fit the data; the negative effects did not occur in the first months after HTMV, but only after 9-10 months of age when the controls had received standard MV (3,26).

3) Could the non-specific benefits of MV be ascribed to long-term excess mortality after measles infection?

Measles deaths are usually considered to be death within one month of a measles rash. However, we speculated that measles cases could have continued to increase mortality from other causes: “delayed excess mortality”. If measles infection had delayed excess mortality, MV would appear to have a much larger overall effect than expected based on just preventing the acute measles mortality (15). Studies of long-term morbidity and mortality after acute measles infection were therefore conducted in Guinea-Bissau, Senegal and Bangladesh (17,28,29).

The epidemiological studies did not find delayed excess mortality after measles infection. In the available studies from Guinea-Bissau, Senegal and Bangladesh, there was rather a trend for lower mortality after the acute phase of measles
infection compared with uninfected, unvaccinated controls (Supplementary Table 2). Noteworthy, the lower mortality after measles infection was not a selection bias due to the frail children having died already of measles infection; the same trend of lower mortality after measles infection was found in situations with no acute measles mortality (28). Hence, these studies suggested that measles infection could have a beneficial effect on survival and they refuted the hypothesis that the mortality reduction after MV was due to prevention of long-term excess mortality after measles infection.

**Summarising the evidence**

The triangulation of all available observations on measles infection, MV and mortality made it inevitable that at least standard-titre MV had such NSEs (Table 3): First, the before-after studies and the studies comparing mortality for vaccinated and unvaccinated children suggested that MV had beneficial NSEs which could not be explained by disease-specific prevention. Second, these effects could not be explained by measles infection having delayed excess mortality. Third, the observations that MV had stronger effect when given early and for females were incompatible with an only-disease-specific-prevention-perspective, but were potentially compatible with the non-specific perspective (32,33). Fourth, the observation that the VED was nearly the same with and without censoring for measles infection in the analysis was incompatible with an only-disease-specific-prevention-perspective but potentially explainable from the perspective that MV as well as mild measles infection may have beneficial NSEs (Supplementary Table 2).

Given the consistency of these observations, we eventually formulated the hypothesis that MV had beneficial NSEs (1). Subsequent studies have strongly supported this perspective. In a review of the potential NSEs on child mortality of BCG, DTP and MV sponsored by the World Health Organization (WHO)’s Strategic Advisory Group of Experts on immunization (SAGE) in 2013-2014, MV was associated with a 46% reduction in mortality (33).

**New questions arising**

The fact that MV had beneficial NSEs raised an interesting question. It could potentially have negative effects to stop MV after eradication of measles infection. Smallpox vaccine was the only vaccine which had been stopped (1980) after disease eradication (1977). We therefore decided to examine whether smallpox vaccine had beneficial effects which could be detected long after smallpox had disappeared. We started studies of smallpox vaccination in Guinea-Bissau in 1997 to document
whether it was likely that there were beneficial NSEs of smallpox vaccine and therefore negative effects of stopping the vaccine.

Non-specific effects of smallpox vaccine

NSEs of smallpox vaccine was noted already in the first decade after the introduction of vaccinia. Dr. Gierl, the first smallpox vaccinator in Bayern, noted that vaccinia seemed to protect against many other diseases: “vaccinated persons are less susceptible to infectious diseases such as measles, scarlet fever, whooping cough than non-vaccinated persons” (7). Historical demographic studies from several European countries have noted that the decline in general mortality in the first part of the 19th century was much larger than could be explained by the absence of smallpox deaths (34).

When we decided to examine the potential NSEs of smallpox vaccination in Guinea-Bissau, no vaccination register covered the population and the assessment was therefore based on reading smallpox scars in both urban and rural areas of Guinea-Bissau. We later identify a small subgroup with documented smallpox vaccination in a public health register and were able to show that nearly everyone with a documented smallpox vaccine had a smallpox scar 30 to 60 years after vaccination.

