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ORIGINAL INVESTIGATION

Exposure to antibiotics in the first 24 months of life and neurocognitive outcomes at 11 years of age

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Abstract

Rationale Antibiotics are commonly prescribed for infants. In addition to increasing concern about antibiotic resistance, there is a concern about the potential negative impact of antibiotics on the gut microbiota and health and development outcomes.

Objective The aim of this study was to investigate the association between early life antibiotic exposure and later neurocognitive outcomes.

Methods Participants were infants born to mothers enrolled in the probiotics study. The initial study was designed to evaluate the effect of two different probiotics on allergy outcomes in childhood. Antibiotic exposure was based on parent report and categorised according to the following timing of the first exposure: 0–6 months, 6–12 months, 12–24 months or not at all. At 11 years of age, children's neurocognitive outcomes were assessed using psychologist-administered, parent-report and self-report measures. The relationship between the timing of antibiotic exposure and neurocognitive outcomes was examined using regression models.

Results Of the 474 participants initially enrolled, 342 (72%) children had a neurocognitive assessment at 11 years of age. After adjustment for mode of delivery, probiotic treatment group assignment, income and breastfeeding, children who had received antibiotics in the first 6 months of life had significantly lower overall cognitive and verbal comprehension abilities, increased risk of problems with metacognition, executive function, impulsivity, hyperactivity, attention-deficit hyperactivity disorder, anxiety and emotional problems.

Conclusions These results provide further evidence that early exposure to antibiotics may be associated with detrimental neurodevelopmental outcomes.

Keywords Antibiotics · Gut-brain-axis · Neurodevelopment · Behaviour · Anxiety · Attention-deficit hyperactivity disorder

Introduction

Antibiotics are widely used in children under the age of 24 months to treat infections (Rogawski et al. 2017). US data show that the antibiotic amoxicillin was the most commonly prescribed treatment for children age 0–23 months (Chai et al. 2012). In addition to increasing concern about antibiotic resistance, there is growing concern about the potential negative impact of antibiotics on the gut microbiota. Studies show that the composition of the infant's gut microbiota changes during antibiotic treatment. A study of eight oral antibiotics in 1- to 3-month old infants found that *Bacteroides, Bifidobacteria* and *Lactobacilli* species were suppressed during treatment in most infants with any of the eight antibiotics (Bennet et al. 2002). Animal work has found that low dose penicillin alters the gut microbiota of infant mice, increases cytokine expression in the frontal region of the brain and alters behaviour (Leclercq et al.

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2017). More specifically, antibiotic exposed male mice showed changes in anxiety-like behaviour on the elevated plus maze. Mice exposed to antibiotics also showed decreases in sociability but these were prevented if supplementation with the probiotic *Lactobacillus rhamnosus* JB-1 was given (Leclercq et al. 2017).

Colonisation of the infant's gut microbiota is thought to begin before birth (Collado et al. 2012). Infancy represents a time when the composition of the microbiota is rapidly changing as the infant's gut is influenced by factors such as method of delivery and infant feeding practices. At the same time as gut maturation and microbiota stabilisation are occurring, the central nervous system is also rapidly developing, and the bidirectional pathways between the gut and brain are being established. Thus, there are neurodevelopmental windows during which the microbial composition of the gut may influence brain development and therefore function (Borre et al. 2014). Antibiotic exposure in infancy has been associated with an increased risk of allergy and obesity later in childhood (Vangay et al. 2015; Lu and Ni 2015). In a previous cohort study of 871 children followed from birth, we found that those exposed to antibiotics in their first year of life had an increased risk of behavioural difficulties and depression symptoms later in childhood (Slykerman et al. 2017). Specifically, those who had received antibiotics during the first year of life had high rates of parent-rated difficulties at 3.5 and 11 years of age, parent and teacher-rated symptoms of ADHD at 11 years on the Conners Rating Scale and higher self-reported symptoms of depression at age 11 years. These associations persisted after adjustment for confounders and were not seen for the group of children who first received antibiotics between 12 months and 3.5 years of age. To our knowledge, this was the first study to report a significant association between early life antibiotic use and neurocognitive outcomes in children, and it is therefore important that the relationship is further examined in other cohorts. Relationships between the gut microbiome and neurodevelopmental outcomes, including the impact of early life exposure to antibiotics, need further investigation (Dinan et al. 2018).

The aim of this study was to determine whether the findings from our previous study could be replicated in a different cohort of children. Furthermore, we sought to expand on the previous study by examining whether the timing of antibiotic exposure within the first year of life was an important factor in cognitive, behavioural or mood outcomes later in childhood. To achieve this objective, we examined whether exposure to antibiotics at different time points in the first 24 months of life was associated with cognitive, behavioural and emotional health outcomes at the age of 11 years. A range of individually administered, observer-report and self-report measures was chosen to comprehensively assess the cognitive, behavioural and mood outcomes relevant to 11-year old children. Many of the assessment measures were the same as those used in the previous study, but additional measures of attention and cognition were added to expand on the previous study. We hypothesised that earlier exposure to antibiotics would be associated with more detrimental neurocognitive outcomes.

