

Association Between Thimerosal-Containing Vaccine and Autism

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HIGH DOSES OF MERCURIC COMPOUNDS are nephrotoxic and neurotoxic.¹ Thimerosal, an organic compound that contains ethylmercury, has been widely used since the 1930s as a preservative in certain vaccines. In the 1990s, an increasing number of different vaccines containing thimerosal were introduced in immunization schedules around the world, and thus the average cumulative exposure to thimerosal in infants has increased in recent years. This has led to the suggestion that childhood vaccination with thimerosal-containing vaccines increases the risk of neurodevelopmental disorders, such as autism, attention-deficit/hyperactivity disorder, and language and speech delay.

In a recent independent review conducted by the Immunization Safety Committee, on behalf of the Institute of Medicine, it was concluded that the evidence was inadequate to accept or reject a causal relationship between thimerosal-containing vaccine and neurodevelopmental disorders.² However, based on comparison with the toxicology of methylmercury, the biological plausibility of a link remained. Further research was recommended. We examined the hypothesized association by comparing children vaccinated with a thimerosal-containing pertussis vaccine with children vaccinated with the same pertussis vaccine formulated without thimerosal and following them with respect to development

Context Mercuric compounds are nephrotoxic and neurotoxic at high doses. Thimerosal, a preservative used widely in vaccine formulations, contains ethylmercury. Thus it has been suggested that childhood vaccination with thimerosal-containing vaccine could be causally related to neurodevelopmental disorders such as autism.

Objective To determine whether vaccination with a thimerosal-containing vaccine is associated with development of autism.

Design, Setting, and Participants Population-based cohort study of all children born in Denmark from January 1, 1990, until December 31, 1996 (N=467 450) comparing children vaccinated with a thimerosal-containing vaccine with children vaccinated with a thimerosal-free formulation of the same vaccine.

Main Outcome Measures Rate ratio (RR) for autism and other autistic-spectrum disorders, including trend with dose of ethylmercury.

Results During 2 986 654 person-years, we identified 440 autism cases and 787 cases of other autistic-spectrum disorders. The risk of autism and other autistic-spectrum disorders did not differ significantly between children vaccinated with thimerosal-containing vaccine and children vaccinated with thimerosal-free vaccine (RR, 0.85 [95% confidence interval {CI}, 0.60-1.20] for autism; RR, 1.12 [95% CI, 0.88-1.43] for other autistic-spectrum disorders). Furthermore, we found no evidence of a dose-response association (increase in RR per 25 µg of ethylmercury, 0.98 [95% CI, 0.90-1.06] for autism and 1.03 [95% CI, 0.98-1.09] for other autistic-spectrum disorders).

Conclusion The results do not support a causal relationship between childhood vaccination with thimerosal-containing vaccines and development of autistic-spectrum disorders.

JAMA. 2003;290:1763-1766

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of autism and other autistic-spectrum disorders.

METHODS

The Danish childhood vaccination program is voluntary and free of charge to the vaccinees. Vaccines against diphtheria, tetanus, polio, measles, mumps, rubella, pertussis, and *Haemophilus influenzae* type b are administered by general practitioners.³ From 1970, the only thimerosal-containing vaccine in the program has been the whole-cell pertussis vaccine. In late March 1992, the last batch of thimerosal-containing whole-cell pertussis vaccine was released and distributed from Statens Serum Institut. Only the whole-cell vaccine produced by Statens Serum Institut

has been used in Denmark. The same vaccine was reformulated without thimerosal and used until January 1, 1997, when it was replaced with an acellular pertussis vaccine.⁴ The whole-cell vaccine was administered at 5 weeks, 9 weeks, and 10 months from 1970 and until it was replaced, irrespective of thimerosal content.³ The thimerosal formulation contained 50 µg of thimerosal (~25 µg of ethylmercury) in the first

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dose and 100 µg (~50 µg of ethylmercury) in each of the succeeding 2 doses.

