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RESEARCH ARTICLE

REVISED Identification and phylogenetic analysis of oral *Veillonella* species isolated from the saliva of Japanese children [version 5; peer review: 2 approved]

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Abstract

Background: As the most frequent infectious disease among children worldwide, dental caries have a strong relationship with oral hygiene status, specifically in the development of infection. However, the study regarding the identification and distribution of oral *Veillonella* are limited. The oral *Veillonella* community may affected by the differences in geographical location, age, diet, lifestyle, socio-economic status and oral hygiene status. Here, we studied the oral hygiene status by examining the composition and proportion of oral *Veillonella* species in saliva of Japanese children.

Methods: Microbial samples collected from 15 Japanese children divided into three oral hygiene groups were cultured under anaerobic conditions after homogenization and dilution, and inoculated onto brain heart infusion and selective medium *Veillonella* agar. Genomic DNA was extracted from each isolate. *Veillonella* species were detected by one-step PCR using *rpoB* species-specific primers. To analyse the phylogenetic properties of the unknown *Veillonella* strains, PCR amplification and sequence analysis of *rpoB* were conducted for 10 representative strains.

Results: Although *V. rogosae* was found as the predominant species among all groups, its prevalence was significantly lower in the children with poor oral hygiene than in those with good oral hygiene. *V. parvula* was the prevalent species in the poor oral hygiene group. Approximately 10% of the isolated *Veillonella* strains were not classified to any established species; the phylogenetic analysis showed that they were most closely related to *V. infantium*

Conclusions: This study demonstrates that the composition and proportion

of oral *Veillonella* species in the saliva of Japanese children is correlated with different oral hygiene status. Changes in detection ratios of *V. parvula* and *V. rogosae* can be useful indicators of oral hygiene status. Furthermore, new strains closely related to *V. infantium* were isolated from the saliva of Japanese children.

Keywords
Oral Veillonella species, dental caries, oral hygiene status, indicator, phylogenetic, saliva, children, Japan

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Introduction

The oral biofilm comprises multiple bacterial species and develops as a result of adhesion of pioneer bacterial species to adsorption of salivary proteins and glycoproteins on the enamel surface. These biofilms are not formed by random simultaneous colonization, but rather by selective, reproducible, and sequential colonization[1,2]. Oral biofilms are a source of bacteria that cause oral infections, for instance dental caries and periodontal disease, and they sometimes lead to or worsen systemic diseases[3].

Saliva is an acknowledged pool of biological markers that range from biochemical molecules changes such as DNA, RNA, and proteins, to those in microbiota structural composition[4]. Furthermore, saliva has an important role in oral biofilm development and maintenance. Recently, metagenomic analysis from saliva samples of Thai children demonstrated that *Streptococcus* and *Veillonella* were the predominant bacterial genera in the samples, and the proportion of *Streptococcus* decreased, while that of *Veillonella* increased in the children with poor oral hygiene status[5].

The genus *Veillonella* consist of multiple gram-negative bacterial species, obligate anaerobic, non-motile, non-spore forming, small cocci belonging to the family Veillonellaceae[6].

No *Veillonella* species ferment carbohydrates or amino acids, except for *V. criceti*, *V. ratti*, and *V. seminalis*. The metabolic end products of *Veillonella* species from trypticase-glucose-yeast extract are mainly acetic acid and propionic acid[6]. *Veillonella* species are present as commensal organisms in the oral cavity, intestinal tract and genitouritary and respiratory systems of humans and animals. Previous studies have reported that *Veillonella* species are rare causative organisms of meningitis, endocarditis, bacteraemia, discitis, vertebral osteomyelitis, and prosthetic joint infection[7–9]. Generally, *Veillonella* species are known to be resistant to tetracycline and sensitive to penicillin and ampicillin. However, some *Veillonella* strains resistant to both penicillin and ampicillin have recently emerged[10].

There are 14 species reported to belong to genus *Veillonella* including *V. infantium* which was assign as a novel species in 2018[11]. Of the 14 documented species, *V. atypica*, *V. denticariosi*, *V. parvula*, *V. rogosae*, *V. dispar*, *V. infantium*, and *V. tobetsuensis* have been found in human saliva or on tongue or dental biofilms[12–17]. Periasamy and Kolenbrander reported that oral *Veillonella* species are an early colonizer during the formation of oral biofilm, along with *Streptococcus* species, which were reported as initial colonizers in developing multispecies communities of oral biofilm[18]. Therefore, it is important to determine the role of oral *Veillonella* species in formation of oral biofilm to improve the prevention and treatment of oral infectious diseases.

Veillonella strains are relatively easy to identify at the genus level, but remain difficult to identify at the species level, since there are no useful phenotypic or biochemical examinations to distinguish them[19]. To resolve this problem, Mashima *et al.* established a novel one-step PCR method with species-specific primer sets based on the variable region of the *rpoB* gene sequences of oral *Veillonella* species[12]. Additionally, 1,442 *Veillonella* strains isolated from the saliva of 107 Thai children were identified by this method as *V. dispar*, *V. parvula*, *V. rogosae*, *V. atypica*, *V. denticariosi*, and *V. tobetsuensis* in our previous study[20]. In that study, *V. parvula* was significantly more prevalent in the poor oral hygiene, and the detection rate of oral *Veillonella* species in the saliva was indicative of the oral hygiene status of Thai children[20]. Additionally, another study suggested that several novel *Veillonella* species may inhabit the human oral cavity[21]. However, the study regarding the identification and distribution of oral *Veillonella* are limited. The oral *Veillonella* community may affected by the differences in geographical location, age, diet, lifestyle, socio-economic status and oral hygiene status.

Therefore, in this study, we examined composition and proportion of oral *Veillonella* species in saliva of Japanese children with different oral hygiene status.

Furthermore, we determined the phylogenetic position of the unknown *Veillonella* strains evaluated by the genus-specific PCR primer set as members of the genus *Veillonella* with a phylogenetic tree.

Methods
Subjects
The 15 children selected to take part in the study were 6 boys and 9 girls, aged 4 to 14 years old. Participants were recruited in-person during appointments at the Dental Hospital, Health Sciences University of Hokkaido. The subjects who had a history of immunosuppression or systemic diseases (e.g. leukemia, hepatitis), or any conditions requiring antibiotic monitoring or treatment procedures (e.g. heart conditions, bone fractures), or those with mucosal lesions, previous chemotherapy, radiation therapy, or medications that can reduce the salivary flow, and those that underwent treatment with antimicrobials within the previous three months were excluded from this study.

