

PROFILING THE TOTAL NUMBER OF BACTERIA IN THE DIGESTIVE TRACT OF CHILDREN WITH STUNTING CONDITIONS

Hasnawati S^{1*}, Syamsuryana Sabar¹, Andi Sulfyana Sumang³

¹ Universitas Megarezky, Makassar, Indonesia

*Email:watihasnawati08@gmail.com

Abstract

Stunting is a failure to thrive in children under five caused by various factors, including lack of nutritional intake, experiencing repeated infections, and inadequate psychosocial stimulation. The excess of bacteria, especially pathogenic bacteria in the gastrointestinal tract, causes inflammation, gut microbiome imbalance, and malabsorption of nutrients; this impacts growth disturbance resulting in stunting. Stunting can occur in the first 1000 days of birth and is related to many factors, including socioeconomic status, food intake, infection, mother's nutritional status, infectious diseases, micronutrient deficiencies, and the environment. Stunting does not only have an impact on individuals who experience it but also on the wheels of the economy and national development. This is because human resources with stunting are of lower quality than human resources without stunting. Using the qPCR method, this study aims to determine the total number of bacteria in the digestive tract of checking toddlers in Bone-bone Village and Pepandungan Village, Baraka District, Enrekang Regency. This study used a molecular method, namely the Quantitative PCR (Q-PCR) method with 16SrRNA primers to detect total bacteria. The subjects of this study were stunted toddlers in Bone-Bone Village and Pepandungan Village, totaling 21 people plus ten controls. This type of research is a quantitative descriptive study using a cross-sectional study design to identify the total number of bacteria present in the feces of stunted toddlers. The results obtained from the q-PCR method showed that the average total number of small children's bacteria was less, namely 2.28 log DNA Copies/gram compared to normal children of 5.95 log DNA Copies/gram with a difference between the two subject groups of 3 .67 log DNA Copies/gram. The results obtained show that bacteria do not cause the incidence of stunting in the two villages.

Keywords: Stunting, Gut Microbiome, 16SrRNA, q-PCR

Introduction

The World Health Organization (WHO) defines stunting as a condition in which a child's growth is impaired due to poor nutrition, repeated infections, and inadequate psychosocial stimulation (Saadah, 2020). Stunting (short) is one of the nutritional problems faced by the world, especially in developing countries such as Indonesia. Stunting has long-term effects on individuals and society, such as decreased cognitive and physical development, reduced productive capacity, poor health, and an increased risk of degenerative diseases such as diabetes, so motor and mental development is hampered (WHO, 2014b).

Stunting can occur in the first 1000 days of birth and is associated with many factors, including socioeconomic status, food intake, infection, maternal nutritional status, infectious diseases, micronutrient deficiencies, and the environment. Stunting does not only have an impact on individuals who experience it but also on the wheels of the economy and nation-building. This is because Human Resources (HR) with stunting have lower quality compared to HR without stunting (WHO, 2018).

Stunting globally affects about 162 million children under the age of 5 years (WHO, 2014b). In 2016 there were 154.8 (22.9%) million children under the age of 5 years suffering from stunting, 87 million stunted children in Asia, 59 million in Africa, and 6 million in Latin America and the Caribbean. Five sub-regions have child stunting rates that exceed 30%,

namely West Africa (31.4%), Central Africa (32.5%), East Africa (36.7%), South Asia (34.1%) and Oceania (38.3%, excluding Australia and New Zealand) (WHO, 2018). In the WHO Southeast Asia/SEAR (South-East Asia Regional) region, of the 11 countries included, Indonesia ranks 3rd after Timor Leste and India, with a stunting rate of 36.4% in 2005-2017. For Indonesia itself, based on Nutrition Status Monitoring (PSG) data in 2015-2017, short toddlers have the highest prevalence compared to other nutritional problems such as malnutrition, thinness, and obesity. In 2016, stunting cases touched 27.5%, increased to 29.6% in 2017 and decreased to 27.67% in 2019 (PuAustin2018; Sudikno *et al.*, 2019).

South Sulawesi Province, in 2018, recorded 33.8% stunting cases and in 2019 there was a decline to 30.1%, where Enrekang Regency had the highest number of 44.8% and the lowest was 16.8% in North Luwu Regency Enrekang district is in the list of 1,000 stunting priority villages in 2018 (Pusdatin, 2018; Sudikno *et al.*, 2019). Based on data obtained at the Baraka Health Center, Enrekang Regency in 2016, Baraka District had a stunting prevalence of 41.06%. In 2017 it decreased slightly to 39.1% and continued to decline until 2018 to 36.4%. In 2019 there was an increase and touched the figure of 46% (PKM Baraka Enrekang, 2021).

