NOT FOR GENERAL CIRCULATION

Review of Guinea Bissau vaccine-related mortality studies:

Report of WHO mission, Bissau, October 9-14 2000

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Background

Following a meeting in Geneva at which Dr. Peter Aaby presented the results of his research, the three members of this mission were contacted at short notice by Dr. P. Duclos at WHO HQ and asked to make a site visit to Bissau to review the study that was about to appear in the British Medical Journal (BMJ) apparently showing a negative impact of DTP vaccine on mortality in Guinea Bissau (Annex 1). One member of the group (KM) had been involved in the review of a draft of this paper in Geneva during June-July 2000; the others had no prior contact with the work. Prior to this site visit, a statistical review of the study had been conducted in Copenhagen by Dr. C. Nelson of WHO (Annex 2). Due to logistical problems the review was started by KM and MB; RB joined the group during the review. Terms of reference were finalised by the team and presented to Dr. Aaby who accepted them. Mr. H. Jensen, the statistician working on the project flew to Bissau for this review and participated throughout the week.

Terms of reference:

- To review the study, soon to be published in the BMJ, which shows unexpected effects of routine immunization on overall mortality – positive effects for BCG and measles vaccines, and negative affects for DTP and possibly OPV vaccines.

- To identify, by visiting the field site and examining the data collection process, and conducting discussions with investigators, any possible unforseen confounding factors that might have led to these conclusions.

- To look for evidence as to the causes of death that may explain the excess mortality observed.

- To review other studies by the same group which examine the indirect effects of vaccines on mortality.

- To suggest alternative analyses of these or other data that may help clarify the situation.

- To prepare a report that summarizes the conclusions of this visit and makes recommendations to WHO as to how to respond appropriately to the findings of this study and other evidence suggesting that vaccines have unexpected effects on overall mortality.

Process

- Presentation of methodology and analysis of main study.
- Review of data collection procedures for main study.
- Presentation of supporting studies including analytical methods.
- Free ranging discussion with investigators about other potential analyses.
- Preparation of draft report.
- Presentation of conclusions to the group.

Background to this study

WHO and many other institutions around the world have based their advocacy for childhood vaccination on the real or perceived reduction of disease-specific morbidity and mortality resulting from the protection afforded by individual vaccines. In many cases, the benefit of vaccination has been estimated by subtracting reported cases and deaths in the vaccination era from estimates of the disease burden during the pre-vaccination era. Few researchers have previously examined the concept that, while an individual vaccine might reduce disease-specific cases and deaths in any given population, it may also have an impact on overall morbidity and mortality in general. Dr. Aaby is unique among researchers for having examined this issue in considerable depth, particularly the potential impact on overall mortality.

Dr. Aaby first began working in Guinea Bissau in the late 1970s. As a young anthropologist his interest was examining the interaction between traditional political structures and the evolution of the new state. However to begin in the country he undertook a nutrition study for the Swedish organisation SAREC. To the dissatisfaction of SAREC, Dr. Aaby showed that the extremely high cumulative under-5 mortality reported in Guinea-Bissau in the 1970s – up to 500 deaths per 1,000 live-births (50%) – was not due to nutritional deficiency, but suggested that it was due to crowding and resulting intense exposure to communicable diseases.

Dr. Aaby convinced a sceptical Ministry of Health to implement a measles vaccination campaign among children 6 months to 6 years of age in December 1979. This campaign was repeated in December 1980 and 1981, with the inclusion of DTP vaccine. Data collection in the Bandim Project area in urban Bissau continued during this period. It was observed that under-5 mortality was 13% in 1979 before the first campaign, but fell to 4-5% in 1980-81. Furthermore, it was noted that non-specific mortality in 1980 was roughly 2% in the measles vaccinated group and 9% in the non-vaccinated group. In 1981, the rates were roughly 3% and 8%, respectively. Dr. Aaby concluded that measles vaccination reduced non-specific mortality and that malnutrition appeared to have no role in this phenomenon. Data from a study in the former Zaire published in the Lancet in 1981 showed similar results.

