1	Association between the incidence of early childhood caries and attending childcare
2	among toddlers in a rural area of Japan: a prospective cohort study
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#### 1 Abstract

Objective: The purpose of this study was to investigate the association between the incidence
of early childhood caries (ECC) and factors of the daytime childcare environment among
toddlers in a rural area of Japan.

5 **Material and methods:** This was a prospective cohort study. Data collected from 2011 to 2015 6 were obtained for initially caries-free, 18-month-old toddlers (N = 640). Dentists recorded the 7 numbers of decayed, missing and filled teeth (caries incidence) of toddlers at baseline (age 18 8 months) and follow-up (age 3 years). In addition, a questionnaire survey was conducted on 9 guardians at baseline to record data on lifestyle.

10 **Results:** In total, 159 (24.8%) toddlers had newly identified ECC at follow-up. Logistic 11 regression analyses indicated that the incidence of ECC was significantly associated with 12 receiving daytime care at a nursery school (odds ratio [OR], 1.55; 95% confidence interval [CI], 13 1.05-2.30; p = .029), prolonged breastfeeding (OR, 1.71; 95%CI, 1.15–2.55; p = .008), and 14 snacking  $\geq$ 3 times a day (OR, 2.39; 95%CI, 1.53–3.74; p < .001). 15 **Conclusion:** These findings suggest that receiving daytime care at a nursery school, prolonged

breastfeeding, and frequent snacking are associated with an increased risk of ECC among Japanese toddlers in rural areas.

<sup>19</sup> Key words: Dental caries, Toddler, Nursery school, Early childhood caries, Childcare

#### 1 Introduction

The incidence of early childhood caries (ECC) in Japan has declined over recent decades; this was closely associated with the increased urbanization of the community [1,2]. The proportion of toddlers with incidence of caries from age 18 months to 3 years was found to be higher in rural areas (42.9%) than in urban areas (24.7%) [2], which suggests that ECC prevention measures remain important in rural areas of Japan.

7Strong positive associations have been reported between the incidence of ECC and both receiving daytime care from a grandmother and grandparental co-residence [2,3]. This situation 8 9 has affected the incidence of ECC in Japan for several decades. Recently, the aspects of daytime caregivers have been changing, with increasing numbers of female workers and decreasing 10 numbers of families co-residing with their grandparents, even in rural areas of Japan [4,5]. In 11 122015, 38.1% of toddlers aged 1–2 years were reported to have attended a nursery school and received daytime care from nursery caregivers, which is substantially higher compared with 13the proportion (22.2%) in 2003 [6,7]. However, the relationship between the incidence of ECC 14after 2010 and daytime caregiving remains unclear. 15

16 Therefore, we hypothesized that the recent change in daytime caregiving affects the 17 incidence of ECC. The purpose of this prospective cohort study was to investigate the 18 association between the incidence of ECC and factors related to daytime childcare among 19 toddlers in a rural area of Japan.

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#### 1 Materials and methods

## 2 **Ethics statement**

The protocol of this prospective cohort study was approved by the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital Ethics Committee (No. 1904-043; April 19, 2019). All procedures were performed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revisions.

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## 9 Study population

The data set used in this study consisted of municipal data from Maniwa city on toddlers aged 10 18 months and 3 years obtained from July 2011 to August 2017 in accordance with the Maternal 11 12and Child Health Law of Japan [8]. Health checkups included questionnaires and medical and dental examinations, which are mandatory for toddlers aged 18 months and 3 years. With a 13population of about 45,000, Maniwa city is a typical and representative rural area in Japan. 14Children under the age of 5 years account for about 4% of the population, while adults over the 15age of 65 years account for about 37%. Residents are primarily engaged in farming, the 16manufacturing industry, and medical and welfare services [9]. The water supply in this area is 17not fluoridated. 18

The inclusion criteria were: received a health checkup for an 18-month-old toddler between July 2011 and August 2015. The exclusion criteria were: i) having dental caries as baseline, ii) missing data at baseline, and iii) not receiving a health checkup for a 3-year-old child between 2013 and 2017, iv) having serious medical problems, systemic disorders or developmental anomalies [10]. In this area, health checkups are conducted at all community centers. More than 320 babies are born every year, and almost all toddlers visit health checkups for 18-monthold. We collected data obtained from 1192 toddlers between July 2011 and August 2015.

