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The presence of zoonotic protozoans and nematodes on contaminated street food dipping Sauce in Tangub City, Mindanao, Philippines

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Abstract

Street food consumption has been a popular staple food in both developed and underdeveloped countries including the Philippines. These street foods were commonly dipped into different sauces like soy sauce, ketchup, and vinegar to improve flavor and moisture. In many cases, these dipping sauces can be contaminated with zoonotic protozoans and nematodes because of improper sanitation practices by the vendors and consumers as well as because of non-hygienic food preparation, storage, and handling. Consequently, these sauces can be a major source of infection for the person ingesting them. This study sought to identify the different zoonotic protozoans and nematodes that can be found in the street food dipping sauces in Tangub City. Five (5) mL of the dipping sauce were collected and brought to the laboratory for analysis and identification of zoonotic protozoans and nematodes. The findings of the study were subjected to statistical analysis like frequency, percentage, and analysis of variance. Zoonotic protozoans and nematodes were found, namely: Entamoeba histolytica and Ancylostoma duodenale. Entamoeba histolytica was the most commonly occurring organism in all the samples examined and causes harm in the form of a disease such as amoebic colitis. On the other hand, Ancylostoma duodenale was also considered a zoonotic organism. The samples contained significant differences in the numbers of Entamoeba histolytica (p-value 0.0025) in soy sauce and vinegar, while Ancylostoma duodenale (p-value 0.0172) in soy sauce and ketchup. The study concludes that the consumption of street foods dipped in contaminated different sauces can be a venue for zoonotic disease transmission and should be carefully monitored by the local authorities.

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Introduction

The street food industry plays an important role in meeting the food needs of city and town dwellers in many developing countries. Every day, it feeds millions of people a varied array of low-cost and easily accessible goods (Muinde *et al*., 2011). In the Philippines, Filipino street food gives us a variety of dishes, which include fish balls, tempura, chicken balls, egg waffles ("*kwek-kwek*"), kikiam, calamares, siomai, fried chicken intestine ("*Isaw*"), etc and these foods had been a staple food for common people and family. Consequently, different kinds of dipping sauces, mainly soy sauce, ketchup, and vinegar, come together with street foods that are available for consumer satisfaction. Street food sauces are a worldwide tradition (Jenkins *et al*., 2013). However, the practice of drenching food with liquid to improve flavor and moisture might lead to contamination (Julian, 2016).

One method of getting sauce on street food is to dip it in a bowl of sauce. Consequently, it may result in food diseases for several reasons if sauces are dipped gradually or twice (Kim *et al*., 2011). At present, local authorities, international organizations, and consumer advocacy groups are becoming more aware of the socioeconomic significance of street foods as well as the dangers of being infected with zoonotic organisms that come with them.

Zoonotic protozoans and nematodes are the major cause of infectious diseases in humans, particularly in developed and underdeveloped countries (Jenkins *et al*., 2013). The most typical means by which protozoans and nematodes cause human illness are transferred through food and drink, environmental pollution, and inadequate hygiene (Giangaspero *et al*., 2019). Almost one in ten people get sick every year from consuming tainted food. According to estimates from the World Health Organization, foodborne illnesses caused 420,000 fatalities and 33 million disability-adjusted life years (DALYs) worldwide in 2010 (WHO, 2015).

In Tangub City, street foods had been a staple food of the families. It was a readily available meal that can be easily purchased anytime, anywhere. Different practices, meal preparation procedures, and handling and storage were observed making them a vulnerable source of zoonotic organisms. There had been reported cases from different health facilities within the city of food poisoning and other related gastrointestinal disease experienced by the populace that may be linked to the consumption of these street-vended foods. Therefore, considering the health issues associated with the consumption of contaminated vended foods, this study was conducted to assess the zoonotic organism contamination of dipping sauces (soy sauce, ketchup, vinegar) in the street foods sold at the Public Market, Tangub City.

