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# The sequence of vaccinations and increased female mortality after high-titre measles vaccine: Trials from rural Sudan and Kinshasa

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**Abstract**

*Objective:* West African studies have hypothesized that increased female mortality after high-titre measles vaccine (HTMV) was due to subsequent diphtheria–tetanus–pertussis (DTP) and inactivated polio vaccine (IPV) vaccinations. We tested two deductions from this hypothesis in HTMV studies from rural Sudan and Kinshasa; first, there should be no excess female mortality for HTMV recipients when DTP was not given after HTMV and second, excess female mortality should only be found among those children who received DTP after HTMV.
*Studies:* The Sudanese trial randomised 510 children to Edmonston–Zagreb (EZ) HTMV, Connaught HTMV or a control vaccine (meningococcal). Both the Connaught HTMV and the control group received standard measles vaccine at 9 months. In the Kinshasa study 1023 children received one dose of HTMV at 6 months or two doses at $3\frac{1}{2}$ and $9\frac{1}{2}$ months of age.
*Findings:* First, the Sudan trial is one of the few randomised studies of measles vaccine; the EZ HTMV group had lower mortality between 5 and 9 months of age than controls, the mortality ratio (MR) being 0.00 ($p = 0.030$). This effect was not due to prevention of measles infection. Second, both studies provided evidence that HTMV per se was associated with low mortality. In a combined analysis comparing both HTMV groups with controls, the HTMV groups had a MR of 0.09 (0.01–0.71) between 5 and 9 months of age. In Kinshasa, the HTMV recipients who did not receive simultaneous DTP had an annual mortality rate of only 1.0% between 6 months and 3 years of age. Third, the female–male MR was related to subsequent DTP vaccinations. In Kinshasa, the female–male MR was only 0.40 (0.13–1.27) among the HTMV recipients who did not receive further doses of DTP. In Sudan, the female–male mortality ratio in the EZ group was 3.89 (95% CI 1.02–14.83) and the female–male MR increased with number of doses of DTP likely to have been given during follow-up (trend, $p = 0.043$). Fourth, in Kinshasa, mortality was higher among children who had received HTMV and DTP simultaneously than among children who had received HTMV alone (MR = 5.38 (1.37–21.2)).
*Conclusions:* Measles vaccine is associated with non-specific beneficial effects. When not given with DTP, HTMV per se was associated with low mortality. Increased female mortality was not found among children who did not receive DTP after HTMV. Hence, our deductions were supported and the sequence or combination of vaccinations may have an effect on sex-specific mortality patterns in low-income countries.


*Keywords:* DTP; High-titre measles vaccine; Female mortality; Sex differences; Standard measles vaccine; Sudan; Kinshasa

## 1. Introduction

WHO rescinded the recommendation of high-titre Edmonston–Zagreb (EZ) measles vaccine in 1992 when studies from Guinea-Bissau, Senegal, and Haiti reported that high-titre measles vaccines (HTMV) were associated with increased female mortality [1,2]. At the time, this tendency was interpreted as HTMV having come too close to the natural disease presumably inducing immune suppression [3]. Instead, we suggested that the effect could be due to HTMV not having the same non-specific beneficial effects as standard measles vaccine, which is particularly good for girls

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[1,4,5]. However, neither of these hypotheses explained why some studies did not observe a negative effect of HTMV and why girls had higher mortality than boys in the high-titre group in areas with high mortality [6]. In the pre-vaccination era, girls did not have higher mortality than boys [7,8].

Studies from West Africa have suggested that inactivated vaccines, including diphtheria–tetanus–pertussis (DTP) and inactivated polio vaccine (IPV), may be associated with increased mortality for girls [4,7,9–11]. We therefore, re-analysed the West African high-titre studies; increased female mortality was observed only among children receiving inactivated vaccines after measles vaccination [12]. For this to be a better explanation than the previous hypotheses, inactivated vaccines should have been given after HTMV in the studies with increased female mortality and there should have been no inactivated vaccine administered after HTMV in studies finding no increase in female mortality. It also follows that in the absence of subsequent DTP vaccination, HTMV would be associated with low mortality. The number of studies of HTMV with long-term ascertainment of survival is limited; to our knowledge there were only two other African studies from Sudan [13,14] and Kinshasa [15]. These studies had increased [13,14] and reduced female mortality [15], respectively. We therefore, tested whether these differences in mortality could be related to different use of inactivated vaccines in these studies. The present hypothesis was not envisioned when the studies were conducted. Data on vaccinations received after enrolment was not collected but the DTP vaccination status was documented at the time of entering the trials. Children who had already received DTP3 at enrolment would not have received additional DTP vaccinations after HTMV, whereas children missing doses of DTP at enrolment would be likely to receive these vaccines subsequently. Hence, we have used "missing DTP vaccinations at enrolment" as an index of being likely to receive DTP after measles vaccination.

