Pak. j. life soc. Sci. (2024), 22(1): 6763-6771 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.1.00493

RESEARCH ARTICLE

Detection of *Salmonella typhi DNA* in Water Samples and Its Relationship with Typhoid Fever Risk Factors

Sukma Saini[1*], Yulianto[2], Rauf Harmiady[3], Mardiana Mustafa[4], Heriansyah[5], Muh Basri[6], Rahmatia[7], Agussalim[8]
[1, 2, 3, 4, 5, 6, 7]Makassar School of Nursing, Makassar Health Polytechnic, South Sulawesi Province, Indonesia
[8]Parepare School of Nursing, Makassar Health Polytechnic, South Sulawesi Province, Indonesia

ARTICLE INFO

Received: May 28, 2024

Accepted: Aug 1, 2024

Keywords

Handwashing

Knowledge

Eating

Drinking

DNA

Salmonella typhi

***Corresponding Author:**

sukmasaini@poltekkes-mks.ac.id

ABSTRACT

Typhoid fever is a systemic infectious disease caused by Salmonella typhi, which is still a significant public health problem because of its spread is closely related to urbanization, population density, environmental health, water sources and poor sanitation and hygiene standards of food processing industry is still low. This study aims to detect the presence of Salmonella typhi DNA in water samples of typhoid fever patient's using the PCR method and determine the relationship of risk factors with the presence of typhoid fever Salmonella typhi DNA in water samples in typhoid fever patients. Research conducted at the Laboratory of Immunology and Molecular Biology Medical Faculty of Hasanuddin University, Makassar, by examining 16 water samples from the research respondent of RS Syech Yusuf Gowa. Sampling method based on positive culture results of S. Typhi (4 respondents) and four respondents as control were conducted randomly. Data were analyzed through cross-tabulation with the chi-square test. Research shows, one respondent out of four respondents with a diagnosis of typhoid fever that drinking water containing DNA S.typhi and none of the respondents from the four respondents suspected typhoid fever that drinking water containing DNA S. Typhi, three out of four respondents with a diagnosis of typhoid fever is the source of water containing DNA S.typhi and only one respondent out of four respondents suspected typhoid fever are the source of water containing DNA S.Typhi. There is a relationship between risk factors [the use of latrines(p=0,014), handwashing with soap (p=0,014), eating and drinking patterns(p=0,014)] and the incidence of typhoid fever. the risk factors (knowledge) it's not related (p=0,214) with the incidence of typhoid fever.

INTRODUCTION

Enteric fever or typhoid fever is an acute and endemic infectious disease caused by *Salmonella typhi* which is a bacillus-shaped gram-negative bacterium and is an obligate intracellular pathogen in humans that infects macrophages and Schwann cells. In fighting pathogenic bacteria, it is necessary to increase the cellular and humoral response in the body (Kwenang, 2007). Typhoid fever is transmitted through the fecal-oral route that infects humans who consume food and drinks contaminated with feces containing *S. typhi* bacteria (Aspx, 2006). This infected person will spread the source of Salmonella germs which will enter the body of a healthy person. Directly if this bacteria is present in the feces, urine or vomit of the sufferer can transmit it to others. Meanwhile, indirectly (90%) through food or beverages (Djauzi, 2005).

This disease is easily transferred from one person to another who does not maintain personal hygiene and the environment, for example people who do not wash their hands after coming from the toilet and can spread to others (De Witt, 2002). Infection process *S. typhi* This is in humans, if *S. typhi* entering with food or drink into the human body will reach the digestive system until it reaches the small intestine and M cell channels and causes lesions and multificates in the Peyer plaque ducts, mesenteric glands and lymph nodes then carried into the bloodstream. It is then carried by the blood to various organs and excreted in the feces (Everest, 2001; Santoso, 2003; Jawetz, 1995).

The source of transmission *of S. typhi* comes from contact with patients with typhoid fever and carriers (carriers), which is a person who is infected, but does not show any usual symptoms. A person is said to have a career if a person has recovered from typhoid fever, but in his body is still developing *S. typhi*. They can infect others through feces or urine, and this can happen for years without realizing it. These two sources are major risk factors, but there are also other contributing factors, namely low education levels, poor environmental conditions, and roadside drinking and food supplies. These bacterial infections do not always provide clinical manifestations. It depends on the number and factors of germ virulence and body immunity (Noer, 1996; Easmon, 2005; Hatta & Smits, 2007).

