CHAPTER II

LITERATURE REVIEW

2.1 S*oil-transmitted helminths*

Soil-Transmitted Helminths are worms that to complete its life cycle requires appropriate soil to develop into the infective form.[1] There are five species of STH (Soil-Transmitted helminths) which are roundworms (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), hookworm (*Necator americanus* and *Ancylostoma duodenale*) and *Strongyloides stercoralis*.[1,2] Four first-mentioned species are endemic worms in Indonesia.[3,4]

2.1.1 *Ascaris lumbricoides* (Roundworm)

Taxonomy of *Ascaris lumbricoides*

Kingdom	: *Animalia*
Phylum	: *Nematoda*
Class	: *Secernentea*
Order	: *Ascaridida*
Family	: *Asarididae*
Genus	: *Ascaris*
Species	: *A. lumbricoides*

Figure 1 . *Ascaris lumbricoides* adult male and female

Ascaririasis is a cosmopolitan parasitic infection. Humans are the only host of *Ascaris lumbricoides*. The disease is caused by this worm called ascariasis.[2]

2.1.1.1 Morphology and life cycle

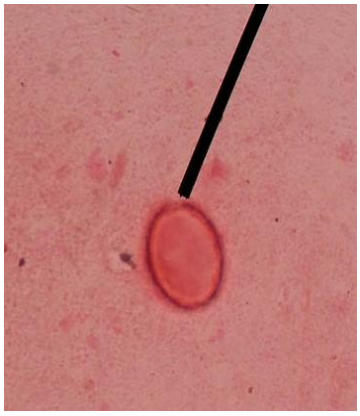

Figure 2. Fertile decorticated egg of *Ascaris lumbricoides*

Figure 3. Infertile corticated egg *of Ascaris lumbricoides*

Ascaris lumbricoides is the largest nematode worm parasites living in human intestine. It's cylindrical shape and red so-called roundworm. Male adult worm sized is 15-25 cm x 3 mm and female is 25-35 cm x 4 mm. Female worm can lay up to 200,000 eggs a day, which can lasted for her lifetime which is approximately 1-2 years. This worm eggs do not hatch in the human body but is realease with the host feces. There are two types of worm eggs, fertilized and unfertilized. Fertilized eggs generally sized 60 x 45 microns, with fertilized-corticated have a cortex and fertilized-uncorticated cortex does not have a cortex. Unfertilized egg ,is not fertilized yet, generally sized 90 x 40 microns and more oval and contains no embrio.[2,20] Here is the life cycle of *Ascaris sp.* :

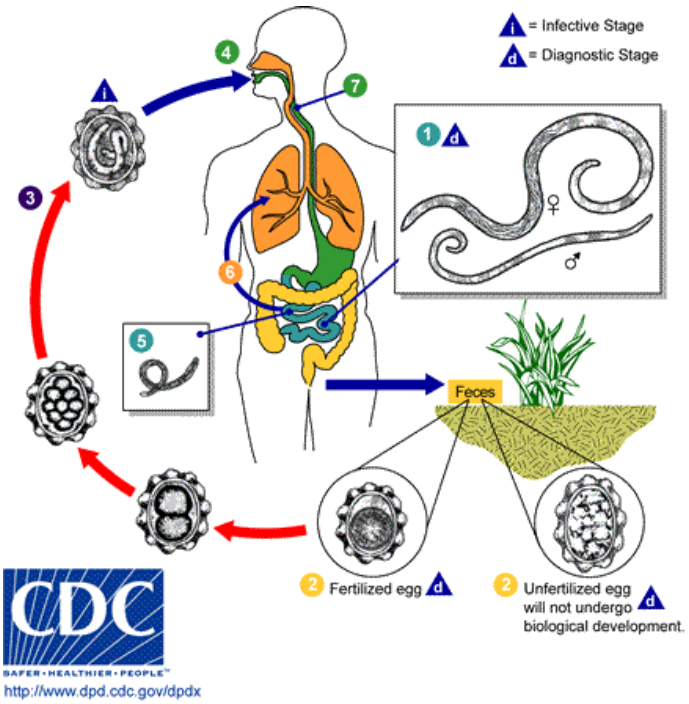

Figure 4. Life cycle of *Ascaris lumbricoides.* Cited from CDC[21]