The evolution of studies associated with high-titre Edmonston-Zagreb (EZ) vaccine does not need to be revisited here. However, the primary conclusion of studies by Dr. Aaby and colleagues in Senegal, the Gambia and Guinea-Bissau and Dr. Halsey in Haiti was that high-titre EZ vaccine given early in infancy was associated with a significant increase in non-specific mortality in female study subjects, and not in males. WHO called a meeting of international experts and a decision was taken to suspend further use of high-titre EZ vaccine. The stated explanation for this phenomenon was that “high-titre vaccine too closely replicated natural disease”, but no serious evaluation was undertaken to elucidate the biological mechanism causing increased gender-specific mortality. However, experts may have overlooked the fact that Dr. Aaby also found that non-

specific (non-measles) mortality was reduced in the normal-titre Schwartz vaccine group, as he had shown previously.

In later studies, Dr. Aaby reported both that non-specific mortality in children following measles vaccination was less than that in unvaccinated children, and that non-specific mortality in surviving children following measles disease was less than that in children with no disease. Furthermore, index measles cases demonstrated a four-fold reduction in non-specific mortality, whereas secondary measles cases (presumably household contacts) demonstrated no significant change in non-specific mortality.

Analysis of data in Bangladesh showed that, in that environment, non-specific mortality in children following measles vaccination was reduced by 49% compared with unvaccinated children, and non-specific mortality in surviving children following measles disease was reduced by 60% compared with children with no disease. This study had a potential bias because it did not utilise a randomised design. However, Dr. Aaby was able to reproduce these results in a small randomized trial in Guinea-Bissau conducted during 1998. In that study, a randomised trial of one dose (at 9 months) versus two dose (6 and 9 months) measles vaccination was interrupted by the outbreak of war. As a result, 433 children received either measles vaccine or placebo at 6 months but did not receive the second dose. During the 3 months of chaos at the beginning of the war 11 children died in the placebo group and four in the vaccine group (one due to measles). The protective effect observed was mainly in girls. Longer observation of the same group has yielded similar results although the numbers are still small.

Dr. Aaby has summarised all the findings of these studies in the attached table from an unpublished review (Annex 3). He claims that these studies show that measles vaccine is associated with an "efficacy to prevent non-specific mortality" of 40-74%. The largest sample size and, hence, the narrowest confidence interval is associated with the study in Bangladesh. In the course of this work he began to look for similar non-specific effects with other vaccines. Unpublished work on the effect of BCG has shown a similar effect to measles, with protection most marked in females and stronger in infants who have a BCG scar and who have a positive tuberculin reaction. Such an effect could not be attributed to prevention of tuberculosis, and indeed in that study a separate analysis was performed excluding all children with contact with known tuberculosis cases. The results were unchanged.

Dr. Aaby believes that the non-specific effects of vaccines may occur with many vaccines, and has found historical evidence suggesting that small pox vaccine may have had a similar impact on survival. In many respects he has been a crusader for vaccination in the most difficult environments, and the finding of a negative effect with high-titre EZ measles vaccine must have been a personal blow. This may be small compared with the impact of DTP that has now been found in several of their studies. The first of these studies is about to appear in the BMJ and is the main subject of this review.

Documents reviewed

Routine vaccinations and child survival: follow up study in Guinea Bissau – This is the paper that will be appearing soon in the BMJ. The presently available version differs slightly from that which was circulated in WHO in May. The general conclusions are unchanged and can be summarized thus:

- In a study that examined survival during a 6 month window period for a cohort of infants 6 weeks to 6 months of age, those who had received one or more doses of DTP + OPV had increased mortality compared to unvaccinated infants when the analysis was controlled for BCG status. (In absolute terms there was little difference between the DTP group and unvaccinated children, but this emerged in the multivariate analysis.)
- In the same study both BCG and measles had the effect of reducing mortality during appropriate 6 month window periods.
- These non-specific effects, both positive and negative, were observed only in girls.

Non-specific effects of routine immunization: Implications for future vaccination policy - This unpublished document prepared by Dr. Aaby summarizes the evidence for indirect positive effects of measles and BCG and indirect negative effects of DTP from various studies. Most of the studies cited come from Dr. Aaby's group in Guinea Bissau or his collaborative group in Senegal. Many are not published yet. For measles there are 5 studies from elsewhere showing greater-than-expected reductions in mortality with the introduction of measles vaccine. For BCG and DTP effects the only study cited from outside this group is a small non-significant study from Benin. The paper discusses these non-specific effects and speculates on mechanisms that could explain the sex-specificity of the effect.