Subjects of this study were divided into three groups based on their evaluation by the Simplified Oral Hygiene Index (OHI-s) into good, moderate, and poor oral hygiene groups, according to the criteria of Greene and Vermillion[22]. Owing to the small number of children with poor hygiene (n=5), it was decided that 5 children would be chosen for each group. The good oral hygiene group (OHI-S score: 0–1.2) was composed of two males and three females. The moderate group (OHI-S score: 1.3–3.0) was composed of 3 males and 2 females. The poor group (OHI-S score: 3.1–6.0) was composed of 1 male and 4 females.

Sample collection
The saliva samples were collected at the Dental Hospital, Health Sciences University of Hokkaido, Japan, over a period between 2016 and 2017. Saliva was stimulated by paraffin chewing for 1 min and was then collected into sterile plastic tubes, and transferred to an anaerobic box (Hirasawa Works, Inc., Osaka, Japan)

containing 10% H_2, 85% N_2, 5% CO_2. These samples (1 ml each) were transferred to 1.5-ml Eppendorf tubes, then homogenized for 1 min with a BioMasher ®II (Nippi, Incoporated Protein Engineering Office, Tokyo, Japan).

Culture conditions

These homogenized saliva samples were serially diluted by 10-fold with sterile phosphate buffer saline (PBS) from 10^{-3} to 10^{-7}. Aliquots (100 µl) of each diluted sample were inoculated into Bacto™ Brain Heart Infusion (BHI, Difco Laboratories, Detroit, MI, USA) supplemented with 5% (volume/volume) defibrinated sheep blood (BHI agar), hemin (10 µg/mL, Wako, Osaka, Japan), menadione (5 µg/ml, Wako), and the selective medium *Veillonella* agar[23]. After inoculation, all media were incubated under anaerobic conditions with 10% H_2, 85% N_2, and 5% CO_2 at 37°C. *Veillonella* agar was incubated for 5 days and BHI agar was incubated for 7 days. The bacterial colonies grown on BHI and *Veillonella* agar were counted as the total number of bacteria and typical *Veillonella* colonies in the saliva sample, respectively. Bacterial cells of typical *Veillonella* colonies were confirmed as gram-negative cocci with light microscopy after gram staining. Standard strains consisted of *V. atypica* ATCC 17744[T], *V. denticariosi* JCM 15641[T], *V. dispar* ATCC 17748[T], *V. parvula* ATCC 10790[T], *V. rogosa* JCM 15642[T], and *V. tobetsuensis* ATCCBAA-2400[T].

DNA extraction

The genomic DNA was extracted from the isolated bacterial cells by using Insta Gene Matrix Kit (Bio-Rad Laboratories, Hercules, CA, USA). The DNA concentration determination was based on fluorescence by using a Qubit 3.0 Fluorometer. (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Additionally, genomic DNA extracted from the standard strains stated above was used as positive control for PCR.

Identification of *Veillonella* species

For the identification of *Veillonella* species at the genus level, a genus-specific PCR primer pair, Veill-rpoBF and Veill-rpoBR, were used according to the protocols described by Arif *et al.* and Mashima *et al.*[12,13,24]. Strains confirmed by PCR as members of genus *Veillonella* were then subject to the one-step PCR method with the species-specific primers sets ATYR, DENR, DISR, PARR, ROGR, TOBR, and VF, performed according to the method reported by Mashima *et al.*, for identification at species level[12].

The PCR products were applied to a 2.0% agarose gel, and after electrophoresis, the gel was stained with SYBR® Safe DNA gel stain (Invitrogen).

Phylogenetic analysis of unknown strains

For phylogenetic analysis of unknown strains, genomic DNA was also extracted from bacterial cells of unknown *Veillonella* strains showing positive PCR reaction with the genus-specific primer, but negative with the species-specific primer sets. In addition, PCR-based amplification and sequence analysis of *rpoB* were performed using the previously described primers for genus *Veillonella rpoB*-forward (5'-GTA ACA AAG GTG TCG TTT CTC G-3') and *rpoB*-reverse (5'-GCA CCR TCA AAT ACA GGT GTA GC-3')[24].

The PCR product contained DNA fragments were purified by using QIAquick® Gel Extraction Kit (Qiagen, Hilden, NW, Germany), according to the manufacturer's instructions. The DNA concentration after purification was determined based on fluorescence using a Qubit® 3.0 Fluorometer dsDNA HS Assay Kit (Invitrogen life Technologies, Carlsbad, CA, USA. The PCR reaction was performed with 15–20 ng/µl of DNA template for cycle sequence.

Purified DNA from PCR was sequenced with an BigDye® Terminator v1.1 Cycle Sequencing kit (Thermo Fisher, Waltham, MA, USA), BigDye® Terminator 5X Sequencing Buffer (Thermo Fisher, Waltham, MA, USA), single primer 1 pM and PCR product in a final volume of 20 µl. Cycle sequencing of the purified DNA was as follows: preheating at 96°C for 1 minutes; followed by 25 cycles of denaturation at 96°C for 10 seconds and annealing with extension at 60°C for 4 minutes[12]. Furthermore, the sequencing of PCR products were purified by using Centri-Sep column (Princeton Separations, Adelphia, NJ, USA), according to the manufacture's instruction and resolved for the sequencing analysis.

DNA sequences were determined using an ABI PRISM 310 Genetic Analyzer (Applied Biosystem) and were aligned and connected using SEQMAN Pro from the LASERGENE program (DNASTAR). The programs MEGALIGN, which includes CLUSTALW and NJPlot were used to compare sequences and to reconstruct an evolutionary tree by the neighbour-joining method[25]. Confidence intervals were also assessed by CLUSTALW with bootstrap analysis. Furthermore, pairwise similarity values were determined with MEGALIGN in the LASERGENE program. The *rpoB* sequences of the unknown *Veillonella* strains were aligned against the sequence of the established *Veillonella* species retrieved from GenBank. Unipro UGENE could be use as free alternative for both sequencing and pairwise similarity values.