## 2. Subjects and methods

### 2.1. Sudan

In the Sudanese trial [13,14], 510 children were randomised at 5 months of age to two high-titre ($10^{4.7}$ pfu) measles vaccination groups, Edmonston–Zagreb (EZ) ($N = 170$) or Connaught ($N = 170$), and a control group receiving meningococcal vaccine ($N = 170$) (Table 1). As WHO defines measles vaccine with a titre of $\geq 10^{4.7}$ pfu as HTMV, both the EZ and the Connaught measles vaccines used in Sudan would qualify as HTMV though being in the lower end of the spectrum of HTMV. At 9 months of age, the meningococcal and the Connaught groups received standard Schwarz measles vaccine and the EZ group received meningococcal vaccine. The "placebo" vaccine was a lyophilised preparation of purified polysaccharides from *Neisseria meningitidis* of groups A and C (SKB).

In contrast to the studies in West Africa, the trial in Sudan did not provide routine vaccinations when the children received the two trial vaccines at 5 and 9 months of age. Hence, the study did not document other vaccinations. However, the physician and two research students registered vaccines received before enrolment from the children's vaccination cards; only 21% (106/510) had received DTP3 and the third dose of oral polio vaccine (OPV3) already. According to the clinicians working in the study area, children missing vaccines at the time of enrolment are believed to have received these vaccines later.

### 2.2. Kinshasa

Between June and August 1990, children coming for routine vaccination at three health centres in Kinshasa, Congo, were recruited for studies of high-titre measles vaccination, a one-dose study with recruitment at 6 months and a two-dose study with recruitment at $3\frac{1}{2}$ months [15].

Table 1
Mortality rates and mortality ratio (MR) according to age, sex, and vaccine group

| Age at vaccination | Mortality rate (deaths/person-years) [*N*] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | Connaught high-titre | | | EZ high-titre | | | Meningococcal A + C | | |
| months | Male [102] | Female [68] | All [170] | Male [90] | Female [80] | All [170] | Male [87] | Female [83] | All [170] |
| 5–9 months | 2.7% | 0% | 1.7% | 0% | 0% | 0% | 12.7% | 6.9% | 9.8% |
| | (1/36.4) | (0/24.1) | (1/60.5) | (0/32.8) | (0/28.5) | (0/61.3) | (4/31.6) | (2/29.6) | (6/61.2) |
| 9 months | Schwarz standard | | | Meningococcal A + C | | | Schwarz standard | | |
| 10–36 months | 1.8% | 2.0% | 1.9% | 1.5% | 5.3% | 3.3% | 1.6% | 2.3% | 2.0% |
| | (4/221.1) | (3/150.6) | (7/371.7) | (3/197.3) | (9/169.1) | (12/366.4) | (3/182.7) | (4/177.1) | (7/359.8) |
| Total | 1.9% | 1.7% | 1.9% | 1.3% | 4.6% | 2.8% | 3.3% | 2.9% | 3.1% |
| | (5/257.5) | (3/174.7) | (8/432.2) | (3/230.1) | (9/197.6) | (12/427.7) | (7/214.3) | (6/206.7) | (13/421.0) |
| Female/male MR[a] | 0.91 (0.21–3.88) | | | 3.89 (1.02–14.83) | | | 1.01 (0.33–3.10) | | |

Sudan 1989–1992. *Note*: Children were censored in the first age group (5–9 months) when they received their second vaccination or became 10 months old if not vaccinated before.

[a] MR adjusted for village.

