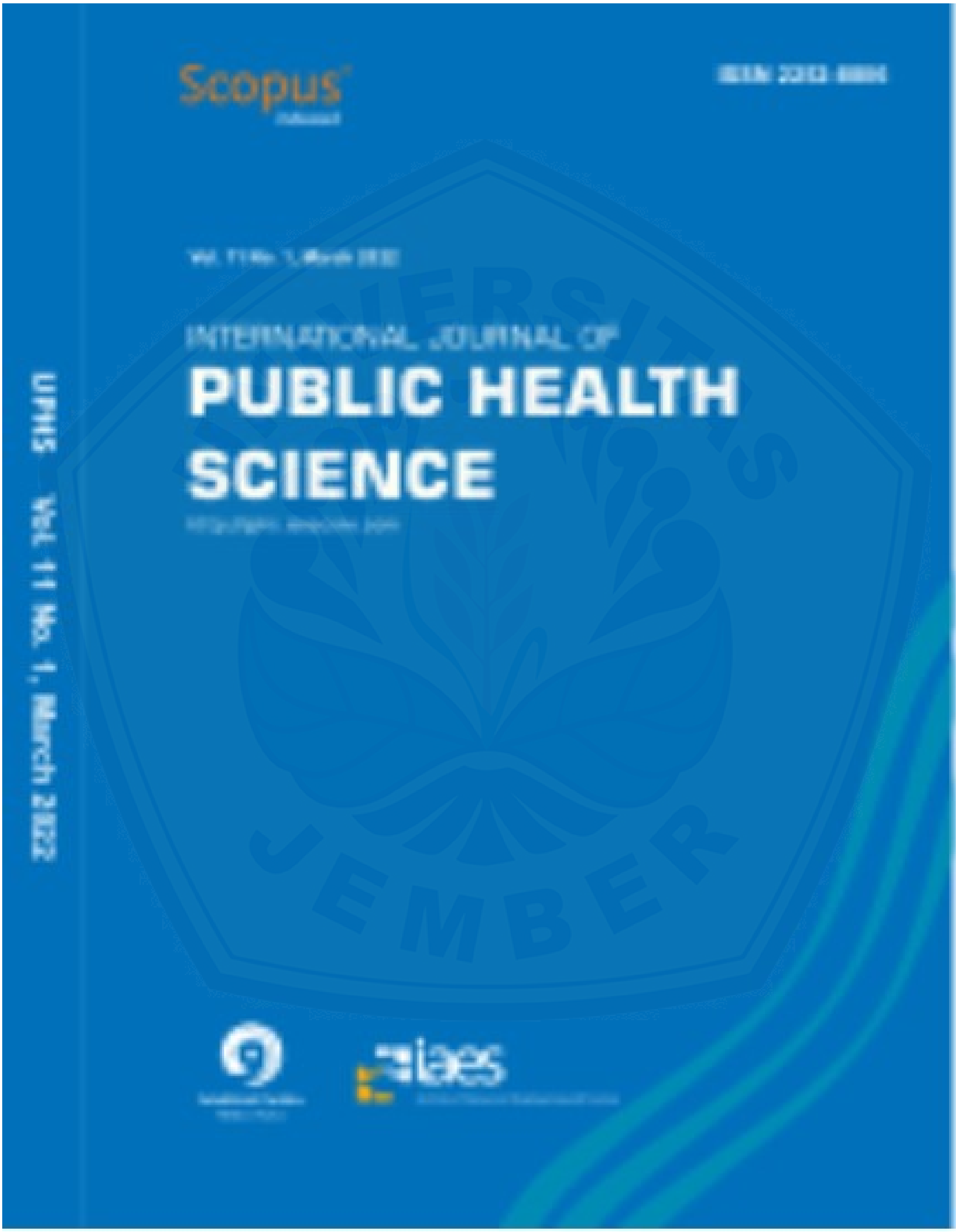
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Bacterial and parasitic contamination of raw vegetable: potential risk for food-borne diseases