## 2 Questionnaire on lifestyles

Guardians were asked to complete a questionnaire at baseline (18 months old). The 3 questionnaire was not piloted, and has been widely used according to the Japanese national 4 survey protocol [8]. The questionnaire consisted of 14 questions, and included the toddler's  $\mathbf{5}$ medical condition (e.g., medical history), demographic characteristics (e.g., age, position in 6 7birth order, gestational age and birth weight), daytime care situation (e.g., daytime caregiver/ who is caring for the child during daytime, co-residing with grandparents), nutritional habits 8 9 (e.g., prolonged breastfeeding and use of feeding bottle, snacking frequency, such as eating cookies, Japanese crackers, and fruits between meals, daily intake of sugar-containing 10 beverages), and dental health practices (e.g., daily tooth brushing by parents, daily tooth 11 brushing habit, use of fluoride varnish). 12

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#### 14 Dental examination

A dental examination was performed at baseline (18 months old) and follow-up (3 years old) by experienced dentists who had undergone training involving a document and visuals describing the ECC criteria and illustrating the condition. A dental examination was conducted by 38 dentists at 8 public community centers. An examiner at baseline did not always check the same toddler at follow-up. The criteria were based on the World Health Organization guideline [11], and the numbers of decayed, missing and filled teeth were recorded using a dental mirror under artificial light.

The incidence of ECC was determined based on the presence of new ECC (> 0) at follow-up among the toddlers who did not have ECC at baseline [12].

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25 Statistical analysis

The sample size was estimated based on a previous study [13]. The expected odds ratio (OR) 1 was 1.9, which indicated that the incidence of ECC was significantly associated with receiving  $\mathbf{2}$ 3 daytime care from a grandmother as compared with the mother in logistic regression analyses. Based on the data, a sample size of at least 168 was needed to provide a power of 95% with an 4 alpha of .05. Sample size determination and power estimation were calculated using statistical  $\mathbf{5}$ software (G\*Power ver. 3.1.9.2; Universität Kiel, Kiel, Germany) [14]. Consequently, the 6 7sample size was determined as 210 in consideration of a dropout rate of 20%. Data analysis was performed using SPSS (versions 15, 20; SPSS Japan, Tokyo, Japan). 8

9 First, the toddlers were divided into two groups based on the presence of ECC at age 3 years: ECC positive (+) and negative (-) groups. Furthermore, the toddlers were divided into 10two or three groups based on the following parameters: birth order (first or  $\geq$ second) [12]; 11 12receiving daytime care from the mother, nursery caregivers, or other caregivers (e.g., grandparents, father, aunt and uncle); co-residing or not co-residing with grandparents; 13prolonged breastfeeding ([+] or [-]); prolonged use of feeding bottle ([+] or [-]); snacking 14frequency (<3 or  $\geq$ 3 times/day) [2]; daily intake of sugar-containing beverages ([+] or [-]); 15daily tooth brushing by parents ([+] or [-]); daily tooth brushing habit ([+] or [-]); and use of 16fluoride varnish ([+] or [-]). Comparisons of other parameters between the ECC (+) and (-) 17groups were analyzed using the unpaired *t*-test and chi-squared test. The level of significance 18was set at p < .05. 19

Second, the ORs and 95% confidence intervals (CIs) were calculated using a logistic regression model with the incidence of ECC as a dependent variable. Birth order, daily caregiver, co-residing with grandparents, prolonged breastfeeding and use of feeding bottle, snacking frequency, and use of fluoride varnish were considered risk factors for dental caries based on previous studies [3,12,15,16], and were entered as independent variables in the multivariate analysis. Finally, differences in parameters regarding care from mothers were compared with daytime nursery caregivers and other caregivers (father, grandparents, etc) using the chi-squared test. The level of significance was set at p < .05.