*2.2.1. One-dose study (6 months of age)*

In the one-dose study, 485 six-month-old children presenting for measles vaccination were randomised to either high-titre ($10^{5.7}$ pfu) or medium-titre ($10^{4.7}$ pfu) Edmonston–Zagreb (EZ-M) measles vaccine; the children were followed for measles infection and survival to the age of 34–37 months. As WHO defines measles vaccine with a titre of $\geq 10^{4.7}$ pfu as HTMV, the medium-titre EZ used in Kinshasa would also qualify as a HTMV. Twenty-four (5%) of the children received DTP at the same time as measles vaccination because they were still missing a dose; however, 461 (95%) children in the one-dose study did not receive any further DTP vaccination as they had already received all three doses of DTP and oral polio and there was no policy of giving a booster dose in Kinshasa.

*2.2.2. Two-dose study*

In the two-dose study, 538 children aged $3\frac{1}{2}$ months of age who presented for DTP and OPV vaccination were randomised to EZ or AIK-C HTMV. Six months later, at $9\frac{1}{2}$ months of age, these children were revaccinated with an EZ vaccine with a titre of $10^{4.8}$ plaque-forming units (pfu). Among the children recruited at $3\frac{1}{2}$ months, 416 (77%) received their DTP3 at the same time as measles vaccination; the remaining 122 (23%) mostly received their first [22] or second dose (52) of DTP, only OPV [14] or no additional vaccine (34) at enrolment. Presumably, these children received the missing DTP vaccinations at a later date; unfortunately, data on such additional vaccinations was not collected. It is not possible to know from the records whether the children who received no DTP together with HTMV had actually received these doses previously or whether DTP was not available.

*2.2.3. Long-term follow-up and statistical analysis*

Excess EZ mortality has previously been noted before 3 years of age [1,13], we therefore, analysed mortality between enrolment at 5 months of age and 3 years of age in the Sudanese trial. Long-term survival could be ascertained for all children in the Sudanese trial. Since the coverage for other vaccinations differed by village in Sudan, we adjusted all estimates for village. In Kinshasa, all children were initially followed to February 1991 [15]. In January 1993, when the children were 34–37 months old, a survey was conducted to assess long-term survival; the interviewer saw 76.6% of study children, another 13.1% were reported to be alive, 4.3% had died, and no information could be obtained for 6%.

Presentation of mortality rates in different groups was calculated as number of deaths divided by observed time-at-risk. The calculation of mortality rate ratios (MR) and 95% confidence interval was done using a Cox regression model with age as underlying time scale. Children were included in the long-term follow-up even if they did not turn up for the second vaccination in the trial. In the comparison of EZ and meningococcal group between 5 and 9 months of age, we used a Fisher's exact test because of no deaths in the EZ group. We have called the test of equal mortality ratios between different groups for "test of homogeneity" and this was done with a Wald test using Cox regression models. In one of the test of homogeneity, one of the mortality ratios was zero and we used a likelihood ratio test instead. The test for trend of the female–male ratio according to the number of DTP vaccinations was carried out in a Cox regression model using number of DTP doses as a continuous covariate.

## 3. Results

### 3.1. Sudan

Between 5 and 9 months of age, the EZ high-titre group had significantly lower mortality than the control (meningococcal) group (mortality ratio (MR) = 0.00 (Fisher's exact test, $p = 0.030$)) (Table 1). This difference was not due to the prevention of measles-related deaths; between 5 and 9 months of age, there was only one case of measles in the control group [13,14] and the child did not die. The Connaught high-titre group had also lower mortality than the control group between 5 and 9 months of age (MR = 0.18 (0.02–1.54)). In a combined analysis comparing the two high-titre groups with controls, the measles vaccine groups had a MR of 0.09 (0.01–0.72).