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ABSTRACT

Food-borne diseases can be transmitted through raw vegetables contaminated with bacteria and intestinal parasites. The study aimed to determine bacteria and intestinal parasites that contaminate raw vegetables in traditional markets. In this study, we collected raw vegetables from eight traditional markets. We chose randomly at each market five samples of vegetables that usually consumed in raw, like lettuce, tomato, cabbage, basil, long bean, and cucumber. The bacteria were identified by culture and microbiological test and the intestinal parasites were identified using sedimentation and floatation methods. This study showed that all of raw vegetables were contaminated with *Escherichia coli* (E. coli) (91%), *Staphylococcus aureus* (84%), and *Vibrio cholera* (79%). Besides bacteria, 36% of samples were contaminated by soil-transmitted helminths (STH), and intestinal protozoa contaminated 27% of samples. Lettuce was the most contaminated vegetable with bacteria and intestinal parasites. The results of this study proved that there was bacterial contamination as well as intestinal parasites in raw vegetables sold in traditional markets which could be a source of spread of food-borne diseases. Therefore, handling raw vegetables properly is needed as an effort to prevent it.

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1. INTRODUCTION

Food-borne disease is a group of infectious diseases transmitted by food that brought the pathogen (viruses, bacteria, or parasites). The main symptom that often occurs from food-borne diseases is diarrhea. Based on world health organization (WHO) report in 2020, 550 million people get sick due to diarrhea and fever. Several cases can cause severe dehydration or systemic infection like sepsis that have a high mortality rate up to 230,000 death every year, especially in vulnerable population such as geriatric or children under five years old [1], [2]. Jember is a Regency in East Java, Indonesia, which has high incidence of food borne illness, like diarrhea and thypoid. From primary healthcare data in Jember Regency, reported cases of diarrhea as much as 48,582 cases, and there is an increase of Thypoid cases in all district, up to 4.3% in 2018-2019 [3]. Diarrhea and typhoid were transmitted through contaminated food with those pathogens.

Fresh vegetable that contaminated with bacteria and intestinal parasite and eaten raw or not yet cooked optimally, can be transmission media for food-borne diseases [4]. Some pathogens had been found in raw vegetables, like bacteria e.g *Escherichia coli* (*E. coli)* O157:H7, Hepatitis A, *Listeria monocytogenes, Norovirus, Salmonella sp., Shigella sp,* also intestinal parasites that include soil-transmitted helminths (STH) e.g hookworm, *Strongyloides stercoralis, and Ascaris lumbricoides, and protozoa (Cyclospora cayetanensis, Entamoeba histolityca, Giardia lamblia, and Cryptosporidium parvum)* [5]. The contamination of raw vegetables occurs through the process of plantation, distribution sale and inappropriate handling food before comsumed. Some studies Study in Malaysia and Thailand have detected the presence of some pathogen in raw vegetables from marketplaces. In Malaysia, with 306 randomly collected samples from marketplaces, showed contamination of *L. monocytogenes* in all samples, with the most prevalence of contamination found in *Cosmos caudatus* (50%), cucumber (43.8%), and Japanese parsley (39.4%) [6]. Other research in Thailand [5] detected egg or cyst from the helminth group in raw vegetable that collected from three center market in a different district, especially hookworm in celery (>50% sample) and egg of *Ascaris lumbricoides* in lettuce, coriander, and celery.

In Indonesia, study on bacterial contamination as well as intestinal parasites on raw vegetables in the market is still lack of data. A study conducted by Purba *et al*. in Medan, North Sumatra, was only looking for *E. coli* and parasite contamination in lettuce, eggplant, basil, and broccoli, but other diarrhea-causing bacteria like *Salmonella sp.* and *Shigella sp.* have not been studied yet [7]. Therefore, a comprehensive study is imprtant to determine all bacteria dan parasites that contaminated the raw vegetables in marketplaces, particularly in traditional market as the main shopping place for the people of Jember. The results of this study may encourage the government to make the better regulation in handling raw vegetables and the society to wash and cook raw vegetable properly to prevent the transmission of food-borne diseases. Furthermore, this study aimed to determine all bacteria and intestinal parasites that contaminated raw vegetables in traditional markets in Jember Regency.