Routine Immunizations and mortality at a paediatric department, Guinea Bissau – This study approaches the same issue from a different perspective. The team reviewed a very early draft manuscript. (At the end of the review Dr. Aaby found a significant programming error in the study and requested that the numbers presented earlier be disregarded.) By examining mortality patterns in a paediatric hospital ward the investigators sought to determine the causes of the excess mortality associated with DTP. In the study, children who were registered in the main Bandim Health Project and therefore whose vaccination status was known, and who were recorded as being admitted in the hospital were analysed. Children who had received measles vaccine had a lower case fatality rate in hospital (risk ratio 0.5). The effect was more marked for girls and for those with a diagnosis of malaria or pneumonia (conditions which may be difficult to distinguish in a poorly functioning hospital ward). Among children who had received DTP, case fatality rates were higher for girls, and there was a trend towards higher case fatality rates compared with unvaccinated children. This study is still undergoing analysis. The study appears to lend support to the concept that immunization status affects outcome of common infections, and hints that this might be limited to malaria and

pneumonia, or even malaria alone. The clinical data available for analysis is quite limited but the final analysis should be viewed very carefully.

The introduction of DTP vaccine associated with increased infant mortality in rural Guinea Bissau – This paper, also unpublished, contains the most concerning evidence that DTP vaccine is causing harm in high mortality communities. Up to 1984 there was little vaccine use in Guinea Bissau. As part of a study initially designed to look at malnutrition rates, Aaby's group began delivering DTP and measles vaccine during their regular, usually 6 monthly, visits to study villages. Initially the government resisted the use of DTP in these visits as 6 monthly visits meant that children could only receive 1 or 2 doses in the first year but, after a devastating pertussis epidemic in 1983, Aaby's group convinced the government that even a single dose of pertussis vaccine was probably useful. Hence, in a sense, nobody has more to lose at the local level from the findings of the study to be published in BMJ than Aaby's group.

BCG was not introduced until 1986 and few children in this study received it. Vaccines were not always available for the visits as supplies were irregular. Thus, during the period 1984-1987 the group maintained a follow-up, and administered most vaccines to, a cohort of children in rural Guinea Bissau. Data from 1655 children followed during this period formed the basis of this analysis. The analysis is not yet complete, but essentially it showed that, during the window between the first two visits, all cause mortality was 11.3 deaths per 100 person years in DTP vaccinated children, compared with 5.1 in unvaccinated children. With further analysis it can be shown that this effect is most marked in children who were older at the time of the beginning of the 6 months period of observation.

It is not yet clear exactly how these data will be presented, but the manuscript is in an advanced stage and provides compelling evidence that, even in a situation where pertussis is still circulating, the introduction of DTP actually increased mortality. The impact of the publication of this paper could be greater than that of the BMJ article that presents a picture of balanced risks with the overall impact of vaccination being seen as positive.

Review process

The focus of this review is the paper about to be published in the BMJ, *Routine vaccinations and child survival: follow up study in Guinea Bissau*, by I Kristensen, P. Aaby and H. Jensen. The first full day of the review, 10/10/00, was devoted almost entirely to detailed discussion of the methodology of that study with Dr. Aaby and Mr. Jensen. The first author is currently working in South Africa and does not appear to be involved in the finalisation of the paper.

The history of this study is of interest. It was started in 1989-1990 with a grant from UNICEF designed primarily to look at the rate of neonatal tetanus. This was conducted in an environment where maternal mortality was 800/100,000 and child mortality 250-

330 per 1000 live births. To ensure that neonatal deaths were not missed the study sought to identify 100 women of childbearing age in 20 clusters in each of 5 regions. In general entire villages were included in the clusters and where there were not sufficient women in a cluster, neighbouring villages were included as well. During the course of the study women becoming of childbearing age or women migrating into the area were included as well, swelling the numbers well beyond the original planned sample of 10,000 women. Because of the design some clusters contained villages that were separated by some distance so that factors such as access to care were not always balanced within the clusters. Visits were made to each cluster at intervals of approximately 6 months, although visits were occasionally delayed or cancelled because of logistical problems.