Enrekang Regency is well known for its abundant agricultural products such as shallots, coffee, and salak. However, the farm sector which the Enrekang mainly cultivate is rice, corn, tubers and beans. Plus fruit plants include mango, orange, avocado, jackfruit, papaya, rambutan, breadfruit, durian and banana (DPMPTSP Susel 2021).

Although food sources are abundant to fulfill nutritional sources in Enrekang Regency, stunting in children under five is still common. Another factor that causes the high stunting in Enrekang Regency is probably due to parenting patterns and poor sanitation (Normaisa *et al.*, 2020).

Gastrointestinal (GI) tract = hosts a large number of microbes, including bacteria, viruses, fungi, and archaea. Interestingly, the microbial cells in the gut (10¹⁴ cells) exceed the number in the human body (10¹³) (Ghoshal & Ghoshal, 2017). Poor sanitation conditions in the living environment can cause problems with gastrointestinal diseases and infections or environmental enteric dysfunction, such as diarrhea. *Shigella* sp., *Salmonella* sp., *Escherichia coli*, *Cryptosporidium*, sp. and *Campylobacter* are microorganisms that often cause (Utami & Luthfiana, 2016). The excess of bacteria, especially pathogenic bacteria in the gastrointestinal tract, causes inflammation, microbiota imbalance and malabsorption of nutrition has an impact on disrupting growth, causing stunting (Helmyati *et al.*, 2017).

Examining the number of bacteria in stool samples can use the culture method. In addition to the culture method, the examination of the number of bacteria can also use a molecular method, namely the PCR examination with the Quantitative-PCR (q-PCR) method (Bakri *et al.*, 2015; Purnamasari, 2019).

Excessive bacteria take up nutrients in the child's body to multiply, causing the absorption of nutrients in the child's digestive tract to be disrupted; this allows slowing the child's growth and development. Some of the presented problems prompted researchers to research the number of bacteria using stool samples in children with stunting.

Methods

This research is quantitative descriptive research with a research design that uses a cross-sectional study approach. The samples in this study that met the inclusion criteria were children aged 1-4 years who were declared stunted based on anthropometric measurements and stool samples taken <3 hours after defecation. While the exclusive the same time, criteria were toddlers who consumed antibiotics <1 month and had diarrhea and feces mixed with urine or toilet water were five samples for bone-bone village and 16 stunting samples for Pepandungan Village. As well as taking samples for children who have not stunted as many as 20 samples as a control material. So that the total sample of this study was 31 samples using the purposive sampling technique, data collection techniques use observation by taking a sample of the phases of each sample.

The data collection procedure is carried out in the following manner Submit an application to conduct research to the Chancellor of Megarezky University. After obtaining a research permit, proceed with submitting a research permit to Balitbang, Enrekang Regency, South Sulawesi; next, submit a research permit to the Enrekang District Health Office, South Sulawesi. After obtaining permission from the Health Office, proceed with applying for a research permit to the

Baraka Health Center, Enrekang Regency, South Sulawesi. Finally, it is continued to the selected respondents after giving informed consent ,and the respondents agree to participate in the research.