2. RESEARCH METHOD

This was a descriptive analytic study. It studied raw vegetable that usually direct consumed by people, there were lettuce, tomato, cabbage, basil, long bean, and cucumber. The sample was collected ramdomly from eight different traditional markets in Jember Regency, East Java, Indonesia. This study was approved by ethics committee in Faculty of Medicine, University of Jember, with No. of Ethical Approval 1432/H25.1.11/KE/2020.

The sample was collected from eight different traditional markets in Jember Regency randomly. We get five samples for each vegetable and each market, as much as 300-500 grams. A total sample of vegetables we used is 241, which lettuce was 41 samples, and other each five vegetables 40 samples. Every sample saved into sterile polythene plastic, and brought into laboratories for testing by a cool box with 4-6 ^{o}C temperature inside the box. In the laboratories, each sample divided into two parts, 25-100 g for microbiology testing, and the remain for parasitology testing.

2.1. Laboratory testing

2.1.1. Bacterial examination procedure

a. Bacteria calculation

The quantitative method was used for calculate colony of bacteria. We used most probable number (MPN) with three tubes multiple dilution test [8]. Dilution media we used in this study was lactose broth with ratio 1:10-1, 1:10-2, and 1:10-3. Each sample to be examined with MPN method, were incubated in two ways, first group was incubated in 37 ^{o}C during 24 hours then continued for 48 hours, and second group was incubated in 44 ^{o}C during 24 hours. Positive result showed by the appearance of bubbles that caught in Durham tubes. The results were interpreted by confirming with a MPN table in bacteriological analytical manual (BAM) Online [9].

b. Bacteria isolation and identification

We used nutrient agar, McConkey agar, salmonella-shigella agar (SSA), and mannitol-salt agar (MSA) as culture and growth media. A 100 µL sample was inoculated in agar plate with spread plate technique. Especially for sample that would be inoculated in SSA, it must be enrich in selenite F broth media at 37 ^{o}C all night. Then all plate incubated at 37 ^{o}C during 24 hours. After culturing process, the bacteria were characterized by gram staining, motility test, oxidase test, and by their growth in triple sugar iron agar (TSIA). Gram positive bacteria were continuous to test with coagulase test [10], and gram negative bacteria were continuous to evaluate with the indole methyl-red voges-proskauer citrate (IMVIC) test.

2.1.2. Parasitic examination procedure

We used two methods to identified parasites in sample, there were sedimentation and flotation method. For pre-analytic, we washed 200-250 grams samples by 1,000 mL aquadest in beaker glass, then shook it for 15 minutes to separating parasite and samples. Wastewater was collected and stand overnight until the precipitate was formed. After that, removed the supernatant, poured the remaining precipitate into a centrifuge tube and then centrifuge with 2,000xg in 15 minutes. For the sedimentation method for helminth group identification, removed the supernatant after centrifugation smoothly, then shook gently the remaining sediment, after that, dropped it into object glass, then covered by cover glass. We made three slides for each sample for increasing parasites finding. Placed the slide under microscope with objective magnitude 10x and 40x [5]. In the other side, for floatation method, after removing the supernatant after centrifugation, the rest of the sediment was shaken and then dripped sucrose saturated solution to full and convex formed on the surface of the tube but did not spill. Closed the surface of the tube using a cover glass and let stand for 30 minutes. Later, took the cover glass, then placed it into an object glass gently, so bubbles were not formed. Then, observed under the microscope, with 10x and 40x objective lens magnitude to find the protozoa.

3. RESULTS AND DISCUSSION

3.1. Bacteria examination results

The first microbiological examination performed was to calculate the number of colony bacteria by MPN 3-3-3 method. Based on the examination, the results of most samples had a large number of bacterial colonies with an MPN index value of >1,100/g. Recapitulation of the results of the calculation of the number of colonies was shown in Table 1.