Method

Participants

Participants in this study were children born to mothers initially enrolled in the probiotics study which was designed to evaluate the effect of probiotic supplementation on allergy outcomes in childhood. The probiotics study design and methodology has been described in detail elsewhere (Wickens et al. 2007). In brief, the study enrolled pregnant women from two centres, Auckland and Wellington in New Zealand. Women were eligible to enrol if they or the child's father had a history of allergic disease. Women were then randomised to receive either *Bifidobacterium animalis* HN019 or *Lactobacillus rhamnosus* HN001 or placebo. Women were instructed to take the capsules from 35 weeks of pregnancy until birth and from birth until 6 months postpartum if they were breastfeeding their infant. Infants also received the same treatment or placebo as their mother had been randomised to from birth until 2 years of age.

Data collection

Demographic data was collected at baseline when mothers were enrolled in the study. Participants were then assessed at 3, 6, 12, 18 and 24 months and at 4, 6 and 11 years for allergy outcomes. Cognitive, behavioural and mood outcomes were assessed when children were 11 years of age using parent-report, self-report and individually administered measures.

Antibiotic exposure

Antibiotic use in the first 2 years of life was assessed using parent report. At 3, 6, 12, 18 and 24 months, parents were asked if their child had received antibiotics orally or by injection since the previous time point. Antibiotic exposure was categorised according to when the child was first exposed to antibiotics as follows: never, between birth and 6 months, between 6 and 12 months, between 12 and 24 months.

Outcome measures

Wechsler Intelligence Scale for Children—fourth edition (WISC-IV)

The WISC-IV is a standardised, psychologist-administered assessment of general intellectual ability. It is comprised of four index scores: verbal comprehension index, perceptual reasoning index, working memory index and processing speed index which combine to give an overall full scale IQ. The index scores and full scale IQ have a mean of 100 and a standard deviation of 15.

Center for Epidemiological Studies for Children (CES-DC)

The CES-DC is a 20-item self-report questionnaire that assesses symptoms of depression in children and adolescents. Scores range from 0 to 60 with higher scores indicative of higher depressive symptomatology. For analysis, CES-DC scores were categorised into those with moderate to severe symptoms of depression (scores ≥ 24) and those with mild symptoms or no symptoms of depression.

Behavior Rating Inventory of Executive Function: parent form (BRIEF)

Parents completed the BRIEF which is a questionnaire-based measure of executive function abilities in everyday home situations. The BRIEF combines two index scores: the behavior regulation index and the metacognition index to give an overall global executive composite score. Scores greater than or equal to 60 are considered indicative of problems and this cutoff was used in analysis.

Conners—third edition: parent form (Conners 3)

The Conners 3 parent form is a questionnaire-based measure asking about common symptoms of attention-deficit hyperactivity disorder (ADHD) in children. A *T* score of greater than or equal to 60 is considered of clinical significance and was used as the cutoff in this study.

Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a 25-item questionnaire completed by parents that asks about a child's functioning in five domains: peer problems, hyperactivity, emotional symptoms and conduct problems and prosocial behaviour. Scores from all of the subscales with the exception of the prosocial subscale combine to give an overall total difficulties score. Scores are then categorised as normal, borderline or abnormal. For this analysis borderline and abnormal scores were combined and compared with normal scores.

Multidimensional Anxiety Scale for Children

The MASC 2 assesses symptoms of anxiety in children and adolescents. It has parent-report and self-report forms. Scores are converted to standardised *T* score which range from 0 to 100. Scores of 60 or above are considered to be of clinical significance when the MASC 2 is used as a screening tool for anxiety.

Conners Continuous Performance Test—third edition (CPT 3)

Conners CPT 3 is a task-oriented computerised assessment of attention-related problems in individuals aged 8 years and older. The Conners CPT 3 provides objective information about an individual's performance in attention tasks. Detectability indicates a subject's ability to accurately detect a given stimulus when it appears, higher scores represent better performance. Overall hit reaction time represents the average time taken to give the correct response; faster response times indicate better performance.

Cambridge Automated Neuropsychological Test Battery (CANTAB)

Designed by Cambridge Cognition, the CANTAB is a battery of cognitive and executive function tests in tablet form. This study used three tasks from the CANTAB battery.

Attention switching test (AST) is a measure of a participant's ability to switch their attention efficiently and ability to filter out distractions. Shorter response latency time represents better performance on this task.

One Touch Stockings of Cambridge (OTS) is a measure of executive function ability particularly spatial planning and working memory. The variable "responses solved in first move" is an accuracy measure where higher scores represent better performance.

Spatial working memory (SWM) is a test that requires the retention and manipulation of visuospatial information. It yields a strategy score where a low strategy score indicates better performance.

Statistical analysis

Generalised linear models and logistic regression models were used to examine the association between early life antibiotic exposure and neurocognitive outcomes using the whole sample of children. Multivariable analysis was used to adjust for potential confounders including treatment group assignment, mode of delivery, breastfeeding and income. Adjusted results were produced for variables that were significantly associated with antibiotic exposure with $p \leq 0.10$. Significant results in adjusted regression analysis were with $p \leq 0.05$. In both unadjusted and adjusted analyses, the comparison group was

those children never exposed to antibiotics in the first 24 months of life.