Between 10 months and 3 years of age, the EZ high-titre group tended to have higher mortality than the control group receiving Schwarz standard vaccine at 9 months, the MR being 1.68 (0.66–4.26) (see Fig. 1). The mortality ratios for the EZ compared with the control group at 5–9 months and 10–36 months, respectively, were significantly inversed (test of homogeneity, using a likelihood ratio test, $p = 0.002$). Mortality in the EZ group was similarly increased in relation to the Connaught group receiving standard measles vaccine at 9 months. As in the West African trials, girls in the EZ high-titre

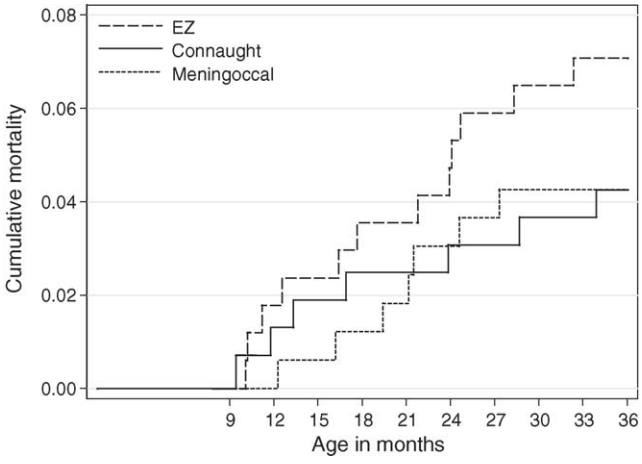


Fig. 1. Cumulative mortality curves from 9 months of age for recipients of high-titre measles vaccine groups (EZ and Connaught) and control group (meningoccal). Sudan 1989–1992.

Table 2
Frequency of having received the third dose of DTP before enrolment in the HTMV trial

| Background factors | Prevalence of DTP3 | DTP3 Prevalence ratio (95% CI) | Prevalence of deaths (%) | Mortality prevalence ratio (95% CI) |
|---|---|---|---|---|
| Type of vaccine | | | | |
| Connaught | 21.8% (37/170) | 1.32 (0.85–2.06) | 4.7% (8/170) | 0.62 (0.26–1.45) |
| EZ | 24.1% (41/170) | 1.46 (0.95–2.25) | 7.1% (12/170) | 0.92 (0.43–1.96) |
| Control | 16.5% (28/170) | | 7.7% (13/170) | |
| Gender | | | | |
| Male | 23.3% (65/279) | 1.31 (0.93–1.86) | 5.4% (15/279) | 0.69 (0.36–1.34) |
| Female | 17.8% (41/231) | | 7.8% (18/231) | |
| Electricity | | | | |
| Yes | 25.1% (66/263) | 1.55 (1.09–2.20) | 5.7% (15/263) | 0.78 (0.40–1.52) |
| No | 16.2% (40/247) | | 7.3% (18/247) | |
| Mother's education | | | | |
| 0-year of schooling | 10.3% (18/175) | | 7.4% (13/175) | 1.24 (0.63–2.44) |
| 1–6 years of schooling | 26.3% (88/335) | 0.39 (0.24–0.63) | 6.0% (20/335) | |
| Village | | | | |
| Largest village | 43.8% (53/121) | 3.21 (2.33–4.43) | 5.8% (7/121) | 0.87 (0.39–1.94) |
| Other villages | 13.6% (53/389) | | 6.7% (26/389) | |
| Having radio | | | | |
| Yes | 22.7% (73/322) | 1.32 (0.91–1.93) | 6.2% (20/322) | 0.75 (0.32–1.76) |
| No | 17.1% (32/187) | | 7.0% (13/187) | |
| Livestock | | | | |
| Yes | 22.0% (96/436) | 1.79 (0.94–3.38) | 6.2% (27/436) | 0.75 (0.32–1.76) |
| No | 12.3% (9/73) | | 8.2% (6/73) | |

Sudan 1989–1992.

group had increased mortality from 5 to 36 month of age compared with boys receiving EZ (MR = 3.89 (1.02–14.83)). In the control group, the female–male ratio was 1.01 (0.33–3.10) (test of homogeneity, $p = 0.131$). The Connaught high-titre group, which also received standard measles vaccine at 9 months, had a mortality pattern similar to the control group (Table 1).

We examined whether missing DTP vaccine was associated with increased female mortality in the high-titre EZ group; the frequency of DTP3 vaccinations according to various background factors is presented in Table 2. Most of the children having received DTP3 came from the largest village in the area and since this village had better socio-economic indicators, the DTP3 prevalence was also associated with these indicators. Control for socio-economic background factors did not modify the inversions between age or vaccine groups.

