

# Cryptosporidiosis Detection Using Immunoglobulin a Serum with Enzyme-Linked Immunosorbent Assay Method

Deany Puteri Naila<sup>1</sup>, Nuzulia Irawati<sup>2</sup>, Malinda Meinapuri<sup>3</sup>, Hasmiwati<sup>2</sup>,  
Rikarni<sup>4</sup>

<sup>1</sup>Ungraduated Student of Faculty of Medicine, Andalas University, Padang, Indonesia

<sup>2</sup>Department of Parasitology, Faculty of Medicine, Andalas University, Padang, Indonesia,

<sup>3</sup>Department of Histology, Faculty of Medicine, Andalas University, Padang, Indonesia

<sup>4</sup>Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Andalas University, Padang, Indonesia

Corresponding Author: Nuzulia Irawati

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## ABSTRACT

**Background:** Cryptosporidiosis is still one of the most common intestinal protozoan infections found in developing countries, affecting children and adults. *Cryptosporidium* sp. infection activates the immune response by secreting high levels of specific *Cryptosporidium* IgA antibodies during the infection process. IgA can be examined by ELISA to establish a diagnosis of cryptosporidiosis.

**Methods:** The purpose of this study was to determine the results of Cryptosporidiosis detection with serum IgA ELISA. This study was a quantitative descriptive study with 39 samples collected from children to adults using consecutive sampling technique. Data obtained from the study results were processed by univariate analysis and presented in tabular form to see the frequency distribution.

**Result:** The results of the study involving 39 respondents revealed that the most prevalent age groups were 6-11 years old and 36-45 years old, with the majority being female. Among the 39 samples examined using the ELISA method, the Optical Density ranged from 0.059 to 1.393. One sample (2.56%) tested positive for IgA *Cryptosporidium* sp., specifically from age 55 years (16.6%) and male participants (20%).

**Conclusion:** The study concluded that the Optical Density value of the positive ELISA examination was 1.393 with one sample found

positive for Cryptosporidiosis from a 55 years old male.

**Keywords:** [*Cryptosporidium* sp., IgA, ELISA]

## INTRODUCTION

Diseases caused by intestinal protozoa are a global health problem, especially in the tropics.<sup>1</sup> The most common types of protozoa that cause infections in the gastrointestinal tract are *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium* sp.<sup>2</sup> *Cryptosporidium* sp. is one of the major intestinal protozoa causing diarrhea with a high mortality rate in the world. The infection caused by *Cryptosporidium* sp. is known as Cryptosporidiosis.<sup>3</sup>

The global prevalence of Cryptosporidiosis was found to be 7.6%, with Mexico having an estimated prevalence of 69.6%.<sup>4</sup> There is no definitive data on the prevalence of Cryptosporidiosis in Indonesia, but reported research conducted in one of the urban villages in Makassar (Jabal et al. 2020), out of a total of 38 fecal samples examined, two were infected with *Cryptosporidium* sp. parasites with a prevalence of 5.26%.<sup>5</sup> Research on *Cryptosporidium* prevalence in West Sumatra Province is also limited.<sup>6</sup> The results of a study on the prevalence of

intestinal protozoa in West Sumatra Province conducted at an elementary school in Andalas, Padang City, found that 2.7% of samples were infected with *Cryptosporidium sp.*<sup>6</sup>

The high infection rate of *Cryptosporidium sp.* is caused by various factors. Epidemiological index include age, individual immune status, geographical distribution, and ethnic groups. Human activities include poor individual hygiene, inadequate drinking water treatment systems and sources, unsanitary eating behaviors such as not washing hands with soap before eating, and waste treatment that does not comply with standard procedures. Social factors include geography and climate, especially in tropical climates.<sup>7</sup>

A person infected with *Cryptosporidium sp.* parasites shows different symptoms. Patients with good immune status will experience symptoms in the form of acute diarrhea that can heal spontaneously, even without symptoms. Infection due to this parasite in patients with poor immune status can result in acute diarrhea to chronic diarrhea that lasts for months to years.<sup>8,9</sup>

Cryptosporidiosis activates the *host* immune response as a defense in eliminating the parasite. The parasite-induced inflammatory process activates epithelial cells and induces the secretion of cytokines and chemokines.<sup>10</sup> Cp23 is a protein expressed by *Cryptosporidium* sporozoites while in the intestinal mucosa, is highly immunogenic and is able to induce B cells to secrete *Cryptosporidium-specific* antibodies IgG, IgA, and IgM with high levels during the infection process and decreases after repair.<sup>11</sup> IgA functions to neutralize the invading pathogen and induce various effectors to clear the pathogen. When there is an inflammatory process due to pathogen infection, B lymphocytes located in the lamina propria will begin to produce IgA which will then be carried to the intestinal lumen to eliminate parasites.<sup>12,13</sup> Ig A acts as the main antibody to the infection response in the digestive tract and parasite clearance.