To determine whether a linear trend was present between earlier timing of first antibiotic exposure and poorer outcomes, we conducted a trend analysis. An ordinal variable of equal spacing was created to categorise the timing of antibiotic exposure. This was used in regression models and a p value of < 0.05 was used to define the presence of a linear trend.

Ethics

The study was approved by the Central Health and Disability Ethics Committee (15/CEN/75/AM02). The parent or guardian and the child gave written consent.

Results

Of the 474 participants initially eligible at birth, 342 (72.2%) children were assessed for cognitive outcomes at 11 years. Table 1 shows the differences between respondents and non-respondents at 11 years of age. Children assessed at 11 did not differ from non-respondents in treatment or placebo group assignment ($p = 0.58$) or mode of delivery ($p = 0.34$). Respondents were more likely to have been breastfed ($p < 0.001$) and to come from a family with income over $60,000NZD ($p = 0.02$) than those who did not respond.

Of the 446 children for whom antibiotic exposure information was available, 70 (15.7%) children were not exposed to antibiotics at all during their first 24 months of life, 89 (20.0%) children received antibiotics between birth and 6 months, 163 (36.5%) children had their first exposure to antibiotics between 6 and 12 months of age and 124 (27.8%) were first exposed to antibiotics between 12 and 24 months.

Table 2 shows the unadjusted and adjusted results from the continuous neurocognitive outcomes and timing of early life antibiotic exposures. Table 3 shows the unadjusted and adjusted results for the categorical outcome variables and early life exposure to antibiotics. Full scale IQ and verbal comprehension index scores from the WISC-IV were significantly lower in those exposed to antibiotics in the first 6 months of life with an evident trend for later exposure to be associated with increasingly higher scores. With respect to behavioural outcomes, children exposed to antibiotics earlier in life were significantly more likely to have abnormally high scores in the following scales from the Conners 3: inattention, hyperactivity, learning problems, executive function, ADHD-inattentive type index, ADHD-hyperactive type index and the following subscales from the SDQ: emotional problems, hyperactivity and peer relationships subscales.

After adjustment for treatment group assignment, mode of delivery, income and breastfeeding, early antibiotic exposure was associated with lower full scale intelligence quotient scores ($p = 0.05$) and lower verbal comprehension index scores ($p = 0.02$). Figure 1 shows the mean difference in full scale IQ between those exposed to antibiotics at different time points in the first 24 months after birth relative to those who were not exposed to antibiotics. Early exposure was also associated with an increased risk of the following: Metacognition problems (BRIEF) ($p = 0.05$), impulsivity/hyperactivity ($p = 0.02$), executive function problems ($p = 0.04$), both ADHD predominantly inattentive type ($p = 0.01$) and hyperactive impulsive type ($p = 0.02$, emotional problems ($p = 0.004$), hyperactivity ($p = 0.01$) and anxiety ($p = 0.05$). Figure 2 shows the odds ratios for risk of ADHD-inattentive type according to the timing of the first exposure to antibiotics. In all cases, where, antibiotic exposure was significantly associated with negative outcomes exposure between birth and 6 months was associated with the most negative outcome, followed in most cases by the first exposure between 6 and 12 months. Results of the trend analysis are also presented in Tables 2 and 3. For all of the variables that were significantly associated with antibiotic exposure in adjusted analysis except

Table 1 Characteristics of respondents and non-respondents at 11 years of age

	Levels	Non-respondents ($N = 132$)	Respondents ($N = 342$)	Total	P value
Treatment group	HN019	40 (30.3%)	118 (34.5%)	158 (33.3%)	0.58
	HN001	48 (36.4%)	109 (31.9%)	157 (33.1%)	
	Placebo	44 (33.3%)	115 (33.6%)	159 (33.5%)	
Mode of delivery	Caesarean	47 (35.6%)	106 (31%)	153 (32.3%)	0.34
	Non-caesarean	85 (64.4%)	236 (69%)	321 (67.7%)	
Breastfeeding	Yes	120 (90.9%)	339 (99.1%)	459 (96.8%)	< 0.0001†
	No	12 (9.1%)	3 (0.9%)	15 (3.2%)	
Income	< $30,000	5 (4%)	8 (2.4%)	13 (2.8%)	0.022
	$30,000–$60,000	25 (20%)	37 (11%)	62 (13.4%)	
	> $60,000	95 (76%)	292 (86.7%)	387 (83.8%)	

†Fisher's exact test used

Table 2 Association between timing of first antibiotic exposure and cognitive, behavioural and mood outcomes at age 11 years