Table 1. MPN results at 37ºC and 44ºC incubation

Vegetable	No. of sample	MPN index (MPN/g)			
		37ºC		44ºC	
		Min	Max	Min	Max
Lettuce	41	>1100	>1100	>1100	>1100
Tomato	40	290	>1100	290	>1100
Cabbage	40	240	>1100	>1100	>1100
Long Bean	40	>1100	>1100	>1100	>1100
Basil	40	>1100	>1100	460	>1100
Cucumber	40	150	>1100	>1100	>1100

On the bacterial identification test, out of the 241 samples examined, there were 100% vegetable samples contaminated by bacteria. The most bacteria found in the sample was *E. coli* with a discovery rate of 91%. Other bacteria identified are *Staphylococcus aureus*, *Shigella sp.*, *Salmonella sp.*, *Vibrio sp.*, and *Proteus sp*. Of the six vegetable samples examined, lettuce was the vegetable commodity with the most contamination. bacteria, followed by tomatoes and basil on second and third place. The results of the observation and distribution of bacteria in vegetable samples was shown in Table 2.

Table 2. Identification of bacteria in sample of raw vegetables

Vegetable	No. of sample	Identified bacteria (%)					
		Salmonella sp.	*Shigella sp.*	*Staphylococcus aureus*	*E. coli*	*Proteus sp.*	*Vibrio sp.*
Lettuce	41	16 (39)	41 (100)	32 (78)	41 (100)	9 (22)	34 (83)
Tomato	40	28 (70)	24 (60)	39 (98)	38 (95)	9 (23)	34 (85)
Cabbage	40	27 (68)	22 (55)	26 (65)	38 (95)	8 (20)	24 (60)
Long bean	40	18 (45)	29 (73)	35 (88)	36 (90)	10 (25)	34 (85)
Basil	40	24 (60)	40 (100)	31 (78)	34 (85)	5 (13)	30 (75)
Cucumber	40	19 (48)	28 (70)	39 (98)	33 (83)	6 (15)	35 (88)
Total	241	132 (55)	184 (76)	202 (84)	220 (91)	47 (20)	191 (79)

3.2. Parasitological examination results

Based on parasitological observations to find intestinal helminths contamination, it was found that 87 samples (36%) were contaminated by worms. The most worm contamination was found in lettuce samples (63%). Of the 241 samples observed, it was found that the hookworm group worms were mostly found (75%) in the vegetable samples studied. In addition, other worm species found were *Strongyloides stercoralis* (4.6%), and egg of *Ascaris lumbricoides* (1.2%), the rest was a combination of the two groups of worms (19.5%). The distribution of worm contamination in the sample vegetables can be seen in Table 3.

Table 3. Distribution of helminth group contamination in sample of raw vegetables

Vegetable	No. of sample	Helminth group				No. of contamination (%)
		Hookworm (%)	*Strongiloydes stercoralis* (%)	*Ascaris lumbricoides* (%)	>1 type of helminth (%)	
Lettuce	41	15 (36.5)	3 (7.3)	0 (0)	8 (19.1)	26 (63)
Tomato	40	4 (10)	0 (0)	0 (0)	1 (2.5)	5 (13)
Cabbage	40	8 (20)	0 (0)	0 (0)	4 (10)	12 (30)
Long bean	40	20 (50)	1 (2.5)	1 (2.5)	2 (5)	24 (60)
Basil	40	13 (32.5)	0 (0)	0 (0)	2 (5)	15 (38)
Cucumber	40	5 (12.5)	0 (0)	0 (0)	0 (0)	5 (13)
Total	241	65 (27)	4 (1.7)	1 (0.4)	17 (7)	87 (36)

Besides worm contamination, parasitological examination was also conducted to discover for protozoa contamination in raw vegetable samples. Based on observations, 66 samples (27%) were contaminated with protozoa, with 79% of them belonging to the *Entamoeba sp.* group. Other protozoa found were *Cryptosporidium sp*. in the form of oocysts (9%), and in other samples found more than one group of protozoa (12%), it could be Balantidium group with *Entamoeba sp.*, or a combination of Balantidium with *Cryptosporidium sp.* The most protozoa were found in basil and cabbage samples with 43% each. The distribution of protozoa contamination can be seen in Table 4. All microscopic image of parasites can be seen in Figure 1.

