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# A Two-Phase Case-Control Study of Autism Risk Among Children Born From the Late 1990s Through the Early 2000s in the United States

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**Background:** This study evaluated the hypothesis that the 1999 recommendation by the American Academy of Pediatrics (AAP) and US Public Health Service (PHS) to reduce exposure to mercury (Hg) from Thimerosal in US vaccines would be associated with a reduction in the long-term risk of being diagnosed with autism.





**Material/Methods:** A two-phase assessment utilizing a case (n=73) -control (n=11,783) study in the Vaccine Adverse Event Reporting System (VAERS) database (for hypothesis generating) and a more rigorous, independent matched case (n=40) -control (n=40) study (hypothesis testing) was undertaken.

**Results:** Analysis of the VAERS database using logistic regression revealed that the odds ratio (OR) for being an autism case in the VAERS database significantly decreased with a more recent year of vaccination in comparison to controls (OR=0.65) from 1998 to 2003. Sex-separated analyses revealed similar significant effects for males (OR=0.62) and females (OR=0.71). Analyses of the matched case-control data revealed, using the t-test statistic, that the mean date of birth among cases diagnosed with an autism spectrum disorder (ASD) (2000.5±1.2) was significantly more in the past than in controls (2001.1±1.3). Logistic regression also revealed that the OR for being diagnosed with ASD significantly decreased with a more recent date of birth in comparison to controls (OR=0.67) from 1998–2003.

**Conclusions:** This study reveals that the risk of autism during from the late1990s to early 2000s in the US significantly decreased with reductions in Hg exposure from Thimerosal-containing childhood vaccines, but future studies should examine this phenomenon in other US populations. Vaccine programs have significantly reduced the morbidity and mortality associated with infectious disease, but Thimerosal should be removed from all vaccines.

**MeSH Keywords:** **Autistic Disorder • Child Development Disorders, Pervasive • Ethylmercury Compounds • Thimerosal**

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## Background

Mercury (Hg) is a ubiquitous environmental neurotoxin with increasing evidence linking it with neurodevelopmental disorders such as autism [1]. As previously described, the 3 modern “faces” of Hg result in billions of people worldwide being exposed to methyl-Hg in fish, Hg vapor from dental amalgam fillings, and ethyl-Hg from Thimerosal used as a preservative in many vaccines [2]. On July 7, 1999, the American Academy of Pediatrics (AAP) and US Public Health Service (USPHS) issued a joint recommendation to remove Thimerosal from all vaccines as soon as possible [3]. It was cited at the time of the recommendation that the amount of Hg exposure some infants received from Thimerosal-containing vaccines exceeded one of the federal guidelines on methyl-Hg, and the link between Thimerosal exposure and neurodevelopmental effects in children was unknown.

The objective of the current study was to epidemiologically determine the consequences of the July 7, 1999 recommendation to reduce exposure to Hg from Thimerosal in vaccines administered in the US during the late 1990s/early 2000s and the long-term probability of being diagnosed with autism. It was hypothesized that the reduction of Hg from Thimerosal-containing infant vaccines during the late 1990s/early 2000s would reduce the risk of being diagnosed with autism. In order to test this hypothesis, the current study employed a two-phase hypothesis-generating/hypothesis-testing case-control methodology.

## Material and Methods

### Phase I – a hypothesis-generating retrospective case-control assessment of the VAERS database

The US Centers for Disease Control and Prevention (CDC) and the US Food and Drug Administration (FDA) have jointly maintained since 1990 the Vaccine Adverse Event Reporting System (VAERS) as an epidemiological surveillance tool to evaluate vaccine safety. As mandated by law, specific vaccine-associated adverse events are required to be reported to the VAERS database, but other vaccine-associated adverse events are passively reported to the VAERS database. It is estimated by the VAERS Working Group of the CDC that less than 5% of total adverse events are reported to the VAERS database by parents. The US CDC and US FDA follow up specific serious adverse events and deaths reported to the VAERS database. Multiple epidemiological studies have been published by the VAERS Working Group of the CDC and the US FDA that analyze the VAERS database for vaccine safety concerns [4,5].

The design flexibility, simplicity of use, and timely availability of data are among the strengths of the VAERS database noted by the VAERS Working Group; however, they also warn that systematic error due to underreporting, flawed reporting, co-administration of multiple vaccines, numerous outcomes, and absence of exact denominators are potential limitations of the VAERS database. They also warn that the VAERS database contains adverse event reports reported following vaccination by an individual as vaccine-associated, be it coincidental or causally related to vaccine exposure [4].