Continuous variables	Antibiotic exposure	Unadjusted			*p* value	Adjusted			*p* value‡	Trend analysis *p* value
		Estimated mean difference (95%CI)				Estimated mean difference (95%CI)‡				
WISC-IV										
Full scale IQ	0–6 months	− 5.7	(− 10.3,	− 1.2)	0.042	− 4.8	(− 9.4,	− 0.3)	0.046	0.003
	6–12 months	− 3.7	(− 7.6,	0.3)		− 3.6	(− 7.5,	0.3)		
	12–24 months	− 1.3	(− 5.4,	2.7)		− 0.6	(− 4.6,	3.4)		
Verbal comprehension index (VCI)	0–6 months	− 8.4	(− 14.0,	− 2.8)	0.019	− 7.4	(− 12.9,	− 1.8)	0.024	0.003
	6–12 months	− 6.8	(− 11.6,	− 1.9)		− 6.8	(− 11.6,	− 2.1)		
	12–24 months	− 5.2	(− 10.2,	− 0.2)		− 4.4	(− 9.3,	0.5)		
Perceptual reasoning index (PRI)	0–6 months	− 3.2	(− 7.8,	1.4)	0.130	− 2.5	(− 7.1,	2.1)	0.085	0.056
	6–12 months	− 1.4	(− 5.4,	2.5)		− 1.5	(− 5.4,	2.5)		
	12–24 months	1.3	(− 2.8,	5.4)		2.0	(− 2.1,	6.2)		
Working memory index (WMI)	0–6 months	− 2.7	(− 7.3,	2.0)	0.684	− 2.2	(− 6.9,	2.5)	0.697	0.241
	6–12 months	− 1.5	(− 5.5,	2.5)		− 1.5	(− 5.5,	2.5)		
	12–24 months	− 0.8	(− 4.9,	3.4)		− 0.2	(− 4.4,	4.0)		
Processing speed index (PSI)	0–6 months	− 3.4	(− 8.6,	1.7)	0.386	− 3.1	(− 8.3,	2.2)	0.459	0.137
	6–12 months	− 1.7	(− 6.1,	2.8)		− 1.3	(− 5.8,	3.2)		
	12–24 months	0.2	(− 4.5,	4.8)		0.4	(− 4.3,	5.1)		

#Reference taken as never

‡ adjusted for probiotic treatment, method of delivery, breastfeeding and income

the BRIEF metacognition index, there was a significant linear trend indicating that earlier timing of first antibiotic exposure was associated with poorer outcomes.

Discussion

This study examined the relationship between antibiotic exposure at different time points in the first 2 years of life and cognitive, behavioural and emotional outcomes at 11 years of age in a cohort of 342 children. Statistically significant relationships were identified between earlier exposure to antibiotics and increased risk of behavioural problems, increased risk of anxiety and lower overall cognitive and verbal abilities. There was an evident timing effect whereby the first exposure to antibiotics before 6 months of age was associated with poorer outcomes. In many cases, first exposure between 6 and 12 months was associated with worse outcomes than first exposure between 12 and 24 months, in all cases, the comparison group was those children who did not receive any antibiotics during their first 2 years of life. It is possible that those children with earlier first exposure to antibiotics also received more courses of antibiotics over the first 2 years of life. Data on the precise number of doses of antibiotics, each child received in each of the time intervals was not available. However, a trend analysis of the timing effect confirmed that in all cases where antibiotic exposures were significantly associated with the outcome in adjusted analysis except the BRIEF metacognition index; there was a significant trend for earlier exposures to be associated with poorer outcome.

The results are strikingly similar to previous findings where children exposed to antibiotics in the first year of life (but not between 12 months and 3.5 years) had significantly increased risk of behaviour problems at the age of 7 and 11 years (Slykerman et al. 2017). The current results extend the previous study by suggesting that within the first year of life earlier exposure leads to greater risk of behavioural problems, anxiety and lower cognitive ability. Analysis of the gut microbiome was not undertaken as part of this study, but a recent study has linked the infant's gut microbiome to early cognitive outcomes. Increased alpha diversity at 1 year of age in human infants has been associated with lower overall cognitive ability at 2 years of age on the Mullen Scales of Early Learning (Carlson et al. 2018). Although increased microbial diversity has typically been associated with more favourable outcomes, the study by Carlson et al. (2018) is the first to propose that different cluster groups of bacterial composition in infants are associated with cognitive outcomes in childhood. This contributes to the emerging research linking neurodevelopmental windows of importance and composition of the gut microbiota in human infants.

The fact that first exposure to antibiotics between birth and 6 months of age was associated with increased risk of behavioural, cognitive and emotional problems is consistent with

Table 3 Associations between the timing of first antibiotic exposure and categorical cognitive, behaviour and mood outcomes