Ethical considerations

All subjects and their parents were made aware of the objectives and procedures of the study and parents of participants provided written informed consent. This study was conducted with the approval of The Ethics Committee of the Health Sciences University of Hokkaido, Japan, under process number of 2016-015

Results

Colony numbers

The average number of colony forming units (CFU)/ml of all bacteria on BHI agar increased with decreased oral hygiene: 1.38E+08, 2.2E+08 and 4.48E+09 in the good, moderate and poor groups, respectively. Raw CFU data are available on Figshare[26].

Species identification

The phenotypic characteristics of *Veillonella* colonies on the selective medium were 2–4 mm in diameter, and slightly domed in shape with an entire edge, opaque, and greyish white. They were composed of small, gram-negative coccal cells, mainly existing as single cells but with short chains visible. In the

good oral hygiene group, a mean number of 1.70E+06 CFU/ml (median, 1.20E+06 CFU/ml) were identified as the genus *Veillonella*, with 12.3% *V. atypica*, 19.3% *V. dispar*, 10.5% *V. parvula*, 49.1% *V. rogosae*, and 8.8% unknown species (Table 1). In the moderate group, 2.08E+07 CFU/ml with median 2.00E+06 were identified as the genus *Veillonella*, with 6.2% *V. atypica*, 29.6% *V. dispar*, 12.3% *V. parvula*, 44.4% *V. rogosae*, and 7.4% unknown species (Table 2). Meanwhile, in the poor oral hygiene group, 4.48E+09 CFU/ml with median 2.20E+06 were identified as the genus *Veillonella*, with 7.3% *V. atypica*, 12.2% *V. dispar*, 31.7% *V. parvula*, 34.1% *V. rogosae*, and 14.6% unknown species proportions (Table 3).

As shown in the results, *V. rogosae* was found as the predominant species in the saliva samples of all oral hygiene groups. However, *V. denticariosi* and *V. tobetsuensis* were not found in all oral hygiene groups (Table 1–Table 3). Figure 1 shows the per cent ratio of the total number of strains of each species to the total number of *Veillonella* isolates from saliva samples of the good, moderate, and poor oral hygiene groups.

Strain characteristics

In this study, 179 strains were identified as *Veillonella* strains, and 162 strains were identified as *V. atypica*, *V. dispar*, *V. parvula* or *V. rogosae*. However, 17 (9.5%) of 179 strains failed to be classified as any of the known oral *Veillonella* species, thus, they were defined as unknown species. Of the 17 unknown *Veillonella* strains 10 (S3-1, S9-1, S10-1, S15-1, S17-1, S21-1, S25-2, S28-1, S29-1 and S30-1) were selected as representative strains from different hygiene groups for phylogenetic analysis. After determination of the *rpoB* sequences of these 10 strains, these sequences were aligned toward to the sequences of *Veillonella* type strains retrieved from GenBank. The evolutionary tree produced by analysing the type strains of the 14 *Veillonella*

Table 1. Ratio of the number of isolates of each species to the total number of *Veillonella* isolate in saliva from the good oral hygiene group. The colony-forming units (CFU) of all anaerobic bacteria on brain heart infusion agar and *Veillonella* strains on *Veillonella* agar (detection limit <0.1% of the total count). The total of *Veillonella* isolates identified by the *Veillonella* genus-specific PCR primer. Individual species as a percentage of the number of isolates identified by one-step PCR with the species-specific primer sets for each subject (*n* = 5) from saliva of the good oral hygiene group.

Subject			Total number		Isolated *Veillonella* species							
Name	Age	Sex	All bacteria	*Veillonella* spp.	Total number	*V. atypica*	*V. denticaruisi*	*V. dispar*	*V. parvula*	*V. rogosae*	*V. tobetsuensis*	Unknown
			CFU/mL	CFU/mL	(100%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
S8	13	F	7.60E+07	3.00E+05	3	0	0	0	1 (33.3)	2 (66.7)	0	0
S9	6	M	2.04E+08	5.00E+06	5	2 (40.0)	0	0	0	1 (20.0)	0	2 (40.0)
S12	6	F	3.52E+08	1.20E+06	12	0	0	4 (33.3)	0	8 (66.7)	0	0
S25	6	F	2.70E+07	2.00E+06	20	2 (10.0)	0	3 (15.0)	4 (20.0)	9 (45.0)	0	2 (10.0)
S28	8	M	3.20E+07	1.70E+02	17	3 (17.6)	0	4 (23.5)	1 (5.9)	8 (47.1)	0	1 (5.9)

Table 2. Ratio of the number of isolates of each species to the total number of *Veillonella* isolate in saliva from the moderate oral hygiene group. The colony-forming units (CFU) of all anaerobic bacteria on the brain heart infusion agar and *Veillonella* strains on *Veillonella* agar (detection limit <0.1% of the total count). The total of *Veillonella* isolates identified by the *Veillonella* genus-specific PCR primer. Individual species as a percentage of the number of isolates identified by one-step PCR with the species-specific primer sets for each subject (*n* = 5) from saliva of the moderate oral hygiene group.

Subject			Total number		Isolated *Veillonella* species							
Name	Age	Sex	All bacteria	*Veillonella* spp.	Total number	*V. atypica*	*V. denticaruisi*	*V. dispar*	*V. parvula*	*V. rogosae*	*V. tobetsuensis*	Unknown
			CFU/mL	CFU/mL	(100%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
S1	9	M	1.04E+08	2.00E+06	20	3 (15.0)	0	11 (55.0)	2 (10.0)	4 (20.0)	0	0
S3	4	M	5.30E+08	1.20E+07	12	0	0	3 (25.0)	1 (8.3)	4 (33.3)	0	4 (33.3)
S10	7	M	4.16E+08	9.00E+07	9	0	0	0	5 (55.6)	3 (33.3)	0	1 (11.1)
S29	6	F	4.46E+07	2.00E+05	20	2 (10.0)	0	5 (25.0)	2 (10.0)	10 (50.0)	0	1 (5.0)
S32	14	F	3.90E+06	2.00E+04	20	0	0	5 (25.0)	0 (0.0)	15 (75.0)	0	0

Table 3. Ratio of the number of isolates of each species to the total number of *Veillonella* isolated in saliva from the poor oral hygiene group. The colony-forming units (CFU) of all anaerobic bacteria on the brain heart infusion agar and *Veillonella* strains on *Veillonella* agar (detection limit <0.1% of the total count). The total of *Veillonella* isolates identified by the *Veillonella* genus-specific PCR primer. Individual species as a percentage of the number of isolates identified by one-step PCR with the species-specific primer sets for each subject (*n*=5) from saliva of the poor oral hygiene group.