Worm's life cycle includes the processes of ascariasis transmission in humans. Eggs released by the worm through human faeces will develop into infertile and fertile eggs. Fertile eggs will develop into embryos and infective larvae if the environment is suitable to growth. When infective form ingested by humans it will hatch into larvae in the small intestine. The larvae then exit through the wall into the circulatory system or lymphatic sytem. The larvae will go to the lungs, trachea, pharynx, esophagus and swallowing enter to the small intestine. In the small intestine the larvae turn into adults. This worm's life cycle lasts 2-3 months.[21,22]

2.1.1.2 Pathology and clinical symptoms

Only a small number of patients showing clinical symptoms, mostly asymptomatic. Symptoms appear usually caused by migration of larvae and adult worms [22]

2.1.2 *Necator americanus* and *Ancylostoma duodenale* (Hookworms)

Taxonomy of Hookworm

Kingdom	:Animalia
Phylum	:Nematoda
Class	:Rhabditea
Order	:Strongylida
Family	:Ancylostomatidae
Genus Species	*:Necator* *americanus*
Genus Species	*:Ancylostoma* *duodenale*

Figure 5. *Necator americanus* adult female

There are two species of hookworms that cause disease in humans, which is *Necator americanus* and *Ancylostoma duodenale*. The first two species are commonly found in Asia and Africa. The disease is caused by a hookworm called necatoriasis and ancilostomiasis.[20]

2.1.2.1 Morphology and life cycle of hookworms

N.*americanus* adult form is a cylindrical or the letter S, while the form of *A. Duodenale* is the letter C. Female worms sized ± 1 cm long and male worms sized 0.8 cm ±, with *A.duodenale* sized slightly larger than *N.americanus*.[2,21,23]

A.duodenale adult worms have two pairs of ventral triangular teeth called *triangular cutting plates*, while *N.americanus* mouth only has a pair of crescent-shaped cutting plates (*semilunar cutting plates*). *N.americanus* is capable to produce 10,000-25,000 eggs per day and *A.duodenale* is 5,000-10,000 eggs per day .[2,21,23]

Figure 6. Hookworm egg

Figure 7. Filariform larvae of hookworm

Forms of hookworm eggs can not be differentiated, it is necessary to distinguish species with Harada Mori technique. Typical morphology of hookworm eggs are transparent walls, usually containing morula, thin-walled and

oval / ellipsoid. Rhabditiform larvae ± 250 microns in length, while the length of filariform larvae ± 600 mikron.[2,23]

Life cycle that occurs in hookworms is as follows:

Eggs→ Rhabditiform larvae → filariform larvae→ penetrate the skin→ blood capillaries→ right heart→ lung→ bronchi → trachea→ larynx → small intestine.[2]