Categorical variables	Antibiotic exposure	Unadjusted				Adjusted				Trend analysis p value[1]
		OR	95% CI	p value		OR	(95% CI)[1]		p value[1]	
BRIEF parent T scores										
Behavior regulation index	0–6 months	1.5	(0.6,	3.8)	0.377	1.8	(0.7,	4.6)	0.215	0.105
	6–12 months	1.5	(0.7,	3.3)		1.6	(0.7,	3.7)		
	12–24 months	0.9	(0.4,	2.2)		0.9	(0.4,	2.2)		
Metacognition index	0–6 months	2.1	(0.8,	5.2)	0.071	2.2	(0.9,	5.5)	0.050	0.142
	6–12 months	0.8	(0.3,	2.0)		0.8	(0.4,	2.0)		
	12–24 months	0.9	(0.4,	2.1)		0.8	(0.3,	1.9)		
Global executive composite	0–6 months	2.3	(0.9,	5.6)	0.140	2.7	(1.0,	6.8)	0.067	0.067
	6–12 months	1.1	(0.5,	2.5)		1.2	(0.5,	2.7)		
	12–24 months	1.1	(0.4,	2.5)		1.0	(0.4,	2.5)		
Conners T scores										
Inattention	0–6 months	2.7	(1.1,	6.9)	0.037	2.4	(0.9,	6.4)	0.063	0.136
	6–12 months	1.0	(0.4,	2.4)		0.9	(0.4,	2.3)		
	12–24 months	1.3	(0.5,	3.1)		1.0	(0.4,	2.6)		
Impulsivity/hyperactivity	0–6 months	2.0	(0.9,	4.8)	0.044	2.1	(0.9,	5.0)	0.020	0.034
	6–12 months	1.1	(0.5,	2.4)		1.1	(0.5,	2.4)		
	12–24 months	0.7	(0.3,	1.6)		0.6	(0.3,	1.4)		
Learning problems	0–6 months	4.2	(1.3,	13.7)	0.023	3.3	(1.0,	11.1)	0.064	0.007
	6–12 months	2.7	(0.9,	8.1)		2.4	(0.8,	7.4)		
	12–24 months	1.4	(0.4,	4.6)		1.2	(0.3,	4.0)		
Executive function	0–6 months	3.9	(1.3,	11.6)	0.038	3.8	(1.2,	11.7)	0.044	0.011
	6–12 months	2.0	(0.7,	5.5)		1.9	(0.7,	5.5)		
	12–24 months	1.5	(0.5,	4.5)		1.4	(0.4,	4.1)		
Defiance/aggression	0–6 months	1.1	(0.4,	3.0)	0.989	1.3	(0.5,	3.5)	0.968	0.717
	6–12 months	1.2	(0.5,	2.7)		1.2	(0.5,	2.7)		
	12–24 months	1.1	(0.5,	2.6)		1.1	(0.5,	2.8)		
Peer relationships	0–6 months	1.2	(0.5,	2.9)	0.275	1.2	(0.5,	2.9)	0.310	0.779
	6–12 months	0.7	(0.3,	1.4)		0.6	(0.3,	1.4)		
	12–24 months	0.7	(0.3,	1.5)		0.7	(0.3,	1.6)		
Conners 3 ADHD-predominantly inattentive type	0–6 months	4.4	(1.6,	12.1)	0.0025	4.0	(1.4,	11.4)	0.006	0.008
	6–12 months	1.4	(0.5,	3.6)		1.3	(0.5,	3.5)		
	12–24 months	1.5	(0.5,	3.9)		1.3	(0.5,	3.6)		
Conners 3 ADHD-impulsive/hyperactive	0–6 months	2.3	(1.0,	5.4)	0.032	2.4	(1.0,	5.9)	0.019	0.029
	6–12 months	1.0	(0.5,	2.3)		1.1	(0.5,	2.4)		
	12–24 months	0.8	(0.3,	1.8)		0.7	(0.3,	1.7)		
Conners CGI	0–6 months	2.9	(1.1,	7.7)	0.188	3.0	(1.1,	8.3)	0.154	0.039
	6–12 months	2.1	(0.9,	5.2)		2.3	(0.9,	5.6)		
	12–24 months	1.9	(0.7,	4.7)		1.7	(0.7,	4.5)		
SDQ parent T score										
Total difficulties score	0–6 months	4.1	(0.8,	20.2)	0.081	4.6	(0.9,	23.8)	0.067	0.032
	6–12 months	1.6	(0.3,	7.9)		1.8	(0.4,	8.9)		
	12–24 months	1.0	(0.2,	5.5)		0.8	(0.1,	5.2)		
Emotional problems	0–6 months	9.0	(1.9,	41.5)	0.005	9.2	(2.0,	43.0)	0.004	0.001
	6–12 months	3.5	(0.8,	15.7)		3.2	(0.7,	14.8)		
	12–24 months	2.6	(0.5,	12.2)		2.4	(0.5,	11.5)		
Conduct problems	0–6 months	4.1	(0.8,	20.2)	0.125	3.6	(0.8,	15.6)	0.141	0.066

Table 3 (continued)