### Determining the population at risk

The VAERS updated through April 14, 2014 was analyzed using the MedAlerts online computer interface (<http://www.medalerts.org/vaersdb/index.php>). This portal provides independent researchers with access to quickly examine up-to-date data in the VAERS database. The VAERS database was analyzed for vaccine-associated adverse events reported following vaccines administered from January 1998 through December 2003. The vaccine recipients had to be between 2 and 6 years old and specify a residence in the USA. A total of 11,856 total adverse event reports were identified and analyzed in the current study.

### Determining cases

Among the total 11,856 adverse event reports examined in the present study, cases were selected by identifying those adverse event reports specifying the outcome of autism (VAERS code: 10003805). A total of 73 autism cases were identified from the adverse event reports examined in the VAERS database. Table 1 summarizes the sex and year of vaccination status for each of the autism cases examined.

### Determining controls

Among the 11,856 total adverse reports examined in this study, the controls were selected by including only those adverse event reports that did not include the case outcome of autism. A total of 11,783 controls were identified. Table 1 summarizes the sex and year of vaccination status of the controls studied.

### Statistical analyses

The StatsDirect (version 3.0.152) statistical software package was employed. A two-sided p value <0.05 was considered to be statistically significant. The data were analyzed using the logistic regression statistic to evaluate trends in autism cases and controls in the VAERS database by year of vaccination from 1998 through 2003. The data were then separated by sex and analyzed using the logistic regression statistic to evaluate trends in sex-specific autism cases and controls in the VAERS database by year of vaccination from 1998 through

**Table 1.** A summary of autism cases and controls examined in phase I.

Sex	Year of vaccination	Cases	Percent	Controls	Percent
Male	1998	17	23.29%	679	5.76%
	1999	14	19.18%	632	5.36%
	2000	6	8.22%	783	6.65%
	2001	10	13.7%	1,037	8.80%
	2002	4	5.48%	1,312	11.13%
	2003	4	5.48%	1,780	15.11%
	Total	55	75.34%	6,223	52.81%
Female	1998	2	2.74%	589	5%
	1999	4	5.48%	582	4.94%
	2000	4	5.48%	672	5.70%
	2001	4	5.48%	899	7.63%
	2002	1	1.37%	1,147	9.73%
	2003	1	1.37%	1,542	13.09%
	Total	16	21.92%	5,431	46.09%
Unknown	1998	0	0%	24	0.20%
	1999	0	0%	18	0.15%
	2000	0	0%	23	0.2%
	2001	1	1.37%	19	0.16%
	2002	1	1.37%	25	0.21%
	2003	0	0%	20	0.17%
	Total	2	2.74%	129	1.1%
Total		73	100%	11,783	100%

2003. The null hypothesis was that there would be no difference in the year of vaccination among autism cases and controls in the VAERS database.

**Phase II – a hypothesis-testing prospective case-control study**

*Institutional Review Board Approval and Human Participants Compliance*

The protocol employed in the current study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas (Dallas, TX, USA). A consent form and a Health Insurance Portability and Accountability Act (HIPAA) form were signed by all parents. One or both parents were present with their children throughout the assessment visit.

**Participants**

Eighty prospectively collected children between 2 years and 6 years of age, who resided in the Dallas, TX metropolitan area were examined in the current study. Half the children were cases confirmed at the time of the study by the principal investigator (JKK) utilizing the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria [6] to be diagnosed with an autism spectrum disorder (ASD) (30% developed normally and then regressed after birth), and the other half were non-ASD-diagnosed matched controls. Children were matched on the basis of age (within 1 year), sex (male/female ratio=3.4), and race/ethnicity (70% white and 30% minorities). The children had received their routine vaccines and none were receiving any unusual diets or supplements. The average amount of fish consumption per household was about 1.2 times per week in both the cases and controls. Fragile X

**Table 2.** A summary of the ASD cases and controls examined in phase II.

Parameter examined	ASD Cases (n=40)	Controls (n=40)	p value
Mean CARS Score $\pm$ SD (range)	35.02 $\pm$ 5.67 (26–46)	15.3 $\pm$ 0.56 (15–17)	<0.001 <sup>1</sup>
Mean age $\pm$ SD (range)	4.2 $\pm$ 1.2 (2–6)	3.9 $\pm$ 1.3 (2–6)	0.2742 <sup>2</sup>
Mean DOB $\pm$ SD (range)	2000.5 $\pm$ 1.2 (1998–2002)	2001.1 $\pm$ 1.3 (1998–2003)	0.0383 <sup>2</sup>

The t-test statistic was utilized. ASD – Autism Spectrum Disorder; CARS – Childhood Autism Rating Scale; DOB – date of birth; SD – standard deviation. <sup>1</sup> Calculated assuming unequal variances; <sup>2</sup> Calculated assuming equal variances.

disorder, tuberous sclerosis, phenylketonuria (PKU), Lesch-Nyhan syndrome, fetal alcohol syndrome, or history of maternal drug use was not present in any of the children examined. Table 2 provides an overview of the demographic breakdown of ASD cases and controls.