Infection caused by *Cryptosporidium sp.* can be diagnosed by several methods, such as microscopic examination, immunodiagnosics, histopathology, or molecularly. Detection of *Cryptosporidium sp.* infection using immunodiagnostic methods has high sensitivity and specificity by detecting parasite antigens or *host* antibodies.<sup>14, 15</sup> Detection using immunodiagnosics can also determine the molecular epidemiologic distribution and possible transmission routes of the parasite. Serologic detection of IgA antibodies is an immunodiagnostic method that is considered more effective than microscopic examination because it is more cost-effective, rapid and reliable in establishing the diagnosis of Cryptosporidiosis. Serological examination is preferred due to its high level of specificity and sensitivity and is more effective and efficient than microscopic examination of feces. The most popular immunodiagnostic examination used is the ELISA method because it is considered to provide faster results and a higher level of sensitivity and specificity.<sup>14</sup> The principle of this ELISA examination assesses the reaction of antigens and antibodies with enzyme labels which are then added to the substrate which later when hydrolyzed produces a colored precipitate that can be detected by reading devices on ELISA.<sup>16</sup>

This research is conducted in Pasié Nan Tigo Village, Koto Tengah Sub-district, Padang City. Geographically, Koto Tengah sub-district is directly adjacent to the Indonesian Ocean from the east.<sup>17</sup> The main livelihood of the community in this sub-district is fishing with the majority of the population living on the beach. The lives of fishermen in this sub-district still face various problems such as financial problems and poverty, as well as inadequate access to health and education services. Lack of awareness and knowledge of the need for clean water sources is also a problem faced by the Pasié nan Tigo community. This puts Koto Tengah Sub-district at high risk of infection with various types of

mycopathogens, including *Cryptosporidium* sp.<sup>18, 19, 20</sup>

## MATERIALS & METHODS

This study used a quantitative descriptive research method by detecting the incidence of Cryptosporidiosis infection in samples from the community of Pasié Nan Tigo Village. This research was conducted at the Biomedical Laboratory of the Faculty of Medicine, Andalas University in Padang. The population in this study were all blood sera from previous studies taken from the community of Pasié Nan Tigo, Koto Tengah District, Padang City which were stored in the Laboratory. The minimum sample size needed in this study was calculated using the formula for estimating the proportion of a population, namely the minimum sample size needed in this study was 38.729, rounded to 39 samples. Samples in this study were taken using *non-probability sampling* technique, namely *consecutive sampling*. This research was conducted from December 2022 to January 2024

The IgA was detected using an ELISA microwell plate reader measuring absorbance at 450 nm. The study utilized

positive and negative controls. Samples were categorized as positive if the sample's optical density (OD) was greater than 0.15 plus the average value of the OD of the negative control samples. Conversely, samples were classified as negative if the sample's OD was less than 0.15 plus the average OD value of the negative control samples. In this study, 39 samples were examined using the ELISA method.

Data analysis involved univariate analysis to observe the frequency distribution of positive samples with Cryptosporidiosis.

## RESULT

### Distribution of Respondents by Age and Gender Characteristics

Table 1 shows the total number of samples collected from 39 respondents with the most age coming from the age group 6-11 years, namely 7 samples (17.9%) and the age group 36-45 years also as many as 7 samples (17.9%). The youngest age was 5 years and the oldest age was 77 years. This table also shows that the total number of female subjects is greater than male subjects, with 34 female and 5 male respondent.

Table 1 Distribution of Respondents by Age and Gender Characteristics

Characteristics	Number (n)	Percentage (%)
<b>Age</b>		
0-5	2	5,1
6-11	7	17,9
12-16	2	5,1
17-25	2	5,1
26-35	6	15,3
36-45	7	17,9
46-55	6	15,3
56-65	3	7,6
>65	4	10,2
Total	39	100
<b>Gender</b>		
Female	34	87,1
Male	5	12,8
Total	39	100

### Optical Density IgA results of serum samples examined by ELISA method

No.	Age	Gender	OD Value
1	49	P	0,083
2	47	P	0,068
3	43	P	0,063
4	8	P	0,177
5	68	P	0,090
6	58	P	0,079
7	25	P	0,079