Data collected related to maternal history, maternal nutrition, birth history, infant nutrition, infant survival and infant vaccination status. In recent years data on other factors such as number of hospitalisations was collected but this was not available for the 1990-96 data. In 1996 the UNICEF grant period ended and an analysis was performed to accompany the report of the 5 year study for UNICEF. This analysis was designed to look at the indirect effects of measles vaccine on child survival (by relating mortality during the period between a child's second and third visit to measles vaccination status at the time of the second visit). Because of the group's interest in this subject and this was expanded to look at indirect effects of the other routine vaccines, BCG and DTP-OPV. (As DTP and OPV were always given together these could not be separated in the analysis.)

The investigators were encouraged by the beneficial effect of measles, which was consistent with their earlier observations on the subject, but alarmed by the finding that DTP was associated with a substantial increase in mortality. A draft paper was prepared in 1997 and this was sent to WHO. Dr. Aaby did not receive a response and soon after civil war erupted in Guinea Bissau, preventing the investigators from following this up with WHO. In 1999 the manuscript was revised, a more sophisticated multivariate survival analysis was performed and in early 2000 the manuscript was sent to the BMJ. After peer review further analyses were requested and a revised manuscript was accepted by the BMJ. The journal plans to publish this paper in 2 forms, a complete version on their website and a simplified version in their paper journal. In the shorter version the methods will be shorter and only Tables 3-5 and Figure 2 will be included.

In the discussion on this paper there was a lengthy discussion about the data collection process. This discussion was focused on a search for potential sources of bias that could explain the results. Although stratification by cluster in the analysis is intended to ensure that the survival of infants is compared with other infants in the same village, this is not always the case and in some cases the clusters contain a number of villages separated by some distance. However a bias that would explain, simultaneously the protective effect of BCG and the harmful effect of DTP is difficult to postulate. As most births occur at home BCG is delivered by the same mechanism as other vaccines. In general women do not travel to deliver their babies so it is difficult to imagine a subgroup who have access to BCG and not DTP, and in any case this would be more likely to be a high risk group.

It is possible that during the age period 2-6 months mothers would be more likely to take a sick infant to a clinic. If this introduced bias in favour of DTP vaccination of the "sickly child" one would expect this to be reflected in nutritional status. In fact mid-upper arm circumference (MUAC) was higher in DTP recipients than controls. Nevertheless, MUAC is a fair guide to nutritional status and this is really the only factor that could potentially produce the results observed. A direct causal association between DTP administration and some fatal complication seems unlikely as the distribution of deaths within the time window was similar in the vaccinated and unvaccinated groups. The possibility that acutely ill children could present to clinic, be inadvertently vaccinated and later die could not explain the findings as vaccination status was recorded "cold" at the routine visits and doses subsequently administered to a child who died soon after vaccination would not be recorded.

This latter point is a drawback of the study, which the authors acknowledge. There is no way to determine the actual vaccination status of the children who died as many will have received vaccines in the period between their last visit and their death. This inevitably leads to misclassification bias and makes the finding of a DTP effect, and the magnitude of that effect, all the more remarkable. Possible ways to obtain these data were discussed, but it is unlikely that this could be achieved in more than a small minority of infants, even with substantial input. The destruction of the EPI offices and all the centrally held records would add to the difficulty of this task. It also should be noted that the recorded date of death is very approximate and may be in error by 1-2 months.

The effect of the government guideline that children weighing less than 2500 grams at birth should not be given BCG was discussed. Theoretically this would bias against vaccination of high risk, low birth weight infants, producing the results observed. However, the investigators believe that this guideline is rarely followed. To guard against this bias they analysed survival in infants who received BCG late, after 4 weeks of age, and found this to be slightly better than those who received BCG early, ruling this out as a source of bias in relation to the observed BCG effect.

Concerns have been raised about the large number of exclusions from the analysis, but observations of the study in the field revealed why this is so. In general a child whose vaccination status could not be determined at the beginning of the 6 months window of observation could not be eligible for this study, even though the vaccination status of survivors could be assessed later as the vaccination status of dead children is lost. We endeavoured to find out more about this group of children who were excluded (Annex 4). They are, as one would expect, a group with slightly poorer overall vaccination status than the others. Their mortality rate is slightly higher than that of children included in the analysis. However, the study is essentially a study of those children for whom the vaccination status could be reliably ascertained at their first visit by the study team during the first 6 months of their life. This is the study group in which survival is being assessed and related to vaccination status. We could not postulate any mechanism for systematic bias resulting from this selection.