Categorical variables	Antibiotic exposure	Unadjusted OR	95% CI	*p* value		Adjusted OR	(95% CI)[1]	*p* value[1]	Trend analysis *p* value[1]
	6–12 months	1.4	(0.3,	7.0)		1.3	(0.3, 5.3)		
	12–24 months	1.5	(0.3,	7.6)		1.4	(0.3, 6.2)		
Hyperactivity	0–6 months	8.3	(1.0,	69.2)	0.004	8.2	(0.9, 73.1)	0.008	0.008
	6–12 months	1.2	(0.1,	11.7)		1.1	(0.1, 11.2)		
	12–24 months	1.0	(0.1,	11.0)		0.9	(0.1, 10.1)		
Peer problems	0–6 months	3.2	(1.0,	10.7)	0.047	2.7	(0.8, 8.8)	0.074	0.036
	6–12 months	1.2	(0.4,	3.9)		1.2	(0.4, 3.7)		
	12–24 months	1.0	(0.3,	3.4)		0.8	(0.2, 2.7)		
Prosocial score	0–6 months	1.4	(0.5,	3.9)	0.187	1.5	(0.5, 4.2)	0.203	0.225
	6–12 months	0.8	(0.3,	2.0)		0.8	(0.3, 2.1)		
	12–24 months	0.4	(0.1,	1.4)		0.5	(0.2, 1.5)		
CES-DC self-report									
Total score ≥ 24	0–6 months	2.6	(0.5,	13.7)	0.705	2.8	(0.5, 16.7)	0.624	
	6–12 months	1.9	(0.4,	9.4)		2.5	(0.5, 11.4)		
	12–24 months	1.5	(0.3,	7.9)		1.5	(0.3, 8.2)		
MASC2 parent *T* score									
Total anxiety score	0–6 months	4.8	(1.3,	18.1)	0.070	5.4	(1.4, 20.7)	0.047	0.007
	6–12 months	3.1	(0.9,	11.0)		3.0	(0.8, 10.7)		
	12–24 months	2.1	(0.6,	7.7)		2.0	(0.5, 7.7)		
MASC2 self-report *T* score									
Total anxiety score	0–6 months	1.7	(0.6,	4.8)	0.469	1.7	(0.6, 4.8)	0.351	
	6–12 months	1.5	(0.6,	3.6)		1.4	(0.6, 3.4)		
	12–24 months	1.0	(0.4,	2.5)		0.8	(0.3, 2.2)		
CPT3 *T* scores									
Detectability	0–6 months	1.4	(0.6,	3.3)	0.68	1.5	(0.6, 3.6)	0.718	
	6–12 months	1.0	(0.5,	2.2)		1.0	(0.5, 2.2)		
	12–24 months	1.4	(0.6,	2.9)		1.2	(0.6, 2.7)		
HIT reaction time	0–6 months	1.1	(0.3,	4.4)	0.53	0.9	(0.2, 3.5)	0.575	
	6–12 months	0.5	(0.1,	1.9)		0.5	(0.1, 1.6)		
	12–24 months	1.0	(0.3,	3.6)		0.9	(0.3, 2.9)		
CANTAB									
AST response latency	0–6 months	1.0	(0.3,	3.1)	0.96	1.2	(0.4, 3.8)	0.95	
	6–12 months	1.2	(0.5,	3.1)		1.3	(0.5, 3.3)		
	12–24 months	1.1	(0.4,	2.8)		1.1	(0.4, 3.0)		
OTS problems solved in first trial	0–6 months	0.9	(0.3,	2.7)	0.98	1.0	(0.3, 2.9)	0.97	
	6–12 months	1.1	(0.5,	2.7)		1.2	(0.5, 2.8)		
	12–24 months	1.1	(0.4,	2.8)		1.1	(0.4, 2.9)		
SWM strategy score	0–6 months	0.8	(0.3,	2.2)	0.88	0.9	(0.3, 2.5)	0.85	
	6–12 months	0.9	(0.4,	2.1)		0.9	(0.4, 2.1)		
	12–24 months	0.7	(0.3,	1.7)		0.7	(0.3, 1.7)		

#Reference taken as never

‡ Adjusted for probiotic treatment, method of delivery, breastfeeding and income

the proposed age centric view of infant's gut development and sensitivity to alterations to the gut microbiome proposed by Vangay and colleagues (Vangay et al. 2015). They propose the following stages of infant's gut development: 0–6 months, 6–12 months, 12–24 months, 24 months and older and suggest that the most sensitive period for immunological development

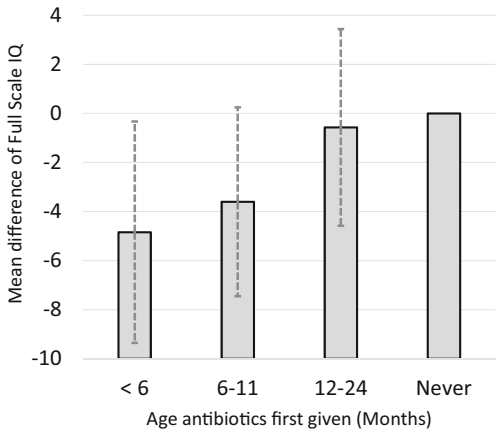

Fig. 1 Mean difference in full scale IQ between those exposed to antibiotics at different time points in the first 24 months after birth relative to those who were not exposed to antibiotics

of the gut is 0–6 months of age. It is possible that antibiotic disruption to the gut microbiome during this time has a more profound effect on later outcomes than at later points in development. Animal models using germ-free mice suggest that early critical periods may play a role in the relationship between the microbiome and mood outcomes. Introducing microbes to germ-free mice at the age of 3 weeks results in an improvement in anxiety-like behaviour (Clarke et al. 2013) while at 10 weeks of age, stabilising the gut microbiome did not alter anxiety phenotype (Neufeld et al. 2011). This is similar to earlier work which also suggests the introduction of bacterial flora to germ-free mice could partly reverse an exaggerated stress response if it was introduced early in development, but the same effect was not seen if colonisation occurred later in development (Sudo et al. 2004).