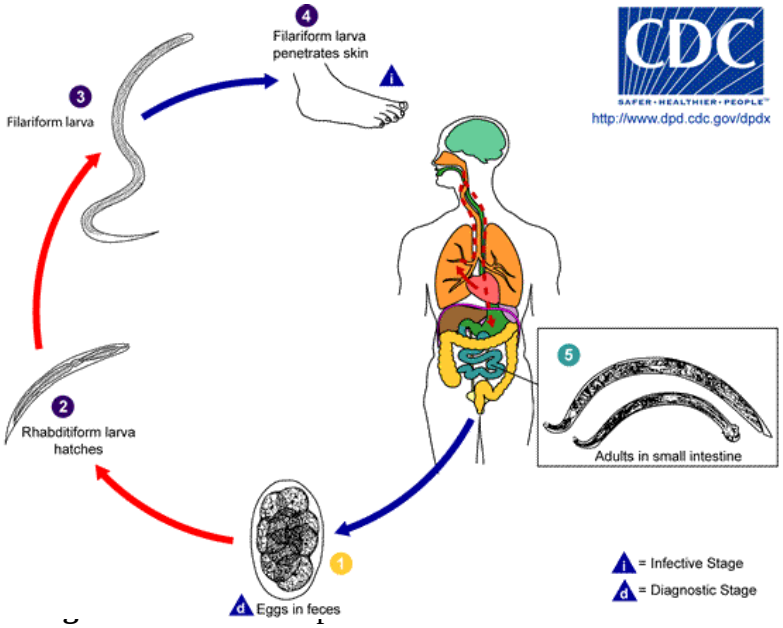

Figure 8. Life cycle of hookworms. Cited from CDC[24]

Infection occurs when filariform larvae of *A.duodenale* penetrate the host skin, and can be dormant in the intestine or muscle. Infection by *A. duodenale* may also occur by ingesting the filariform larvae or through oral route and through breast milk. *Necator americanus* has transpulmonal phase. [23,24]

2.1.2.2 Pathology and clinical symptoms of hookworms

Only a small number of patients showing clinical symptoms, mostly asymptomatic. Clinical manifestations of necatoriasis and ancilostomiasis related to the degree of infection. Tissue damage and disease symptoms can be caused either by larvae and adult worms.

2.1.3 *Trichuris trichiura* (whipworms)

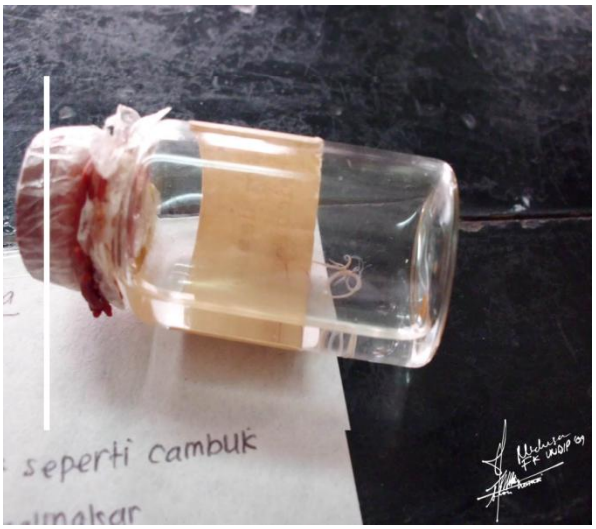

Figure 9. *Trichuris trichiura* adult male and female

Taxonomy of *Trichuris trichiura*

Kingdom	: *Animalia*
Phylum	: *Nematoda*
Class	: *Adenophorea*
Order	: *Trichocephalida*
Family	: *Trichuridae*
Genus	: *Trichuris*
Species	: *T.trichura*

Trichuriasis is a disease caused by *Trichuris trichiura* or known as whipworm. Humans are the definitive host and its main habitat in the large intestine although also can be found in caecum and appendix.[22,23]

2.1.3.1 Morphology and life cycle of *Trichuris trichiura*

Trichuris trichiura is a small worm with wip-shaped. Female worm length of approximately 5 cm, while the male worms of approximately 4 cm. Anterior part of worm is slender whip-like and posterior part fatter, in female worms it shape more blunt rounded. Adult worms live in ascending colon with anterior portion of it,which is whip-shaped, immersed in the intestinal wall to sucking blood.[2,2]