Process of data analysis in this study using univariate, bivariate analysis. Furthermore, it passed the ethics test process with letter number: 018.E/07.091056/2021 by Hasanuddin University Research Ethics. Commission Sample working procedure Sample handling Respondents were asked to collect feces into a sample pot as much as and then freeze the sample ($\pm 2-4^{\circ}\text{C}$) and put it in a coolbox to prepare for sending samples to the laboratory. Sample Extraction In a tube containing a bead, 200 mg of sample was added and, 1000 L of Lysis Buffer L and 100 L of Lysis Additive A were added and then vortexed for 10-15 seconds until homogeneous. Then it was homogenized in a magnetic device at a speed of 6000 for 4 minutes. Then centrifugation was carried out for 4 minutes at a speed of 12,000 rpm. Transfer the clear supernatant to a 1.5 mL microtube for 600 L. Add 100 L of Binding Buffer I, then vortex for 5-10 seconds and incubated at cold temperature (4°C) for 10 minutes, then centrifuge at 12,000 rpm for 4 minutes. Transfer 500 L of supernatant to a new 1.5 mL microtube, and add 500 L of 96% ethanol and vortex for 5-10 seconds. Transfer 500 L of lysate to a spin column tube that has been paired with a collection tube, centrifuge at 12,000 rpm for 2 minutes, discard the supernatant in the collection tube and then reinstall it, transfer the remaining lysate and centrifuge again at 12,000 rpm for 2 minutes. , discard the supernata and reinstall the collection tube. Add 500 L of Binding Buffer C then centrifuge at 12,000 rpm for 2 minutes, remove the supernatant and reinstall the collection tube. Next, add 500 L Wash Solution A, centrifuge for 2 minutes at 12,000 rpm, remove the supernatant and reinsert the collection tube, then repeat the steps again. Then centrifuged at 12,000 rpm for 2 minutes without adding reagents. Transfer the spin column to the elution tube and add 100 L of Elution Buffer B, incubate for 1 minute at room temperature, then centrifuge for 2 minutes at 12,000 rpm,; theDNA is ready for use in the next stage or stored in the freezer at -21°C . DNA amplification Make a concentration of 10 pmol from a concentration of 100 pmol of the primary solution by transferring 10 L of the reverse and forward primers to separate tubes and adding 90 of the solution (nuclease-free water/ddH₂O). Add 1 L of forward and reverse primers, 5 L of master mix, 1 L of H₂O and 2 L of extracted DNA in microtube PCR. Set of q-PCR equipment with a temperature of 95°C for 10 minutes for preheating, 39 cycles with a denaturation temperature of 95°C for 15 seconds, annealing 60°C for 1 minute and 1 cycle of melting curve with a temperature of 65°C and 95°C for 5 respectively. second. Select the tube rack on the screen where the PCR microtubes are placed, set to the settings shown on the screen for a volume per tube of 10 L and run the tool.

Result

Table 1. Characteristics of Respondents' Mother

| Objective Criteria | Stunting Group | | Control Group | |
|------------------------|----------------|----------------|---------------|----------------|
| | Frequency (f) | Percentage (%) | Frequency (f) | Percentage (%) |
| Age | | | | |
| 15- 25 | 9 | 29% | 3 | 9% |
| 26- 35 | 7 | 23% | 5 | 16% |
| 35- 39 | 5 | 16% | 2 | 6% |
| Education | | | | |
| primary school | 13 | 42% | 2 | 6% |
| Junior high school | 7 | 23% | 2 | 6% |
| Senior High School | 1 | 3% | 4 | 13% |
| Bachelor D1/ D2/ D3 | - | - | 2 | 6% |
| Work | | | | |
| Doesn't work | 5 | 16% | 6 | 19% |
| Working | 16 | 52% | 4 | 13% |
| Economic Status | | | | |
| \geq Rp. 2.500.000 | - | - | 10 | 32% |
| \leq Rp. 2.500.000 | 21 | 68% | - | - |

Table 1, the age criteria for the stunting group were mostly 9 people (29%) aged 15-25 years and 5 people (16%) for the control group aged 26-35 years. In terms of maternal education criteria for the stunting group, most of them had graduated from elementary school, namely 13 people (42%) and the control group had graduated from high school, 4 people (13%). The job criteria for the stunting group were dominated by working criteria, namely 16 people (25%). For the control group, the criteria for not working were six people (19%). As for the economic status of the stunting group respondents, 21 people (68%) had income \leq Rp. 2,500,000, and for the control group respondents, name ten ten a person (32%) had income \geq Rp. 2,500,000.

Table 2. Characteristics of Respondents

| Objective Criteria | Stunting Group | | Control Group | |
|--------------------|----------------|----------------|---------------|----------------|
| | Frequency (f) | Percentage (%) | Frequency (f) | Percentage (%) |
| Gender | | | | |
| Male | 14 | 45% | 4 | 13% |
| Woman | 7 | 23% | 6 | 19% |
| Age | | | | |
| One year | 6 | 19% | 5 | 16% |
| Two years | 3 | 10% | 2 | 6% |
| Three years | 7 | 23% | 1 | 3% |
| Four years | 5 | 16% | 2 | 6% |

Table 2. Characteristics of respondents based on gender: 14 people (45%) were male for the stunting group, while six people (19%) were female for the control group. Based on the age of children under five, the highest was seven people (23%) aged three years for the stunting group and as many as five people (16%) aged one year for the control group.