Materials and methods

Research Design

The researchers used a quantitative method to determine the number of zoonotic protozoans and nematodes found in the contaminated dipping sauce of the street food, while qualitative research was used to interpret the data collected from the informal survey interview of the respondents using a descriptive statistic. These methods were chosen since the study involves collecting samples and conducting surveys to identify zoonotic protozoans and nematodes present in the contaminated street food dipping sauce and document the food safety practices of the street food vendors in Tangub City.

Study Area

The survey was conducted at the Public Market, Barangay 3, Tangub City in the Province of Misamis Occidental, Mindanao, Philippines with the coordinates of 8.064149N, 123.748587E. The city has a land area of 162.78 square kilometers or 62.85 square miles, accounting for 7.92 percent of the total area of Misamis Occidental. The study was conducted in the food stall area of the Old Public Market where several vehicles and people passed by and was the busiest street in Tangub City.

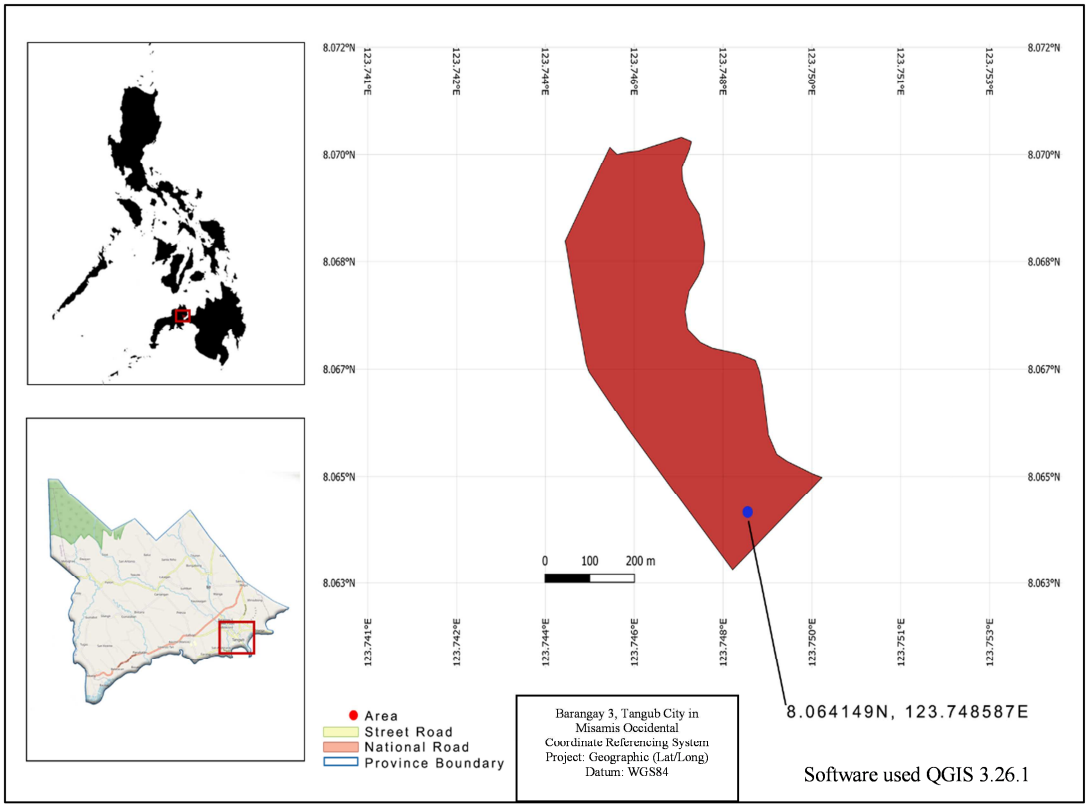


Fig. 1. Map showing the location of Public Market, Barangay 3, Tangub City, Misamis Occidental, Mindanao, Philippines. Source QGIS 3.26.1.

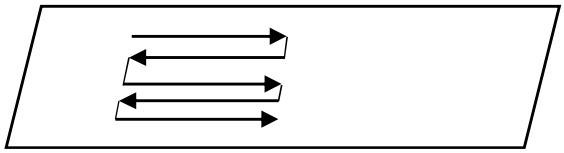


Fig. 2. Pattern and Movement for Counting Parasites.

