

## **CHAPTER IV**

### **RESEARCH METHOD**

#### **4.1 Research fields**

The field of this research is Diagnostic Medical Parasitology.

#### **4.2 Research location and period**

The period of research is around March-June 2014. The research will be conducted at the Rumah Pemotongan Hewan Penggaron Semarang, SD Negeri Pedalangan 02, and Desa Genting RW.6 Kel.Meteseh for the stool collected and preserved. Processes of diagnostic Soil-Transmitted Helminths using flotation method and effectiveness measurement ( number of eggs on microscope slide) will be done at the Laboratory of Parasitology Faculty of Medicine Diponegoro University , Semarang Indonesia.

#### **4.3 Research design**

This research is quasi-experimental analytic. Research design is using two independent variable as treatments. Independent variable 1 is flotation material consisting of saturate NaCl, saturated  $MgSO_4$  and saturated  $ZnSO_4$ . Independent Variable 2 is optional period consisting of five levels of 15,30,45,60 and 70 minutes. Each combination of treatments repeated and performed on minimum 8

samples so that the whole experiment:  $8 \times 5 \times 3 = 120$  times observations. The result of this experiments can be seen in table below :

**Table 6.** Research design

No.	Code Of Sample	FS1					FS2					FS3				
		op 15	op 30	op 45	op 60	op 75	op 15	op 30	op 45	op 60	op 70	op 15	op 30	op 45	op 60	op 70
1		X														
2																
3																
4																
5																
6																
7																
8																
<b>Total</b>																
<b>Average</b>																

FS1 = Flotation method using saturated NaCl

FS2 = Flotation method using saturated  $MgSO_4$

FS3 = Flotation method using saturated  $ZnSO_4$

OP = Optional Period ( in minutes)

X = Number of soil-transmitted helminths eggs

T = Total numbers of soil-transmitted helminths eggs on microscope slide  
(10x10 magnitude)

A = Average of total numbers of soil-transmitted helminths eggs on  
microscope slide (10x10)

#### 4.4 Sample

Determination of amount sample are calculated by Federer's formula:

$$(n - 1) (t - 1) \geq 15$$

With :  $n$  = number of sample

$t$  = total number of treatment groups

This research are using 3 groups of treatment so that :

$$(n - 1) (3 - 1) \geq 15$$

$$(n-1) 2 \geq 15$$

$$2n - 2 \geq 15$$

$$2n \geq 17$$

$$n \geq 8,5$$

$$n \approx 8$$

Each of groups will have minimum 8 number of sample. Each treatments need 8 sample of faeces meaning that each treatments group needs  $8 \times 5 \times 3 = 120$  times observations and will have optional period at 15,30,45,60 and 70 minutes. The counting and microscopic observations was done twice by 2 different people in order to reduce the bias in counting

##### 4.4.1 Inclusion criteria

Samples are taken with inclusion criteria :

- 1) Stool positive infected with STH
- 2) Stool samples took less than 48 hours
- 3) Stool took from people in risk area or in risk groups, in this research from butchers at Rumah Pematongan Hewan Penggaron Semarang, elementary

students of SD Negeri Pedalangan 02, and children in Desa Genting RW.6 Kel.Meteseh .

#### **4.4.2 Drop out criteria**

Samples are dropped out with criteria such as stool contaminated with soil, urine , or other contaminants during research process .

#### **4.4.3 Sampling method**

Consecutive sampling method.

#### **4.5 Research variables**

##### **4.5.1 Independent variable**

- 1) Flotation solution ( saturated NaCl, saturarated MgSO<sub>4</sub>, and saturated ZnSO<sub>4</sub> ).
- 2) Optional Period (15 minutes, 30 minutes, 45 minutes, 60 minutes,70 minutes ).

##### **4.5.2 Dependent Variable**

Total number of eggs on microscope slide which is drawing the effectiveness of flotation.