Table 3. Characteristics of Respondents Based on Anthropometric Index

| Objective Criteria | Stunting Group | | Control Group | |
|---------------------------------|----------------|----------------|---------------|----------------|
| | Frequency (f) | Percentage (%) | Frequency (f) | Percentage (%) |
| Height by Age (TB/U) | | | | |
| Very short | 11 | 33% | - | - |
| Short | 12 | 36% | - | - |
| Normal | - | - | 5 | 16% |
| Tall | - | - | 5 | 16% |
| Weight by Height (BB/TB) | | | | |
| Very thin | 12 | 38% | - | - |
| Thin | 9 | 29% | - | - |
| Normal | - | - | 5 | 16% |
| Fat | - | - | 5 | 16% |
| Nutrition State | | | | |
| Malnutrition | 12 | 38% | - | - |
| Less nutrition | 9 | 29% | - | - |
| Normal | - | - | 5 | 16% |
| Obesity | - | - | 5 | 16% |

Table 3, based on height for age for the stunting group, the highest criteria were 12 people (36%) for short, while for the control group, five people (16%) were ordinary and tall. Criteria based on body weight for height for the stunting group found that the most criteria were very thin as many as 12 people (38%), while for the control group the criteria were normal and fat each as many as 5 people (16%). Finally, criteria based on nutritional status for the stunting group obtained 12 people (38%) as bad nutrition criteria, while for the control group, 5 people (16%) had good nutrition and overnutrition criteria respectively.

Table 4. Characteristics of Respondents' Nutritional Intake

| Objective Criteria | Stunting Group | | Control Group | |
|-------------------------------------|----------------|----------------|---------------|----------------|
| | Frequency (f) | Percentage (%) | Frequency (f) | Percentage (%) |
| Exclusive breastfeeding Breast Milk | | | | |
| No breast milk | 2 | 6% | 6 | 19% |
| | 29 | 94% | 4 | 13% |
| Complementary Foods (MPASI) | | | | |
| Well | 5 | 16% | 10 | 32% |
| Not good | 16 | 52% | - | - |
| Feeding Practice | | | | |
| Well | 2 | 6% | 10 | 32% |
| Not good | 9 | 61% | - | - |

Table 4 shows the criteria based on exclusive breastfeeding for the stunting group with the most being 29 people (94%) who were not exclusively breastfeeding, while for the control group there were 6 people (19%) who were exclusively breastfeeding. For the provision of complementary food (MPASI) for the stunting group, 16 people (52%) had unfavorable criteria, while for the control group, 10 people (32%) had good criteria. As for the practice of feeding the most stunting group, 9 people (61%) had unfavorable criteria, while for the control group, 10 people (32%) had good criteria.

Table 5. Characteristics of Stool Samples

| Objective Criteria | Stunting Group | | Control Group | |
|--------------------|----------------|----------------|---------------|----------------|
| | Frequency (f) | Percentage (%) | Frequency (f) | Percentage (%) |
| Color | | | | |
| Yellow | 5 | 16% | 4 | 13% |
| Chocolate | 13 | 42% | 1 | 3% |
| Yellowish-brown | 2 | 6% | 3 | 10% |
| Greenish yellow | 1 | 3% | 2 | 6% |
| Texture | | | | |
| Congested | 18 | 58% | 6 | 19% |
| Soft | 3 | 10% | 4 | 13% |

Table 5 above shows the criteria for the color of the faeces that were mostly brown in 13 samples (42%) for the stunting group. For the control group, the color of the stool was mostly yellow, namely 4 samples (13%). Criteria based on stool texture for the stunting group obtained 18 samples with a solid texture (58%), while for the control group, the most samples with a solid texture were 6 samples (19%)

Table 6. Average Total Bacterial Count

| Category | (Log DNA Copies/gram) | | |
|----------------|-----------------------|---------|------|
| | Minimum | Maximun | Mean |
| Stunting group | 0,25 | 8,95 | 2,28 |
| Control group | 0,03 | 9,23 | 5,95 |

Table 6 above shows the results of the examination using the q-PCR method, the average value of the total number of bacteria in the digestive tract of the stunting group was 2.28 log DNA Copies/gram and the control group was 5.95 log DNA Copies/gram

Discussions

Based on the data in table 1, it can be seen that the incidence of stunting is more common in the education of parents who only graduated from elementary school, and it has decreased successively as the education of parents is getting higher, which is in accordance with research conducted by Akombi *et al.* (2017) in Sub-Saharan Africa who found that parental education, especially mothers, was the most consistent factor associated with the occurrence of stunting in children. Higher maternal education can have an effect on utilization of health care and more attention to family health, which in turn influences health-related decisions that improve child nutritional outcomes. Likewise, the father's high education can have an effect on household income and family food security.