### Measurements

All children were examined by the principal investigator (JKK) using CARS testing. Quantitative evaluations of autism severity were assessed using the 15-item behavioral rating scale in CARS testing [7]. Table 2 reveals the overall mean CARS scores  $\pm$  standard deviation for the cases diagnosed with an ASD and controls examined.

### Statistical analyses

The StatsDirect (version 3.0.152) statistical software package was employed. A two-sided p value <0.05 was considered to be statistically significant. The mean CARS scores, mean age, and mean date of birth among cases diagnosed with autism in comparison to controls were evaluated using the parametric t-test statistic (assuming equal or unequal variances as appropriate). The null hypothesis was that there would be no differences in the mean CARS scores, mean age, or mean date of birth among cases diagnosed with an ASD in comparison to controls. The data were also analyzed using the logistic regression statistic to evaluate trends in ASD cases and controls by date of birth from 1998 through 2003. The null hypothesis was that there would be no alteration in trends of date of birth among ASD cases and controls.

## Results

### Phase I

Analysis of the VAERS database showed significant differences for the chronological date of vaccination among cases with the outcome of autism in comparison to controls. The logistic regression statistic revealed that the odds ratio for being an autism case in the VAERS database significantly decreased

with a more recent year of vaccination in comparison to controls (odds ratio=0.65 per more recent year of vaccination, 95% confidence interval=0.567–0.746, p value <0.001) from 1998 through 2003. When the data were separated by sex, the logistic regression analysis also revealed the odds ratio for being a male autism case in the VAERS database significantly decreased with a more recent year of vaccination in comparison to male controls (odds ratio=0.62 per more recent year of vaccination, 95% confidence interval=0.525–0.725, p value <0.001) and the odds ratio for being a female autism case in the VAERS database significantly decreased with a more recent year of vaccination in comparison to female controls (odds ratio=0.71 per more recent year of vaccination, 95% confidence interval=0.534–0.940, p value <0.05).

### Phase II

Analyses revealed significant differences among cases diagnosed with an ASD in comparison to controls. Using the t-test statistic, we found that the mean date of birth among cases diagnosed with an ASD (2000.5 $\pm$ 1.2) was significantly more in the past than in controls (2001.1 $\pm$ 1.3). In addition, using the logistic regression statistics, we observed that the odds ratio for being diagnosed with ASD significantly decreased with a more recent date of birth in comparison to controls (odds ratio=0.67 per more recent year of birth, 95% confidence interval=0.459–0.968, p value=0.0329) from 1998 through 2003.

## Discussion

The results of this two-phased epidemiological study appear to offer important insights into the association between exposure to Hg from Thimerosal in vaccines during the late 1990s/early 2000s and the risk of being diagnosed with autism in the US. In the first phase of this study, it was observed, based upon assessment of the VAERS database, that there was a significant overall yearly decreasing risk for autism in comparison to controls for vaccines administered from the late 1990s (a time period of greater Thimerosal exposure) through early 2000s (a time period of lower Thimerosal exposure). Similar significant effects were observed when the data were separated by

sex, but the effects were of a larger magnitude among males than females. Similarly, it was observed in the second phase that there was a significant decrease in the risk for autism in comparison to controls for children born from the late 1990s (i.e., a time period of greater Thimerosal exposure) than the early 2000s (a time period of lower Thimerosal exposure) than in controls. Furthermore, we observed that the size and magnitude of the decreases in autism risk with more recent years were similar in phase I=0.65 per year of vaccination and phase II=0.67 per year of birth.

The results of this two-phase case-control epidemiological study are consistent with several other previous ecological epidemiological studies that evaluated autism time-trends in relation to the reduction in Thimerosal-containing vaccine administration in the US from 1999 onwards. In one study, it was observed that there was a significant reduction in the proportion of autism adverse event reports reported to the VAERS from the mid-1990s onwards [8]. Another study revealed, on a quarterly basis, significant decreasing trends in new autism reports from mid-2002 through 2005 in the VAERS and the California Department of Developmental Services (DDS) databases [9].