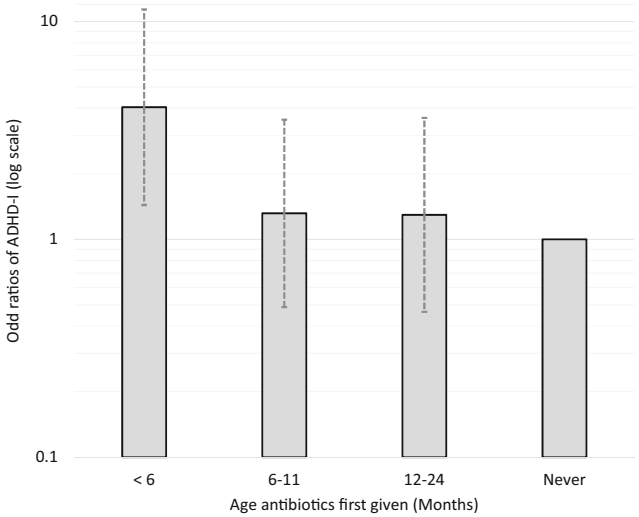

Fig. 2 Odds ratios for risk of ADHD-inattentive type by the timing of first exposure to antibiotics relative to those children not exposed to antibiotics

Research using germ-free mice provides a possible model for how the microbiome-gut-brain axis is associated with cognitive development and outcomes. The hypothalamic-pituitary-adrenal axis (HPA) modulates the stress response system. To examine the relationship between the postnatal gut microbiota and the endocrine system, germ-free mice were compared with specific-pathogen–free mice and gnotobiotic mice. Germ-free mice were found to have an exaggerated stress-response when restrained as measured by biochemical markers and to show increased anxiety-like behaviour. Introduction of *Bifidobacterium infantis* to the germ-free mice partially reversed the exaggerated stress response (Sudo et al. 2004). The HPA axis has been studied in relation to ADHD in children with findings suggesting that in non-stress situations reduced cortisol levels are evident in ADHD children and overall studies are suggestive of a hypo-functioning of the stress response system. (Ma et al. 2010; Angeli et al. 2018). Given that the gut microbiome plays a role in the HPA axis development in animal models, it is possible that the early development of the stress response system in humans is influenced by events in the gut and results in observable changes in behaviour later in childhood.

Previously, a small randomised trial of supplementation with *L. rhamnosus* GG for the first 6 months of life found that the 6/35 children who received the placebo had a diagnosis of ADHD or Asperger's syndrome compared with none of the children who received the probiotic (Pärtty et al. 2015) thereby providing evidence that influencing the early gut microbiome may influence neurodevelopmental outcomes in children. However, we found that in this cohort of children, probiotic supplementation with either probiotic did not improve cognitive, behavioural or mood outcomes (Slykerman et al. 2018).

There are some limitations of the current study. Significant associations were found between parent-reported measures of behaviour problems, particularly behaviours commonly associated with ADHD. However early antibiotic exposure was not significantly correlated with formal measures of attention and executive function (CANTAB and CPT3). The CANTAB and CPT3 measure very specific aspects of executive function in an assessment setting while questionnaire-based measures more broadly assess a child's functioning in everyday situations. It is possible that the CPT3 and CANTAB did not measure the aspects of executive function affected by antibiotic exposure.

This study is not a randomised controlled trial of exposure to antibiotics, and for this reason, a causal effect of antibiotics on later development cannot be proven. It is possible that antibiotic exposure in early life is a marker for illness in childhood and that this is associated with the outcomes measured. We did not collect information about the reason for antibiotic use. A total of 56% of our cohort had received their first dose of antibiotics within the first year of life which we believe means it is unlikely that antibiotic use is a marker for

significant ill health sufficient to impact development. The issue of adequate socioeconomic confounder control in observational studies such as this one is a limitation of this type of study design. Participants in this study were a population of relatively high-income families with mothers who had a high breastfeeding initiation rate and lower maternal smoking during pregnancy rate. The significant associations found between antibiotic exposure and behavioural and cognitive outcomes in this relatively socially advantaged and homogenous group would suggest the results are less likely to be due to residual socioeconomic confounding. Replication of these findings in other cohorts of children will help to better understand the impact of antibiotic exposure and neurocognitive outcomes.

With respect to specific cognitive function that may be influenced by the gut microbiome, human studies have not advanced to the point that specific functions are implicated. Preclinical studies provide some direction. Exaggerated stress response in germ-free mice has been associated with expression of brain-derived neurotrophic factor (BDNF) in the hippocampus (Sudo et al. 2004). A study of the impact of intragastric antibiotic exposure on mice at the microbial, metabolite, cerebral and behavioural levels reported antibiotic exposed mice showed gut dysbiosis, changes in expression of BDNF and reduced novel object recognition (Fröhlich et al. 2016). These studies would suggest that memory may be particularly associated with gut dysbiosis; however, in this study, spatial working memory was not associated with antibiotic exposure. The complex combination of cognitive functions that contribute to learning and recall of information in children may make it less likely that memory measures specifically would be affected by gut microbiota health.

Conclusion

This study confirms the findings of a previous study and suggests that early life exposure to antibiotics is associated with an increased risk of behavioural and emotional problems and lower overall cognitive and verbal ability later in childhood. Further studies of the impact of antibiotics on the infant's gut, the importance of timing of first exposure to antibiotics and the type of antibiotic given in relation to neurodevelopmental outcomes will facilitate a better understanding of the relationship between antibiotics and neurocognitive outcome.

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Compliance with ethical standards

The study was approved by the Central Health and Disability Ethics Committee (15/CEN/75/AM02). The parent or guardian and the child gave written consent.

Conflict interest The authors declare that they have no conflict of interest.