| Subject			Total number		Isolated *Veillonella* species							
Name	Age	Sex	All bacteria	*Veillonella* spp.	Total number	*V. atypica*	*V. denticaruisi*	*V. dispar*	*V. parvula*	*V. rogosae*	*V. tobetsuensis*	Unknown
			CFU/mL	CFU/mL	(100%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
S15	7	M	4.00E+09	1.40E+08	14	0	0	3 (21.4)	1 (7.1)	8 (57.1)	0	2 (14.3)
S16	9	F	4.64E+09	3.00E+08	3	0	0	0	0	3 (100.0)	0	0
S17	10	F	7.84E+09	5.00E+08	5	3 (60.0)	0	0	0	1 (20.0)	0	1 (20.0)
S21	8	F	1.50E+09	1.00E+08	10	0	0	0	8 (80.0)	0	0	2 (20.0)
S30	8	F	4.41E+09	9.00E+08	9	0	0	2 (22.2)	4 (44.4)	2 (22.2)	0	1 (11.1)
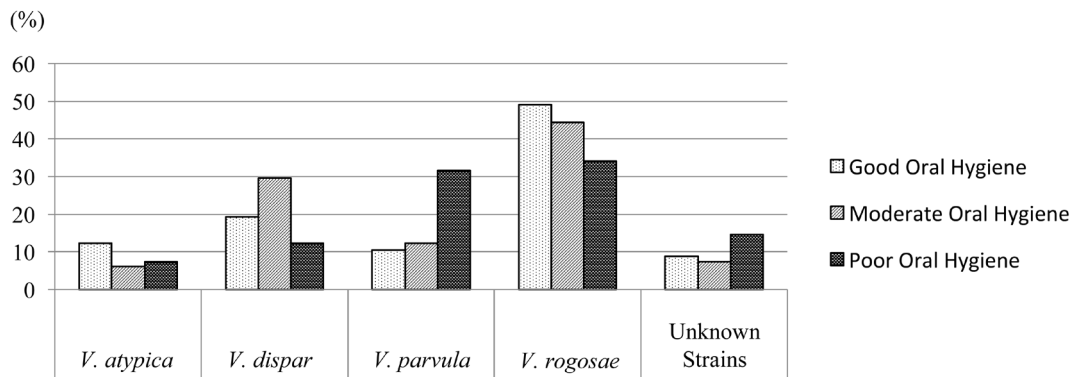
Figure 1. Total isolated number of each *Veillonella* species isolated. Data expressed as percentage of total the total isolated number as *Veillonella* in samples from saliva in good, moderate and poor oral hygiene groups.

species and the 10 unknown strains is shown in the Figure 2. According to this data, the most closely related species was *V. infantium*, although the 10 unknown strains formed three clusters. The DNA sequence similarity of the 10 unknown *Veillonella* strains to *V infantium* JCM 31738[T] (LC191258) ranged from 97.1 to 99.7%.

Discussion

It was previously reported that a higher number of anaerobic bacteria was detected on BHI agar in saliva from Thai children with poor oral hygiene than those with good and moderate oral hygiene[20]. This prior study demonstrated that oral *Veillonella* isolates were detected at a twofold higher frequency in the saliva of Thai children with poor rather than good or moderate oral hygiene[20]. Here, it was demonstrated that the number of anaerobic bacteria on BHI agar and *Veillonella* species on the selective medium increased in saliva of Japanese children with worsening oral hygiene status. Therefore, the detection level of anaerobic bacterial strains and oral *Veillonella* strains in saliva from Japanese children with good, moderate and poor oral hygiene status was similar to that from Thai children.

Using the Illumina MiSeq platform, Mashima *et al.* demonstrated that *Streptococcus* and *Veillonella* species were the predominant bacterial species in the saliva microbiome of Thai children, but that the proportion of *Streptococcus* decreased while that of *Veillonella* increased with poor oral hygiene status[5]. They also found that *Veillonella* species were detected predominantly in the tongue microbiome of Thai children with poor oral hygiene status compared to those with good or moderate oral hygiene status[5]. Taken together with the results of the present study, it is possible that *Veillonella* species could be a biomarker of oral hygiene status for Thai and Japanese children.

This study demonstrated that *V. rogosae* was the predominant species detected in all groups of Japanese children (Figure 1). Beighton *et al.* reported *V. rogosae* as one of the predominant *Veillonella* species in tongue biofilms of healthy adults in the UK[24]. A previous study also showed that *V. rogosae* was the predominant *Veillonella* species in tongue biofilms of the children in Thailand[12]. Recently, Theodorea *et al.* isolated 1,609 *Veillonella* strains from saliva samples of Thai children divided
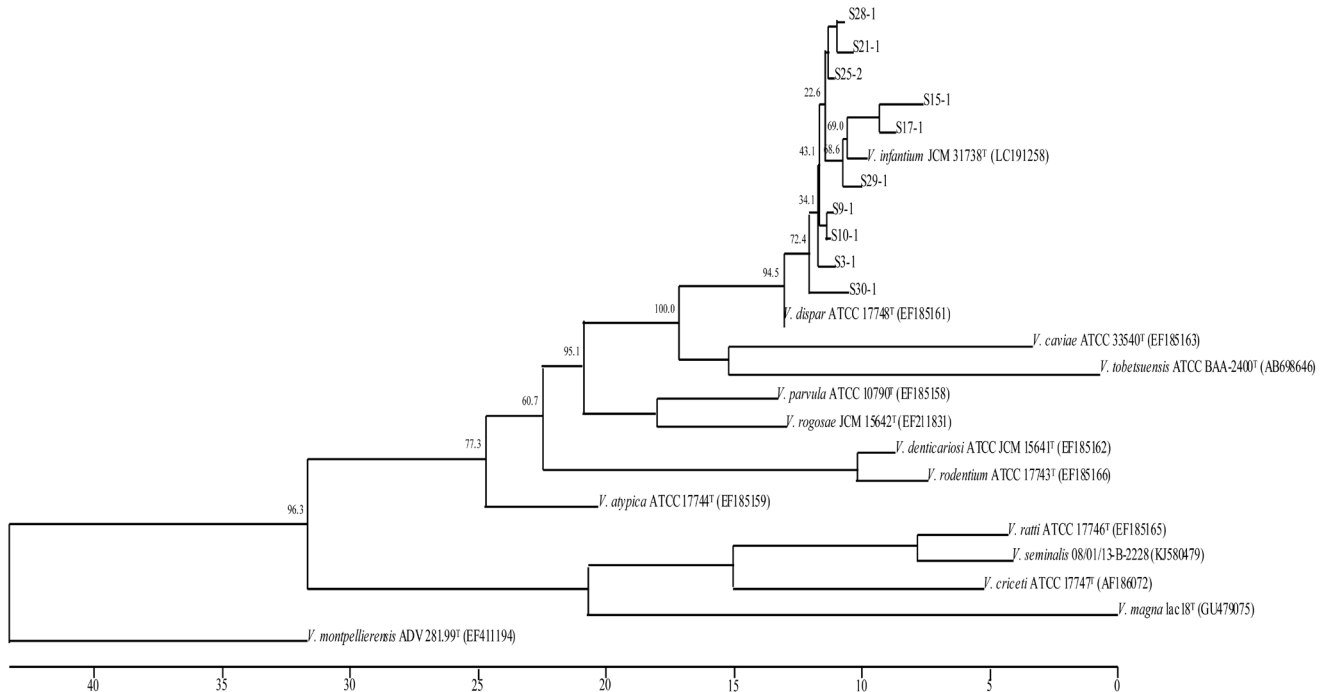
Figure 2. Neighbor-joining tree showing the relationship between the 10 unknown *Veillonella* strains and the type strains of established species of genus *Veillonella*, with accession numbers. Bootstrap values were indicated at corresponding nodes. The scale bar indicated the number of nucleotide substitutions per 100 residues.