Mortality was not increased among children missing doses of DTP at enrolment (27/404) compared with those having received DTP3 (6/106) (MR = 1.03 (0.40–2.65)), the mortality ratio being 0.69 (0.20–2.33) for boys and 1.65 (0.37–7.36) for girls. The average number of doses of DTP at enrolment was 1.5 for girls and 1.4 for boys. For all children in the trial, the female–male mortality ratio was not increased for children who had received DTP3 before enrolment, the ratio being 0.75 (0.14–4.16) (deaths/children: F: 2/41, M: 4/65). The female–male mortality ratio increased with the number of missing DTP doses, being 0.92 (0.26–3.19) for those missing one dose (F: 5/81, M: 5/72), 1.57 (0.35–7.04) for those missing two doses (F: 4/51, M: 3/57), and 3.95 (1.01–15.45) for those missing all three doses (F: 7/58, M: 3/85) (test for trend, $p = 0.090$). After 9 months of age, having received less than DTP3 was not associated with increased female mortality in groups receiving Schwarz standard vaccine (Table 3). Censoring for measles vaccination at 9 months of age, the female–male mortality ratios for children who had received three, two, one or no dose of DTP prior to enrolment were 0.47 (0.05–4.77), 0.91 (0.18–4.62), 1.81 (0.16–20.8), and 10.6 (0.99–114), respectively (test for trend, $p = 0.043$). Compared with the control group receiving Schwarz at 9 months of age, the mortality ratio after 9 months of age for girls in the EZ group was 2.33 (0.84–6.45) whereas the ratio was 0.77 (0.20–3.05) for boys (Table 3).

### 3.2. Kinshasa—one-dose study

The HTMV given at 6 months of age was not associated with higher mortality than medium-titre EZ vaccine, the mortality ratio being 0.55 (0.18–1.64) for high-titre compared with medium-titre (Table 4). There was no indication of excess female mortality in this study (Table 4), the female–male mortality rate ratio being 0.40 (0.13–1.27). It should be noted that the children who received HTMV and had received DTP3 had a mortality rate of merely 1.0% between 6 months and 3 years of age (Table 5). Twenty-four children received DTP simultaneously with their 6-month measles vaccination. The DTP and measles vaccinated children had significantly higher mortality than the children who

Table 3
Mortality rates between 10 months and 3 years of age in relation to sex, type of vaccine received and number of DTP vaccinations before enrolment

| Vaccines received before enrolment | Mortality rate (deaths/person-years) [*N*] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Connaught high-titre + Schwarz standard | | | EZ high-titre + meningococcal A + C | | | Meningococcal A + C + Schwarz standard | | |
| | Male [101] | Female [68] | All [170] | Male [90] | Female [80] | All [170] | Male [83] | Female [81] | All [170] |
| DTP3 | 1.9% (1/52.0) [24] | 0% (0/29.2) [13] | 1.2% (1/81.2) [37] | 1.9% (1/51.9) [24] | 2.8% (1/35.9) [17] | 2.3% (2/87.8) [41] | 0% (0/33.6) [15] | 4.2% (1/23.6) [11] | 1.8% (1/57.2) [26] |
| DTP0–2 | 1.8% (3/169.1) [77] | 2.5% (3/121.3) [55] | 2.1% (6/290.4) [132] | 1.4% (2/145.4) [66] | 6.0% (8/133.1) [63] | 3.6% (10/278.5) [129] | 2.0% (3/149.1) [68] | 2.0% (3/153.6) [70] | 2.0% (6/302.6) [138] |
| Total | 1.8% (4/221.1) | 2.0% (3/150.5) | 1.9% (7/371.6) | 1.5% (3/197.3) | 5.3% (9/169.0) | 3.3% (12/366.3) | 1.6% (3/182.7) | 2.3% (4/177.2) | 1.9% (7/355.9) |

Sudan 1989–1992.

received measles vaccine while having received DTP3 previously (Table 5). Adjusted for sex, years of maternal schooling, potency of EZ, and health centre of recruitment, the mortality ratio for receiving DTP and measles vaccine simultaneously was 5.38 (1.37–21.15).