Entry Protocol

Before conducting the study, the researchers obtained authorization from the LGU of Tangub City. The study's progress was also communicated to the City Health Officer. Upon permission to proceed, the researchers went to the eligible respondents to get their permission in taking part in the study and collect the needed samples.

Collection of Samples

Sauces such as soy sauce, ketchup, and vinegar were gathered from street food vendors near Tangub City's Public Market. There were a lot of people buying street food in the late afternoon, and the sauce had been used since the morning. For ten days, sample sauce was collected from the street food vendors with three different varieties of sauce each day. The samples were obtained by purchasing them from a street vendor and storing them in sterile bottles. A volume of approximately five (5) ml is collected to be analyzed immediately at the laboratory. The samples were divided into two groups: one was analyzed immediately, and the other was refrigerated for one night before being analyzed. This is done to further confirm the occurrence of zoonotic protozoans and nematodes over time.

Microscopy Analysis

A pipette was used to transfer the samples from the sterile bottles to the sterile slides. One drop (0.007 ounces) of sample is placed in the middle of the slides and then spread across the surface, this procedure is repeated until the samples in the sterile bottles were all examined. Furthermore, the samples were examined in a compound microscope with 30x oculars

(eyepiece) with 10x, 40x, and 100x objectives. The samples were photographed, and movement was videotaped to aid in species identification.

Parasite Counting

Starting at the top left of the slide and moving downward in a zigzag manner to the bottom. An extraction pin was used to separate the counted organisms from the non-counted organisms, associated with the Parasite Counting Procedures by World Health Organization (Bruber, 2016)

Identification of Zoonotic Protozoans and Nematodes

Several manuals were used to identify zoonotic protozoans and nematodes. The manuals used were Medical Microbiology for Protozoa: Structure Classification, Growth, and Development (Baron, 1996), Training Manual on Diagnosis of Intestinal Parasites (Geneva, 2004), and Medical Laboratory World: Parasites Under Microscope (Kamram, 2018).

Survey Interview

The researchers used a Key Informant Interview (KII) with a structured questionnaire to gather the respondents' data to establish the vendor's food safety practices.

Data Analysis

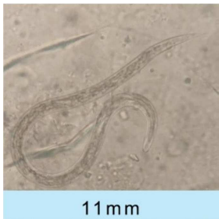
ANOVA was also used to identify the relationship between the number of protozoans and nematodes in dipping sauces. Moreover, descriptive statistics analysis using Microsoft Excel was (Frequency & Percentage) used to analyze the data collected from the survey interview of the respondents.

Results and discussions

Zoonotic Protozoans and Nematodes Found in the Street Food Dipping Sauce

Two zoonotic protozoans and nematodes were found in the dipping sauce collected for the analysis from several street food stalls in Tangub City's Public Market. The organisms were identified by their scientific names using the identification manuals and thorough observation. The species *Entamoeba histolytica* belongs to the Phylum *Amoebozoa,* while *Ancylostoma duodenale* belongs to the Phylum *Nematoda*. Table 1 shows the description and photo documentation of the zoonotic protozoans and nematodes found in the dipping sauce samples.

Table 1. Zoonotic organisms found in the food samples examined in the study.

Microscopic Organism	Description
Entamoeba histolytica 	Phylum: *Amoebazoa* Family: *Entamoebidae* Genus: *Entamoeba* Species: *histolytica* These organisms were observed in x300 and x1200 magnification. Its locomotion is more of a sliding or crawling-like form with a semi-oval to round shape
Ancylostoma duodenale 	Phylum: *Nematoda* Order *Rhabtidia*. Family *Ancylostomatidae* Genus: *Ancylostoma* Species: *duodenale* The organisms were characterized by their slow crawling movement, the organism has a rod structure with a pointed head and tail. It has an S-shaped worm because of its flexure at the frontal end.