Since April 1968, all persons in Denmark have been given a unique identification number in the Danish Civil Registration System.⁵ Based on this registry, we constructed a cohort consisting of all children born in Denmark in the period from January 1, 1990, to December 31, 1996. Using the unique personal identification number, we were able to link information on vaccinations, diagnoses of autism, diagnoses of other autistic-spectrum disorders, other relevant diagnoses, and potential confounders to the children in the cohort. The dates of vaccination with 1, 2, or 3 doses of whole-cell pertussis vaccine were obtained from the National Board of Health. We have published details of this process in a study of autistic-spectrum disorders and measles-mumps-rubella vaccine.⁶ Doses administered before June 1, 1992, were considered to contain thimerosal, and doses administered after June 1, 1992, were considered thimerosal-free. Children who received thimerosal-free vaccine after 1 or 2 doses of thimerosal-containing vaccine were classified only according to receipt of thimerosal-containing vaccine.

Information on autism and other autistic-spectrum disorder diagnoses was obtained from the Danish Psychiatric Central Register.^{6,7} Child psychiatrists make the diagnosis and assign diagnostic codes for this register. In the period 1991-1993, the *International Classification of Diseases, 8th Revision (ICD-8)* was used. In the period 1994 through 2000, the *International Classification of Diseases, 10th Revision (ICD-10)* was used. All cases of autism and other autistic-spectrum disorders in our study have been ascertained using *ICD-10*. Autism was defined by *ICD-10* code F84.0, which is similar to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)* code 299.00, and other autistic-spectrum disorders were defined by *ICD-10* codes F84.1-F84.9, which are similar to *DSM-IV* codes 299.10 and 299.80.

In 1991-1994, only inpatients were included in the Danish Psychiatric Cen-

tral Register. From 1995, both inpatients and outpatients were included. Information on diagnoses of tuberous sclerosis, Angelman syndrome, fragile X syndrome, and congenital rubella, conditions associated with autism, was obtained from the National Hospital Discharge Register.⁸ Information on possible confounding factors was obtained from the Danish Civil Registration System and the Danish Medical Birth Registry,⁹ as follows: child's sex, child's place of birth (Copenhagen, Copenhagen suburbs, area with $\geq 100\,000$ population, area with population of $<10\,000$), birth weight (<2500 , 2500-2999, 3000-3499, 3500-3999, ≥ 4000 g), 5-minute Apgar score (0-7, 8-9, 10), gestational age (<37 , 37-41, ≥ 42 weeks), mother's age at birth of child (<20 , 20-24, 25-29, 30-34, 35-39, and ≥ 40 years), and mother's country of birth (Danish or not). The percentage of missing values for the variables birth weight, gestational age, 5-minute Apgar score, mother's country of birth, and child's place of birth were 6.6%, 6.9%, 6.9%, 0.3%, and 0.03%, respectively.

Children in our cohort contributed person-time to follow-up from 1 year of age or January 1, 1991, whichever occurred last, until a diagnosis of autism, other autistic-spectrum disorder, tuberous sclerosis, Angelman syndrome, fragile X syndrome or congenital rubella, possible death, disappearance or emigration, 11 years of age, or until December 31, 2000, whichever occurred first. Follow-up was begun at 1 year of age because indications for an evaluation of a possible case of autistic-spectrum disorder typically occur after the first year of life. The resulting incidence rates for autism and other autistic-spectrum disorders were analyzed with Poisson regression, producing estimates of rate ratios (RRs) according to vaccination history.¹⁰ Vaccination history was considered a time-varying variable. We estimated the dose-response relationship between thimerosal-containing vaccine and autism and other autistic-spectrum disorders as the increase in RR per 25 µg of ethylmercury. We adjusted

all RRs for age (1-9 years of age, 1/2-year intervals; 10 years of age, 1-year interval) and calendar period (1991-1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000). We further adjusted our estimates for the potential confounding variables previously listed. Statistical analysis was performed using PROC GENMOD in SAS version 6.12 (SAS Institute Inc, Cary, NC).