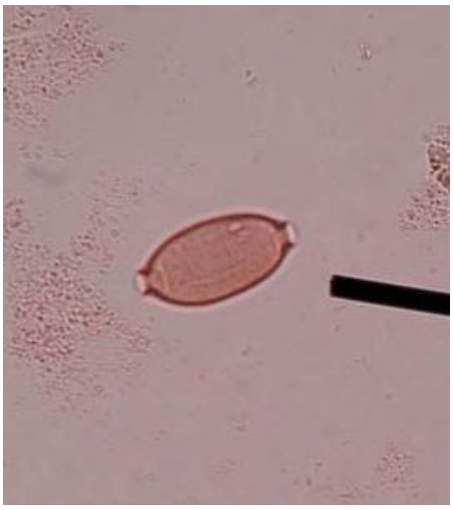

Figure 10. *Trichuris trichiura* egg

Figure 11. Adult male *Trichuris trichiura*

An adult female worm is capable of producing 3000-20000 eggs per day. *Trichuris* eggs typical shaped like a barrel / can and have two distinct mucoid polar plugs. Eggs that passed out with faeces in the immature state and non-infective. The eggs will mature in soil that is conducive within 3-6 weeks to form infective eggs containing embrio.[2,25]

Infective eggs are swallowed (oral route) by humans, and then the eggs will hatch into larvae that will penetrate the mucosa of the small intestine for 3-10 days. After that larvae will down slowly to ascending colon and become mature there, especially in the cecum. In the cecum, the worms can live for years. The worms will lay eggs in the cecum and the eggs out with human faeces. The time required from infective eggs swallowed to adult worms laid eggs ± 30-90 days. It is obvious that these worms have no lungs cycle.[2,21,23]

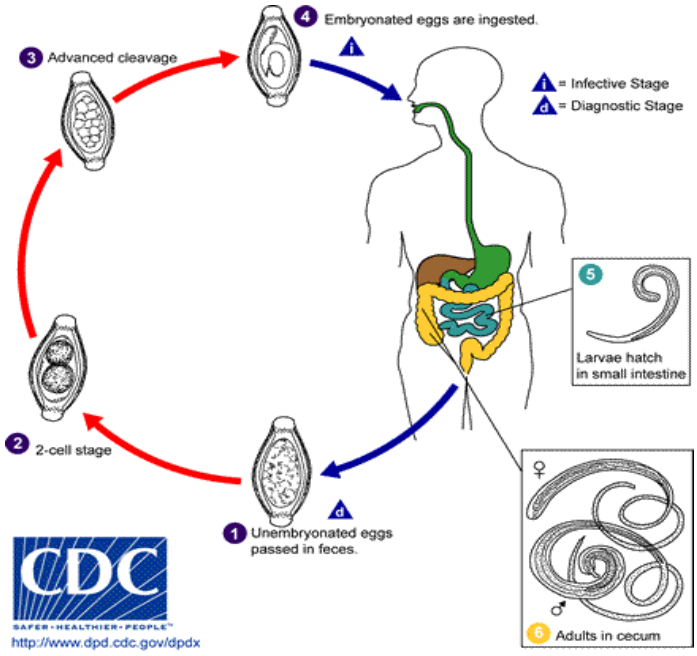

Figure 12. Life cycle of *Trichuris trichiura*. Cited from CDC[25]

2.1.3.2 Pathology and clinical symptoms of *Trichuris trichiura*

Whipworm disease is usually asymptomatic. Chronic and severe infection showed symptoms of severe anemia and bloody diarrhea. Low hemoglobin can decline to 3gr% as a whipworm sucking ± 0.005 cc of blood per day.[2,22]

Trichuris trichiura infection sometimes occurs with other intestinal parasitic infections (polyparasitism) such as *Ascaris lumbricoides*, hookworm, and *Entamoeba histolytica*.[20,23]

2.2 Diagnosis of *Soil-Transmitted Helminths*

In general, the diagnosis of worm infection can be made clinically and epidemiologically. Clinical diagnosis of worms disease, especially hookworm (*Ancylostoma duodenale* and *Necator americanus*) can not give the right diagnosis because of hookworm not provide clear clinical symptoms, thus to assist the diagnosis required laboratory examination.[13] Diagnosis of soil-transmitted helminths can be done by finding worm eggs or adult worms in the faeces examination.[9] The order of process is the preparation of the patient,collection a fresh specimen, preservation specimen if it needs, delivery of specimens, and last macroscopic and microscopic examination of stool specimens in laboratory.[14] Best specimen is fresh specimens that fixed immediately after defecated. Some laboratories require both fresh specimens or with preservatives.