### 3.3. Kinshasa—two-dose study

In the two-dose study, the female–male MR was 0.82 (0.40–1.70) (Table 4). Children not receiving DTP3 together with HTMV at $3\frac{1}{2}$ months of age had higher mortality than those receiving HTMV and DTP3 simultaneously (Table 5). Controlling for background factors, the mortality ratio from $3\frac{1}{2}$ months to 3 years of age was 2.13 (0.93–4.89) for children not receiving DTP3 with HTMV compared with children receiving HTMV and DTP3 (Table 5); mortality tended to increase with number of missing doses of DTP (MR = 1.40 (0.99–1.99), trend estimate). Between $3\frac{1}{2}$ and $9\frac{1}{2}$ months of age – before the second dose of measles vaccine in the two-dose study – the mortality ratio for the same two groups was 2.45 (0.78–7.69), slightly higher for girls (MR = 3.06 (0.58–16.09)) than for boys (MR = 2.06 (0.46–9.23)).

Mortality tended to be higher in the two-dose study than the one-dose study. Though the children were recruited at the same health centres, during the same three-month period, and were followed for the same length of time, mothers of children in the two-dose study had better schooling (average 8.5 years) than mothers of children in the one-dose study (average 8.0 years) ($p = 0.011$). Controlling for health centre, mother's schooling, and age, the MR for the two-dose study compared to the one-dose study was 1.87 (0.95–3.70) after 6 months of age, and was slightly higher for girls (MR = 3.06 (0.96–9.78)) than for boys (MR = 1.41 (0.61–3.25)).

## 4. Discussion

The studies in Sudan and Kinshasa had very different designs. The Sudan trial belonged to the early high-titre studies comparing high-titre measles vaccination before 9 months of age with standard measles vaccination at 9 months of age whereas the Kinshasa study was implemented later when high-titre EZ measles vaccine had become official policy and the study tested different strains and different doses of vaccine. The Sudan study was similar to the West African trials with a HTMV or control vaccination at 4–5 months of age and a subsequent vaccination at 9 months of age to provide the standard measles vaccine to the control group and the "control" vaccination to the high-titre recipients [12]. As in the West African studies [1,12], there was no increased mortality for the HTMV groups between 5 and until 9 months of age when the controls received standard measles vaccine.

Both in vitro and in vivo differences between vaccine-strain measles viruses have been identified (e.g. growth characteristics in cell lines, induction of apoptosis in thymocytes, and minor differences in side-effect profiles and the induction

Table 4
Mortality rates according to vaccine type received and sex

| Vaccine type | Mortality rate (deaths/person-years) [*N*] | | | Female–male mortality ratio |
|---|---|---|---|---|
| | Female | Male | All | |
| I. One-dose: 6 months | | | | |
| EZ-H at 6 months | 0.6% (2/315.8) [130] | 1.1% (3/279.1) [116] | 0.8% (5/594.9) [246] | 0.61 (0.10–3.63) |
| EZ-M at 6 months | 0.7% (2/283.9) [117] | 2.4% (7/290.1) [122] | 1.6% (9/574.0) [239] | 0.30 (0.06–1.44) |
| Total | | | | 0.40 (0.13–1.27)[a] |
| II. Two-dose: $3\frac{1}{2}$ and $9\frac{1}{2}$ months | | | | |
| Follow-up $3\frac{1}{2} - 9\frac{1}{2}$ months | | | | |
| AIK-C-H at $3\frac{1}{2}$ months | 4.4% (3/68.9) [128] | 2.8% (2/70.5) [131] | 3.6% (5/139.4) [259] | 1.55 (0.26–9.31) |
| EZ-H at $3\frac{1}{2}$ months | 4.5% (3/66.3) [134] | 9.4% (7/74.6) [145] | 7.1% (10/140.9) [279] | 0.52 (0.13–2.09) |
| Total | | | | 0.78 (0.27–2.25)[b] |
| Follow-up after $9\frac{1}{2}$ months | | | | |
| EZ-M at $9\frac{1}{2}$ months (AIK-C-H at $3\frac{1}{2}$ months) | 0.9% (2/235.8) | 1.7% (4/241.0) | 1.3% (6/476.8) | 0.52 (0.10–2.82) |
| EZ-M at $9\frac{1}{2}$ months (EZ-H at $3\frac{1}{2}$ months) | 2.0% (5/253.8) | 1.5% (4/265.6) | 1.7% (9/519.4) | 1.25 (0.34–4.67) |
| Total | | | | 0.89 (0.32–2.45)[b] |
| Total two-dose study | | | | 0.82 (0.40–1.70)[b] |

Kinshasa 1990–1993. *Notes*: The children were followed to January 1993 when the cohorts were 34–37 months old; EZ-H and AIK-C-H: high-titre measles vaccines, EZ-M: medium titre measles vaccine.