According to the data, out of 10 samples taken from various street food sellers, *Entamoeba histolytica* was discovered in soy sauce and vinegar but not in ketchup (see table 2). In an article titled *Entamoeba Histolytica*: Updates in Clinical Manifestation, Pathogenesis, and Vaccine Development, a trophozoite enters the portal vein system and travels to various areas of the human organs (Zulfiqar *et al*., 2018). It then targets galactose and N-acetyl-D-galactosamine residues found on the O-linked sugar side chains of mucins. As a result, even if a single Entamoeba histolytica ingested causes harm in the form of a disease such as amoebic colitis (Azanza *et al*., 2019), how much more if multiple is ingested? On the other hand, *Ancylostoma duodenale* is found in soy sauce and ketchup but not in vinegar (table 2). According to Ross *et al*. (2017), a single appearance of this parasite can be lethal and is highly risky to health.

Table 2. Presence and absence of zoonotic protozoans and nematodes in the dipping sauce.

Zoonotic Organism	Type of Sauce		
	Soy Sauce	Ketchup	Vinegar
Entamoeba histolytica	+	-	+
Ancylostoma duodenale	+	+	-

According to the data (Table 3), *Entamoeba histolytica* predominated over *Ancylostoma duodenale* in the soy sauce. Additionally, ketchup contains 85 *Ancylostoma duodenale* in total, whereas *Entamoeba histolytica* is completely absent. Furthermore, there were 159 *Entamoeba histolytica* identified in the vinegar but none in *Ancylostoma duodenale*. According to Julian (2016), one way to contaminate dipping sauces is by drenching street foods in sauces. The most common foods that consumers dip into sauces are "*Isaw*" (chicken intestine), "*atay*" (chicken liver), and chicken skin (Zabala, 2015). Because the meat used in street foods can carry zoonotic protozoans and nematodes (Chhabra *et al*., 2010), there is a risk that the dipping sauces will be contaminated (Kim *et al*., 2011). It was also noted that due to the reproduction rate of *Entamoeba histolytica* there were more of this species rather than *Ancylostoma duodenale*. According to Robinson (2014), *Entamoeba histolytica's* cysts divide cytoplasmically to create eight trophozoites, but *Ancylostoma duodenale's* cysts can only produce one trophozoite.

Table 3. Total Number of Organisms Observed in the Samples of Dipping Sauce.

Zoonotic Organism	Type of Sauce			
	Soy Sauce	Ketchup	Vinegar	Pathogenic
Entamoeba histolytica	501	0	159	Yes
Ancylostoma duodenale	144	85	0	Yes

Table 4. ANOVA of *Entamoeba histolytica* and *Ancylostoma duodenale* in Different Dipping Sauce.

Groups	*E. histolytica*	*A. duodenale*
	Mean	Mean
Soy sauce	50.1	14.4
Vinegar	15.9	-
Ketchup	-	8.5
F value	12.275	6.8870
P-value	0.0025	0.0172

Table 5. Personal Hygiene of Street Food Vendors.

Personal Hygiene	YES		NO	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Wearing of hairnet	2	20	8	80
Wearing of apron	2	20	8	80
Wearing of plastic gloves	2	20	8	80
Trimming of nails	1	10	9	90
Washing of hands	3	30	7	70

Furthermore, pH levels can also contribute to the rise of zoonotic protozoans and nematodes. According to Barcelon (2015), vinegar and ketchup have a pH level of 3–5, and soy sauce has a pH level of 4-5. Since zoonotic protozoans and nematodes can flourish in an acidic environment (depending on pH level) and considering that protozoans have a higher

tolerance to acid rather than nematodes (Rahman, 2020), the chance for these zoonotic protozoans and nematodes to survive in vinegar and ketchup is lower compared to soy sauce. And as seen by the researchers' results, there is a higher number of zoonotic protozoans and nematodes in soy sauce than in vinegar and ketchup.

Table 6. Food Preparation and Food Handling.