#### 1 Results

Among the toddlers who lived in Maniwa city between July 2011 and August 2015, more than 90% received a health checkup at 18 months of age; the proportion was especially high (96.1%) between 2014 and 2015. Finally, 1192 toddlers received health checkups for 18-month-old. After excluding 28 toddlers who had caries at 18 months old and 358 toddlers with missing data, 806 toddlers were enrolled in this study. Furthermore, 33 toddlers did not participate in health checkups at 3 years old, and data from 133 had missing data. Finally, a total of 640 toddlers were analyzed in the study (Figure 1), with a follow-up rate of 79.4%.

9 Overall, 262 (40.9%) toddlers received daytime care from nursery caregivers, 314 (49.1%) toddlers from their mother. The numbers of toddler with prolonged breastfeeding and use of 10 feeding bottle more than 18 months old were 178 (27.8%) and 66 (10.3%), respectively. 126 11 12(16.6%) toddlers had a snack more than 3 times a day and 269 (42.0%) toddlers lived with grandparents. No toddlers have serious medical problems, systemic disorders or developmental 13anomalies. The most common medical histories were injury (1.6%), pneumonia (0.6%) and 14Kawasaki disease (0.6%). New ECC was observed in 159 (24.8%) toddlers at follow-up. The 15average number of decayed and filled teeth was 0.8 ( $\pm$ 2.1). There were no missing teeth. 16

Table 1 shows a comparison between the ECC (+) and ECC (-) groups. Significant differences were found between the two groups in daytime caregiver (p = .019), prolonged breastfeeding (p = .005), and snacking frequency (p < .001). No other significant differences were observed with respect to the other parameters.

The results of the logistic regression analyses indicated that the incidence of ECC was significantly associated with receiving daytime care from nursery caregivers (OR, 1.55; 95%CI, 1.05-2.30; p = .029), prolonged breastfeeding (OR, 1.71; 95%CI, 1.15–2.55; p = .008), and snacking  $\geq$ 3 times/day (OR, 2.39; 95%CI, 1.53–3.74; p < .001) (Table 2).

25 Table 3 shows the differences in parameters regarding care from mothers compared with

#### 1 **Discussion**

In this study, we focused on the influence of daytime care on ECC incidence. To the best of our  $\mathbf{2}$ knowledge, this is the first study to report daytime care at a nursery school as a contributing 3 risk factor for ECC among Japanese toddlers in the past decade. In 1997, only 17% of Japanese 4 toddlers aged 1–2 years received care from daytime nursery caregivers [13,17]. By 2015, this  $\mathbf{5}$ percentage had risen to 38.1% [6,7]. This societal change might have had an effect on the 6 7incidence for ECC in Japan. Our results showed that the incidence of ECC was significantly associated with receiving daytime care from nursery caregivers (OR, 1.55; 95%CI, 1.05–2.30; 8 9 p = .029), thereby suggesting that this is a significant risk factor for ECC.

It has long been accepted that toddlers receiving daytime care from nursery caregivers are 10 associated with a lower incidence ECC because their daytime life is appropriately supervised. 11 12In fact, Sakuma et al. reported that toddlers receiving daytime care from nursery caregivers were at a lower risk of ECC compared with toddlers receiving daytime care from the mother 13[13]. This result conflicts with our findings. In our study, the toddlers receiving daytime care 14from nursery caregivers had a significantly higher frequency of snacking (22.5%) and 15significantly lower frequency of daily tooth brushing (21.0%) compared with the toddlers 16receiving daytime care from the mother (both p < .05) (Table 3). These results might be affected 17by increasing numbers of working mothers. A previous population survey reported that 18working parents spend less engagement time in primary childcare compared with their non-1920working counterparts [18]. The other studies reported that children with a working mother 21consume more unhealthy foods and have meals and snacks irregularly [19,20]. The results of 22these studies suggest that the toddlers with a working mother might have harmful diet at home 23after getting back from a nursery school, nevertheless they can have more regulated diet at a nursery school. The parents of toddlers receiving daytime care from nursery caregivers instead 24of a working mother should pay attention to their toddlers' oral health care at home. 25

A significant association was also observed between ECC and snacking frequency (*p* <.001). Habitual snacking has been regarded as an important factor affecting caries</li>
 development [2,16]. Sankeshwari et al. reported that the prevalence of ECC was significantly
 associated with sucrose exposure between meals and the overall frequency of sucrose exposure
 [21]. The findings from these previous studies support our results.