Data collection

As this study is continuing in a slightly revised form at the present time, the group accompanied the field staff on one of their field visits. Within the cluster area, each compound was visited sequentially. At each compound visit the field worker sat in a central place in the compound surrounded by the women of the compound. Then, one by one he produced a form for each woman of childbearing age. The basic data were recorded. Samples of the data collection instruments are attached (Annex 5). Where a woman was found to be pregnant a form completed describing the relevant details. The woman's arm circumference and foot size was measured. Children were then called. If a child was present, the vaccination card was sought, a series of simple questions asked about the child and the arm circumference measured. At times the child was present, but the mother away. In such cases most questions could be answered by other compound members, but usually the vaccination card could not be physically seen as these tend to be kept locked away. In many cases both the mother and the child were absent, either locally at the market or in the field, or in Bissau city. In such cases questions were asked to ensure that child was still alive. No attempt was made to chase up any mother, even those who may be working in the field nearby. It was the impression of the team members who observed this process (KM, MB) that these simple, and rather crude data are collected faithfully and carefully, and in particular it is highly unlikely that any child deaths are not discovered and recorded.

Where a child is found to have died during the previous 6 months this is recorded and the field worker asks when the child died. In most cases this is probably accurate to within a month or so, as the families relate such events to important events in village life. They ask about the cause of death, but in general this is recorded either as a single word (most often "fever") or nothing at all. No verbal autopsies have been conducted in this study, probably because of the substantial cultural reluctance to speak about dead children. The vaccination cards are invariably destroyed, as are other reminders of the child's existence. Thus, within this study there are no means by which the causes of death in the two groups can be studied short of conducting late verbal autopsies on deaths that are now 5-10 years in the past. This is likely to be unacceptable and unlikely to produce useful results. (Of interest in the urban study in the Bandim Project one experienced nurse is attempting to conduct verbal autopsies. The results of this exercise have not yet been examined but Dr. Aaby is uncertain about the quality of the data.)

The data used for the retrospective analysis of the mortality impact of DTP during the period 1984-87 was also reviewed. A photocopy of one of the cards used in attached (Annex 6) and this shows the limit of the data available. Some of the results of that study appear to be strange (eg. surprisingly low mortality in the younger age cohort in the post vaccine introduction period) and further careful analysis is needed. There was a discussion of possible sources of bias in that study. Like the more recent study, most possible sources of bias would appear to work against the results observed. There was apparently one village in the study, which is attached to a military barracks, and where the mortality rate is lower than expected. This village could be excluded from the analysis if necessary, but it is impossible to imagine how this could introduce bias that

would lead to the major conclusions drawn. It is important to note that this study was not intended to be the focus of this review, and discussions about the study were initiated by the review team, one of whom had obtained an early draft of the paper from a collaborator of Dr. Aaby prior to the visit.

Data management

The hard data records were reviewed by the group. The forms used for the data collection are very simple, but it appears that data are collected carefully and most of the forms reviewed appeared to be filled in reasonably well. The data manager for the study enters all the data at the end of each visit. Data entry is performed using a Dbase structure and data are single-entered in an interactive manner, with new information being appended to the files as it emerges. This is a simple data entry structure that does not contain the file linkages and range checks that automatically clean data and are the feature of modern data entry programs.

Although the group had no reason to suspect that the data management had been performed in anything but a completely open and honest manner, it was decided to review the original forms of a random selection of 50 infants out of the 290 who were 6 weeks to 6 months at the beginning of the observation period (and therefore eligible for inclusion in the DTP analysis), and who died. This sample would include a significant proportion of the infants who formed the basis of the DTP conclusions as well as many who, in accordance with their age, could have been in that analysis but who were excluded. The sample was taken from the original database using SAS software. For this procedure a random seed number was defined. The number was chosen by one of the reviewers. A print out with the most important variables was generated and the original forms related to these children were retrieved one by one from the files where the forms were kept. All the characteristics of the forms suggest that they were old, indicating that have been used in field conditions.

The records of all the 50 cases were found. The detailed check of the data in the forms related with the data in the printouts (Annex 7) showed 3 errors. One vaccinated child was entered as not vaccinated, one female child was entered as male in the database and for the other child there was a 2 months error in the birth date. The one major mistake observed would increase the DTP effect once corrected, and the others had only minor implications for the results. As a result of this exercise, the team concluded that the quality of the database is satisfactory, although it contains some minor errors. In particular, the review process was completely transparent and the investigators gave it their full co-operation without hesitation.