The impact of smallpox vaccination on survival, controlled for known confounders, was much stronger than expected, the reduction in adult mortality being 40% or more even though there was no longer any smallpox infection (Table 4). Subsequently we were able to show similar results in Denmark using Danish school health registers with documented vaccination status at school entry and follow-up of all individuals through public registers. From school entry at 7 years of age to 45 years of age, being smallpox and BCG vaccinated was associated with 46% (95% CI 19-64%) lower mortality from natural causes of death whereas there was no link to accidents, suicides and murders (37). The data from Guinea-Bissau and Denmark suggested also that smallpox vaccination had provided some protection against HIV-1 infection. Since smallpox vaccine down-regulates the CCR5 receptor, there may indeed be biological basis for such an effect (38).

Non-specific effects of BCG vaccine

Calmette wrote in 1931 about BCG that “The general mortality of 8,075 vaccinated children exposed to tuberculous infection, aged from one month to one year ... has been 4.6%, whereas in non-vaccinated children of the same age, living under similar conditions, it is at least 16%, and often exceeds 25%. ... Can it be that tuberculous infection plays a more important part in infant mortality than we have supposed? ... Or does the harbouring of BCG, followed by its digestion and elimination, confer on
the organism a special aptitude to resist those other infections which are so frequent in young children? (8) [At the time, BCG was administered orally.] Similar observations and interpretations were made by Näslund, the Swedish physician responsible for introducing BCG in northern Sweden in the 1920s (9). Näslund may have been the first to have used the concept of “non-specific” immunological effects (9).

While a concept of non-specific immunity continued to be mentioned in immunological studies (39) it does not seem to have been investigated in public health studies of BCG or smallpox vaccine after the 2nd World War (Supplementary Table 1). The overwhelming focus was on the specific protection against TB and NSEs may have been perceived to be irrelevant. It may also have been linked to declining enthusiasm for BCG after the Lübeck catastrophe, where BCG got contaminated with infectious tuberculosis bacilli.

In the 1970s, BCG was used in clinical studies of non-specific immunotherapy for a number of human malignancies. After some successful reports with melanoma and leukemia, results were more variable and the use of BCG was limited. However, since its first use in 1976, BCG has continued to be used for superficial bladder cancer (40).

Inspired by the studies of MV, in the mid-1990s we started to examine whether BCG might also have beneficial NSEs for child survival in low-income countries (13). The initial studies suggested a beneficial non-specific effect. To make sure that the beneficial effect was really due to BCG-vaccinations and not to selection biases between vaccinated and unvaccinated children, we examined in several studies of BCG-vaccinated children whether having or not having a BCG-scar or a positive tuberculin skin test (TST) was associated with better survival. That was the case. Among BCG-vaccinated children having a BCG-scar or a positive TST was associated with around 40% lower mortality in several studies (42). Numerous observational studies have subsequently supported that BCG is associated with much lower all-cause mortality. In the review of the potential NSEs on child mortality of BCG, DTP and MV sponsored by the SAGE in 2013-2014, BCG vaccination was associated with 47% (33) reduction in child mortality.

It is difficult to get permission to test in RCTs vaccines already recommended by WHO. However, it became possible to test BCG-Denmark among low-birth-weight (LBW) children because Guinea-Bissau recommended late BCG vaccination for LBW children. In the LBW group, BCG-at-birth reduced neonatal mortality by 38% (17-
54%) in three RCTs (41). BCG strongly reduced the risk of neonatal sepsis (43). Two subsequent RCTs in India using BCG-Russia found no benefit among extreme LBW children (<2,000 kg) (44). This may relate to strain of BCG, as several studies have found the BCG-Denmark is much better in inducing a BCG-scar but also provide more adverse events than BCG-Russia (45).

**Non-specific effects of OPV**

Having shown beneficial NSEs of three other live vaccines, we became interested in whether OPV, the other commonly used live vaccine, had beneficial NSEs. The literature provides some historical support for this idea.