into three groups: good, moderate and poor oral hygiene status[20]. Then, 1,442 of 1,609 strains were detected by the one-step PCR method with the species-specific primer sets for *V. atypica*, *V. denticariosi*, *V. dispar*, *V. parvula*, *V. rogosae*, or *V. tobetsuensis*. They reported that *V. rogosae* was the predominant species detected in all groups[20]. These results of the previous and present studies indicate that *V. rogosae* is the predominant oral *Veillonella* species in the human saliva and tongue biofilm.

Furthermore, this study showed that the detection rate of *V. rogosae* decreased as oral hygiene quality decreased: 49.1%, 44.4%, and 34.1% in the good, moderate, and poor oral hygiene groups, respectively (Figure 1). Similar results were obtained from saliva samples of Thai children were also reported by Theodorea *et al.*[20]. Based on these results, it was demonstrated that the detection rate of *V. rogosae* decreased with aggravation of oral hygiene status in Japanese and Thai children. Additionally, Arif *et al.* detected *V. rogosae* only in carious-free lesions of dental plaques[13]. All these data suggest that a human oral cavity with good hygiene status is suitable habitat for *V. rogosae*.

Conversely, the detection rate of *V. parvula* in the poor (31.7%) oral hygiene was significantly higher than that in the good (10.5%) and moderate (12.3%) oral hygiene groups, in this study with Japanese children. This result is conformed with data from another study, in which *V. parvula* was most frequently detected in saliva of Thai children with poor oral hygiene status[20]. Previously, it was also reported that *V. parvula* was frequently detected in periodontal pockets[27] and active carious-lesions[28]. These data

suggest that oral cavities with poor hygiene status are suitable environments for *V. parvula*.

In this study, 179 strains were isolated members of the genus *Veillonella* from saliva of 15 Japanese children, *V. denticariosi* and *V. tobetsuensis* were not found in any samples. In the case of saliva samples from Thai children, the detection rate of *V. denticariosi* (0.4%) and *V. tobetsuensis* (1.7%) were very low. In this study 1,609 *Veillonella* strains were isolated from 107 Thai children[20]. Similarly, it was reported that *V. denticariosi* was not detected in any of the tongue biofilms of Thai children[12], and *V. denticariosi* was detected in tongue biofilm of only one young Japanese adult[17]. Therefore, *V. denticariosi* may be the least prevalent oral *Veillonella* species in the saliva and tongue microbiome. On the other hand, *V. tobetsuensis*, was not detected in saliva from Thai children with good oral hygiene status. However, the detection rate of *V. tobetsuensis* was 14.3% and 17.8% in the saliva of Thai children with moderate and poor oral hygiene, respectively (20). Similarly, it was demonstrated that the prevalence of *V. tobetsuensis* ranged from 7.6% to 20.0% in tongue biofilm samples from Japanese adults[16]. Therefore, these data suggest that *V. tobetsuensis* may be potential to co-occur with other *Veillonella* species in saliva and tongue biofilms.

In the present study with saliva samples of Japanese children, 17 (9.5%) of 179 strains confirmed as member of genus *Veillonella* were not belong to any established *Veillonella* species as unknown species. Theodorea *et al.*, also reported

that 167 (10.4%) of 1,609 *Veillonella* isolates from saliva of Thai children could not be assigned to any species of the genus *Veillonella*[20]. Furthermore, it was reported that 43 (9.7%) of the 442 *Veillonella* isolates from periodontal pockets and gingival sulcus could not be identified as any of the known *Veillonella* species. These data may indicate that other novel *Veillonella* species inhabit human oral cavities, although only six species were detected as oral *Veillonella* species up to this point. Further phylogenetic studies are needed to evaluate the possibilities of novel *Veillonella* species.

In 2018, Mashima *et al.*[11] proposed *V. infantium* as a novel species isolated from saliva of Thai children, representing a seventh oral *Veillonella* species. Therefore, for phylogenetic analysis of the unknown *Veillonella* strains isolated in this study, the *rpoB* sequences of type strains of the established *Veillonella* species, including *V. infantium* JCM 31738[T], were examined. Consequently, 10 unknown *Veillonella* strains analysed in this study formed three clusters distinct from *V. dispar*, the most closely related species was *V. infantium*. Further studies are required to assign these strains most accurately.