For data, parents in the group of stunting children mostly work, based on interviews from nutritionists and midwives when conducting data studies in Bone-bone Village and Pepandungan Village, that stunting occurs mostly due to poor parenting because children who are still toddlers are often brought along, gardening or being left by parents to garden because they feel that their children can be left at home and can be left in the care of neighbors or family if someone is guarding at home, so that aspects of food and cleanliness of children when playing and before eating are less attention, this is what makes it possible occurrence of stunting. In a study conducted by Bella *et al.* (2020) also found that poor parenting has a 6.62 times greater tendency to have stunted children and poor feeding parenting styles have an 8.8 times greater chance of having stunting children.

This is also further supported by the lack of knowledge about good parenting, such as the results of research conducted by Kusumawati *et al.* (2015) who found that knowledge of mothers who were less at risk increased the incidence of stunting 3.27 times greater than mothers who had better knowledge. This is most likely due to the lack of related information sources due to road access which is quite difficult and far from the city center plus very minimal network access to find information independently. Even in the two villages, especially in Bone-bone Village, it is very difficult to make a normal telephone call.

The economic status data for the stunting child group are all in the low income category (<Rp. 2,500,000) which indicates that economic status can be one of the factors causing stunting in the family. This opinion is supported by the results of Divine research (2017) in Bangkalan Regency, East Java which found that 34 out of 62 families with stunting children had incomes of less than Rp. 1,414,000. Research conducted by Asrianti *et al.* (2019) which was conducted in Samarinda City also found that families with lower middle income families had a 4 times greater risk of having stunting children compared to families with upper middle income.

From the data it was also found that the number of family members 4 people had a greater incidence of stunting than the number of family members 4 people. This is contrary to previous research which found that family members consisting of 2-4 people had a stunting incidence rate of 32.5% and increased to 35.1% in the number of family members of 5-7 people (Titaley *et al.*, 2019). This is because the source of food nutrition is getting less and less for each family member because there is a lot that must be shared, especially supported by the lack of parenting because there are many family members that must be considered so that the focus on paying attention to the health aspects of individuals in the family is decreasing plus the factor of low education. A relatively large number of families have poor quality food consumption (Illahi, 2017).

In terms of sanitation, the higher incidence of stunting occurs in unsanitary conditions, this is in line with the research Erik *et al.* (2020) dan Danaei *et al.* (2016) who found that poor environmental sanitation is one aspect of stunting. Although from the sample data obtained, all houses already have their own latrines, other sanitation habits such as environmental hygiene, the habit of washing hands using soap in running water, and processing household waste have not been implemented properly. From research conducted by Bella *et al.* (2020) also revealed that mothers' poor hygiene habits for their children were 7.19 times more likely to cause their children to experience stunting.

Based on the data in table 2, it is found that the incidence of stunting in Bone-bone Village and Pepandungan Village is more common in boys with a percentage of 45% compared to girls as much as 23%. This is contrary to the results of research conducted by Rukmana *et al.* (2016) who get the percentage of stunting children in Bogor City are more experienced by girls (50.4%) than boys (49.4%). Research conducted by Candra M *et al.* (2016) also contradicts where girls suffer from stunting more (60.4%) compared to boys (39.6%).

Boys are more susceptible to malnutrition in the early days of life due to physiological factors, boys are bigger than girls, so they need more nutritional intake. If these nutritional needs are not met for a long time, it can affect growth (Hidayat & Pinatih, 2017). When viewed in terms of age, the results of the study showed that children aged 3 years were more likely to suffer from stunting, namely 23% and followed by 1 year old as much as 19%, 4 years old as much as 16% and 2 years old as 10%. This is contrary to the research conducted by (Hidayat & Pinatih, 2017). where stunting is more experienced by children with an age range of 0-2 years. He explained that children aged 0-2 years are a golden period to improve their body condition, nutrition and become the right time to provide nutrition improvement interventions. So that the incidence of stunting in children aged > 2 years can be caused because at the age of < 2 years, children are not given nutrition improvement interventions quickly, precisely and correctly which causes children > 2 years to experience stunting conditions.