From the 1950s and onwards, USSR virologist Voroshilova worked with the immune training potential of live enterovirus vaccines (LEV), including OPV. In huge studies involving hundred thousands of individuals, it was shown that LEV/OPV significantly reduced the risk of influenza and acute respiratory infection (46,47).

When OPV was first introduced in South America in the early 1960s, Contreras showed that OPV interfered with the replication of other enteroviruses and suggested that this resulted in fewer diarrhoea deaths in Chile and Brazil. Hence, he proposed that massive vaccination with OPV could be used as a non-specific measure to combat infant diarrhoea (48,49). It is unclear why these effects were not pursued in subsequent decades.

No study at the time disproved the beneficial NSEs. OPV has usually been co-administered with DTP in the first months of life, and it has therefore been difficult to separate the effects of OPV and DTP. However, in some studies either DTP or OPV was missing for logistic reasons and it then became possible to assess their relative importance. All these studies have indicated that OPV-only is associated with lower mortality than OPV+DTP or than DTP-only (50,51).

Furthermore, from the late 1990s numerous national OPV campaigns were organised as part of a plan to eradicate polio and in response to regional outbreaks of polio infection. Though few studies have been made, campaigns with OPV appeared to be associated with major reductions in mortality. In HDSS studies in Guinea-Bissau, Ghana and Burkina Faso, where it has been possible to follow the general mortality rate before and after campaigns, campaign-OPV have been associated with 10-30% reduction in the overall mortality rate (52-54). Boosting with campaign-OPV is also beneficial, each additional campaign is further enhancing the beneficial NSEs (52). Given the large number of campaigns which have been
organised the last 20-25 years, the campaigns have contributed very significantly to the reduction in child mortality in many low-income countries.

In a large RCT of OPV-at-birth (OPV0), receiving BCG+OPV0 versus only BCG was associated with a 32% reduction in infant mortality or until they received campaign OPV (55). In RCTs, OPV have also been compared back-to-back with inactivated polio vaccine (IPV) in randomised trials in Bangladesh and Finland and been found to be associated with less morbidity (56, 57).

Given that there has been virtually no polio infection in most countries in the last 20 years, these effects of OPV are clearly beneficial NSEs.

The HTMV enigma

Four live vaccines have been associated with strong beneficial NSEs. From this perspective, it was an enigma that HTMV, also a live vaccine, had deleterious NSEs; furthermore, standard MV had stronger beneficial NSEs for females, but HTMV was associated with increased female mortality. Initially, it was hypothesised that standard MV had beneficial NSEs in addition to the preventive specific-disease effects (1), and that HTMV, in contrast, did not have beneficial NSEs. Since females no longer experienced the beneficial NSEs of standard MV, female recipients of HTMV appeared to have higher mortality (58). This was a poor explanation. It did not consider that the trend in all HTMV studies was that female HTMV recipients not only had higher mortality than female controls, but also had higher mortality than male HTMV recipients (26). In the pre-vaccination era in West Africa, girls did not have higher mortality than boys (59).

NSEs of non-live vaccines: Resolving the HTMV enigma

In the pursuit of the non-specific effects of vaccines, in the 1990s, the Bandim Health Project in Guinea-Bissau therefore initiated new studies, not only of smallpox vaccine, BCG and OPV, but also of DTP, hepatitis B vaccine (HBV), and IPV (2, 59-62). As of today, beneficial NSEs have been shown for four live vaccine (41,35,36,52, 55,63). Unfortunately, the studies of DTP and other non-live vaccines found these vaccines to be associated with deleterious NSEs for females (64,65). This opened a new explanation of why the live HTMV had been associated with deleterious NSEs. HTMV had been given so early at 4 to 5 months of age in the RCTs that nearly all children in the HTMV groups had received DTP after HTMV; this was not the case in the control groups receiving standard MV at 9-10 months of age. DTP after MV is associated with increased female mortality (65) and therefore, HTMV ended up
being associated with increased female mortality. This explanation fitted all studies on MTMV and HTMV (26). In fact, before the HTMV recipients received DTP they had significantly lower mortality than controls who had not received MV (26).