Typhoid fever continues to be a health problem worldwide, with an estimated 16 million cases per year (Ivanoff 1995). Typhoid fever is endemic in countries where there is no proper supply of clean water or sanitation (Hornick et al 1970;. Cabello & Springer 1997). This disease is an important public health problem in Indonesia (Simanjuntak et al 1987.). In Indonesia's Central Java province, typhoid fever was ranked fourth among the 10 most common diseases in hospitals, between 1984 and 1988. Most adult and juvenile typhoid cases with mortality varying 3.1 - 10.4% among hospitalized patients (Hadisaputro 1990). Typhoid fever is spread through contamination of feces, water, and food (Egoz et al 1988; King et al 1989;. Stroffolini et al 1992; Al-Quarawi et al 11 995), either by the patient or as a result of excretion by a typhoid fever carrier.

Some of the factors responsible for the increase in typhoid fever cases as well as the failure to complete this disease include; (1) the clinical symptoms displayed are very varied, (2) routine blood tests do not give specific results, (3) the process of laboratory examination using blood culture, is long enough, so there is a delay in the establishment of the diagnosis (Anonymous 2000). In addition to the factors mentioned above, there are several factors that can increase the risk of typhoid fever, namely; environmental sanitation factors, the provision of drinking water, personal hygiene and the habit of consuming drugs without a prescription from a doctor.

In developing countries, the identification of risk factors and transmission routes relevant to diseases such as typhoid fever is essential for the development of rational control strategies. Therefore, resources can be allocated to areas with high risk factors, for example, by building or expanding water distribution networks or wastewater systems, chlorination of drinking water, ensuring food safety, hygiene education, mass vaccination campaigns, and/or identification of carriers inside or outside the patient's household.

Based on Indonesia's health profile in 2009, it was found that of the 26,507,302 families (45.86%) examined out of a total of 57,803,765 families throughout Indonesia, there were 29,423,382 families who had clean water facilities in the form of tap water, hand pump wells, dug wells, rainwater reservoirs, bottled water and others. And specifically in South Sulawesi, of the 1,312,944 families examined, there are 1,184,421 families who have clean water facilities, with details: tap water 279,932 (23.63%) families, hand pump wells 37,892 (3.20%) families, dug wells 607,628 (51.30%) families, rainwater reservoirs 10,827 (0.91%) families, bottled water 7,841 (0.66%) families and other sources 240,312 (20.29%) families.

Meanwhile, based on the proportion of the population with access to safe drinking water by province and region in 2009, data was obtained that in Indonesia, of the total number of urban communities that exist and have access to 49.82, rural communities are 45.72. In South Sulawesi itself, the proportion of urban people who have access to healthy drinking water is 63.38 and the proportion of rural people who have access to healthy drinking water is 43.74. (Indonesia Health Profile, 2009)

From the aspect of owning basic sanitation facilities, the number of families in South Sulawesi who have latrines is 822,136 families with a healthy category of 131,997 families (64.14%), who have garbage cans as many as 126,488 with a healthy category of 80,308 families (63.49%) and who have wastewater management as many as 156,527 families with a healthy category of 92,672 families (42.36%). (Indonesia Health Profile, 2009)

Similarly, based on the percentage of healthy public places and food management (TUPM), out of a total of 24,191 TUPM in South Sulawesi, and 15,920 TUPM have been inspected, 9,958 (62.55) TUPM are included in the healthy category. (Indonesia Health Profile, 2009)

From the description above, the magnitude of public health problems with various possible risks for the occurrence of diseases mediated by vectors and predisposed by unhealthy habits and patterns both from individuals or the community itself as well as from environmental management and sanitation that does not support health.

Based on facts and data, research was conducted on the risk factors of typhoid fever in relation to the presence of *Salmonella typhi* DNA in water samples as a vector of typhoid fever.

METHOD OF RESEARCH

The design of this study is observational analytical research with a *cross sectional study* design. The tools used in this study are**:** water bath, vortex, shaker, 1.5 ml eppendorf tube, eppendorf tube rack, *centrifuge flush*, incubator, freezer, beaker, micropipette, PCR machine, *PCR tube* (PCR tube), PCR tube rack, plate, comb, *electrophoresis chamber*, *hot plate*, beakers, erlenmeyer, scale spoons, magnetic stirres, analytical balances, power supplies, UV transilluminators, parafilms, cameras and polaroid films.