Based on Anthropometric Index Based on the data in table 4, it can be seen that breastfeeding can reduce the risk of stunting in children. Toddlers who did not receive exclusive breastfeeding were higher in the stunting group by 94% compared to normal children as much as 13%. This is in line with the research of Ni'mah & Nadhiroh (2015), Akombi *et al.* (2017), Budiastutik & Rahfiludin (2019) dan Erik *et al.* (2020) that breastfeeding can help children avoid stunting. Followed by a pattern of giving MPASI that is not good which also greatly affects the incidence of stunting, giving MPASI too early can cause stunting causes diarrhea in children because the child's digestive system is not ready to process food other than breast milk. The delay in giving MPASI, MPASI that is not nutritionally sufficient, the frequency and pattern of giving MPASI according to age can be a factor in the occurrence of stunting (Aridiyah *et al.*, 2015).

To determine the amplification result unit, a standard curve setting is used so that the number of bacterial DNA copy logs per gram can be determined from the results of the Ct value in the amplification curve. Based on the results obtained, the average total number of bacteria for normal children is greater at 5.95 log DNA copies/gram and for stunted children the average total number of bacteria is 2.28 log DNA copies/gram.

This can be influenced by illegible amplification results in some samples, the number of intestinal bacteria can also be influenced by several other factors such as food intake, environmental exposure and antimicrobial therapy. Food intake to meet nutritional needs is a factor that can be controlled in quantity and this will last throughout life. This condition will have an effect on the number of gastrointestinal microbiota populations that will be in charge of processing the food substances that have been consumed (Kurniati, 2016). The gut microbiota begins to increase in the early stages of life in the first 2-3 years, during the introduction to solid food. When entering adolescence, the increase in gut microbiota is influenced by sex hormones and when entering adulthood it will be influenced by the influence of food, lifestyle and drug consumption (Kundu *et al.*, 2017).

From the results of interviews with parents, nutrition assistants and seeing directly the conditions in the field, indeed children with stunting tend to have less and irregular eating patterns, from the nutrition side also said that because they have many children, sometimes parents do not really pay attention to whether their children eat well or not because the focus of parents is divided by taking care of a large number of children, related to fields and also household chores, so parents usually say their children eat regularly because they see their children eating but in reality children only eat little and do not finish their food.

Based on the research results Helmyati *et al.* (2017) regarding the state of the gastrointestinal microbiota of stunted elementary school children in West Lombok found that good bacteria such as Lactobacillus bacteria and Bifidobacterium (6.96 log CFU/g and 8.19 log CFU/g) in the stunted child group were less compared to normal children (7.38 log CFU/g and 8.22 log CFU/g). For the pathogenic bacteria Enterobacter and E. coli, the numbers were higher in the stunting group (7.82 log CFU/g and 7.03 log CFU/g) than in the normal group (7.71 log CFU/g and 6.96 log CFU/g). The results of the research by Khan Mirzaei *et al.* (2020) showed that the phylum Actinobacteria was most abundant in younger children regardless of health status, although the relative abundance was slightly higher in normal children. The second most common phylum in younger children is Firmicutes. In contrast to older offspring, Firmicutes were the most abundant followed by Actinobacteria. Stunting children carried more phyla Proteobacteria compared to their non-stunted counterparts, with younger stunted children exhibiting the highest proportion of Proteobacteria (9%). At the species level, non-stunted youths had more Bifidobacterium adolescents (6%), whereas stunted children had a slightly higher proportion of Bifidobacterium longum (25%) and especially a higher proportion of E. coli (19%).

The results of the research of Toro Monjaraz *et al.* (2021) regarding the gut microbiota in children in Mexico with acute diarrheal conditions found a diverse composition of pathogenic bacteria, namely *Campylobacter jejuni* (20%), *Clostridium difficile* toxin A/B (17%), Enterotoxigenic *Escherichia coli* (10%), *E. coli* O157 (7%), *Shigella* spp. (7%), *Salmonella* spp. (3%). Diarrhea experienced by children, especially in the early days of life until the age of 2 years can cause stunting. This is in line with the research conducted by Sutarto *et al.* (2021) with the results showing that the incidence of diarrhea has a tendency of 4.3 times greater to cause stunting in children.

Excessive bacterial growth, especially if it is dominated by pathogenic bacteria, is associated with poor sanitation conditions so that the incidence of diarrhea and stunting can occur. Excessive colonization of pathogenic bacteria in the gastrointestinal tract due to infection from poor sanitation conditions and food hygiene, plus immature immunity in children causes gastrointestinal probiotics to decrease. These pathogenic bacteria will cause inflammation and result in malabsorption of nutrients that causes stunting (Helmyati *et al.*, 2017).