**Beneficial NSEs of non-live vaccines: DTP and Rabies vaccine?**

A number of WHO-sponsored reports have reported large reductions in child mortality after DTP vaccine (Table 5). These reductions clearly suggest beneficial NSEs since the reductions are far larger than can be explained by prevention of pertussis, tetanus and diphtheria infections. However, the reports have a number of problems. First, the data have been collected with “survival bias”, meaning that data was better for those who survived than those who died (71). This may happen because it can be difficult to get accurate information from the children who had already died, e.g. the parents threw the vaccination card away after the child died. If such children are classified as unvaccinated, it may exaggerate the benefit of the vaccine because some vaccinated deaths were misclassified as “unvaccinated”.

Second, in many studies which reported beneficial NSEs of DTP, DTP had actually been co-administered with BCG (66). In the official WHO schedule BCG should be given at birth and DTP only from 6 weeks of age. However, in many rural areas in low-income countries BCG is often delayed and therefore become co-administered with DTP. There is now substantial evidence that mortality is lower when BCG is co-administered with DTP than when DTP is administered after BCG (72-74). When studies with survival bias or co-administration of BCG and DTP were excluded, DTP-vaccinated children had higher mortality than DTP-unvaccinated children (64). Hence, there are no valid studies to suggest that DTP has beneficial NSEs.

Recently, it was hypothesized that rabies vaccine may have beneficial NSEs (75,76). The key observation leading to this suggestion is the RCTs of RTS,S malaria vaccine which found that RTS,S was associated with a higher incidence of meningitis and cerebral malaria (75) and higher female mortality (77). Rather than ascribing this to a deleterious effect of RTS,S, it was suggested that rabies vaccine, which had been used as a control vaccine in one of the two age groups, in which RTS,S was tested, had beneficial NSEs (75). Furthermore, epidemiological studies of rabies vaccine to dogs in South Africa suggesting strong beneficial NSEs were taken as support of the hypothesis (78). Against the hypothesis speaks that RTS,S was also associated with increased female mortality in the age group in which conjugated meningococcal vaccine had been used as control vaccine (77). The reported beneficial NSEs of
rabies vaccine among dogs (78) may be due to survival bias, since the collection of vaccination information was not as good for the dogs that had died.

Hence, as of now there is no study supporting beneficial NSEs of non-live vaccines.

**Implications and conclusions**

Most trends noted in the development of the concept of the NSEs of MV starting from 1979 turned out to be consistent in later studies. Several RCTs of MV, BCG and OPV have found strong beneficial NSEs (41,55,63), though the results have not been universally consistent (44,79).

The search for beneficial NSEs have generated several tentative generalisations which have been remarkably consistent: First, live vaccines have beneficial NSEs. Second, early vaccination enhances the beneficial NSEs. Third, boosting with live vaccines enhances the beneficial NSEs. Fourth, maternal priming with a similar antigen may also enhance the beneficial NSEs in the offspring. Fifth, so far all studies have shown that non-live vaccines are associated higher female than male mortality. Sixth, sequence of vaccinations is important with the latest vaccine having the strongest NSEs. Seventh, a non-live vaccine administered after a live vaccine will reduce the beneficial NSEs of the live vaccine.

These generalisations do not mean that beneficial NSEs are invariable effects which will always occur. The NSEs are generated through the immune system, and other immune training interventions, conditions or infections may therefor interfere to produce unexpected results (79). Most health research is still conducted within a one-problem-one-solution paradigm, e.g. one-infection-one-vaccine or one-deficiency-one-supplementation. It is rarely examined whether these solutions interact but they often do. For example, when BCG was co-administered with DTP it reduced negative effect of DTP (72); when DTP was administered after HTMV that had a strong negative effect for female survival(26); when campaigns with OPV are implemented it will reduce the differential effects of other exposures that the children have experienced (53). With the increase in new vaccines and other interventions the number of interactions will increase. To optimise the benefits of public health interventions it is essential that the potential interactions are studied systematically, so that negative interactions can be limited or prevented.