### 4.5.3 Confounding variable

- 1) Enviromental Hygiene
- 2) Delay between stool collection and preserved (changes in stool consistency)
- 3) Polyparasitism

### 4.6 Operational definitions

**Table 7.**Operational definitions

No	Variable	Unit	Scale
1.	Flotation Solution  The solution that used to floating the helminth eggs on the basis of differences in specific gravity. The solution used in this study are saturated NaCl, saturated MgSO <sub>4</sub> and saturated ZnSO <sub>4</sub> with a certain specific gravity obtained from the dilution process.	gm/L	Ratio
2.	Optional Period  Time or period of time starting when the floating solution was added and then stirred until homogeneous, until the cover glass is placed on top of the	minute	Ratio

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test tube and raised again to put on the object glass. Periods Optional in this research are 15 minutes, 30 minutes, 45 minutes, 60 minutes and 70 minutes.

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3.	Effectiveness of Flotation Method	Number of	Ratio
	Effectiveness measurement performed by counting total number of Soil-Transmitted helminth eggs on microscope slide at 10x10 magnitude.	eggs	

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## 4.7 Data collection

### 4.7.1 Lab work materials

- 1) Collected and handling stool sample : 70% ethanol for antiseptic
- 2) Preserved stool sample : formalin 10%
- 3) Making flotation solution : aquadest, sodium chloride crystals, magnesium sulfate crystals, zinc sulfate crystals.
- 4) Flotation method : Stool sample, aquadest, lugol 1%, saturated NaCl, saturated MgSO<sub>4</sub>, and saturated ZnSO<sub>4</sub>.

### 4.7.2 Lab Work Equipments

- 1) Collected and handling stool sample : face mask, disposable gloves, disposable pot stool 100 gm, tissue paper, permanent marker pen for labelling, plastic spoons, and plastic bag.

- 2) Preserved of stool sample : stirring stick, pipettes
- 3) Making flotation solution : analytical balance (kitchen scale), beaker glass 600ml, stirring stick, hydrometer.
- 4) Flotation method :face mask, disposable gloves,timer,petri dish, dispossable centrifuge tube 15 ml, disposable sput 3ml and 5 ml, stirring sticks, object glass 25.4 x 76.2 mm , deck glass 20x20 mm, microscope model CX21-FS1, dispossable transfer pipettes, centrifuge model 0412-1,and tissue paper.

#### **4.7.3 Type of data**

The type of data is primary data, which is directly obtained from the result of experiment.

#### **4.7.4 Laboratory work**

##### **4.7.4.1 Collected and preserved stool sample**

- 1) Stool samples is taken with a plastic spoon from a probandus which is fulfill the inclusion criteria. Stool that taken must be fresh stools (less than 2 hour until 48 hours after it defecated). Part of stool that are taken on the upper-middle surface of stool and must be uncontaminated with soil or urine.
- 2) Stool samples is put on a pot/clean container with volume 100 gm..  
Specimen container will be clearly labeled with information:
  - Name or code of probandus
  - Age of probandus

- Address of probandus
  - Date and time of sampling
- 3) If its needed the samples is preserved by mixing with formalin 10% with ratio 3:1 ( 3 part of formalin and 1 part of stool).
  - 4) The samples is transported to the Laboratory of Parasitology, Faculty of Medicine Diponegoro University Semarang and kept in the stored room with enough lighting and normal room temperature.
  - 5) Suspension fecal slurry created by mixing aquadest and stool with a 2:1 ratio in the glass beaker. The mixture is stirred until homogeneous.
  - 6) The samples are divided into 3 groups, with simple randomized allocation and treatment as :
    - a. Treatment I Group (T1): consist of 1 part of stool sample from each probandus, which are receiving Flotation method using saturated NaCl.
    - b. Treatment II Group (T2): consist of 1 part of stool sample from each probandus, which are receiving Flotation method using saturated MgSO<sub>4</sub>.
    - c. Treatment III Group (T3): consist of 1 part of stool sample from each probandus, which are receiving Flotation method using saturated ZnSO<sub>4</sub>.

This treatment will be conducted in 1 week after collected of stool sample. Termination, microscope examination and effectiveness measurement will be done in the same day of treatment.