RESULTS

A total of 467 450 children were born in Denmark between January 1, 1990, and December 31, 1996. During 2 986 654 person-years of follow-up, we identified 440 cases of autism and 787 cases of other autistic-spectrum disorders. The mean (SD) age at diagnosis was 4.7 (1.7) years for autism and 6.0 (1.9) years for other autistic-spectrum disorders. The follow-up of 5770 children was prematurely terminated because of death (n=579), emigration (n=5035), disappearance (n=87), tuberous sclerosis (n=51), Angelman syndrome (n=17), or congenital rubella (n=1).

In our cohort, only 20 755 (4.4%) children did not receive any whole-cell pertussis vaccine, 446 695 (95.6%) were vaccinated at least once, 416 081 (89.0%) were vaccinated twice, and 293 186 (62.7%) received 3 doses of whole-cell pertussis vaccine. Among those who received at least 1 thimerosal-containing pertussis vaccine (n=1 389 53), 1 185 93 received 1 subsequent dose and 65 725 received 2 subsequent doses of thimerosal-containing vaccine. Furthermore, 42 032 children who received at least 1 dose of thimerosal-containing vaccine subsequently received at least 1 dose of thimerosal-free vaccine. In those receiving at least 1 dose of whole-cell pertussis vaccine, there were 407 cases of autism (303 receiving thimerosal-free and 104 receiving thimerosal-containing vaccine) and 751 cases of other autistic-spectrum disorders (430 receiving thimerosal-free and 321 receiving thimerosal-containing vaccine).

Comparing children vaccinated with at least 1 dose of thimerosal-containing whole-cell pertussis vaccine with

children vaccinated with a thimerosal-free formulation of the same vaccine, we found a fully adjusted RR of 0.85 (95% confidence interval [CI], 0.60-1.20) for autism and an RR of 1.12 (95% CI, 0.88-1.43) for other autistic-spectrum disorders (TABLE). Furthermore, we found no evidence of a dose-response association between the dose of ethylmercury received and autistic-spectrum disorders (increase in RR per 25 µg of ethylmercury, 0.98 [95% CI, 0.90-1.06] for autism and 1.03 [95% CI, 0.98-1.09] for other autistic-spectrum disorders).

Although doses administered after June 1, 1992, were considered thimerosal-free, it is conceivable that a few thimerosal-containing doses may have been administered during the months after this date. To assess whether misclassification of vaccine type might have biased our estimates, we reestimated the RRs, omitting children vaccinated from June 1, 1992, through December 31, 1992. We found a fully adjusted RR of 0.87 (95% CI, 0.61-1.23) for autism and an RR of 1.15 (95% CI, 0.90-1.47) for other autistic-spectrum disorders and no evidence of a dose-response association (increase in RR per 25 µg of ethylmercury, 0.98 [95% CI, 0.90-1.07] for autism and 1.04 [95% CI, 0.98-1.09] for other autistic-spectrum disorders).

In a further analysis we evaluated the robustness of our results by restrict-

ing our cohort to children born in 1991-1993, a presumably more homogeneous group with respect to diagnosis, length of follow-up, and factors not included in this study (eg, mercury exposure through food) and found a fully adjusted RR of 0.86 (95% CI, 0.53-1.39) for autism and an RR of 1.05 (95% CI, 0.77-1.44) for other autistic-spectrum disorders and no evidence of a dose-response association (increase in RR per 25 µg of ethylmercury, 0.97 [95% CI, 0.85-1.10] for autism and 1.04 [95% CI, 0.96-1.13] for other autistic-spectrum disorders).

Finally, we evaluated the impact of missing values by the method of single imputation, replacing a missing value with the most common value of the relevant variable, and found a fully adjusted RR of 0.85 (95% CI, 0.60-1.20) for autism and 1.13 (95% CI, 0.89-1.44) for other autistic-spectrum disorders.