Historically, ideas about NSEs of a vaccine have been suggested many times, but there is usually no clear documentation why the interest faded again or the studies were dismissed. The current studies of NSEs have been based on the perceived need
to measure the effect on overall mortality, not merely on the targeted disease. Hopefully, the interest will not disappear this time. There are huge potentials in studying systematically - in RCTs, in natural experiments, and in observational studies - the NSEs of different vaccines in different disease environment and with different interacting interventions with a view to further improve overall health and not just specific-disease immunity (31).

So far, the only major policy implication of the NSEs of vaccine has been that HTMV was withdrawn by WHO in 1992 because it was associated with increased female mortality (3). However, based on what we know now, it would seem appropriate to test how NSEs of vaccines could possibly improve the current vaccination schedule and child survival. Since BCG is often delayed, the median age of vaccination being more than one month in Africa (80), providing BCG vaccination within the first days of life consistently would probably reduce mortality very considerably. The relative benefit of different strains of BCG should be determined (41,44). It should be examined how the negative effects of DTP can be reduced, possibly through co-administration with BCG (72). If MV could be given earlier (81) it would increase the period with a beneficial live vaccine as the last vaccination. Furthermore, it should not be recommended to give a non-live vaccine after MV as this has consistently produced increased female mortality (26). Can other live vaccines replace the beneficial NSEs of OPV, when OPV is removed and substituted with IPV as planned (82)? We need to consider whether it is optimal for both females and males to have the same vaccination schedules.

Once we start measuring the NSEs of vaccines systematically many more patterns that may improve child survival will emerge.
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Conflict of interest: Nothing to declare

Ethics: The study is a literature review.

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Transparency declaration: The lead author (the manuscript’s guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.
Legend:

Figure 1. Time lines in the study of the non-specific effects of vaccines
<table>
<thead>
<tr>
<th>Study</th>
<th>Design; age at vaccination; who compared; follow-up period</th>
<th>Mortality rate (deaths/person-years)</th>
<th>Vaccine efficacy against death (95% CI)</th>
<th>Measles death among unvaccinated deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bissau, urban (15)</td>
<td>6-36 months; MV campaign participants vs absent; 12 months</td>
<td>Unvaccinated 14.2% (10/70.5) Vaccinated 1.9% (7/361.0)</td>
<td>86% (65-95%) 0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Bissau, Rural (14)</td>
<td>6-36 months; MV campaign participants vs absent; 13 months</td>
<td>Unvaccinated 6.6% (5/75.3) Vaccinated 4.1% (7/170.3)</td>
<td>38% (-95-80%) 0% (0/5)</td>
<td></td>
</tr>
<tr>
<td>Congo (Zaire) (12)</td>
<td>7-9 months; different areas; 24 months</td>
<td>Unvaccinated 3.4% (66/1811.2) Vaccinated 1.7% (6/348.8)</td>
<td>53% (-9-80%) NA</td>
<td></td>
</tr>
<tr>
<td>Bissau, “Natural experiment” (16)</td>
<td>7-24 month; effective MV vs ineffective MV; 24 months</td>
<td>Unvaccinated 7.5% (7/92.8) Vaccinated 2.5% (6/244.6)</td>
<td>67% (3-89%) 29% (2/7)</td>
<td></td>
</tr>
<tr>
<td>Senegal (17)</td>
<td>9-18 months; vaccinated vs unvaccinated same area; 23 months</td>
<td>Unvaccinated 5.3% (86/1610.5) Vaccinated 3.2% (90/2806.7)</td>
<td>40% (19-55%) 22% (11/51)</td>
<td></td>
</tr>
<tr>
<td>Burundi (18)</td>
<td>9-23 months; vaccinated vs unvaccinated same community</td>
<td>Unvaccinated 4.7% (51/1083.4) Vaccinated 1.2% (14/1201.2)</td>
<td>75% (55-86%) 3% (3/86)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Mortality rate ratios for measles-vaccinated and measles-unvaccinated children with and without measles cases/measles deaths in the survival analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design; age at vaccination; who compared; follow-up period</th>
<th>Vaccine efficacy against death (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Including measles cases in survival analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Study Design; age at vaccination; who compared; follow-up period]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[Vaccine efficacy against death (95% CI)]</td>
</tr>
<tr>
<td>Bissau, “Natural experiment” (16)</td>
<td>7-24 month; effective MV vs ineffective MV; 24 months</td>
<td>67% (3-89%)</td>
</tr>
<tr>
<td>Guinea-Bissau (19)</td>
<td>9-23; vaccinated vs unvaccinated in same area; 19 months</td>
<td>64% (37-79%)</td>
</tr>
<tr>
<td>Senegal (17)</td>
<td>9-18 months; vaccinated vs unvaccinated same area; 23 months</td>
<td>40% (19-55%)</td>
</tr>
<tr>
<td>Burundi (18)</td>
<td>9-23 months; vaccinated vs unvaccinated same community</td>
<td>75% (55-86%)</td>
</tr>
</tbody>
</table>
Table 3. Epidemiological observations on measles vaccination measles infection in relation to the “specific-disease-prevention hypothesis” and the “beneficial non-specific effects hypothesis”.