Food Preparation & Handling Practices		Frequency	Percentage (%)
Cleaning of cooking wares	1 -	0	0
	2 -	0	0
	3 -	5	50
	4 -	1	10
	5 -	4	40
Weekly change of dishwashing sponge	1 -	8	80
	2 -	1	10
	3 -	1	10
	4 -	0	0
	5 -	0	0
Changing of table rug	1 -	0	0
	2 -	0	0
	3 -	0	0
	4 -	10	100
	5 -	0	0
Daily washing of food containers	1 -	0	0
	2 -	0	0
	3 -	0	0
	4 -	10	100
	5 -	0	0
Covering the container of the dipping sauce	1 -	0	0
	2 -	8	80
	3 -	2	20
	4 -	0	0
	5 -	0	0
Daily checking of food spoilage	1 -	0	0
	2 -	0	0
	3 -	5	50
	4 -	4	40
	5 -	1	10
Throwing away of left overs dipping sauce	1 -	0	0
	2 -	0	0
	3 -	0	0
	4 -	10	100
	5 -	0	0

Legend: 1- Never, 2- Rarely, 3- Once in a while, 4- Sometimes, 5- Always

Number of Zoonotic Protozoan and Nematode Per Sample

Entamoeba histolytica has a mean value of 50.1 in soy sauce and 15.9 in vinegar. This suggests that there is a difference in the quantity of *Entamoeba histolytica* between the two condiments, and data show that there are more *Entamoeba histolytica* in soy sauce. Recent research in the Philippines found that soy sauce might foster the growth of zoonotic protozoans and nematodes compared to vinegar (Azanza *et al*., 2019). Additionally, Beyhan *et al*. (2016) said that vinegar is utilized to destroy *Entamoeba histolytica*, resulting in cyst and trophozoite reduction. Also, data shows a significant difference in the amount of *Entamoeba histolytica* between dipping sauces, with a p-value of 0.0025.

On the other hand, *Ancylostoma duodenale* has a higher mean value in soy sauce. With a mean of 14.4 in the soy sauce and 8.5 in the ketchup, this means that there is a difference in the number of *Ancylostoma duodenale* between dipping sauces. *Ancylostoma duodenale's* preferred pH to survive is 6-8 pH (Hotez *et al*., 2010). Hence, soy sauce has a more preferable pH for *Ancylostoma duodenale* to survive than ketchup (which has 3-5 pH).

In Kintampo North Municipality, Ghana, 76.2 percent of children tested positive for *Ancylostoma duodenale* (Humphries, 2013). The accumulation of this organism has the potential to inflict disease on people. *A. duodenale* was detected in a baseline of 61% of the respondents from the ages of 7–30 in a similar study in Leyte, Philippines (Shaw, 2010). Also, a p-value of 0.0172 between dipping sauces shows that the presence of *Ancylostoma duodenale* has a significant difference.

Personal Hygiene of the Vendors

Maintaining a clean, healthy external body requires good personal hygiene. One practice is to keep the hair tidy, but according to the data collected, 80% of the respondents do not wear a hairnet. The human scalp's hair is a potential source of zoonotic organism exposure (DeSousa, 2017). This also indicates the dangers of cross-contamination between humans and condiments.

Furthermore, the data indicated that only 80% of the respondents use an apron. In a similar study conducted by Bodmer (2013), clothes are a shelter for parasites, and if infested with such organisms, they can easily be transmitted to the food that is cooked. Wearing an apron, for example, can help to reduce this danger significantly.

Most of the respondents do not wear gloves or trim their nails. According to Nerin (2016), using plastic gloves when preparing food provides further defense against potentially harmful zoonotic protozoans and nematodes. However, the data indicated that only 20% of the respondents wear plastic gloves and 10% of the respondents trim their nails. Data also indicates that the respondents wash their hands whenever they are going to handle money and raw ingredients. Seventy percent of the respondents did not wash them. This adds another risk of contamination to the street food dipping sauce. According to Haque *et al*. (2016), the transmission of zoonotic protozoans and nematodes is considerably aided by bare hands coming into contact with food. Employees who are ill can quickly shed viral infections, and they can do so even before they realize they are sick. Most of these zoonotic protozoans and nematodes have a low pathogenic dosage, which means that humans do not need to consume a large amount of them before becoming ill (Grace *et al*., 2012).