Conclusion

Based on the research that has been done, it can be concluded that the total number of bacteria for stunting children is very diverse with an average total of 2.28 log DNA copies/gram. The average total number of bacteria in children without stunting is 5.95 log DNA copies/gram. Based on the final results obtained, it can be seen that there is no relationship between the total number of gastrointestinal microbiota bacteria in children in Bone-bone Village and Pepandungan Village, Baraka District, Enrekang Regency to the incidence of stunting that occurs.

Recommendations

It is recommended to study more deeply about other aspects that can cause stunting in children in Bone-bone Village and Pepandungan Village, Baraka District, Enrekang Regency such as parenting, exclusive breastfeeding practices, good complementary foods, not too early weaning, age parents, parents' knowledge level, varied feeding practices with fulfilled nutritional needs, good and correct daily sanitation practices, and other aspects that may not have been detected. Enlarge the scale of the number of samples, because the larger the number of samples, the more likely it is to get a more definite picture of the results of the research conducted. It is also a good idea to proceed to a more specific stage such as the sequencing stage to find out what type of bacteria is more dominant in the gastrointestinal tract of children with stunting.

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References

- Adak, A., & Khan, M. R. (2019). An insight into gut microbiota and its functionalities. *Cellular and Molecular Life Sciences: CMLS*, 76(3), 473–493. <https://doi.org/10.1007/s00018-018-2943-4>
- Adib, A., Wahid, M. H., Sudarmono, P., & Surono, I. S. (2013). *Lactobacillus plantarum* pada Feses Individu Dewasa Sehat yang Mengonsumsi *Lactobacillus plantarum* IS-10506 dari Dadih. *Jurnal Teknologi Dan Industri Pangan*, 24(2), 154–160. <https://doi.org/10.6066/jtip.2013.24.2.154>
- Asrianti, T., Afiah, N., Mulyana, D., & Risya. (2019). Tingkat Pendapatan, Metode Pengasuhan, Riwayat Penyakit Infeksi dan Risiko Kejadian Stunting pada Balita di Kota Samarinda. *JNIK*, 2(1), 1–8. <https://journal.unhas.ac.id/index.php/jnik/article/download/6503/3724>
- Bakri, Z., Hatta, M., & Massi, M. N. (2015). Deteksi Keberadaan Bakteri *Escherichia coli* O157:H7 pada Feses Penderita

Diare dengan Metode Kultur dan PCR. *JST Kesehatan*, 5(2), 184–192.

Bella, F. D., Fajar, N. A., & Misnaniarti. (2020). Hubungan antara Pola Asuh Keluarga dengan Kejadian Balita Stunting pada Keluarga Miskin di Palembang. *Jurnal Epidemiologi Kesehatan Komunitas*, 5(1), 15–22. <https://ejournal2.undip.ac.id/index.php/jekk/article/download/5359/3746>

BPS Kabupaten Enrekang. (2021). *Kabupaten Enrekang Dalam Angka (Enrekang Regency in Figures) 2021*. <https://doi.org/1102001.7316>

Budge, S., Parker, A. H., Hutchings, P. T., & Garbutt, C. (2019). Environmental Enteric Dysfunction and Child Stunting. *Nutrition Reviews*, 77(4), 240–253. <https://doi.org/10.1093/nutrit/nuy068>

Budiastutik, I., & Rahfiludin, M. Z. (2019). Faktor Risiko Stunting pada Anak di Negara Berkembang. *Amerta Nutrition*, 3(3), 122–129. <https://doi.org/10.2473/amnt.v3i3.2019.122-129>

Candra M, A., Subagio, H. W., & Margawati, A. (2016). Determinan Kejadian Stunting pada Bayi Usia 6 Bulan di Kota Semarang. *Jurnal Gizi Indonesia*, 4(2), 82–88. <https://ejournal.undip.ac.id/index.php/jgi/article/download/16302/11942>

Dinas Penanaman Modal dan Pelayanan Terpadu Satu Pintu Provinsi Sulawesi Selatan. (2021). *Profil Kab/Kota: Enrekang*. <https://dpmpstp.sulselprov.go.id/publik-profil-kabkota?id=6>