<table>
<thead>
<tr>
<th>Observation</th>
<th>MV prevents only acute measles infection and its long-term consequences</th>
<th>MV has non-specific beneficial effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low mortality after MV</td>
<td><strong>Does not support</strong>: The vaccine efficacy against death is nearly the same with and without censoring for measles infection in the analysis; No long-term excess mortality after measles infection</td>
<td><strong>Support</strong>: There is clear reduction in mortality even when all measles cases have been censored</td>
</tr>
<tr>
<td>MV has a stronger beneficial effect for females than males</td>
<td><strong>Does not support</strong>: Measles infection is equally severe for females and males so effect of MV should not differ by sex</td>
<td><strong>Compatible</strong>: Beneficial immune training may differ for females and males</td>
</tr>
<tr>
<td>HTMV associated with higher mortality</td>
<td><strong>Does not support</strong>: HTMV was protective against measles infection so should not be associated with higher mortality</td>
<td><strong>Support</strong>: See text. HTMV had beneficial NSEs until they received non-live vaccine after the MV.</td>
</tr>
<tr>
<td>The negative HTMV only seen after 9 months of age</td>
<td><strong>Does not support</strong>: Cannot be explained</td>
<td><strong>Support</strong>: Standard MV given in the control group at 9-10 months of age</td>
</tr>
<tr>
<td>Mild measles infection associated with beneficial effects on survival</td>
<td><strong>Does not support</strong>: We are vaccinating to prevent the disease</td>
<td><strong>Compatible</strong>: This is compatible with MV also having a beneficial effect</td>
</tr>
<tr>
<td>Stronger effect when given early</td>
<td><strong>Does not support</strong>: When given early MV should have less effect due to interference from maternal antibodies</td>
<td><strong>Support</strong>: Other studies have found stronger beneficial NSEs after early vaccination</td>
</tr>
<tr>
<td>The vaccine efficacy against death is nearly the same with and without censoring for measles infection</td>
<td><strong>Does not support</strong>: Since MV prevents measles death this observation in contradictory</td>
<td><strong>Compatible</strong>: While MV will reduce the measles mortality some of the unvaccinated children may have benefitted from a natural measles infection. The net impact of measles infection on mortality may therefore be less than the deaths</td>
</tr>
<tr>
<td></td>
<td>classified as being due to acute measles infection.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Screening for scars</td>
<td>Age group</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>---------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Guinea-Bissau: Epidemiological study of scar and adult mortality</td>
<td>Urban area: 1893</td>
<td>25 years or older</td>
</tr>
<tr>
<td></td>
<td>adults</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea-Bissau: Epidemiological study of scar and adult mortality</td>
<td>Rural area: 367</td>
<td>25 years and older</td>
</tr>
<tr>
<td></td>
<td>adults</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Studies with survival bias assessing the effect of DTP-vaccination compared with no DTP vaccination. 
Studies used by SAGE