2.2.1 Collected and preserved spesimen

Accuracy of various coromicroscopic (copro = faeces) techniques is very dependent on proper technique in both collection and preservation of samples. If possible, it is recommended to collect faeces material in a fresh state and without other contaminants (eg fly larvae, mites, etc.). Preservation feces using 10% formalin or PVA solution.[14]

2.2.2 Diagnostic techniques of soil-transmitted helminths eggs

Before microscopic stool examination conducted in the laboratory, first do macroscopic examination that includes consistency, presence of other materials in stool (mucus, blood, adult worms), as well as odor and color of the stool. If the consistency of the stool can be determined (solid, semi-solid, soft, or liquid), it can be estimated the types of organisms there. Worm eggs can be found in each type of consistency, but the chance of finding an egg in liquid stools will reduce.[14] Macroscopic examination is followed by microscopic examination of stool, directly or indirectly (sedimentation and culture) .[13]

Stool examination aims to establish a definitive diagnosis,to know the presence or absence of helminth infections, light or weight of infections, and to know the type of worm eggs.[11] Examination of stool can be done directly or indirectly. Examination of worm eggs from stool with concentration method is one of indirect method.[13]

Concentration method of stool examination is part of a complete routine procedure of parasite examination to detect a small number of parasites that may not be found on direct examination. The principle is to separate the eggs of worms and protozoan organisms from stool debris through the difference of specific gravity.[14]

Concentration technique consists of two types, which are the qualitative and quantitative method. Qualitative concentration technique is the examination based on finding eggs in microscope slide ,to determine the type of parasites

without a counting amount per gram (Eggs per gram/EPG). Quantitative examination of stool is based on finding eggs in each gram of faeces (EPG) .[26] Qualitative examination conducted by using native methods (*direct slide),* floating method, formol ether sedimentation method and the method of Harada Mori. While the quantitative examination used Kato Katz sedimentation and egg count techinique (Stool).[14] Here is the level of infection intensity assessed from calculations EPG:

Table 3. Classification intensity of worm infections, WHO[26]

Worm	Infection Intensity	Eggs per gram/EPG
Ascaris lumbricoides	Light	1-4999
	Moderate	5000-49.999
	Heavy	≥50.000
Trichuris trichiura	Light	1-999
	Moderate	1000-9999
	Heavy	≥10.000
Hookworms	Light	1-1.999
	Moderate	2.000 – 3.999
	Heavy	> 4.000

Several laboratories are using this concentration techniques, including Kato Katz sedimentation and Flotation. Each laboratory has its own standart procedure, which method is used, often based on the type of procedure requested by the physician and the time interval between specimen collection to specimen delivery to the laboratory. Nowadays the most widely used technique in the operational diagnosis of parasitic either within the scope of the clinical and epidemiological survey was flotation method.[14]

2.3 Flotation method

Flotation method is a technique of flotation and centrifugation with a specific solution. Flotation procedure is useful to find protozoan cysts and worm eggs.[14] This method successfully finding *Nematode* eggs, *Schistosoma*, *Dibothriocephalus*, porous eggs from the family of *Taenidae*, *Achantocephala*, and *Ascaris* infertile eggs, but not for the unfertilized eggs of *Ascaris lumbricoides* and stool specimens that contain large amount of fat.[10,14,15]