The materials used in the study were water samples consumed by people with typhoid fever, (Guadinium thyocianate GuSCN, TE buffer (Tris EDTA), Celite suspension, L2 buffer solution, TE buffer (tris – EDTA (Ethylene Diamine Tetra Acetat), triton X, ethanol 70%, acetone, sterile aquades, DNA extract consisting of: 10x PCR buffer (Roche), Taq polymerase enzyme, triphosphate deoxynucleotide (dNTPs) (Takara) : dATP, dGTP, dTTP, dCTP, dH2O (Beker), pure destillated water (Takara), paraffin/mineral oil, agarose gel 2%, marker (Smart Ladder SF), buffer loading 6x (Takara), buffer TBE (Tris Borate EDTA) 0.5X and EtBr (Ethidium Bromide), paraffin paper, label paper, Primer: ST1 (5' – ACT GCT AAA ACC ACT ACT – 3'), ST2(5' – ACT GCT AAA ACC ACT ACT –3'), ST3(5' – AGA TGG TAC TGG CGT TGC TC – 3'), ST4(5' – TGG AGA CTT CGG TCG CGT AG – 3'), air.

The population in this study is all water samples in drinking water reservoirs and water sources found in the homes of typhoid fever patients. The samples of this study are water samples from water sources and drinking water of typhoid fever patients (8 samples) and suspected typhoid fever (8 samples), so the total number of samples is 16 samples. The data from the interview results were connected with the data from the PCR test results for the detection *of S.typhi* based on the band pattern contained in the electrophoresis gel which was presented with a descriptive approach using tables and pictures and statistical analysis with the help of the SPSS program.

Water samples taken from the water reservoir of typhoid fever patients are put into sterile bottles for further isolation of S germs. typhi from the sample which was then followed by DNA amplification by the Nested-PCR method. In addition, blood samples of 6 ml each from patients suffering from typhoid fever were also taken and then cultured in bactec and then incubated for 24 hours at a

temperature of 35 – 37oC. 1 ml of culture on *bactec* medium was inoculated into *Salmonella Shigella Agar* (SSA) medium, then incubated for 24 hours at 37oC, and observed for growth. *Salmonella typhi* shows a circular colony shape, flat colony edges (*entire*), convex colony surfaces (*convex*) with clear colony colors and black centers on SSA medium.

DNA extraction from the sample was sorted by the Boom method, A 100µl water sample was inserted into 900 µl of "L6" solution consisting of 120 g of Guanidium thyocianate (GuSCN) (Fluka Chemie AG, Buchs, Switzerland, paint no. 50990) in 100 ml of 0.1 M Tris HCl, pH 6.4; 22 ml 0.2 M Ethylene Diamine Tetra Acetate (EDTA) pH 8.0 and 2.6 g Triton X-100 (Packard, Instruments) with a final concentration of 50 mM Tris HCl, 5 M GuSCN, 20 mM EDTA, 0.1 % Triton X-100. Next, it was rotated at a speed of 12,000 rpm and the sediment was taken. Add 20µl of diatom consisting of a diatom suspension consisting of 50ml of H2O and 500 µl of 32% (w/v) "Celite" ("diatom") (Jansen Chimica, Beerse, Belgium, 10.846.79). Where 20 µl of this diatom suspension can bind 10 µg of bacterial DNA. Then the divortex was used to homogenize and centrifuge in a 1.5 ml efendorphic tube at 12,000 rpm for 15 minutes.

The supernatant was removed and the sediment was washed with a "L2" solution consisting of 120 g of GuSCN in 100 ml of 0.1 M Tris HCl, pH 6.4, i.e. by adding 900 µl of "L2" solution. Next, divortex and centrifuge at a speed of 12000 rpm for 15 minutes, the washing is repeated 2 times using "L2" solution and continued with 70% ethanol and acetone.