Analysis

Routine vaccinations and child survival: follow up study in Guinea Bissau –

We were aware that the analysis has been reviewed in detail by Dr. Nelson in Copenhagen (Annex 2), but we felt that it was also within our brief to examine this as well. There was a lengthy discussion of the multivariate analysis on which the conclusions are based. The initial analysis was a simple Mantel-Haenszel test performed by Dr. Aaby and controlling for other vaccines. This produced a result similar to the final conclusion. Later a more appropriate Cox Proportional Hazards model was used in which cluster, other vaccines and age group (in 2 months brackets) were used. With this analysis the risk associated with DTP 1 was significant (hazard ratio 1.8, significant at the $p<0.05$ level) as was the protective effect of BCG and measles. Two or three doses of DTP also were associated with increased risk but these were not significant (also at the $p<0.05$ level). If a Cox model were used in which age is controlled exactly (ensuring that each child is compared with children of exactly the same age, rather than in the same bracket) the DTP1 effect was reduced such that the 95% confidence limits include 1.0. In some respects this approach is less valid as confidence of vaccination status will be quite variable according to where in the time window children were when they reached the age in question. In addition the confounding effect of measles vaccine, given towards the end of the time window for the older infants, would be stronger in this type of analysis. Whilst in general the group was satisfied with the methods chosen and the conduct of the analysis, we felt that it would be worthwhile to ask a statistician with particular expertise in this type of analysis to look again at the methods used.

Routine Immunizations and mortality at a paediatric department, Guinea Bissau

This study, which was designed to try and understand what form is taken by the increased or decreased mortality associated with vaccines, was presented and discussed. A visit was made to the paediatric department of the hospital. This revealed a crowded ward in which many basic commodities such as oxygen are virtually absent. Drug supplies are erratic and in most cases mothers are required to purchase drugs and supplies. The general situation is significantly worse than when one member of the group (KM) visited the same department 7 years ago. Damage during the civil war has clearly been a factor in this deterioration. Laboratory services are virtually non-existent, apart from malaria films (of uncertain quality) and diagnostic accuracy is probably limited to the broad categories used for the analysis. In particular the diagnosis of malaria probably includes a wide range of conditions associated with a positive blood film. Despite the problems at the hospital this is an interesting and relevant study. Such studies could be replicated easily at other paediatric clinical research units, such as Kilifi in Kenya.

Conclusions

In every respect the study about to be published in the BMJ is what it appears to be. A dedicated group of investigators, with long experience of working in a difficult country on a limited budget, have conducted a longitudinal cohort study in which the main variables collected are birth and pregnancy details, vaccination status, nutritional status (MUAC) and survival. The methods are simple and practical, but imperfect and this makes the analysis of the data a little complex. The study draws the conclusion that

measles and BCG vaccines provide a beneficial effect on survival that cannot be explained on the basis of prevention of measles and tuberculosis, but DTP is associated with an increased risk of mortality. On the basis of what we have seen the only conclusion that can be drawn is that this is a simple, honest study whose alarming conclusion, with respect to DTP, cannot be attributed to any detectable bias or imperfections in the study.

The study does not allow any analysis of the specific causes of the excess deaths. The hospital inpatient mortality study hints that this may be due to malaria and/or pneumonia but this remains an open question. If data from non-malarious areas do not show such an effect it will be essential to examine closely the possibility that the DTP effect observed is related to some disturbance of the development of malaria immunity.

The investigators have looked hard at other data for evidence that would support or refute their conclusions. Analysis of other data sets appears to provide compelling supportive evidence. In particular, the study of infant mortality rates, during similar 6 months observation "windows" around the time when DTP was first introduced into Guinea Bissau, provides evidence that in this environment that vaccine is associated with increased mortality, substantially increased mortality if the data from that study accurately reflect the true picture. In some respects that study is more crude with very simple data collection instruments initially designed for a nutritional study, but it has the advantage that during that period pertussis was still circulating, and all vaccines were given by this team so exact vaccination status could be determined for all children who died.