In conclusions, this is the first study to identify oral *Veillonella* at the species level in the saliva of Japanese children divided into three oral hygiene status groups: good, moderate and poor group. Although *V. denticariosi* and *V. tobetsuensis* were not found in any groups in this study because of small number of subjects, the distribution and frequency of *V. atypica*, *V. dispar*, *V. parvula* and *V. rogosae*, were mostly the same as those in the saliva from Thai children divided into the aforementioned oral hygiene status groups. Additionally, the results of this study demonstrate that changes in the ratio of some *Veillonella* species, such as an increase of *V. parvula* and decrease of *V. rogosae* in those with poor oral hygiene, can be a useful indicator of oral hygiene status, as with results obtained in the study of saliva taken from Thai children. The present study also showed that approximately 10% of the isolated *Veillonella* strains were not classified to any *Veillonella* species, and that they will be assigned to *V. infantium* or novel *Veillonella* species after further studies.

Data availability
16S rRNA sequences of the 10 unknown *Veillonella* strains are available from GenBank, accession numbers: LC467206 (S9-1), LC467207 (S28-1), LC467208 (S25-1), LC467209 (S17-1), LC467210 (S15-1), LC467211 (S29-1), LC467212 (S10-1), LC467213 (S30-1), LC467214 (S21-1), LC467215 (S3-1).

Figshare: Raw Data Saliva Japanese Children.xlsx. https://doi.org/10.6084/m9.figshare.7856657.v1[26].

This project contains the number of colony-forming units and total number of *Veillonella* strains isolated from each child.

Data on Figshare are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgments

References

1. Bjarnsholt T, Alhede M, Alhede M, *et al.*: **The *in vivo* biofilm.** *Trends Microbiol.* 2013; **21**(9): 466–474.
   PubMed Abstract | **Publisher Full Text**

2. Nyvad B, Kilian M: **Microbiology of the early colonization of human enamel and root surfaces *in vivo*.** *Scand J Dent Res.* 1987; **95**(5): 369–380.
   PubMed Abstract | **Publisher Full Text**

3. Maddi A, Scannapieco FA: **Oral biofilms, oral and periodontal infections, and systemic disease.** *Am J Dent.* 2013; **26**(5): 249–254.
   PubMed Abstract

4. Zhang CZ, Cheng XQ, Li JY, *et al.*: **Saliva in the diagnosis of diseases.** *Int J Oral Sci.* 2016; **8**(3): 133–137.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

5. Mashima I, Theodorea CF, Thaweboon B, *et al.*: **Exploring the salivary microbiome of children stratified by the oral hygiene index.** *PLoS One.* 2017; **12**(9): e0185274.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

6. Jean-Philippe C: **Genus *Veillonella* Prèvot 1933, 118[AL] emend. Mays, Holdeman, Moore, Rogosa and Johnson 1982, 35.** In *Bergey's Manual of Sysematic Bacteriology.* Second Edition, Edited by Paul DV, George MG, Dorothy J, Noel RK, Wolfgang L, Fred AR, Karl-Heinz S & William BW. Springer: Dordrecht, Heidelberg, London & New York, 2009; **3**: 1059–1065.
   Reference Source

7. Isner-Horobeti ME, Lecocq J, Dupeyron A, *et al.*: ***Veillonella* discitis. A case report.** *Joint Bone Spine.* 2006; **73**(1): 113–5.
   PubMed Abstract | **Publisher Full Text**

8. Liu JW, Wu JJ, Wang LR, *et al.*: **Two fatal cases of *Veillonella* bacteremia.** *Eur J Clin Microbiol Infect Dis.* 1998; **17**(1): 62–4.
   PubMed Abstract | **Publisher Full Text**

9. Marriott D, Stark D, Harkness J: ***Veillonella parvula* discitis and secondary bacteremia: a rare infection complicating endoscopy and colonoscopy?** *J Clin Microbiol.* 2007; **45**(2): 672–4.
   PubMed Abstract | **Publisher Full Text** | **Free Full Text**

10. Ready D, Bedi R, Mullany P, *et al.*: **Penicillin and amoxicillin resistance in oral *Veillonella* spp.** *Int J Antimicrob Agents.* 2012; **40**(2): 188–9.
    PubMed Abstract | **Publisher Full Text**

11. Mashima I, Liao YC, Miyakawa H, *et al.*: ***Veillonella infantium* sp. nov., an anaerobic, Gram-stain-negative coccus isolated from tongue biofilm of a Thai child.** *Int J Syst Evol Microbiol.* 2018; **68**(4): 1101–1106.
    PubMed Abstract | **Publisher Full Text**

12. Mashima I, Theodorea CF, Thaweboon B, *et al.*: **Identification of *Veillonella* Species in the Tongue Biofilm by Using a Novel One-Step Polymerase Chain Reaction Method.** *PLoS One.* 2016; **11**(6): e0157516.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

13. Arif N, Do T, Byun R, *et al.*: ***Veillonella rogosae* sp. nov., an anaerobic, Gram-negative coccus isolated from dental plaque.** *Int J Syst Evol Microbiol.* 2008; **58**(Pt 3): 581–584.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

14. Byun R, Carlier JP, Jacques NA, *et al.*: ***Veillonella denticariosi* sp. nov., isolated from human carious dentine.** *Int J Syst Evol Microbiol.* 2007; **57**(Pt 12): 2844–2848.
    PubMed Abstract | **Publisher Full Text**

15. Mashima I, Kamaguchi A, Miyakawa H, *et al*.: ***Veillonella tobetsuensis* sp. nov., an anaerobic, gram-negative coccus isolated from human tongue biofilms.** *Int J Syst Evol Microbiol.* 2013; **63**(Pt 4): 1443–1449.
    PubMed Abstract | **Publisher Full Text**

16. Mashima I, Nakazawa F: **Identification of *Veillonella tobetsuensis* in tongue biofilm by using a species-specific primer pair.** *Anaerobe.* 2013; **22**: 77–81.
    PubMed Abstract | **Publisher Full Text**

17. Mashima I, Kamaguchi A, Nakazawa F: **The distribution and frequency of oral *veillonella* spp. in the tongue biofilm of healthy young adults.** *Curr Microbiol.* 2011; **63**(5): 403–407.
    PubMed Abstract | **Publisher Full Text**

18. Periasamy S, Kolenbrander PE: **Central role of the early colonizer *Veillonella* sp. in establishing multispecies biofilm communities with initial, middle, and late colonizers of enamel.** *J Bacteriol.* 2010; **192**(12): 2965–2972.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