ECC incidence was significantly associated with prolonged breastfeeding at 18 months of age (p = .008). Prolonged breastfeeding has been identified as risk factor for ECC worldwide [16,22]. In Japan, Tanaka et al. reported that breastfeeding for 18 months or longer was positively associated with the prevalence of ECC [15]. Human milk is significantly more cariogenic than cow milk because it has lower mineral content and higher lactose levels [23]. The lactose in human milk is rapidly fermented by cariogenic bacteria and might contribute to caries development. The findings from these studies also support our results.

No significant difference in the incidence of ECC was observed between the children co-13residing or not co-residing with grandparents (p = .366). However, Sakuma et al. reported that 14children co-residing with and receiving daytime care from grandparents were at a higher risk 15of ECC [13]. It is generally considered that grandparents bring up children indulgently and give 16them sweets more frequently, thereby creating a higher risk for dental caries [13]. In this study, 17the number of toddlers receiving daytime care from grandparents was small (N = 57, 8.9%). 18Thus, the results of this study do not fully clarify the effects of grandparents on ECC. In 1920addition, because of the increased interest in oral health promotion [24,25], grandparents nowadays may not give sweets to their grandchildren as readily as in the past. In fact, the 21percentages of toddlers with a snacking frequency  $\geq 3$  times/day were 18.8% for toddlers 2223receiving care from others, including grandparents, and 11.1% for those receiving care from the mother; however, this difference was not statistically significant (Table 3). 24

25 It is important to focus on the incidence of ECC in rural areas. The prevalence of new cases

of ECC in this study (24.8%) was substantially higher than that in a previous study conducted in an urban area of Japan (16.4% from 2006 to 2009) [26]. ECC has also been found to be strongly associated with caries in permanent teeth [27]. Reducing the incidence of ECC in rural areas could also contribute to reducing the incidence of dental caries in adults.

The participants in this study did not represent a specific population. The prevalence of new cases of ECC seen in this study (24.8%) was similar to that reported in a previous study conducted from 2007 to 2011 on 3-year-old toddlers in a rural area (30.6%) [12]. Furthermore, the ratio of toddlers attending a nursery school (40.9%) was similar to that in a national survey in 2015 (38.1%) [28].

10 This study has several methodological strengths. First, the follow-up rate (79.4%) was high. 11 Second, ECC data were obtained from dental examinations given by dentists who were blinded 12 to the study protocol. Finally, a prospective cohort design was used, which allowed us to 13 identify the risk factors for ECC development more clearly than would have been possible 14 using a cross-sectional design.

This study has several limitations. First, we did not consider other important confounding 15factors, such as family income or maternal level of education [22]. However, in Japan, 16researchers tend to refrain from asking these questions, as municipalities do not allow such 17data to be collected [16]. Second, inter- and intra- examiner reliabilities could not be 18ascertained, because we used the data set which had been already collected in a community 1920dental service. However, all examiners were preliminarily informed of diagnostic criteria by 21public health service personnel. Thus, there might be overall high reliability and validity. Third, 22although using of fluoridated toothpaste is associated with ECC [13], the information of using 23of fluoride toothpaste at baseline could not be included. Forth, we could not investigate the changes in oral health behaviors and dietary habits between baseline and follow-up. These 24factors might be changed because guardians got some advices from dentists, dental hygienists 25

and/or public health nurses at baseline. Finally, the type of liquid in the bottle according to the
 prolonged use of feeding bottle remained unclear.

3 In conclusion, receiving daytime care at a nursery school, prolonged breastfeeding, and a

- 4 high frequency of snacking were significantly associated with ECC risk in Japanese toddlers.
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# 1 Acknowledgments

- 2 We gratefully acknowledge the contributions of the Maniwa City Office and Mimasaka Public
- 3 Health Center staffs.
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# 5 **Declaration of interest statement**

- 6 Aya Yokoi, Noriko Takeuchi, Daisuke Ekuni, and Manabu Morita declare that they have no
- 7 conflict of interest.

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- 1 Figure caption
- 2 Figure 1. Flowchart of the recruitment process