Table 4. Distribution of protozoa group contamination in sample of raw vegetables

Vegetables	No. of sample	Protozoan group				No. of contamination (%)
		Cyst *Entamoeba sp.* (%)	Cyst *Balantidium sp.* (%)	Oocyst *Cryptosporidium sp.* (%)	>1 type of protozoa (%)	
Lettuce	41	13 (32)	0 (0)	0 (0)	2[a] (5)	15 (37)
Tomato	40	3 (7.5)	0 (0)	1 (2.5)	0 (0)	4 (10)
Cabbage	40	14 (35)	0 (0)	0 (0)	3[b] (7.5)	17 (43)
Long Bean	40	9 (22.5)	0 (0)	0 (0)	0 (0)	9 (23)
Basil	40	12 (30)	0 (0)	2 (5)	3[b] (7.5)	17 (43)
Cucumber	40	1 (2.5)	0 (0)	3 (7.5)	0 (0)	4 (10)
Total	241	52 (21.5)	0 (0)	6 (2.4)	8 (3.3)	66 (27)

a. Balantidium sp. and Entamoeba sp. (n=1), Balantidium sp. and Cryptosporidium sp. (n=1)
b. Entamoeba sp. and Cryptosporidium sp. (n=3)

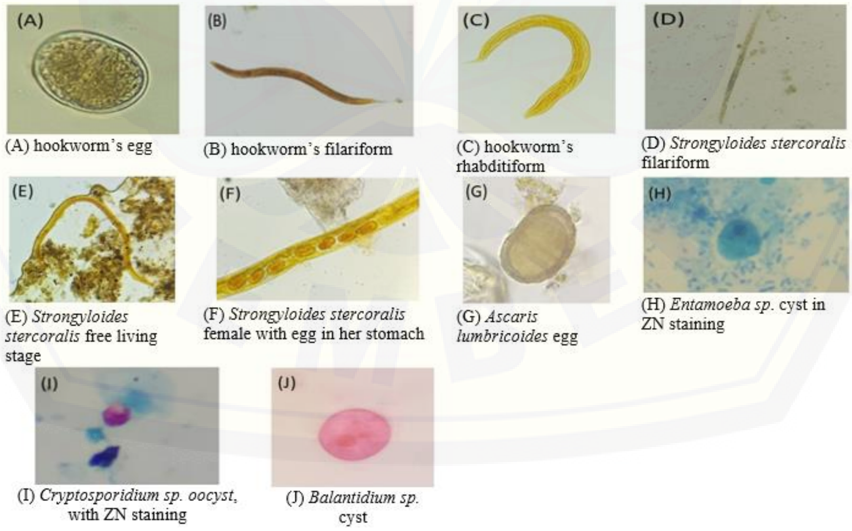

Figure 1. Microscopic view of parasites found in samples

3.3. Discussion

This study was the first research conducted in Jember Regency to find all intestinal bacteria and parasites that contaminate raw vegetables in traditional markets. The finding of intestinal infection-causing bacteria e.g *Salmonella sp.*, *Shigella sp.*, *E. coli* and *Vibrio sp.* proved the contamination of raw vegetables. This study also found intestinal parasite contamination in raw vegetables that include egg and larva of STH, also the cyst and oocyst of protozoa. This contamination could be a potential source for transmission of food-borne diseases if the handling of raw vegetables is not properly before consumed. Finally, the contamination will have an impact on society's health in the short and long term.

In this study, the most common bacteria found were *E. coli*. The finding of *E. coli* shows the high level of faecal contamination in the plantation area or organic contamination during the post-harvest process. This finding is also in line with research in India [11]. It found that the results of the examination on 480 samples from eight types of vegetables, the most common bacteria found was *E. coli* (16.7%). Another study in Arba Minch Town, Southern Ethiopia [12] also mentioned that the discovery rate of *E. coli* was the highest compared to other microbes, which was 31.4% of the 347 vegetable samples studied. Another bacterium that occupies the second position of the highest findings in this study is *Staphylococcus aureus*, 86% of the total sample. Almost the same as the research conducted at the Fako division, Cameroon [10] showing a high rate of *S. aureus* discovery in the fresh vegetable samples studied as much as 35.4%. Although better known as pathogenic bacteria in the airway tract, *S. aureus* can contaminate food at the stage of food preparation and processing [13] such as contact with hands, contamination via droplets, poor environment, or cross-contamination with other foodstuffs.