19. Kolenbrander PE, Moore LVH: **The genus *Veillonella*.** In: *The Prokaryotes.* (2nd edn), Springer, New York, USA. 1992; 2034–2047.
    Publisher Full Text

20. Theodorea CF, Mashima I, Thaweboon B, *et al*.: **Molecular detection of oral *Veillonella* species in the saliva of children with different oral hygiene statuses.** *Int J of Curr Microbiol Appl Sci.* 2017; **6**(7): 449–461.
    Publisher Full Text

21. Theodorea CF, Mashima I, Nakazawa F: **Prospects of novel species of oral *Veillonella* in human saliva.** *Adv Biotech Microbiol.* 2017; **5**(4): 1–3.
    Publisher Full Text

22. Greene JC, Vermillion JR: **The Simplified Oral Hygiene Index.** *J Am Dent Assoc.* 1964; **68**(1): 7–13.
    PubMed Abstract | **Publisher Full Text**

23. Rogosa M, Fitzgerald RJ, Mackintosh ME, *et al*.: **Improved medium for selective isolation of *Veillonella*.** *J Bacteriol.* 1958; **76**(4): 455–456.
    PubMed Abstract | **Free Full Text**

24. Beighton D, Clark D, Hanakuka B, *et al*.: **The predominant cultivable *Veillonella* spp. of the tongue of healthy adults identified using *rpoB* sequencing.** *Oral Microbiol Immunol.* 2008; **23**(4): 344–347.
    PubMed Abstract | **Publisher Full Text**

25. Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees.** *Mol Biol Evol.* 1987; **4**(4): 406–25.
    PubMed Abstract | **Publisher Full Text**

26. Djais AA: **Raw Data Saliva Japanese Children.xlsx.** *figshare.* Dataset. 2019.
    http://www.doi.org/10.6084/m9.figshare.7856657.v1

27. Mashima I, Fujita M, Nakatsuka Y, *et al*.: **The Distribution and Frequency of Oral *Veillonella* spp. Associated with Chronic Periodontitis.** *Int J Curr Microbiol App Sci.* 2015; **4**(3): 150–160.
    Reference Source

28. Arif N, Sheehy EC, Do T, *et al*.: **Diversity of *Veillonella* spp. from sound and carious site in children.** *J Dent Res.* 2008; **87**(3): 278–282.
    PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 5

Reviewer Report 16 September 2019

https://doi.org/10.5256/f1000research.22657.r53912

✔ **Takuichi Sato**

Division of Clinical Chemistry, Department of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Japan

OK

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 4

Reviewer Report 08 August 2019

https://doi.org/10.5256/f1000research.22192.r52215

✔ **Takuichi Sato**

Division of Clinical Chemistry, Department of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Japan

I have recognized and agreed with the revised parts in the Introduction.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

Reviewer Report 01 August 2019

? **Takuichi Sato**
Division of Clinical Chemistry, Department of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Japan

[Suggestions]
I recognized the revised parts in the Abstract (Background) as follows;

"However, the study regarding the identification and distribution of oral Veillonella are limited. The oral Veillonella community may affected by the differences in geographical location, age, diet, lifestyle, socio-economic status and oral hygiene status."

I think that the above-mentioned parts should be included in the Introduction, but I was unable to find the revised parts. The authors need to revise the Introduction, like as in the Abstract.

[Typographical errors]
Introduction (6th paragraph)
"difficult to idnetify" should read "difficult to identify".

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Aug 2019
Ariadna Djais, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia, Jakarta, Indonesia

The authors would like to thank the area editor and the reviewers for their precious time and valuable comments. We have carefully addressed all the comments.

Reviewer: Takuichi Sato, Division of Clinical Chemistry, Department of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Japan [Suggestions]

I recognized the revised parts in the Abstract (Background) as follows;

"However, the study regarding the identification and distribution of oral Veillonella are limited. The oral Veillonella community may affected by the differences in geographical location, age, diet, lifestyle, socio-economic status and oral hygiene status."

I think that the above-mentioned parts should be included in the Introduction, but I was unable to find the revised parts. The authors need to revise the Introduction, like as in the Abstract.

[Typographical errors]
Introduction (6th paragraph)
"difficult to idnetify" should read "difficult to identify".

Authors: Thank you very much for this direction.
1. We have changed the wording of the relevant in the Introduction 6th Paragraph, L 11, like as in the Abstract.
2. [Typographical errors] Introduction (6th paragraph) "difficult to idnetify" into "difficult to identify".
3. Introduction 7th Paragraph, L 2 was removed because in this study we are focused on demonstrating that the composition and proportion of oral *Veillonella* species in the saliva of Japanese children is correlated with different oral hygiene status.

The revised version of this paper has been submitted.

Thank you for taking the time and energy to help us improve this manuscript. We hope that you will find that these revisions rise to your expectation.

Competing Interests: No competing interests were disclosed.

Version 2

Reviewer Report 28 June 2019

?

Takuichi Sato

Division of Clinical Chemistry, Department of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Japan

The authors have described the following in the Response, but I am unable to find the differences in the abstract (and/or introduction).

"We have changed the wording of the relevant in the abstract to express the reason why did we want to know the detection rate and distribution of oral *Veillonella* species in Japan. The reasons are the study regarding the identification and distribution of oral *Veillonella* are limited; also the oral *Veillonella* community may affected by the differences in geographical location, age, diet, lifestyle, socio-economic status and oral hygiene status."

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 27 June 2019

https://doi.org/10.5256/f1000research.21331.r50156

Juni Handajani 🆔

Department of Oral Biology, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia

Thank you for the revision. I now understand the analysis statistic correctly, no more changes are required and I approved the article.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Area of my expertise are oral biology and immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 23 May 2019

https://doi.org/10.5256/f1000research.20250.r48113

? **Takuichi Sato**

Division of Clinical Chemistry, Department of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Japan

Suggestions:

Abstract and Introduction:
I think that the manuscript, in the Abstract and Introduction, needs to express rationales of this study in more detail, such as, why did the authors want to know the detection rate and distribution of oral *Veillonella* species in saliva of Japanese children? Why did the authors want to compare the results with those of Thai children?

Figure 1 and Tables 1-3:
I think that the data in Figure 1 and Tables 1-3 are overlapping, and Tables 1-3 could be deleted from the manuscript, because the authors mainly stated the total and the mean (proportions) of the isolates of *Veillonella* species in the Results and Discussion.