[a] Adjusted for type of EZ.

[b] Adjusted for type of vaccine given at $3\frac{1}{2}$ months.

of sero conversion) [16–19]. However, a variety of vaccine strains (EZ, Schwarz, Biken, Connaught) were used in the original HTMV trials with very similar effects in terms of mortality. In the Sudanese trial, recipients of high-titer EZ and Connaught vaccines responded in a very similar fashion between 5 and 9 months of age. Because the Connaught group was revaccinated at 9 months of age, these groups were no longer comparable after this age. As a result, measles vaccine strain differences are unlikely to account for the effects that we report.

The present reanalysis of HTMV trials for Sudan and Kinshasa provided several important observations. First, the Sudanese trial clearly documented the non-specific beneficial effects of measles vaccine; between 5 and 9 months of age, at which age the control group received standard measles vaccine, the two HTMV groups had a 10-fold reduction in mortality which could not be explained by prevention of measles infection. This is one of the few randomised studies comparing the survival of measles-vaccinated and measles-unvaccinated children. Two previous randomised or blind, but unplanned studies have provided similar results for standard measles vaccine [5,20].

Second, HTMV was withdrawn for being associated with increased mortality, but both the Sudan and the Kinshasa studies indicated that mortality was low when HTMV was not given together with DTP. In the Sudanese trial, the children did not get other routine vaccines at recruitment and mortality was significantly lower for the high-titre recipients than controls in the interval until 9 months of age [14]. In the Kinshasa study there was no control group, but the children who received EZ-high-titre at 6 months of age and no simultaneous DTP had a mortality rate of only 1.0% till 3 years of age (Table 5). This level cannot be reconciled with the hypothesis that HTMV should have a negative effect due to immune suppression [3].

Third, both studies supported that excess female mortality after HTMV might have been due to subsequent DTP vaccinations. In Kinshasa, the majority received the third DTP at the same time as their first measles vaccination (two-dose study) or had already received DTP3 when receiving measles vaccination (one-dose study). Therefore, these children did not receive additional DTP vaccinations after HTMV.

The girls had lower mortality than the boys in these studies, the female–male ratio being similar to the ratio reported

Table 5
Mortality rates according to simultaneous administration of DTP and measles vaccination

| Age vaccination | Mortality rate (deaths/person-years) | | Mortality ratio (MR) | Adjusted MR[a] |
|---|---|---|---|---|
| One-dose: 6 months | MV + DTP<br>5.4% (3/55.2) | MV + no DTP<br>1.0% (11/1113.8) | 5.14 (1.43–18.45) | 5.38 (1.37–21.15) |
| Two-dose: $3\frac{1}{2} + 9\frac{1}{2}$ months | MV + DTP1/2[b]<br>4.3% (12/280.6) | MV + DTP3<br>1.8% (18/995.8) | 2.41 (1.16–5.01) | 2.13 (0.93–4.89) |

Kinshasa 1990–1993. *Notes*: The children were followed to January 1993 when the cohorts were 34–37 months old; MV: measles vaccine; DTP: diphtheria–tetanus–pertussis vaccine; OPV: oral polio vaccine.

[a] Adjusted for sex, years of maternal schooling, type of measles vaccine, and health centre of recruitment.

[b] This group received DTP1 [22], DTP2 (52), OPV alone [14] or no other vaccine (34) together with MV at age $3\frac{1}{2}$ months.

in several studies of standard-titre measles vaccines from Africa [5,8,21,22]. Likewise, in the Sudan study, there tended to be lower female mortality among the HTMV children who had received DTP3 prior to enrolment in the trial and who would not received additional doses of DTP during follow-up. In contrast, female mortality was increased among children missing doses of DTP at enrolment (Table 3) and the female–male MR increased significantly with number of missing doses of DTP. Hence, both studies are compatible with the hypothesis that DTP/IPV and not HTMV per se may have caused the increased female mortality in the high-titre trials [3,13].