The advantage of this technique is easy to perform, produce a cleaner preparation so that the eggs are easily observed, also good for the type of infection either mild or severe. Flotation disadvantages compared to other techniques are delay in examination can result in distortion, larvae and some fluke eggs do not concentrate,and frequent checking of specific gravity.[26]

In general, the effectiveness of stool flotation examination is affected by the type of flotation solution, specific gravity of eggs and solution, flotation time (flotation period), and the homogeneity of the solution after the centrifugation process.[16]

2.3.1 Flotation solution

The flotation solution plays an important role in causing the worm eggs can float so it will easy to observe. How it works is based on the difference in specific gravity of certain chemical solutions (s.g 1120-1210) and worm eggs (s.g 1050-1150) so that the eggs floating on the surface and also to separate it from

large particles contained in the stool. Flotation solutions commonly used in the flotation method are saturated NaCl, glucose, $ZnSO_4$, $MgSO_4$ pro analysis, and *millet jelly*.[16,26]

Flotation solution has an important role in determining the effectiveness in terms of analytical sensitivity, precision, and accuracy in knowing the type of worm and the calculation of the number of eggs in the entire field of view of the microscope. The specific gravity of of a solution will be specific to a certain type of worm eggs.[17] Solutions that commonly used in Indonesia are saturated NaCl, glucose and $ZnSO_4$ because its cheap and easy to find. $ZnSO_4$ flotation is often used and it is usually better than the sugars flotation, sodium chloride, or saturated salt solution.[16,17] By equating the specific gravity ,its buoyancy will be the same and the effectiveness of the method obtained would not be much different.[16,17] Here is the difference of flotation solution that used in floattion method :

Table 4. Comparison of flotation solution[27]

Salt/ Sugar	Directions	Specivic Gravity	Cost	Comments
Sugar	454 gm in 355 water,6 ml formaldehyde	1.27		Sticky,Mold Sheather's
Salt-sodium chloride	500 gm in 1 liter water	1.18-1.20		Corrosive,crystals
Epsom salts-Magnesium Sulfat	450 gm in 1 liter water	1.2		Corrosive, cystals
Zinc Sulfate	330 gm in 500 ml	1.18-1.20		Purchased,Vet

	water		Solutions Zinc Sulfate
Sodium nitrate	338 gm 1 liter water	1.18-1.20	Purchased,fecasol

2.3.1.1 Sodium chloride (NaCl)

The characteristics of Sodium Chloride, which are:

1. Molecular formula: NaCl
2. Molecular weight: 58.45 g / mol
3. Boiling point: 1413 ˚ C at 1 atm
4. Freezing Point: 800.4 ˚ C at 1 atm
5. Forms : cubic crystalline solid
6. Color: white
7. Density: 2.163 g / ml

Sodium chloride, also known as salt and table salt, an ionic compound with the formula NaCl and a specific gravity (s.g) 1,200. Sodium chloride is usually clear and odorless solids and soluble in glycerol, ethylene glycol, and formic acid, but insoluble in HCl.

Sodium chloride is the most influence on the salinity of the ocean and of the extracellular fluid of many multicellular organisms. As the main ingredient in table salt, and is commonly used as a spice and food preservative.

Sodium chloride is sometimes used as a drying agent that is cheap and safe because it has hygroscopic characteristic, making salting become one of the effective methods for food preservation (Anonima, 2010).

Production of sodium chloride is commonly performed by evaporation of seawater or brackish water from various water sources, such as wells and salt lakes, and by mining of salt rocks called *halite*. Besides being used in cooking, sodium chloride is also used in many applications, such as in the manufacture of pulp and paper, to adjust the color levels in textiles and fabric, and to produce soaps, detergents and other products. Sodium chloride is a major source of industrial chlorine and sodium hydroxide, and used in almost every industry.