According to Pascual (2019), who conducted a similar observation in Cabanatuan City, the vendors show little concern for personal cleanliness, appropriate work clothes, or hand hygiene measures. Furthermore, according to Hilario's (2015) research on the street food sellers near Far Eastern University in the Philippines, they lack training in food preparation and management as well as knowledge of personal cleanliness. 74 percent of the respondents said they only learned how to prepare food by seeing others do it at food stands, where it is messy, oily, and infested with insects. Leftovers are also left out in the ambient temperature, or what is termed the Temperature Danger Zone (TDZ). If street food vendors have poor personal hygiene, it can contribute to the contamination of street food and street food dipping sauces (Estrada *et al*., 2012).

Food preparation, storage, and handling practices

Another factor that can increase the prevalence of zoonotic protozoans and nematodes is the food preparation, storage, and handling practices of the vendors (Ekanem, 2015). Data shows that 50% of respondents selected the answer "once in a while" in cleaning their cooking wares. Moreover, 80% of the respondents never changed their dishwashing sponge every week. According to a study conducted by Bredbenner (2013), one of the most common sources of bacteria buildup is the kitchen sponge. Sponges quickly become heavily contaminated with a variety of dangerous germs, allowing the illness to spread to hands, kitchen equipment, foods, and other contact surfaces. According to Muinde (2011), sponges can get into contact with the utensils that were used in the preparation of street foods, and these sponges can contain different zoonotic protozoans and nematodes that can cause debilitating diseases when transferred to the dipping sauce.

If there was negligence in cleaning the containers, this would increase the risk of foodborne diseases (Mauinde, 2011). Furthermore, 100% of respondents confirmed that they keep the containers clean. In addition to cleaning their containers, a good usable rug is needed to wipe the containers when dirty, and the respondents change the table rug sometimes. Covering food with plastic cellophane/foil or putting it in a closed container is one of the practices in preparing and handling food, but data shows that 80% of the respondents rarely practice such preparation and food handling techniques. According to Samapundo (2015),

consumers and vendors did not know that foodborne diseases coming from zoonotic protozoans and nematodes could contaminate the food, so to prevent such organisms; it is needed to be covered. Nonetheless, 50% of the respondents once in a while checked for food spoilage, and after being inspected, 100% of the respondents sometimes throw away the leftovers and unsold dipping sauces. Foods that are still good are reheated, and spoiled food is thrown away (Ryan *et al*., 2019). Zoonotic protozoans and nematodes are spread by foodborne, waterborne, or person-to-person transmission, with foodborne being the most prevalent. The rapid growth of these zoonotic protozoans and nematodes is caused by infrequent sauce changes; it has been recorded that they change the sauce twice to three times every two weeks. However, changing the sauce twice or three times does not change the fact that zoonotic protozoans and nematodes will still accumulate on the food due to low environmental conditions and food preparation.

According to Hilario's (2015) research the street food sellers near Far Eastern University in the Philippines, the conditions of the food sold are a Temperature Danger Zone (TMZ), which only indicates that the food is susceptible to foodborne diseases. Contrary to the researcher's results in terms of hygienic procedures, food preparation and processing, and food contamination prevention, street food sellers in Dipolog City observed food safety (Van & Olmogues, 2021).

Conclusion

Based on the results gathered in this study it was shown that there is a great concern about the consumption of street foods in Tangub City. Strategies and actions should be crafted and implemented to reduce the risk of the consumers being infected with the diseases brought about by zoonotic organisms contaminating the vended foods. Furthermore, it is also necessary to have stern monitoring of the compliance of the vendors to the said strategies and actions. On the other hand, intensive education campaigns and awareness raising should be made to improve sanitary practices in food preparation, handling, and storage, hence reducing the risk of food contamination and further spread of zoonotic diseases.

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