The result was then heated in a "waterbath" at 56°C for 10 minutes and 60 µl of "TE" solution consisting of 1 mM EDTA in 10 mM Tris HCL pH 8.0 was then vortex followed by centrifugation at 12,000 rpm for 30 minutes and the supernatant was transferred into a new efendorphage tube. Then 40 µl of TE solution is added back to the sediment and incubated for 10 minutes at 56°C. Then a vortex and re-centrifuge were carried out for 30 minutes at a speed of 12,000 rpm and supernatant was taken. Supernatants from this process will get DNA extraction results and store them at a temperature of 20°C for PCR analysis.

This procedure was performed on isolated DNA samples, *Salmonella typhi* DNA extract as a positive control and aquadest as a negative control. Previously, a reaction mixture was made for PCR, namely 196 µl of dH2O, 2.5 µl of 10X PCR buffer (consisting of 10 mM Tris Hcl pH 8.3 + 50 mMKcl, 15 MMgCl2 + 0.01 % gelatin), 0.1 µl of dNTPs, 0.1 µl of ST1 and ST2 primers, and 0.1 µl of Taq polymerase and 2.5 µl of DNA Template. Next, a 0.5 ml tube is prepared (according to the number of samples to be amplified). then each sample tube is filled with 40 µl of PCR reaction mixture. Tubes 1 to 16 were filled with 0.25 µl *of Salmonella typhi* DNA extract and tube 17 as a negative control.
Amplification is carried out using a PCR (DNA thermal Cycler) machine. This amplification consists of 40 cycles, where each cycle consists of denaturation at 94oC for 1 minute, annealing at 57oC for 1 minute and 15 seconds, and polymerization at 72oC for 3 minutes. Furthermore, each 9 µl of PCR amplification product is transferred into a tube that mixes the reaction to make nested PCR. The reaction mixture for nested PCR is almost the same as the PCR reaction mixture, the difference is the primer for nested PCR, namely ST3 and ST4.
This second amplification is 40 cycles, where each cycle consists of denatured at 94o C for 1 minute, annealing at 680C for 1 minute and 15 seconds, and polymerization at 720 C for 3 minutes.
Agarose is made with 2 g of agarose (Sigma, type II, medium EEO) in 15 ml of 10 Tris borate (TBE) consisting of 100 g of Tris base, 27.5 g of boric acid, 20 ml of 0.5 M EDTA pH 8.0 in 1 liter of water. Then 135 ml of water is added and heated to a boil. Next, 7.5 µl of ethidium bromide was added and inserted in a gel printer containing a solution of TBE and ethidum bromide with a final concentration of 5 ug/ml TBE. Put the DNA of the sample mixed with a loading liquid containing 4 grams of sucrose, 25 mg of bromephenol blue in 10 ml of water.
The results of the PCR product are passed through electrophoresis of 2% agarose gel to see if there is a DNA band from the sample. Each 8 µl of amplification product is mixed with 2 µl of the loading

solution. Once well mixed, each is put into a well of 2% agarose gel submerged in a tank containing a TBE buffer. Next, electrophoresis is run for 1 hour with a constant voltage of 75 volts. After 1 h, electrophoresis is stopped with the gel being lifted to be observed under UV light. The result is positive if there is a DNA band and negative if there is a DNA band on the gel.

RESULT

A study has been conducted on the relationship between the risk factors of typhoid fever and the presence of *Salmonella typhi DNA* in water samples of typhoid fever patients using the Nested-PCR method. The results of the PCR amplification of the water sample can be seen in the following table

Table 1. Nested PCR results on water samples of typhoid fever patients

Sample No	**Hasil Nested-PCR**			
	Water sample (SM)		**Drinking water sample (AM)**	
	Code on gel sample	**Result**	**Sample code on gel**	**Result**
GW01	SM9	Positive	AM13	Negative
GW04	SM2	Positive	AM6	Positive
GW06	SM6	Positive	AM8	Negative
GW07	SM1	Negative	AM5	Negative
GW08	S3	Positive	AM7	Negative
GW11	S12	Negative	S16	Negative
GW17	S11	Negative	S15	Negative
GW23	S10	Negative	S14	Negative
	4 Negative samples and 4 positive samples		7 Negative samples and 1 positive sample	

	Age	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	11-20	1	12.5	12.5	12.5
	21-30	1	12.5	12.5	25.0
	31-40	3	37.5	37.5	62.5
	41-50	2	25.0	25.0	87.5
	51-60	1	12.5	12.5	100.0
	Total	8	100.0	100.0	

In table 1 above, of the 8 water samples examined, 4 samples were positive for *S.typhi* DNA (50%) and 4 (50%) samples were negative (unamplified). The results of Nested PCR amplification obtained a DNA target of 343bp.