It is a limitation of the present study that the suggested association with subsequent DTP vaccinations [12] had to be tested indirectly using "missing DTP vaccinations at enrolment" as an index of the likelihood of receiving DTP after HTMV. In both studies having received DTP3 prior to enrolment was associated with somewhat lower female–male MR. There was no indication of DTP vaccination policy being different for boys and girls since they had received the same average number of doses before enrolment. It is certainly the children who were missing doses of DTP at enrolment who would be likely to receive such doses during follow-up. The physicians working in the area clearly had the impression that all children eventually received all routine vaccinations. Should the assumption be wrong for a few children, this should only make it more difficult to establish an association because the children who did not received DTP after HTMV had lower female–male MR. Being behind with the vaccination schedule could be an indicator of maternal competence or socio-economic status and be associated with higher child mortality. However, in Sudan, boys missing DTP vaccinations did not have higher mortality than boys having received all three doses of DTP and missing DTP vaccinations at enrolment was not associated with increased mortality in the control and Connaught groups which received MV at 9 months of age (Table 3). It seems therefore, unlikely that increased female mortality in the HTMV groups is due merely to characteristics of mothers bringing their children too late for vaccination (Table 2). In Kinshasa, receiving less than DTP3 vaccination with HTMV in the two-dose study was also associated with increased mortality. This trend was also strongest for girls before the children received a second dose of measles vaccine. Hence, it does seem most likely that the increased female mortality in the Sudan trial is indeed due to the missing doses of DTP being provided after enrolment in the trial.

Fourth, in Kinshasa, mortality was increased when DTP was given at the same time as HTMV compared with children receiving HTMV alone. In Sudan, measles vaccination had not been given simultaneously with DTP. A possible increase in mortality associated with simultaneous MV and DTP vaccinations has not been reported before. We have subsequently made the same observation in additional studies from Bissau, The Gambia [23], Senegal, and Malawi [24]. Furthermore, independent confirmation has been provided by a study from Bangladesh [25]. The MR of MV-vaccinated compared with MV-unvaccinated children was 0.93 (0.65–1.34) but when the authors censored children who had received DTP and BCG after 9 months of age together with MV, the beneficial effect of MV became 0.61 (0.44–0.85). Though the authors did not make the point, the only way this result can be obtained is that the DTP, BCG and MV vaccinated children censored in the analysis had strong excess mortality. This is potentially an important observation which might have direct implications for vaccination policies and child health. It should be important to examine the effects of both simultaneous and sequential administration of measles vaccine and DTP in future observational and randomised studies.

Little immunological research has examined non-targeted effects of vaccinations. There are, however, animal studies suggesting that prior infection may influence the response, both positively and negatively, to subsequent unrelated infections [26–28]. There would seem good reasons to examine the immunological consequences of simultaneous and sequential administration of routine vaccinations in areas with high mortality in which differences in immune profile could have major consequences for child survival [12].

In conclusion, the studies from Sudan and Kinshasa supported all deduction from our hypothesis of DTP administered after HTMV being the cause of increased female mortality in the HTMV trials. In both Kinshasa and Sudan, there was no indication of increased female mortality among children who did not receive DTP after measles vaccination. In the Sudan study, female mortality was increased in the high-titre group among children missing DTP doses before enrolment and the female–male MR was associated with the number of missing doses of DTP. Furthermore, mortality was low among HTMV recipients who did not receive DTP simultaneously with or after MV. These analyses were obviously not planned when the study was initiated and important information on vaccination status is therefore, missing. Nonetheless, the data collected for other purposes was consistent with our non-trivial hypothesis of increased female mortality after HTMV [12].

Should these observations turn out to be repeatable, we have to assess not only the specific effects of the vaccines but also the potential non-specific effects due to the sequence of vaccinations. It will be essential to consider the possible interactions between measles vaccine and inactivated vaccines, including DTP, should new attempts be made to vaccinate early with measles vaccine [12].

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