<table>
<thead>
<tr>
<th>Country and study reference</th>
<th>Age group</th>
<th>Assessment of ‘unvaccinated’; % excluded for no information</th>
<th>Mortality rate per 1000 person-years (deaths/follow-up time)</th>
<th>Bias index # (i.e. MRR for unvaccinated vs vaccinated children)</th>
<th>MRR (95% CI) for DTP-vaccinated versus DTP-unvaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh @ (66)</td>
<td>6 weeks-9 months</td>
<td>Default; none</td>
<td>67 (329/1783651 days)</td>
<td>3.40 (2.93-3.95);</td>
<td>0.52 (0.31-0.87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 (362/6677421 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burkina Faso (67)</td>
<td>0-7 months, 6 months follow-up</td>
<td>Default; none</td>
<td>120 (281/28128 person-months)</td>
<td>2.29 (1.74-3.00)</td>
<td>1.00 (0.60-1.67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52 (64/14662 person-months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papua New Guinea (68)</td>
<td>29 days to 5 months</td>
<td>Default; none</td>
<td>233 (92/144285 days)</td>
<td>7.52 (5.15-10.97)</td>
<td>0.48 (0.22-1.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31 (38/448418 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>India &amp; (69)</td>
<td>6 weeks to 8 months</td>
<td>Health centre vaccinations; none</td>
<td>200 (23/1381 person-months)</td>
<td>6.82 (4.42-10.52)</td>
<td>0.28 (0.20-0.40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29 (183/74937 person-months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghana (70)</td>
<td>0-59 months</td>
<td>Default; none</td>
<td>Data not provided; since estimates for some vaccines versus no vaccine were 0.14 (0.13-0.16) the MRR must be at least 7-fold higher for unvaccinated than for vaccinated children</td>
<td>7.14 (6.25-7.69)</td>
<td>0.15 (0.14-0.16)</td>
</tr>
</tbody>
</table>

Notes: # The bias index measures the mortality rate ratio for unvaccinated versus vaccinated (any vaccine) children. When vaccinated dead children gets misclassified as “unvaccinated” the bias index will become very high. It will seen here that is was > 2.0 in all studies. In studies with assessment of status for both vaccinated and unvaccinated and prospective follow-up the bias index has been <= 1.7.