Table 2 Age of respondents in suspected typhoid fever patients in Gowa Regenc

Table 3 Gender of research respondents in suspected typhoid fever patients in Gowa Regency

gender					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	man	4	50.0	50.0	50.0
	woman	4	50.0	50.0	100.0
	Total	8	100.0	100.0	

Table 3 shows that the number of male and female respondents is the same, namely 4 people each (50%).

Table 4 Relationship between risk factors and the presence of *Salmonella typhi DNA* in water samples of typhoid fever patients

Risk Factor Variables	Hasil Nested-PCR	
	Water sample (n=8)	
	Positive (n=4)	Negative (n=4)
Knowledge of typhoid		
I don't know	4	2
Already	0	2
Drinking patterns		
Risk	4	0
No risk	0	4
Diet		
Risk	4	0
No risk	0	4
Hand washing habits		
Often	4	0
Never	0	4
Use of latrines		
Often	4	0
Never	0	4

DISCUSSION

The examination of water samples of suspected typhoid fever patients in this study used the nested-PCR method. *Nested-PCR*, this process makes it possible to reduce contamination of the product during amplification from unneeded primary unification. Two sets of primers are used to support this method, the second set amplifies the second target during the first process. The target DNA sequence of one set of primers called *the inner primer* is stored between the target sequences of the second set of primers referred to as *the outer primer*. In practice, the first reaction of PCR uses *an outer primer*, then the second PCR reaction is carried out with *an inner primer* or *nested primer* using the results of the first reaction product as an amplification target. *The nested primer* will fuse with the first PCR product and produce a shorter product than the first product.
From the results of PCR amplification on water samples found in the water reservoir at the home of a suspected typhoid fever patient, 4 (50%) samples with positive results were obtained as shown by the appearance of bands at 343 bp after agarose gel electrophoresis (figure 1). This indicates that the sample contains S*. typhi* DNA. Likewise, drinking water samples were found to have 1 sample (12.5%) out of a total of 8 samples.
The results of this PCR amplification compared to risk factors (table 3) show a relationship. Statistical analysis using spss showed that there was a relationship between the presence of S*. typhi DNA* and the use of latrines, the habit of washing hands before eating, especially using soap, diet and drinking patterns. ($p<0.05$). This is in accordance with previous research conducted by Faraqui A. regarding the relationship between the incidence of typhoid fever and the quality of the water used.

Based on the results of the analysis of the relationship between risk factors and the presence of S. typhi DNA in water samples with typhoid incidence, it was found that there was a relationship between toilet use and typhoid incidence ($p=0.014$), there was a relationship between hand washing habits and typhoid incidence ($p=0.014$), there was a relationship between hand washing habits using soap and typhoid incidence, there was a relationship between diet and typhoid incidence ($p=0.014$) and there was a relationship between drinking patterns and typhoid incidence. Based on this, it is proven that in fact individual hygiene factors, diet-drinking patterns and environmental sanitation of individuals/families are the main factors in the transmission/spread of typhoid fever which can be assumed to be the cause of typhoid fever. This is in accordance with the theory that typhoid fever is spread through the fecal-oral route which infects humans who consume food and drinks contaminated with feces containing *S. typhi* bacteria (Aspx, 2006). And the process of transferring this disease occurs from one person to another who does not maintain personal hygiene and the environment, for example, people who do not wash their hands after coming from the toilet and can spread it to others (De Witt, 2002).

On the other hand, the absence of a statistically related relationship between knowledge and typhoid incidence ($p=0.214$) does not mean that knowledge factors do not have a positive influence on efforts to prevent typhoid fever transmission. In this case/study, the relationship does not form a linear relationship because in addition to the knowledge aspect, there are still other aspects that affect a person can suffer from typhoid fever, namely the aspect of risk factors owned and the aspect of the availability of healthy drinking water facilities/sources.