To evaluate whether the incidence of autistic-spectrum disorders was increasing in Denmark in the study period, we calculated time period trends from our cohort. We found statistically significant increases in age-adjusted RR per calendar year for both autism and other autistic-spectrum disorders during the study period (RR, 1.24 [95% CI, 1.17-1.31] for autism; RR, 1.21 [95% CI, 1.16-1.27] for other autistic-spectrum disorders). In the period from January

1, 1995, to December 31, 2000, a period where outpatients were included, we found similar trends (RR, 1.24 [95% CI, 1.16-1.32] for autism; RR, 1.20 [95% CI, 1.13-1.26] for other autistic-spectrum disorders).

COMMENT

We found no evidence of an association between thimerosal-containing vaccine and autism in children who received thimerosal-containing vaccine compared with children who received the same vaccine formulated without thimerosal. Furthermore, there was no indication of a dose-response association between autism and the amount of ethylmercury received through thimerosal.

The hypothesis of an association between thimerosal and autism has primarily been based on biological plausibility through analogies with methylmercury.² Ethylmercury, however, is thought to have a shorter half-life in the human body than methylmercury, and no controlled studies of low-dose ethylmercury toxicity in humans have been conducted.¹¹ Pichichero and colleagues¹² measured the concentration of mercury in the blood, urine, and stool of infants who received thimerosal-containing vaccines and concluded that vaccination did not raise the blood concentration of mercury above safe limits, and that ethylmercury was rapidly

Table. Rate Ratio of Autism and Other Autistic-Spectrum Disorders Comparing Children Vaccinated With a Thimerosal-Containing Vaccine to Children Vaccinated With a Thimerosal-Free Formulation of the Same Vaccine

	Person-Years at Risk	Autism			Other Autistic-Spectrum Disorders		
		No. of Cases	RR (95% CI)*	RR (95% CI)†	No. of Cases	RR (95% CI)*	RR (95% CI)†
Vaccinations							
All thimerosal-free	1 660 159	303	1.00	1.00	430	1.00	1.00
Any containing thimerosal	1 220 006	104	0.85 (0.60-1.20)	0.85 (0.60-1.20)	321	1.12 (0.88-1.43)	1.12 (0.88-1.43)
Doses of thimerosal-containing vaccine							
None	1 660 159	303	1.00	1.00	430	1.00	1.00
1 dose (25 µg eHg)	169 920	18	0.99 (0.59-1.68)	1.01 (0.60-1.71)	40	0.96 (0.67-1.39)	0.95 (0.66-1.37)
2 doses (75 µg eHg)	447 973	33	0.71 (0.46-1.09)	0.70 (0.46-1.09)	130	1.20 (0.92-1.56)	1.20 (0.92-1.56)
3 doses (125 µg eHg)	602 113	53	0.96 (0.63-1.46)	0.96 (0.63-1.47)	151	1.11 (0.83-1.48)	1.13 (0.84-1.51)
Trend (increase in RR per 25 µg eHg)			0.98 (0.90-1.06)	0.98 (0.90-1.06)		1.03 (0.97-1.09)	1.03 (0.98-1.09)

Abbreviations: CI, confidence interval; eHg, ethylmercury; RR, rate ratio.

*Adjusted for confounders: age and calendar period.

†Fully adjusted: age, calendar period, child's sex, child's place of birth, birth weight, 5-minute Apgar score, gestational age, mother's age at birth of child, and mother's country of birth.

eliminated via the stools. They estimated the blood half-life of ethylmercury at 7 days (95% CI, 4-10 days), although their study was not designed as a formal pharmacokinetic study of ethylmercury.