References

Angeli E, Korpa T, Johnson EO, Apostolakou F, Papassotiriou I, Chrousos GP, Pervanidou P (2018) Salivary cortisol and alpha-amylase diurnal profiles and stress reactivity in children with attention deficit hyperactivity disorder. Psychoneuroendocrinology 90: 174–181. https://doi.org/10.1016/j.psyneuen.2018.02.026

Bennet R, Eriksson M, Nord CE (2002) The fecal microflora of 1–3-month-old infants during treatment with eight oral antibiotics. Infection 30:158–160. https://doi.org/10.1007/s15010-002-2140-z

Borre YE, O'Keeffe GW, Clarke G, Stanton C, Dinan TG, Cryan JF (2014) Microbiota and neurodevelopmental windows: implications for brain disorders. Trends Mol Med 20:509–518. https://doi.org/10.1016/j.molmed.2014.05.002

Carlson AL, Xia K, Azcarate-Peril MA, Goldman BD, Ahn M, Styner MA, Thompson AL, Geng X, Gilmore JH, Knickmeyer RC (2018) Infant gut microbiome associated with cognitive development. Biol Psychiatry 83:148–159. https://doi.org/10.1016/j.biopsych.2017.06.021

Chai G, Governale L, McMahon AW, Trinidad JP, Murphy JD (2012) Trends of outpatient prescription drug utilization in US children, 2002-2010. Pediatrics 130:23–31. https://doi.org/10.1542/peds.2011-2879.

Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, Dinan TG, Cryan JF (2013) The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry 18:666–673. https://doi.org/10.1038/mp.2012.77

Collado MC, Cernada M, Baüerl C, Vento M, Pérez-Martínez G (2012) Microbial ecology and host-microbiota interactions during early life stages. Gut Microbes 3:352–365. https://doi.org/10.4161/gmic.21215

Dinan TG, Cryan JF, Stanton C (2018) Gut microbes and brain development have black box connectivity. Biol Psychiatry 83:97–99. https://doi.org/10.1016/j.biopsych.2017.11.005

Fröhlich EE, Farzi A, Mayerhofer R, Reichmann F, Jačan A, Wagner B, Zinser E, Bordag N, Magnes C, Fröhlich E, Kashofer K, Gorkiewicz G, Holzer P (2016) Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. Brain Behav Immun 56:140–155. https://doi.org/10.1016/j.bbi.2016.02.020.

Leclercq S, Mian FM, Stanisz AM, Bindels LB, Cambier E, Ben-amram H, Koren O, Forsythe P, Bienenstock J (2017) Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. Nat Commun 8:15062. https://doi.org/10.1038/ncomms15062

Lu C, Ni Y (2015) Gut microbiota and the development of pediatric diseases. J Gastroenterol 50:720–726. https://doi.org/10.1007/s00535-015-1082-z

Ma L, Chen Y, Chen H, Liu Y, Wang Y (2010) The function of hypothalamus–pituitary–adrenal axis in children with ADHD. Brain Res 1368:159–162. https://doi.org/10.1016/j.brainres.2010.10.045

Neufeld KM, Kang N, Bienenstock J, Foster JA (2011) Effects of intestinal microbiota on anxiety-like behavior. Commun Integr Biol 4: 492–494. https://doi.org/10.4161/cib.15702

Pärtty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E (2015) A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatr Res 77:823–828. https://doi.org/10.1038/pr.2015.51

Rogawski ET, Platts-Mills JA, Seidman JC, John S, Mahfuz M, Ulak M, Shrestha SK, Soofi SB, Yori PP, Mduma E, Svensen E, Ahmed T, Lima AA, Bhutta ZA, Kosek MN, Lang DR, Gottlieb M, Zaidi AK, Kang G, Bessong PO, Houpt ER, Guerrant RL (2017) Use of antibiotics in children younger than two years in eight countries: a prospective cohort study. Bull World Health Organ 95:49–61. https://doi.org/10.2471/BLT.16.176123

Slykerman RF, Thompson J, Waldie KE, Murphy R, Wall C, Mitchell EA (2017) Antibiotics in the first year of life and subsequent neurocognitive outcomes. Acta Paediatr 106:87–94. https://doi.org/10.1111/apa.13613

Slykerman RF, Kang J, Van Zyl N, Barthow C, Wickens K, Stanley T, Coomarasamy C, Purdie G, Murphy R, Crane J, Mitchell EA (2018) Effect of early probiotic supplementation on childhood cognition, behaviour and mood a randomised, placebo-controlled trial. Acta Paediatr 107:2172–2178. https://doi.org/10.1111/apa.14590

Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, Koga Y (2004) Postnatal microbial colonization programs the hypothalamic-adrenal-pituitary system for stress response in mice. J Physiol 558: 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Vangay P, Ward T, Gerber J, Knights D (2015) Antibiotics, pediatric dysbiosis, and disease. Cell Host Microbe 17:553–564. https://doi.org/10.1016/j.chom.2015.04.006

Wickens K, Black P, Stanley T, Mitchell E, Fitzharris P, Crane J (2007) A differential effect of two probiotics in the prevention of eczema and atopy. World Allergy Organ J :S316. DOI: https://doi.org/10.1097/01.WOX.0000301532.49363.c1.