Similarly, the absence of a relationship between the existence of S.typhi DNA in water sources and drinking water consumed by individuals/families with typhoid incidence, shows that even though there is S.typhi DNA in water sources, but because the individual/family uses drinking water for family consumption not from that source (gallon water/bottled water), or processes it first (e.g. cooked) before consumption, So that the incidence of typhoid fever was not found. This is the opposite of the opposite, that is, even though the source of water or drinking water for consumption is not found to have S. typhi DNA, but because individuals have risk factors such as poor/unhealthy hygiene patterns or unhealthy eating and drinking patterns inside and outside the home, the potential to suffer from typhoid fever is also likely to occur.

Apart from the above, some of the things that are limitations of the research and can be a confounding factor in the results of the study are the lack of control in the sampling process, both water samples from water sources and drinking water samples, namely the sampling process that is not carried out by the researcher himself, but is carried out by the respondent's family, so that there is a possibility of inconsistency with the techniques and procedures that should be. In fact, it is likely that it does not come from a source that should be in accordance with the purpose and purpose of the research.

ACKNOWLEDGMENT

We would like to express our great gratitude to the Director of the Makassar Ministry of Health Polytechnic and the deputy director who provided the opportunity to conduct this research. And we also don't forget to thank the participants who participated in this research.

CONFLICT OF INTEREST

There is no conflict of interest from this study.

FUNDING

There is no funding from this study

REFERENCES

Al-Quarawi SM, El Bushra Fontaine RE, Bubshait SA & El Tantawy NA., 1995. Typhoid fever from water desalinized using reverse osmosis. Epidemiology and Infection 114, 41±50.

Anonymous. 1993. Textbook of Medical Microbiology. Teaching Staff of the Faculty of Medicine, University of Indonesia. Binarupa Aksara. Jakarta.

Anonim, 2000. Web Health Centre Typhoid, J. Clin. Microbiol; 23; pp. 1-3

Aspx. 2006. Typhoid Fever. (Online). (http :// nhs.direct.nhs.uk/ articles/ article.aspx? articled : 380&Sectionid.12570 14k.

Burrows, W. 1993. Text Book of Mikrobiology. 20th edition. W.B. Saunders Company. Mexico.

Cabello F & Springer AD., 1997. Typhoid fever in Chile. 1977±90: an emergent disease. Revista Medica de Chile 125, 474±482.

Davis, BD, *et al.* 1998. Microbiology 3th edition. Harper and Row International Edition.

DeWitt, Rosalyn C. 2002. Typhoid Fever. (Online). (http :// healthatoz.com/ healthatoz/ atoz/typhoid_fever.jsp

Djauzi, S. 2005. Prevent typhoid fever. (Online). (http://www.kompas. com/kesehatan/news/0503/27/090454.htm

Easmon, C. 2006. Typhoid Fever and Paratyphoid Fever. (Online). http://www. hmc.psu.edu/ healthinfo/t/typhoidfever.htm.

Edelman R and Levine MM., 1986. Summary of an international workshop on typhoid fever. Rev. Infect Dis; 8: 329-349.

Egoz N, Shihab S, Leitner I & Lucian M., 1988. An outbreak of typhoid fever due to contamination of municipal water supply in Northern Israel. Israel Journal of Medical Science 24, 640±643.

Everest, P., Wain, J., Roberts, M., et.al. 2001. The Molecular Mechanisms of Severe Typhoid Fever. Trends in Microbiology. Vol.9 No.7.

Fauza, V., Hatta, M. 2003. DNA Extraction Method. Department of Clinical Pathology & Microbiology FK Unhas Perjan Hospital dr. Wahidin Sudirohusodo . Makassar.

Hadisaputro S., 1990. Factors infuencing the development of intestinal bleeding and or perforation in typhoid fever. P.hD Thesis, Diponegoro University, Semarang, Indonesia.

Hatta, M. 2002. DNA Isolation and Measurement Techniques. Paper. Presented at the DNA Isolation Engineering Seminar. Hasanuddin University. Makassar.

Hattta, M., Mubin, H., Abdoel, T., Smits., Henk. L. 2002. Antibody Response in Typhoid Fever in Endemik Indonesia and the Relevance of Serology and Culture to Diagnosis. Southeast Asian Journal Tropical Medicine and Public Health. Vol.33.

Hartini, R. 2000. Comparison of 3 Ways to Breed *S. typhosa, S. paratyphosa A,* and *S. paratyphosa B* as Supporting the Diagnosis of Typhoid Fever. NEXUS vol. 13 no.1.