Sodium chloride is used as a flotation solution because it's easily available and relatively cheap,althought it is more expensive than $MgSO_4$.vIn this study, a specific gravity of 1.200 NaCl solution will be made.[15,28]

2.3.1.2 Magnesium sulfat ($MgSO_4$)

Magnesium Sulfate is one kind of salt. Magnesium Sulfate has many types where each type has a specific function. It depends on *hydrate* contained. The types of Magnesium Sulfate based on Hydrate contained can be seen in this table :

Table 5. The types of Magnesium Sulfate based on Hydrate contained

No	*Hydrat*	Mineral	Molecular Formula
1.	*Monohydrate*	*Kieserit*	$MgSO_4.H_2O$
2.	*Tetrahydrate*	*Starkeyite*	$MgSO_4.4H_2O$
3.	*Pentahydrate*	*Pentahydrite*	$MgSO_4.5H_2O$

4.	*Hexahydrate*	*Hexahydrite*	$MgSO_4.6H_2O$
5.	*Heptahydrate*	*Epsomite*	$MgSO_4.7H_2O$

Epsom salt is one type of Magnesium Sulfate that considered potential. This salt is known as very important and can be used in industries, such as: the coloring aniline, for the production of cotton clothing. Along with the development of industry, especially in the fields of pharmacology, other advantages of Epsom salts can be use as a purgative (to treat of functional constipation and can not cope with constipation caused by intestinal pathological condition before radiological examination, examination of recti and colon operation also to eliminate toxins in poisoning patients). In the manufacturing process, Magnesium Sulfate made from raw material of Magnesium Carbonate and Sulfuric Acid. (Asril et al, 1986).

Chemical reaction of $MgSO_4$ which is :

$$MgCO_3 + H_2SO_4 \rightarrow MgSO_4 + CO_2 + H_2O$$

Magnesium sulfate is used as a flotation solution because it's easily available and relatively inexpensive. Disadvantage of $MgSO_4$ as flotation solution are if the preparations / slide ignored for a certain time before reading, the liquid will crystallize and eggs may be distorted.[28] In this study, the specific gravity will be made to 1,200. Actually magnesium sulfate is used for soil examination, but in this study these substances will tested and compare their effectiveness with zinc sulfate and sodium chloride using the flotation method with stool samples.[15,28]

2.3.1.3 Zinc sulfat ($ZnSO_4$)

Zinc sulfate is an inorganic salt with the chemical formula $ZnSO_4$. This substances are solid and colorless. This substance can be mixed with water and can not be burned. This substance is very toxic to aquatic organisms (organisms that live in water) , may cause adverse effects in the aquatic environment for long periods of time. Therefore ,its required a special handling for its disposal, by following the procedures disposed of as hazardous waste. Avoid using freely in environment.[15,28]

This substances also give an accute effects. This substance also harmful if swallowed by oral route and the risk of causing serious eye damage if the substance is exposed to the eye. First aid is given by forcing for vomiting if swallowed, wash eyes with running water if it's attached to the skin or eyes.[15,28]

Zinc sulfate is used as a flotation solution because easily available and relatively inexpensive. In this study, the specific gravity will be made to 1,200.

2.3.2 Optional period

Floating time is closely related to the optional period. Optional period is a time or period of time starting when the floating solution is added and then stirred until homogeneous, until the cover glass is placed on top of the test tube and raised again to put on the object glass. Some studies indicate that the optional period ranged between 20 and 60 minutes.[16] As differences in specific gravity affects the type of eggs that will floating, specific gravity of the solution also affects the optional

period. The best optional period will determine the best effectiveness of flotation method. Studies conducted by Ketut Nugraha (1996) showed the effectiveness of experiment obtained from both $MgSO_4$.7H2O also $MgSO_4$ pro analysis slightly different for every level of an optional period, but the difference variance test was not significant ($P>0.05$). Further research is required to determine the optional period that gives the best effectiveness from three commonly used flotation solutions, that are saturated NaCl, $MgSO_4$, and $ZnSO_4$.[9,16]