@ The Bangladesh study is based on community registers and it is assumed that all information is available since no child is excluded for lack of information. However, there is no documentation that ‘unvaccinated’ was actively verified. The study had problems with registration of early events since children who subsequently moved had highly significantly lower vaccination coverage.
& Health centre provided all vaccinations. Vaccinations coverage was very high. Hence, unvaccinated children would be a small group of frail children in the 6-8 week age range whereas DTP vaccinated children would tend to be 5-6 months old on average. Hence, the comparison is between different age groups and between healthy and frail children but not between different vaccination groups. The researchers themselves did not make a comparison between DTP-vaccinated and DTP-unvaccinated children, but the SAGE reviewers included this age-unadjusted comparison as an estimate of the effect of DTP. This comparison is “unnatural” both because it is age-unadjusted and because the control group does not reflect the general population of children (26).
<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Number of studies</th>
<th>Immunology; Methods; Discussion papers</th>
<th>Case reports; Adverse events</th>
<th>Other vaccines were the focus</th>
<th>NSEs epidemiology (first year)#</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox vaccine</td>
<td>7</td>
<td></td>
<td>3</td>
<td></td>
<td>4 (2004)</td>
<td>See text</td>
</tr>
<tr>
<td>BCG</td>
<td>139</td>
<td>73</td>
<td>9</td>
<td>10</td>
<td>47 (1979)</td>
<td>In the 1970s studies explored the used of BCG in cancer treatment. More recently many studies have examined the effect of BCG on child mortality, morbidity and allergic diseases.</td>
</tr>
<tr>
<td>OPV</td>
<td>24</td>
<td>3</td>
<td></td>
<td>10</td>
<td>11 (2004)</td>
<td>See text</td>
</tr>
<tr>
<td>Measles</td>
<td>78</td>
<td>11</td>
<td>4</td>
<td>14</td>
<td>49</td>
<td>Studies summarized in text</td>
</tr>
<tr>
<td>Rubella</td>
<td>11</td>
<td>1</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mumps</td>
<td>14</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pertussis</td>
<td>69</td>
<td>18</td>
<td></td>
<td>14</td>
<td>37</td>
<td>Nearly all studies are DTP; associated with increased female mortality; see text</td>
</tr>
<tr>
<td>Tetanus</td>
<td>82</td>
<td>26</td>
<td>1</td>
<td>55</td>
<td></td>
<td>Nearly all studies are DTP; associated with increased female mortality; see text</td>
</tr>
<tr>
<td>Disease</td>
<td>First Year NSEs</td>
<td>Second Year NSEs</td>
<td>Third Year NSEs</td>
<td>Fourth Year NSEs</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>------------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Diphtheria</td>
<td>64</td>
<td>12</td>
<td>1</td>
<td>51</td>
<td>Nearly all studies are DTP; associated with increased female mortality; see text</td>
<td></td>
</tr>
<tr>
<td>Varicella</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>6</td>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabies</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>1 (2017)</td>
<td>Hypothesis - see text</td>
<td></td>
</tr>
<tr>
<td>Meningitis</td>
<td>7</td>
<td></td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumococcus (PCV)</td>
<td>18</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza</td>
<td>28</td>
<td>17</td>
<td>3</td>
<td>5 (2010)</td>
<td>Non-live vaccine associated with increased non-influenza morbidity in two studies</td>
<td></td>
</tr>
<tr>
<td>RTS,S malaria vaccine</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>See rabies vaccine</td>
<td></td>
</tr>
</tbody>
</table>

Notes: # First year NSEs were mentioned
<table>
<thead>
<tr>
<th>Country, time, reference</th>
<th>Age group</th>
<th>Follow-up period after measles</th>
<th>Groups being compared</th>
<th>Measles infected vs. non-measles infected (95%CI)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea-Bissau, 1979-80 (21)</td>
<td>0-6 years</td>
<td>6-18 months</td>
<td>Previous measles inf. vs not vaccinated</td>
<td>0.62 (0.16-2.30)</td>
<td></td>
</tr>
<tr>
<td>Guinea-Bissau, 1988 (29)</td>
<td>Under 3 years</td>
<td>1 to 60 months</td>
<td>Measles cases vs controls</td>
<td>0.50 (0.22-1.16)</td>
<td>Adjusted age, sex, immunizations</td>
</tr>
<tr>
<td>Senegal, 1983-1986 (30)</td>
<td>0-9 years; censored Dec 1986</td>
<td>1 to 48 months</td>
<td>Unimmunized measles cases vs unvaccinated, uninfected controls</td>
<td>Index cases: 0.27 (0.09-0.85) Secondary cases: 1.10 (0.80-1.51) (p=0.018, interaction test)</td>
<td></td>
</tr>
<tr>
<td>Senegal, 1992-1996 (28)</td>
<td>0-6 years; censored Dec 1996</td>
<td>1-48 months</td>
<td>Exposed with clinical or subclinical measles vs exposed uninfected</td>
<td>0.20 (0.06-0.74)</td>
<td>Adjustment had no effect</td>
</tr>
<tr>
<td>Bangladesh, 1982-1985 (24)</td>
<td>Under 5 years</td>
<td>3-12 months post measles</td>
<td>Measles cases vs uninfected controls</td>
<td>0.40 (0.16-0.98)</td>
<td>Adjusted age, sex, siblings, maternal education, area</td>
</tr>
<tr>
<td>All studies</td>
<td></td>
<td></td>
<td></td>
<td>0.49 (0.26-0.91)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Table from (31)