Hornick RB, Griesman SE, Woodward TE et al. (1970) Typhoid fever: pathogenesis and immunological control. New England Journal of Medicine 283, 686±691.

Ivanoff B., 1995 Typhoid fever: global situation and WHO recommendations. Southeast Asian Journal of Tropical Medicine and Public Health 26 (Suppl. 2), 1±6.

Jawetz, M and Adelberg. 1995. Medical Microbiology 20th edition. EGC Medical Book Publisher. Jakarta

Jesudason MV, Sridharan G, Arulsevan R, Babu PG John TJ., 1998. Diagnosis of typhoid fever by the detection of anti-LPS and anti-flagellin antibodies by ELISA. Indian J Med Res; 107: 204-207.

Ministry of Health of the Republic of Indonesia, 2010. Indonesia Health Profile 2009.

King CC, Chen CJ, You SL et al., 1989. Community-wide epidemiological investigation of a typhoid outbreak in a rural township in Taiwan, Republic of China. International Journal of Epidemology 18, 254±260.

Kresno, SB. 2001. Immunology: Laboratory Diagnosis and Procedures, Edition IV. Faculty of Medicine, University of Indonesia. Jakarta.

Kwenang, O. A. 2007. Serologic and Molecular Analysis On Thypoid Endemic Population To Determine The Endemic Level In Jeneponto, South Sulawesi. Disertasi of Hasanuddin University, Makassar.

Luby SP, Faizan MK, Fisher-Hoch SP, Syed A, Mintz ED, Bhutta ZA *et al.* 1998., Risk factors for typhoid fever in an endemic setting, Karachi, Pakistan. Epidemiol Infect. 1998; 120: 129-138.

Mansjoer A., Kapita Selekta Med. Typhoid fever. Jakarta. FK-UI, 2000.

Muladno. 2002. About Genetic Engineering Technology. Young Entrepreneur Library, Bogor.

Myvrik, Quentin N., Russel S. Weiser, 1998. Fundamental of Medical Bacteriology and Mycology, Lea & Febiger, Philadelphia.

Noer, Sjaifoellah. 1996. Textbook of Internal Medicine, Volume I, Third Edition. FK-UI. Jakarta.

Otegbayo JA, Daramola OO, Onyegbutulem HC, Balogun WF and Oguntoye OO., 2003. Retrospective analysis of typhoid fever in a tropical tertiary health facility. Trop Gastroenterol; 23:9-12.

Otegbayo, 2005. Typhoid Fever: The Challenges of Medical Management. Annals of Ibadan Postgraduate Medicine. Vol.3 No1 June.

Pang T., 1995. Molecular biology as a diagnostic tool in Salmonellosis. Dalam : Sarasombath S, Senawong S, Eds. Second Asia-Pacific symposium on typhoid fever and other Salmonellosis. Thailand : SEAMEO Regional Tropical Medicine and Public Health Network, 1995:213-6

Santoso, Sanarto. 2003. Salmonella typhi Adhesin Protein as a Virulence Factor with Immunogenic Potential in Protective S-Iga Production. Airlangga University, Surabaya

Simanjuntak CH, Hoffman SL, Punjabi NH et al., 1987. Epidemiology of typhoid fever in a semi urban area, Paseh, West Java. Cermin Dunia Kedokteran 45, 16±18.

Steen, S.W. 1999. Handbook for DNA Isolation, RAPD-PCR, and PCR-RFLP Botanical garden and Museum. University of Oslo.

Stroffolini T, Manzillo G, De Sena R et al., 1992. Typhoid fever in the Neapolitan area: a case-control study. European Journal of Epidemiology 1, 539±542.

WHO and UNICEF, 2000. Global assessment of water supply and sanitation.

Wikipedia, 2010. DNA Replication. http://en.wikipedia.org/wiki/DNA_Replication.

Wulandari F dan Yayan Akhyar Israr, 2008. Demam Tifoid (Tyfoid Fever). Faculty of Medicine,-University of Riau. Pekanbaru-Riau. http://www.Belibis17.blogspot.com.

Yuwono Tribowo, 2006. Theory and Application of Polymerase Chain Reaction, PCR Experiment Guide to Solving the Latest Biological Problems, Andi Publishers, Yogyakarta.