The results of this two-phase case-control epidemiological study are different than several other previous ecological epidemiological studies that failed to find a significant relationship between Thimerosal-containing vaccine administration and time-trends in autism diagnosis in Denmark [10] and California [11].

The Danish study was previously criticized for numerous methodological flaws (e.g., changing sources of data from inpatient to inpatient and outpatient, and changes in diagnostic coding) in attempting to discern changing time-trends in autism diagnosis following the reduction of Thimerosal-containing vaccines in Denmark [12]. Also, it is interesting to compare data from the original Danish study [10] with a subsequent Danish study that provided more complete information on the prevalence rate of ASD by birth cohort year from 1980 through 2004 [13]. The new Danish study revealed that the prevalence of ASD birth cohort year significantly increased between 1980 through 1995 (from a low of 0.3% for children born from 1980 to 1984 to a high of 1.5% for those born from 1994 to 1995). The prevalence of ASD significantly decreased from 1995 through 2004 (to a low of 1.0% among children born between 2002 and 2004). This observation is consistent with decreased time-trends in the birth cohort prevalence of diagnosed ASD following the removal of Thimerosal-containing vaccines in Denmark.

The California study was potentially flawed because it attempted to trace time-trends in autism diagnosis in the California DDS by examining the prevalence of autism by age for different birth cohort years from 1989 through 2003 without taking into account factors influencing changes in practices for autism

diagnosis in the DDS system. It was previously estimated that changes in practices for autism diagnosis have substantially affected caseloads in the DDS system between 1992 and 2005 [14]. Further complicating the study design, the DDS data examined was only updated through March 2007. As a consequence of the ecological study design to evaluate the prevalence rate of autism by birth year, the potential birth years examined in the study that received reduced levels of Thimerosal from childhood vaccines (2000–2003) were only followed until these children were less than 6 years old. In the extreme, children in the 2003 birth cohort were evaluated at a maximum age of 3 years old for their prevalence of autism, to attempt to accurately determine their prevalence of autism in comparison to other birth cohort years. Finally, since the study was ecological in design, no actual examinations of individual patients was undertaken to determine the accuracy or consistency of the diagnoses made across the many birth cohort years examined.

The results of the present study are supported by numerous studies revealing the biological plausibility for how Hg in general, and specifically Thimerosal, can induce autism. The similarities between autism symptoms and Hg toxicity were previously compared [15,16]. In addition, individuals diagnosed with autism were observed to be susceptible to the toxic effects of Hg, and particularly to the toxic effects of Hg exposure from multiple bolus doses from Thimerosal in routine infant vaccines [17]. A significant overall association between environmental Hg exposure during prenatal or infant periods and autism risk was found in a recently published meta-analysis (odds ratio=1.66 95% confidence interval=1.14–2.17) [18]. Finally, a recent critical review of the more than 90 studies published from 1999 to 2016 that examined the potential relationship between Hg and autism found that 74% supported the link [19]. By contrast, there are studies that did not link Hg exposure with the long-term risk of a child being diagnosed with autism, but these studies have been criticized for their potential methodological limitations [12] and potential conflicts of interest [20].

### Strengths/limitations

An overall strength of this study was that it utilized a previously established epidemiological framework for how to evaluate potential vaccine safety concerns by the US FDA [21]. The US FDA epidemiological framework postulated that vaccine safety risks may be identified through reports to the VAERS, and then examined for consistency with other epidemiological studies using more structured study designs. It was observed in both phases of this study that there were consistent results of similar magnitude. The consistency and magnitude of the results observed argues against the phenomena detected being the result of a mere statistical chance or the result of some unknown bias/confounder present in the data driving the effects. Finally, epidemiological studies as early as



2003 hypothesized that Thimerosal reduction in US vaccines in the late 1990s/early 2000s would be associated with a reduced risk of autism [22], again, further supporting the validity of the results observed.

A potential limitation of this study was the sources and types of Hg exposures from Thimerosal-containing vaccines that each individual case or control received were not examined. This study examined Thimerosal exposure in aggregate terms on a yearly basis. This means that it was assumed that Thimerosal was removed/reduced from vaccines in the US on a gradual basis from July 7, 1999 onwards, and that the potential effects would be observed over the next several years. The assumption made regarding Thimerosal in this study after July 7, 1999 is supported by the gradual introduction of newly licensed Thimerosal-free or Thimerosal-reduced vaccines in the US after July 7, 1999, changes to the recommended vaccine schedule to allow for reduced or less rigorous exposures to Hg from Thimerosal-containing vaccines for some infants, and the known expiration of most vaccines within about 1 year after delivery at healthcare facilities [8]. Future studies should investigate the actual Thimerosal content of specific vaccines given to specific children to evaluate whether this would change the phenomena observed in this study.