Dr. Aaby has examined several other data sets for mortality by sex. Typically, and in the pre-vaccination era in Bissau, boys suffer more mortality in infancy. However after the introduction of DTP this situation is reversed with more girls dying. Aaby has found data from other countries drawing the same conclusion. In an interesting study of twins, 21 pairs of boy/girl twins in Guinea-Bissau in which one twin died were identified. In 12 pairs who received DPT and OPV vaccines, 10 girls and 2 boys died. In 9 pairs who received measles vaccine, 3 girls and 6 boys died. In the study of inpatient mortality in the main paediatric hospital in Guinea Bissau, increased mortality in female DTP recipients and decreased mortality in female measles vaccine recipients lends support to his hypothesis.

Put together, all these bits of evidence support the authors' main conclusion that, in the high mortality, malarious environment of Guinea Bissau, the use of measles and BCG vaccines provides mortality savings that are substantially greater than could be explained on the basis of prevention of measles and tuberculosis, while the use of DTP is associated with a substantial increase in risk of infant mortality. The latter point is of grave concern for the WHO EPI program. There is an urgent need for substantive action in this regard, a need that arguably was present three years ago when these findings were first communicated with WHO. The recommendations below reflect the course of action that this review group believes should now be undertaken by WHO.

To some extent, Dr. Aaby's earlier findings with relation to measles vaccine have not attracted the attention they deserve because experts in the vaccine field and vaccine manufacturers cannot imagine a biological mechanism that might explain the results. Now with the BCG and DTP findings, the effects occur in different directions depending upon the vaccine, but still primarily affect female children. Dr. Aaby has begun to address this issue by postulating that BCG and measles vaccines strengthen the non-specific immune response in female children, whereas DTP vaccine weakens it. He does not believe that OPV has any effect in either direction. He has suggested that the aluminium hydroxide adjuvant used in DTP vaccine is responsible for weakening the immune response in female children through a complex mechanism associated with the gender-specific cell-mediated immunological response. Immunologists in Copenhagen have recently initiated discussions on ways in which this phenomenon might be studied in animal models. Whether or not these findings are associated with alum is a question that demands urgent attention as it has implications for the use of other alum containing vaccines such as hepatitis B and *Haemophilus influenzae* type b (Hib).

It is essential at this stage to appreciate the true meaning of what the findings of these investigators suggest. Widespread use of DTP in the developing world began two decades ago with the beginning of the EPI programme. Technical leadership was assumed by WHO. At that time many parts of the developing world had mortality patterns similar to those seen in Guinea Bissau today. As is pointed out by the writer of the editorial that accompanies the BMJ paper, at the time of the introduction of DTP, and in the 20 years since, there has been little work to evaluate objectively its overall impact on child health in high mortality areas, although the virtual disappearance of pertussis from areas with good DTP coverage is well documented. If the findings of the Guinea Bissau investigators prove to be generalisable the true cost of this omission will be measured in the deaths of millions of children over the past two decades. The gravity of this is only minimally offset by the unexpected positive effects of measles and BCG, as the true question that should be asked is, how would things have been different had careful evaluation of the safety and effectiveness of DTP introduction been undertaken at the time. This has important implications for the role of WHO to ensure the safety and effectiveness of the interventions it promotes. Whether or not the findings prove to be generalisable, the publication of this paper may lead some countries to drop DTP from their schedules, with the inevitable reappearance of pertussis and pertussis mortality. It is essential that WHO determines the most appropriate response for developing countries to these findings.

In summary, following a weeklong review by members of this review team, we conclude that the study reported in the BMJ has been honestly conducted and faithfully reported. No major sources of bias were detected. The study is supported by the findings of the retrospective study of the introduction of DTP into Guinea Bissau. At this stage, the findings of this group of researchers lead by Dr. Aaby should be regarded as serious, even alarming, but should not be generalised. There still remains the possibility of some undetectable bias. The need to evaluate the reproducibility of these findings in other settings is now urgent to contest or generalize the findings from Guinea Bissau.

Recommendations

The publication of this paper is now imminent. Despite some methodological weaknesses, the study was well conducted, is internally consistent and is supported by several other studies of the same group. Bearing in mind that the WHO response will be a process that needs to be addressed with great urgency, the review team traced some basic scenarios and proposed action to be taken under different scenarios. These are incorporated into the following recommendations.

- A Task Force should be formed to co-ordinate the response of WHO to this problem. Sufficient funds must be made available for the work that will be needed.