In 1999, when thimerosal was still widely used, children in the US childhood immunization program would have received 187.5 µg of ethylmercury by the age of 6 months and 237.5 µg of ethylmercury by the age of 2 years.² In Denmark, children would have received 125 µg of ethylmercury by the age of 10 months. However, in the Danish program, children received larger doses of ethylmercury per vaccine (50 µg compared with 25 µg in the United States) so that at 3 months, Danish children would have received the same amount of ethylmercury as US children (75 µg).²

To our knowledge, our study is the first population-based cohort study to examine the association between thimerosal and autism. In Denmark since 1970, only the whole-cell pertussis vaccine was formulated with thimerosal, and this vaccine was the only one used for pertussis immunization until it was replaced with an acellular pertussis vaccine in 1997. The unique situation has allowed a direct comparison of children vaccinated with a thimerosal-containing whole-cell pertussis vaccine with children vaccinated with the same vaccine formulated without thimerosal, and thus we have avoided confounding by contraindication and other selection bias associated with unvaccinated children. Furthermore, we have no reason to believe that the 2 groups of children differ with respect to other potential risk factors for autism.

All data used in this study were collected prospectively, eliminating concerns about recall bias. Madsen and colleagues⁶ reviewed the medical records of 40 children with autism from the Danish Psychiatric Central Register and found that 37 children met the operational criteria for autism according to a systematic coding scheme developed by the Centers for Disease Control and Prevention.¹³ Furthermore,

Madsen and colleagues⁶ found Danish prevalence rates for autism and other autistic-spectrum disorders comparable to prevalence rates found in other studies. Thus we conclude that the validity and completeness of the autism and other autistic-spectrum disorder diagnoses in the Danish Psychiatric Central Register is high. However, it is possible that the National Hospital Discharge Register is not complete with respect to a diagnosis of tuberous sclerosis, Angelman syndrome, fragile X syndrome, and congenital rubella. However, these conditions are rare in the general population and since we have compared only vaccinated children, lack of completeness is unlikely to seriously confound an association between thimerosal content and autistic-spectrum disorder.

We found statistically significant increased rates over time for both autism and other autistic-spectrum disorders. These results are compatible with a dramatic increase in the number of diagnosed cases of autistic-spectrum disorders during the study period, similar to what has been observed in other countries (eg, the United States).

In Denmark, general practitioners administer all childhood vaccinations and are reimbursed when reporting these to the National Board of Health, thus ensuring a high degree of completeness. In our cohort we found that 96%, 89%, and 63% of children were vaccinated at least once, at least twice, and 3 times with whole-cell pertussis vaccine. The low uptake of 3 doses is unexpected but can be partially explained by the transition to acellular pertussis vaccine in January 1997. Furthermore, for each dose there is a small chance of either missing the dose or the vaccination not being recorded. Even small probabilities for each dose can, if they are statistically independent, result in a significant reduction in the calculated uptake of all 3 doses.

A possible weakness of this study is that the date of diagnosis used as the incidence date may differ significantly from the "onset of symptoms" date. A diagnosis of autistic-spectrum disorder

can be a lengthy process; this is reflected in the mean ages of diagnoses in this study (4.7 years for autism and 6.0 years for other autistic-spectrum disorders). However, this is more likely to be a problem in an incidence study than in a risk factor study.

In conclusion, our results are not compatible with the hypothesis of a causal association between thimerosal and autistic-spectrum disorders.

Author Contributions: *Study concept and design:* Hviid, Stellfeld, Wohlfahrt, Melbye. *Analysis and interpretation of data:* Hviid, Wohlfahrt. *Drafting of the manuscript:* Hviid. *Critical revision of the manuscript for important intellectual content:* Stellfeld, Wohlfahrt, Melbye. *Statistical expertise:* Hviid, Wohlfahrt. *Obtained funding:* Hviid, Melbye. *Study supervision:* Wohlfahrt, Melbye. **Funding/Support:** This study was supported by grant 11 from the Danish National Research Foundation and grant 22-02-0293 from the Danish Medical Research Council.

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