8	5	L	0,061
9	10	P	0,060
10	32	P	0,068
11	40	P	0,080
12	7	P	0,059
13	18	P	0,076
14	15	P	0,070
15	55	P	0,075
16	63	P	0,076
17	38	P	0,065
18	54	P	0,062
19	31	P	0,059
20	44	P	0,071
21	35	P	0,122
22	7	L	0,089
23	77	P	0,099
24	51	P	0,083
25	41	P	0,064
26	70	P	0,169
27	30	P	0,145
28	30	P	0,061
29	29	P	0,072
30	9	L	0,094
31	29	P	0,082
32	5	L	0,088
33	55	L	1,393
34	33	P	0,065
35	8	P	0,059
36	66	P	0,076
37	15	L	0,060
38	10	P	0,068
39	63	P	0,074
Average			0,115
Average positive control			1,216
Average negative control			0,070

Table 2. Optical Density of IgA Serum Samples

Table 2 shows the Optical Density ELISA results on 39 samples examined. The highest Optical Density level obtained in this study was 1.393 in one sample with the age of the sample owner was 55 years old and male. The lowest Optical Density value in this study was 0.059 obtained in three samples with each sample owner aged 7

years, 31 years, and 8 years and the three sample owners were female. The average Optical Density value of the 39 samples was 0.115

### Distribution of samples detected Cryptosporidiosis by IgA examination

Table 3. Distribution of Samples Detected Cryptosporidiosis by IgA Examination

Cryptosporidiosis	Total	Percentage (%)
Cryptosporidium antigen positive	1	2,56
Cryptosporidium antigen negative	38	97,4

Table 3 shows that of the 39 samples examined for Cryptosporidium sp. antigens using IgA by ELISA method, 1 sample (2.56%) was positive. There were 38 samples (97.4%) with negative serum antigens for Cryptosporidiosis

### Frequency Distribution of Cryptosporidiosis Detected Samples by Age and Sex Characteristics

Table 4. Distribution of Cryptosporidiosis Detected Samples by Age and Sex Characteristics

Characteristics	Cryptosporidiosis			
	Positive		Negative	
	f	%	f	%
Age				
0-5	0	0	2	100
6-11	0	0	7	100

12-16	0	0	2	100
17-25	0	0	2	100
26-35	0	0	6	100
36-45	0	0	7	100
46-55	1	16,6	5	83,3
56-65	0	0	3	100
>65	0	0	4	100
Gender				
Female	0	0	34	100
Male	1	20	4	80

Table 4 above shows the results of the age group detected Cryptosporidiosis is the age group 46-55 years, namely 55 years old. One of the six respondents in this age group was detected positive for Cryptosporidiosis with a percentage of 16.6%, while other age groups were not detected positive for Cryptosporidiosis in this study. Cryptosporidiosis distribution based on gender was found positive in one sample with male gender. One out of five male respondents was positive for Cryptosporidiosis with a percentage of 20%. The other four samples who were also male were negative for Cryptosporidiosis, which amounted to 80%. No positive Cryptosporidiosis was found in the female gender in this study.

## DISCUSSION

### Distribution of Respondents by Age and Gender Characteristics

This study obtained results showing that the age of the most respondents based on the samples examined was the adult age group 36-45 years, namely 7 respondents (17.9%) and the children's age group 6-11 years with 7 respondents (17.9%). This study also shows that there are more female respondents than male respondents, with 34 (87.1%) female respondents and 5 (12.8%) male respondents.

Based on statistical data released by the Central Bureau of Statistics of Padang City in 2018, the Pasie Nan Tigo area has a population of 9,444 people with a population under the age of 20 totaling 3,479 people and a population over the age of 20 totaling 5,965 people. The age groups 36-45 years and 46-55 years are the most numerous age groups in this area. The 36-45 year age group totals 1219 people and the

46-55 year age group totals 1124 people. The elderly group aged 56-65 years only numbered 678 people, and the elderly who were more than 65 years old numbered 336 people. In the age group of children to adolescents, the age group of 5-14 years has a total of 1795 people.<sup>21</sup> This is in accordance with the distribution of respondents by age in this study, where the age group 36-45 years is the respondent with the largest age group in the adult group and the age group 6-11 years in the child age group.

### Optical Density IgA results of serum samples examined by ELISA method

Immunoglobulin A examination using the ELISA method with blood serum samples was carried out at the Biomedical Laboratory of the Faculty of Medicine, Andalas University. This examination uses positive control and negative control to ensure the examination goes well and the examination results can be trusted. The results of this ELISA examination are assessed qualitatively and quantitatively. Qualitative examination is characterized by a color change from blue to yellow in the ELISA examination well. This study found one ELISA well that changed color from blue to yellow. Quantitative examination is obtained by reading the ELISA reaction results with an ELISA reader that reads at an absorbance of 450 nm. The results of quantitative examination obtained the average value of Optical Density positive control is 1.216 and the average value of Optical Density negative control is 0.070. The cut off value obtained to determine the positive result of Cryptosporidiosis is  $\geq 0.220$ . The OD results of the 39 samples examined obtained one sample with an OD

value exceeding the cut off, namely 1.393, while the other 38 samples had an OD value below the cut off.