Another potential limitation of this study was that a previous study warned of potential sources of bias in longitudinal studies examining autism adverse events in the VAERS database [23]. These investigators claimed that an undisclosed rise in the VAERS database of the number of reports in the database for conditions such as autism were related to pending litigation for vaccine injury. Obviously, it is of great importance to consider this potential phenomenon in any study attempting to examine trends in reported adverse events in the VAERS database. Interestingly, contrary to these investigators' previous assertion that the VAERS database is biased, the results of the present study actually revealed significant decreasing trends in the risk of autism adverse event reports to VAERS following vaccines administered in the early 2000s, a time when pending litigation related to autism was peaking in US courts [24], in comparison to the late 1990s. In addition, in further refutation of these previous investigators' assertion of bias in the VAERS database, this study revealed quantitatively similar results from analysis of the VAERS database with a second phase of the study undertaking a more rigorous case-control study design.

A further potential limitation of this study was that limited population samples were analyzed. As a consequence, it is possible that the observations made may not be generalizable to larger populations and to the US population as a whole. In considering this potential, it is important to consider that the US CDC has been monitoring the prevalence of autism among children 8 years old through the Autism and Developmental

Disabilities Monitoring (ADDM) Network since 2000. In 2007, the US CDC published their first estimates of the prevalence of autism among children born in 1992 and 1994 within the ADDM Network, and it was estimated that overall prevalence of autism was about 1 in 150 children [25,26]. It was observed that in each subsequent birth year examined (1998, 2000, and 2002) there was an increase in the prevalence of autism [26–29]. Interestingly, in the most recently published data from the ADDM Network from children born in 2004, there was no increase in the prevalence rate of autism relative to children in the birth year of 2002 (1 in 68 children) [30]. This is the first time in the ADDM Network history, since the US CDC published their original autism prevalence estimates from children born in 1992 and 1994, that the prevalence rate of autism did not increase by birth year. The lack of an increase between the 2002 and 2004 birth years is even more remarkable when one considers that in January 2004, the AAP and US CDC issued for the first time their Autism A.L.A.R.M., a broad-based notification to the healthcare community to increase autism awareness and facilitate increased autism diagnosis [31]. Similarly, another US CDC study examined autism prevalence by age group, and revealed a lower prevalence rate for autism among children born in late 1990s/early 2000s (1 in about 208 children) in comparison to children born in in the mid- to late-1990s (1 in about 135 children) [32]. It is also noteworthy that both of the US CDC reported results concerning apparent changing directions in the prevalence of autism correspond to the period of Thimerosal reduction in some US vaccines and correspond to the reduced risk of autism observed in this study. This may suggest the results observed in this study have applicability to apparently changing prevalence rates in the broader US population.

## Conclusions

This study provides important new evidence revealing that the risk of autism during the late 1990s through the early 2000s apparently significantly decreased as the amount of Hg exposure children received from Thimerosal-containing vaccines also decreased. The effects observed were of consistent size and magnitude across 2 different phases of the study, and are also consistent with several previous epidemiological studies and other studies supporting a link between Hg exposure and the risk of autism. In addition, when the data were separated by sex, similar effects were observed for both males and females. Future studies should further evaluate the phenomena observed in this study by examining other US populations. These studies should be careful to consider that there are several potential changing variables that need to be considered among US populations, such as the recommendation to routinely administer influenza vaccines (most of which still contain Thimerosal) to pregnant women and infants in 2003–2004 [16]; the change from the DSM-IV to the Diagnostic

and Statistical Manual of Mental Disorders, 5<sup>th</sup> Edition (DSM-5) criteria for autism diagnosis on May 18, 2013; and the change from the International Statistical Classification of Disease and Related Health Problems, 9<sup>th</sup> Revision (ICD-9) coding to the International Statistical Classification of Disease and Related Health Problems, 10<sup>th</sup> Revision (ICD-10) coding for autism on October 1, 2015. A failure to consider these variables may result in these future studies generating false-negative results. Finally, while vaccine programs have significantly reduced

infectious disease-associated morbidity/mortality [33], the results of the current study suggest Thimerosal should be eliminated from all vaccines.

### Potential conflict of interest

All of the authors have been involved in vaccine/biologic litigation relating to autism.

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