#### **4.7.4.2 Making flotation solution**

##### **4.7.4.2.1 Saturated Sodium Chloride solution (NaCl, s.g. 1.800)**

- 1) Combine 1000 ml of warm aquadest and about 500 grams of salt until no more salt goes into solution and the excess settles on the bottom of container.
- 2) Dissolve the salt in the aquadest by stirring stick.
- 3) To ensure that the solution is fully saturated, stand it overnight at room temperature.
- 4) Check the s.g. with a hydrometer, recognizing that the s.g. of saturated solution will vary slightly with environmental temperature.

##### **4.7.4.2.2 Saturated Magnesium Sulfate Solution (MgSO<sub>4</sub>, s.g 1200 )**

- 1) Combine 1000 ml of aquadest and 450 grams of magnesium sulphate
- 2) Dissolve the magnesium sulphate in the aquadest by stirring stick.
- 3) Add aquadest to reach a final volume of 1000 ml.
- 4) Check the s.g. with a hydrometer.

##### **4.7.4.2.3 Saturated Zinc Sulphate Solution (ZnSO<sub>4</sub>-7H<sub>2</sub>O, s.g 1.200 )**

- 1) Combine 500 ml of aquadest and 330 grams of zinc sulphate.
- 2) Dissolve the zinc sulphate in the aquadest by stirring stick. ‘

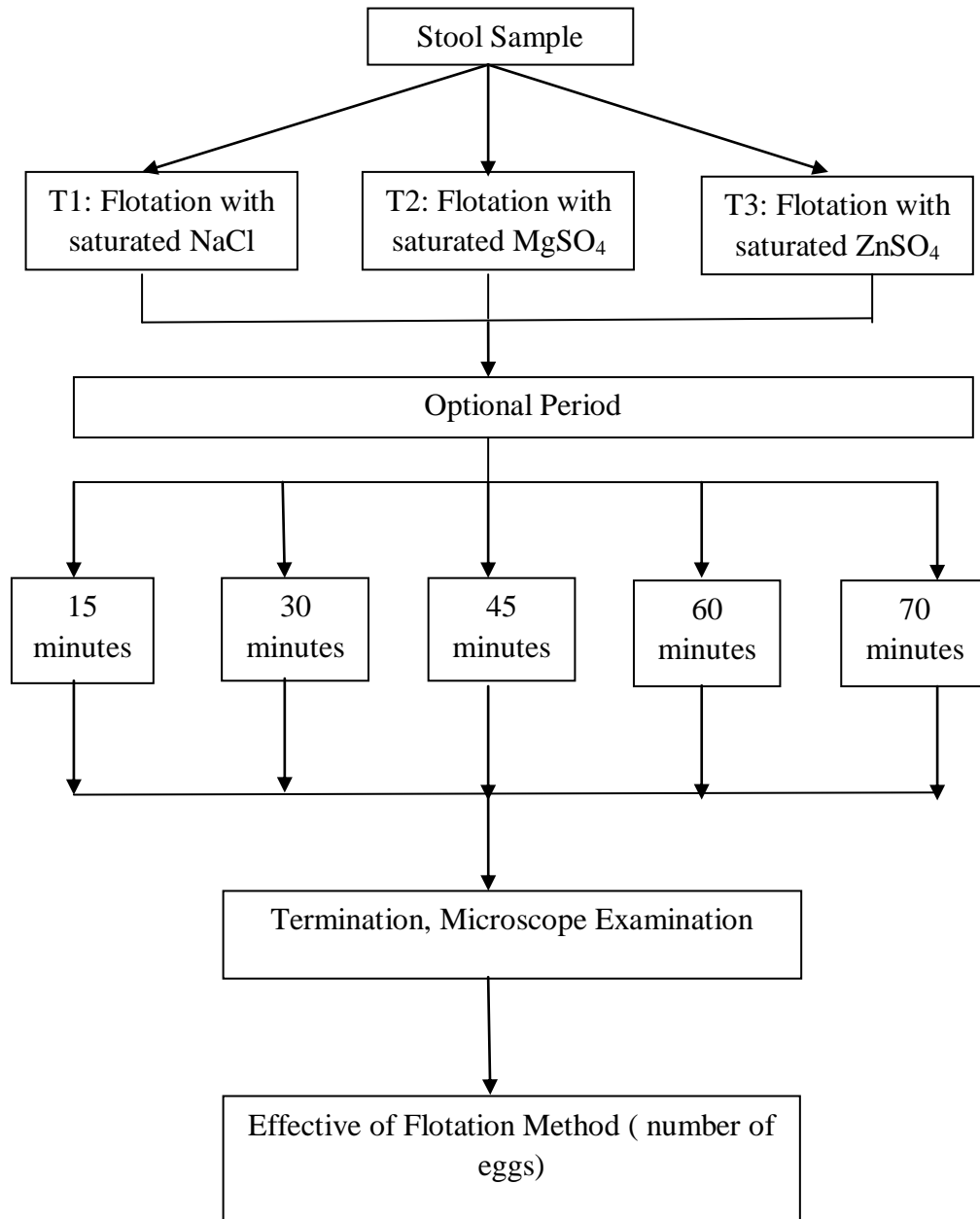
- 3) Add aquadest to reach a final volume of 1000 ml.
- 4) Check the s.g. with a hydrometer.