Contamination in fresh vegetables can come from the whole process from planting until before serving. Vegetables that use organic fertilizer from animal or planted in areas that had faecal contamination from humans or animals in their water and soil sources will be at risk of carrying pathogens such *E. coli*, *Vibrio sp.*, *Salmonella sp.*, *Shigella sp.*, or other microbial pathogens. During the packaging process, there is a risk of botulinum bacteria breeding in canned food, or also during cooking, cross-contamination can occur from bacteria present in animal flesh that is being transferred to vegetables or fruit. Alternatively, in the process of distribution from a seller to a consumer, bacterial transmission occurs from the seller's hand to vegetables or fruit, for example, the transmission of Streptococcus bacteria or Staphylococcus. Poor washing processes and inadequate cooking could infect people who consume these agricultural products [14].

Other bacteria found in the research sample were *Vibrio sp.*, *Shigella sp.*, *Salmonella sp.*, and *Proteus sp. Vibrio sp.* which is quite widely found in samples (79%), also needs to be a concern, considering cholera disease has relatively high severity, especially in children. Transmission from the vibrio group generally comes from seafood such as fish, shrimp, or oyster but can also contaminate fresh vegetables and fruits that often consumed raw. The contamination process occurs during plantations irrigation with water polluted by this vibrio group [15]. Salmonella contamination is commonly found in beef or chicken products, but some studies also reported salmonella contamination in ready-to-eat vegetables, such as cabbage, tomatoes, lettuce, and othersm [16]. While Shigella does not have a particular vector and is closely related to water pollution and caused by poor hygiene and environmental sanitation [17] contamination in raw vegetables can occur during the harvesting process the time of washing vegetables if washed with poor-quality water. The least bacteria found in our samples were *Proteus sp.*, as much as 20% of total samples. *Proteus sp.* is an opportunistic bacteria in the human and animal gastrointestinal tract, which can cause food poisoning in raw meat, seafood, vegetables, and canned food when the food does not serve hygienically [18]. When referring to national food safety guidelines issued by the national agency of drug and food control, Republic of Indonesia [19] the number of bacterial colonies found in all research samples showed results exceeding the safe limit, potentially becoming an outbreak in the future.

From a total sample of 241 fresh vegetables, our study obtained 87 (36%) samples contaminated by eggs and larvae of STH and 66 (27%) contaminated by intestinal protozoa with a total prevalence of parasitic contamination reached 63%. These findings are higher than previous studies in Ethiopia [20] which obtained a prevalence of fruit and vegetable parasite contamination of 56%. Similarly, for contamination by eggs and larvae of STH, this study's results are slightly higher than a study in Thailand that found STH parasite contamination of 35.1% in fresh vegetables purchased in the market [5]. Contamination by parasites in the soil can occur through organic fertilizer from animal manure or associated with unhealthy defecation habits, not in latrines but on plantation soil. Research conducted on coffee plantation workers in Jember Regency found that workers infected with hookworms had defecation in the plantation area (32.47%) so that the plantation soil was contaminated with hookworm larvae [21]. Research in India on tea plantation workers also obtained the same results that female tea picker workers defecate in the trenches between tea trees [22].

Among the six vegetables studied, lettuce was the vegetable most contaminated with STH eggs and larvae (63%), and the second one was long beans (60%). At the same time, the most contaminated samples with intestinal protozoa were cabbage (43%), basil (43%), and lettuce (37%). Vegetables least contaminated with STH (13%) and intestinal protozoa (10%) are tomatoes and cucumbers. This study's results are identical with previous studies in Sudan that also obtained lettuce as a vegetable with the highest contamination rate (36.3%) (Mohamed *et al.* 2016). Another study in the markets of the Jimma City in Ethiopia also found lettuce had high contamination by parasites (55.6%) after salad (77.8%) and cabbage (68.9%) [20]. Lettuce, cabbage, and basil are herbaceous plant, so the leaves are close to the ground. It causes these vegetables' leaves to be easily contaminated by soil or dust containing STH or protozoa eggs and larvae. Parasites attached to vegetable leaves will not disappear if not appropriately washed; consequently, lettuce, cabbage, basil, and long beans, which are commonly eaten raw by Indonesians, must be washed properly before consumption, i.e., washed using running water. Variations also influence different types of contaminated vegetables in geographic location, climatic and environmental conditions, type and size of samples, sampling techniques, methods used to detect intestinal parasites, and socioeconomic status [20].