Optical Density is a value resulting from ELISA examination by measuring the color changes that occur in the examination well using an ELISA plate reader at a certain absorbance. In this study using an absorbance of 450 nm. The Optical Density value obtained is then processed using the OD standard value curve. This standard OD value is obtained from positive and negative controls which is useful for converting the Optical Density value of the test sample into the concentration of the tested substance. Furthermore, the Optical Density value of each sample examined is plotted into the standard curve so that the line equation is obtained from the curve. The line equation obtained is then used to convert the Optical Density value of the sample into a grade that can be compared. From this grade value comparison, a sample is said to be positive or negative for Cryptosporidiosis.<sup>22</sup>

Distribution of samples detected Cryptosporidiosis by IgA examination

The examination results of 39 samples in this study, found one positive sample of IgA Cryptosporidiosis (2.56%) with an Optical Density value of 1.393, while the other 38 samples were negative for IgA Cryptosporidiosis (97.4%) with an Optical density value below 0.220.

The incidence of Cryptosporidiosis infection in this study is still quite high.<sup>23</sup> This is due to a variety of factors, such as immunocompromised conditions, contact with animals, transmission through food and beverage consumption, and direct contact with infected humans. Population density, personal and environmental hygiene levels, and inappropriate livestock and waste management are also risk factors for Cryptosporidiosis, especially in developing countries.<sup>24, 25</sup>

Pasie Nan Tigo is a village in West Sumatra that is located on the coast, so the majority of the population works as fishermen.<sup>18</sup> The majority of the Pasie Nan Tigo community still faces various challenges and obstacles,

such as financial problems, limited access to education and health services, and public services. In addition, this area is still often hit by a lack of clean water. This is because Pasie Nan Tigo is an area that often experiences flooding when there is high rainfall, so it is often difficult to get clean water supplies.<sup>19</sup> The lack of knowledge and awareness of the people of this area of the importance of clean water needs, also makes people still use many water sources that are not suitable for use. Another problem faced by the Nan Tigo Patient community is that the use of proper sanitation access such as healthy latrines is still low.<sup>37</sup> This is why it is important to detect Cryptosporidiosis in the Pasie Nan Tigo area, because this area is a high risk area for people infected with Cryptosporidiosis.

Frequency Distribution of Cryptosporidiosis Detected Samples by Age and Sex Characteristics

The results of the examination in this study stated that the 46-55 years age group was the age group detected Cryptosporidiosis infection, which was 51 years old (16.6%). This study shows that the adult age group is more susceptible to infection than children. This could be due to adults traveling more often, including traveling to endemic areas, a decreased state of immunity due to age than children, besides that adults are also more often in contact with infected humans and livestock, and more often consume food and drinks that are unwittingly contaminated with *Cryptosporidium* sp. parasites.<sup>23, 26</sup>

The results of this study also showed that the group detected with Cryptosporidiosis was male (20%). No positive results were found in the group with female gender. This is based on the statement that men have a lifestyle that is more often exposed to parasites, such as from the type of work and work environment so that they are more susceptible to infection with parasites, including *Cryptosporidium* parasites.<sup>27</sup>

The majority of men in the Pasie Nan Tigo area work as fishermen, because this area is

close to the coastal area.<sup>18</sup> This is explained in the study (Roberts et al. 2007) which suggests that fishermen are at high risk of being infected with Cryptosporidiosis through the route of ingestion of Cryptosporidium sp. parasites after skin contact with fish or water surfaces and the habit of eating undercooked fish.<sup>28</sup> This is consistent with the results of this study, where the male gender (20%) was the group with the most types of Cryptosporidiosis detected compared to the female gender.

## CONCLUSION

Based on research on the examination of Cryptosporidiosis using IgA by ELISA method, it is concluded that the age of the most research respondents is the age group 6-11 years and 36-45 years with the most gender is female. The Optical Density results of serum IgA samples examined by the ELISA method were between 0.059 to 1.393. Of the 39 serum samples examined, one sample (2.56%) was detected positive for Cryptosporidium sp. with the characteristics of the research sample detected Cryptosporidiosis is male with the age of 55 years.

## Declaration by Authors

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