#### **4.7.4.3 Flotation method**

- 1) According to the rules of randomization, the specimen (stool slurry) in one pot is taken as 5 x 2 ml, put in 5 centrifuge tubes (each tube filled with 2 ml of stool slurry).
- 2) Each of tube is added with 5 ml of aquadest, stirring for 1 minute until homogeneous.
- 3) The four tubes are centrifuged at 2000 rpm for 2 minutes.
- 4) Four centrifuge tube are taken out of the centrifuge machine, then removed the supernatant, added flotation solution to it sediment (according to the standard of treatment) until filled 3 cm below the surface of the tube.
- 5) The solution in each tube is stirred until homogeneous for 1 minute. Carefully with pipette, the tube is filled again with the same solution to form level convex on the surface of the tube.
- 6) In a certain time interval (according to the standards of treatment, optional period ) cover glass is placed over the surface of the tube so that no liquid is spilled.

- 7) Carefully, the cover glasses are taken from each tube, placed on a glass object that already contains 1 drop of Lugol 1%.
- 8) The experiment is started again for the other specimen (according to the rules of randomization) until all stool slide finished. Stool slide that have been prepared is placed in a petri dish with wet tissue paper to keep humidified.
- 9) All of these experiments resulted in 120 stool slide that have been prepared to examined under a microscope magnification of 10 x 10.

#### 4.8 Research protocol



**Figure 15.**Research protocol framework

Eight Stool samples that positive infected by Soil transmitted helminths are collected and then sample consecutively. In this research the stool samples are divided into several groups. Treatment 1 (T1) Flotation method using saturated

NaCl , Treatment 2 ( T2) Flotation method using saturated  $MgSO_4$  and Treatment 3 (T3) Flotation method using saturated  $ZnSO_4$ . All of treatment groups treating with different of Optional Period (15 minutes,30 minutes,45 minutes,60 minutes and 70 minutes) .Each combination of treatments repeat performed minimum on eight samples so that the whole experiment:  $5 \times 8 \times 3 = 120$  times experiment.

#### 4.9 Data analysis

Data is analyzed with the steps :

- a. Editing the collected data
- b. Data cleaning, to re-check the mistakes during data taking
- c. Data tabulation, present the data in table
- d. Data analysis

Descriptive analysis present the mean, median, mode and deviation index. The result is made into box-plot. Normality test using the Saphiro-Wilk test. The abnormal distributed data is using the Kruskall Wallis tes continued with Mann Whitney test. Normally distributed data is tested with two ways analysis of variance (ANOVA).

1. If  $p < 0.05$  it means there is significant difference
2. If  $p > 0.05$  it means there is no significant difference

Data continued with post hoc test with the paired Duncan test if there is significant difference of two ways ANOVA.

#### 4.10 Research ethics

All of data collections and research will be done under permission of the Commission of Health Research Bioethics Faculty of Medicine Diponegoro University/dr. Kariadi General Hospital Semarang Indonesia.

#### 4.11 Research schedule

**Table 8.** Research schedule

Activities	1 <sup>st</sup> Month		2 <sup>nd</sup> Month				3 <sup>rd</sup> Month			4 <sup>th</sup> Month
	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4
Research Proposal										
Research Preparation										
Research										
Data Analysis										
Research Report										
Report Presentation										