The most contaminating types of STH worms were hookworm eggs and larvae (74.7%), while the least was found eggs of *Ascaris lumbricoides* (1.1%). This study's results followed previous research in Thailand, which also found hookworm as the parasite with the most frequency, 42.9% [5]. Other studies in several countries got different results, such as in Pakistan, hookworm is the second parasite after *Ascaris lumbricoides* that contaminate many vegetables commonly eaten raw [23]. Research in Southern Ethiopia also showed that raw vegetables were the most contaminated by *Ascaris lumbricoides*, which was 12.6%, while hookworm occupied the lowest position, which was only 2.6% of the 270 samples studied [24]. Previous research in Jember showed that many plantation workers are infected with hookworm and are associated with poor sanitation and frequent contact with soil [21]. Therefore, people who work in plantation areas, including vegetable plantations and hookworm infection, can be sources of hookworm pollution in the environment. Sources of contamination can also come from animals, such as dogs and cats, as reservoir animals. Research in Ethiopia found *Strongyloides sp.* larvae to be the most frequently detected parasites apart from other STH worms, including hookworm [25]. The larvae of hookworm and *Strongyloides sp.* in the rhabditiform stage have morphologies that are difficult to distinguish under a microscope compared to filariform larvae. Therefore, the rhabditiform larval stage allows misidentification between hookworm and *Strongyloides sp*. This study also found larvae and adult worms of *Strongyloides stercoralis* with a low prevalence of only 4.5%, with adult worms being more dominant than other stages. The adult worm *Strongyloides stercoralis* can be found in the soil because it has two life cycles, parasitic in the human body and living freely in nature. Soil contamination by *Strongyloides sp.* can also come from reservoir animals, such as infected dogs [25]. If the contaminated vegetables are eaten raw without being appropriately washed, the infective form of STH worms will enter the human body and infect. *Strongyloides stercoralis* infection needs to be watched in immunocompromised humans such as human immunodeficiency virus (HIV) patient because superinfection syndrome can occur [26].

Intestinal protozoa that were mainly detected were *Entamoeba sp.* cysts found in all types of fresh vegetables. These results support previous research conducted by Mohamed *et al.* in Sudan and found *Entamoeba histolytica* to be the most dominant parasite (42.9%) [27]. Another study in Ethiopia also detected Entamoeba histolytica as the second most common parasite that contaminates fruits and vegetables [25]. Intestinal protozoan contamination of raw vegetables can be caused by the increasing use of organic fertilizers, which often come from faeces, and contaminated water for planting and irrigation of rice fields or plantations [28]. Research in Sudan has shown that 14% of water samples used to water the vegetables being sold contain parasites [27]. Apart from contamination of water and soil during planting, parasites' contamination can also occur during the post-harvest process, namely storage and sale [29]. Research in Ethiopia shows that parasite contamination is significantly associated with unclean nails and vegetables that are not washed before placing them in the stall [20], [25]. Other intestinal protozoa found are *Cryptosporidium sp.* oocysts in tomato, basil and cucumber vegetables. *Cryptosporidium sp*. is a cause of diarrhea, especially in immunocompromised and zoonotic individuals. Livestock, domesticated animals, and wild animals can be reservoirs for *Cryptosporidium sp.* [30]. Organic fertilizers from cow dung for vegetable crops can be a source of contamination with *Cryptosporidium sp.* in raw vegetables.

4. CONCLUSION

This research proved that raw vegetables sold in traditional markets can be a source of food-borne diseases because they contain bacteria and intestinal parasites. The level of discovery of bacteria and parasites in raw vegetables, usually directly consumed without cooking, in the Jember Regency is classified as high. It is important to make preventive measures in microbial control of foodstuffs, especially in vegetables that are often consumed raw, to prevent food-borne illness in the future. This study's limitation is that no research was carried out to find the source of contamination and the risk factors from distributors and traders related to contamination of vegetables by bacteria and intestinal parasites. Infection by bacteria and intestinal parasites can be avoided by good food handling practice, washing vegetables properly, avoiding eating them raw and cooking vegetables before eating.

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