Minor:
The authors should check the meaning of the phrase "although":

P. 7, Discussion: in the 6th paragraph
L. 1-2: "although 179 strains were isolated members of the genus *Veillonella* from saliva of 15 Japanese children, …".

P. 7, Discussion: in the 6th paragraph
L. 6-7: "although in this study 1,609 *Veillonella* strains were isolated from 107 Thai children".

P. 8, Discussion: in the 8th paragraph
L. 7-8: "although 10 unknown *Veillonella* strains analysed in this study formed three clusters distinct from *V. dispar*, …".

Typographical errors;
P. 4, Results: Species identification
L. 6: "numberof" should read "number of". (Put a space in between.)

P. 7, Discussion: in the 5th paragraph
L. 4: "conforms" should read "conformed".

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 May 2019
Ariadna Djais, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia, Jakarta, Indonesia

Abstract and Introduction:
I think that the manuscript, in the Abstract and Introduction, needs to express rationales of this study in more detail, such as, why did the authors want to know the detection rate and distribution of oral *Veillonella* species in saliva of Japanese children? Why did the authors want to compare the results with those of Thai children?
Response: Thank you very much for this direction. We have changed the wording of the relevant in the abstract to express the reason why did we want to know the detection rate and distribution of oral *Veillonella* species in Japan. The reasons are the study regarding the identification and distribution of oral *Veillonella* are limited; also the oral *Veillonella* community may affected by the differences in geographical location, age, diet, lifestyle, socio-economic status and oral hygiene status.
Figure 1 and Tables 1-3:
I think that the data in Figure 1 and Tables 1-3 are overlapping, and Tables 1-3 could be deleted from the manuscript, because the authors mainly stated the total and the mean (proportions) of the isolates of *Veillonella* species in the Results and Discussion.
Response: Thanks for raising this important point. However, we consider to state
- the Table 1-3 as Ratio of the number of isolates of each species from each subject.
- Figure 1 was indicated to express the Total isolated number of each *Veillonella* species from each group of oral hygiene.

Minor:
The authors should check the meaning of the phrase "although":

P. 7, Discussion: in the 6th paragraph
L. 1-2: "although 179 strains were isolated members of the genus *Veillonella* from saliva of 15 Japanese children, …".
Response: the word of "although" has been removed

P. 7, Discussion: in the 6th paragraph
L. 6-7: "although in this study 1,609 *Veillonella* strains were isolated from 107 Thai children".
Response: the word of "although" has been removed

P. 8, Discussion: in the 8th paragraph
L. 7-8: "although 10 unknown *Veillonella* strains analysed in this study formed three clusters distinct from *V. dispar*, …".
Response: the word of "although" has been removed
Typographical errors;
P. 4, Results: Species identification
L. 6: "numberof" should read "number of". (Put a space in between.)

Response: the word of "numberof" has been revised to "number of"

P. 7, Discussion: in the 5th paragraph
L. 4: "conforms" should read "conformed".

Response: the word of "conforms" has been revised to "conformed"
Thank you for taking the time and energy to help us improve this manuscript. We hope that you will find these revision rise to your expectation.

Competing Interests: No competing interests were disclosed.

Reviewer Report 20 May 2019

? **Juni Handajani** (iD)

Department of Oral Biology, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia

This study is good for identification and phylogenetic analysis of oral *Veillonella* species isolated from saliva samples of children in Japan. The aim of this study was to analyze the composition and proportion

of oral *Veillonella* species in the saliva of Japanese children compared to previous studies in Thailand.

The manuscript is certainly well written but I have some major concerns on the data analysis:
1. The number of samples in this study was fewer (15 children) compared to the number of samples in the previous study from Thailand (107 children). It is suggested that the analysis uses proportions so that it can describe the oral *Veillonella* species according to the number of samples.

2. To find out the comparison of the results of this study with the results of a previous study from Thailand, a correlation analysis is suggested.

3. It is also necessary to add the results of a correlation analysis between the results of *Veillonella*'s oral identification and oral health status.

Minor comment:
1. Some references used are older than 10 years. I suggest to use current references from at least the last 10 years.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Area of my expertise are oral biology and immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 May 2019

Ariadna Djais, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia, Jakarta, Indonesia

This study is good for identification and phylogenetic analysis of oral Veillonella species isolated from saliva samples of children in Japan. The aim of this study was to analyze the composition and proportion of oral *Veillonella* species in the saliva of Japanese children compared to previous studies in Thailand.

Response: Thank you for your valuable comment. The aim of this study was focus to analyze the composition and proportion of oral *Veillonella* species in the saliva of Japanese children. Furthermore, we compared the results with some reports such as in Thailand. The revise version has been submitted.

The manuscript is certainly well written but I have some major concerns on the data analysis:

1. The number of samples in this study was fewer (15 children) compared to the number of samples in the previous study from Thailand (107 children). It is suggested that the analysis uses proportions so that it can describe the oral *Veillonella* species according to the number of samples.

Response: These children visited to the Dental Hospital, Health Sciences University of Hokkaido for dental examination, over a period between 2016 and 2017. Based on the evaluation by the Simplified Oral Hygiene Index, they were divided into three groups, good, moderate and poor. But, among many children, only five children had poor oral hygiene status. Therefore, we used these five children as poor group. And we have selected five children from good and moderate groups, without distinction. As the results, the total subject : 15 subjects.

1. To find out the comparison of the results of this study with the results of a previous study from Thailand, a correlation analysis is suggested.
2. It is also necessary to add the results of a correlation analysis between the results of Veillonella's oral identification and oral health status.

Response: Thank you for another good point at No. 2 and No.3. The research of oral *Veillonella* were very limited. The analysis was use in this study : descriptive analysis with mean value to determine the distribution and proportion of oral *Veillonella*, then we compare the characteristic of oral veillonella distribution with the previous study.

Minor comment:

1. Some references used are older than 10 years. I suggest to use current references from at least the last 10 years.

Response: As we mentioned before, that the research of oral *Veillonella* were very limited. Therefore, some of references were used older that 10 years.

Thank you for taking the time and energy to help us improve this manuscript. We hope that you will find these revision rise to your expectation.

Competing Interests: No competing interests were disclosed.

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