Erik, Rohman, A., Rosyana, A., Rianti, A., Muhaemi, E., Yuni, E. E., Fauziah, F., Nur'azizah, Rojuli, Abdi R, Y., & Huda, N. (2020). Stunting Pada Anak Usia Dini (Study Kasus di Desa Mirat Kec Lewimunding Majalengka). *Etos: Jurnal Pengabdian Masyarakat*, 2(1), 24–36. <https://media.neliti.com/media/publications/328005-stunting-pada-anak-usia-dini-d97ccdb3.pdf>

Garrido-Cardenas, J. A., Garcia-Maroto, F., Alvarez-Bermejo, J. A., & Manzano-Agugliaro, F. (2017). DNA Sequencing Sensors : An Overview. *Sensors*, 17(3). <https://doi.org/10.3390/s17030588>

Ghoshal, U. C., & Ghoshal, U. (2017). Small Intestinal Bacterial Overgrowth and Other Intestinal Disorders. *Gastroenterology Clinics of North America*, 46(1), 103–120. <https://doi.org/10.1016/j.gtc.2016.09.008>

Hegar, B. (2017). Kesehatan Saluran Cerna pada Awal Kehidupan untuk Kesehatan pada Masa Mendatang. *EJournal Kedokteran Indonesia*, 5(2), 73–77. <https://doi.org/10.23886/ejki.5.8339>

Helmyati, S., Atmaka, D. R., Wisnusanti, S. U., & Wigati, M. (2020). *STUNTING: Permasalahan dan Tantangannya*. Gadjah Mada University Press.

Helmyati, S., Yuliati, E., Wisnusanti, S. U., Maghribi, R., & Juffrie, M. (2017). Keadaan Mikrobiota Saluran Cerna pada Anak Sekolah Dasar yang Mengalami Stunting di Lombok Barat. *Jurnal Gizi Dan Pangan*, 12(1), 55–60. <https://doi.org/10.25182/jgp.2017.12.1.55-60>

Hidayat, M. S., & Pinatih, G. N. I. (2017). Prevalensi Stunting pada Balita di Wilayah Kerja Puskesmas Sidemen Karangasem. *E-Jurnal Medika*, 6(7), 1–5. <http://ojs.unud.ac.id/index.php/eum>

Illahi, R. K. (2017). Hubungan Pendapatan Keluarga, Balita Lahir, dan Panjang Lahir dengan Kejadian Stunting Balita 24–59 Bulan di Bangkalan. *Jurnal Manajemen Kesehatan*, 3(1), 1–14. <https://media.neliti.com/media/publications/258449-hubungan-pendapatan-keluarga-berat-lahir-669eb155.pdf>

Kurniawati, M. D., Sumaryam, & Hayati, N. (2019). Aplikasi Polymerase Chain Reaction (PCR) Konvensional Dan Real Time-PCR Untuk Deteksi Virus VNN (Viral Nervous Necrosis) Pada Ikan Kerapu Macam (*Epinephelus fuscoguttatus*). *Journal TECHNO-FISH*, 3(1), 19–30

Normaisa, Mahsyar, & Sudarmi. (2020). Strategi Dinas Kesehatan Dalam Menekan Laju Penderita Stunting di Kabupaten Enrekang. *Kajian Ilmiah Mahasiswa Administrasi Publik (KIMAP)*, 1(3), 907–920.

<https://jurnal.unismuh.ac.id/index.php/kimap/article/view/3760>

Puskesmas Baraka Enrekang. (2021). *Data Stunting Wilayah Kerja Puskesmas Kecamatan Baraka Kabupaten Enrekang*

Rosselo, J., Kandarina, I., & Kumorowulan, S. (2019). Faktor Risiko Stunting di Daerah Endemik Gaki Kabupaten Timor Tengah Utara. *MGMI*, 10(2), 125–136. <https://doi.org/https://doi.org/10.22435/mgmi.v10i2.598>

World Health Organization. (2018). Reducing Stunting In Children: Equity considerations for achieving the Global Nutrition Targets 2025. In *World Health Organization. License: CC BY-NC-SA 3.0 IGO*. <https://apps.who.int/iris/bitstream/handle/10665/260202/9789241513647-eng.pdf?sequence=1>

World Health Organization, United Nations Children’s Fund (UNICEF), & World Bank. (2021). Levels and Trends in Child Malnutrition: UNICEF / WHO / The World Bank Group Joint Child Malnutrition Estimates: Key Findings of The 2021 Edition. In *World Health Organization*. <https://apps.who.int/iris/handle/10665/341>