- A detailed literature search should be undertaken to identify any studies that might help to address this issue. Dr. Aaby suggested that the INCAP report by Scrimshaw and Gordon shows DTP to have a negative impact on growth. Two unpublished studies from the islands of Cebu and Bohol in the Philippines have been identified and both show better survival in DTP recipients.)

- All studies/databases from developing countries that record children's immunization status and survival should be identified. Studies/databases which record admission details including nutritional status, outcome and immunization status for children admitted to hospitals in developing countries should be identified, as well as other studies that might shed light on this issue (such as Simondon's wDTP vs aDTP study from Senegal). The Task Force should call an urgent meeting for senior investigators responsible for the main studies to agree on a uniform plan for analysis to determine whether or not these studies support the findings of Dr. Aaby's group with regard to the effect of vaccines on survival.

- Case-control studies should be initiated urgently to evaluate the risk of DTP for the occurrence of cases or deaths due to specific causes (e.g. pneumonia, diarrhoea and malaria). Multi-centre approaches will speed the conclusion of such studies. Attention should be paid to the malaria epidemiology in proposed study sites.

- Support should be provided for Dr. Aaby to allow him to undertake analysis of the remaining data in his possession, which may help support or refute these findings. This should include funding for a data manager-statistician to work in Bissau for a period of 6 months to work on the completion of the following projects:

    - Completion of the analysis of the 1984-1987 study of DTP introduction in Guinea Bissau
    - Analysis of the 1986-1990 data from the same study site

- Analysis of Bandim Project, a similar study in urban Bissau in which children are visited at 3-monthly intervals
- Analysis of the verbal autopsy data from the Bandim Project
- Analysis of early data prior to 1984 and between 1987 and 1990 in the rural study
- Completion of the analysis of the association of inpatient case fatality with vaccination status

- Studies should be conducted in settings in which DTP is used in schedules other than the WHO recommended 6, 10 and 14 weeks to observe possible variation in the negative effects under different circumstances.

- In view of the fact that there may be a need to suspend DTP use globally for safety reasons, the Task Force should evaluate the implications of suspending DTP use and prepare guidelines for countries under these circumstances.

- Immunological studies should be initiated urgently to investigate the biological plausibility of the indirect effects attributed to DTP, particularly the possible role of aluminium hydroxide adjuvant in this process. It is important to note that the same adjuvant is used in hepatitis B and *Haemophilus influenzae* type b vaccines. Such studies should include immunological studies of the effect of DTP at different age schedules in human infants.

In addition the following 2 scenarios should be considered:

Scenario 1 – If, in the coming months, Aaby's findings are ***not*** confirmed by studies done in other settings:

1- The results of all the future studies need to be scrutinised very closely. The Guinea Bissau studies were done in a high mortality, malarious setting. Studies done in other settings with different epidemiologic patterns must be evaluated carefully and should not be regarded as immediate evidence against Aaby's findings. In particular data should be sought from another site with intense malaria transmission as interaction with the development of immunity to malaria could be a possible mechanism for the effect.

2- Studies to observe and explain the positive effects of measles and BCG need to be stimulated. If these are confirmed they will be welcomed as they will strengthen advocacy for those vaccines.

Scenario 2 – If, in the coming months, Aaby's findings ***are*** confirmed by studies done in other settings:

1- Emergency and detailed plans need to be made ready regarding the consequences of suspending DTP use globally. WHO should have clear and defendable recommendations on this issue ready for broad dissemination.

2- The evaluation procedures for vaccine safety need to be reviewed and the non-specific effects need to be considered as major outcomes. Post-marketing evaluation will need more structured rules and this must always include longitudinal observational studies.

List of Annexes:

Annex 1 – the paper soon to be published in the BMJ, which was the main subject of this review.
Annex 2 – the report from the analysis of statistical methods conducted by Dr. C. Nelson in Copenhagen, and the note for the record following Dr. Aaby's presentation in Geneva..
Annex 3 – the table of studies supporting Dr. Aaby's hypothesis that vaccines are associated with indirect affects that affect mortality in ways other than by prevention of specific diseases.
Annex 4 – some further analyses conducted by Mr. Jensen during the review in response to questions from the team.
Annex 5 – data collection instruments currently in use.
Annex 6 – copy of one of the cards from the data collected during the early 1980s
Annex 7 - forms used for the review of outcome data and photocopies of the original forms that contained errors.