Nutritious food and public health risks: Insights on foodborne pathogens in edible land snails

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A thesis

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of the requirements for the Degree of

Doctor of Philosophy

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by

Tanyitiku Mary Nkongho

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“Customer! Customer! Cus! You want buy Nyama? I tire your own for how much?”
(Snail meat market seller, July 20, 2019, Buea, Cameroon)

“Congo meat! Congo meat! Buy one chop two, buy two chop bucket!”
(Snail meat street hawker, September 12, 2019, Buea, Cameroon)

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The edible part of a snail is the 'foot' commonly termed snail meat. It contains high-quality protein, low fat, high calcium, magnesium, iron, and little or no cholesterol. Researchers believe that a continuous consumption of snail meat could support healthy immune systems, thus, preventing acute to severe health conditions such as obesity, type 2 diabetes, cardiovascular diseases, and certain cancers. People are becoming informed of these health benefits and their consumption of snail meat is gradually increasing especially in many communities in Africa and Europe.

However, snail farming or heliciculture is still under-developed around the world. Snail-consuming populations have often picked land snails from locations that include, swamps, forests, gardens, footpaths, farmlands, household wastes, stones, and logs of wood or termed 'anywhere'. The close association of land snails with the soil and decaying vegetation, coupled with its uncontrolled free-living and feeding patterns, could make them susceptible to microbial contamination. Consequently, humans could easily get exposed to foodborne pathogens in these 'snail picking' locations or during the handling and/or consumption of snail meat. The aim of this PhD thesis was to understand foodborne disease health risks in the handling and/or consumption of snail meat, which is currently a desirable source of food among rural communities in sub-Saharan Africa.

Specifically, this research focused on land or terrestrial snails where I sought to 1) explore human foodborne pathogen exposures in snail handling and/or consumption practices, 2) determine the prevalence of potential foodborne pathogens (Shigatoxigenic *E. coli* (STEC),

Campylobacter spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp.) in edible land snails collected from different natural habitats (farms and within dwellings) and local markets, and 3) evaluate the survival characteristics of STEC and non-STEC strains in the faeces of edible land snails.

To achieve these research objectives, a methodological framework was developed (in Chapter 1), and the results are organized in three results chapters (Chapter 2, 3 & 4), using peer-reviewed journal formats.

In Chapter 2, a field work-based study is described. It was conducted in Buea (Cameroon) where in-depth face-to-face interviews, participant observation, and focus group discussions were used to explore participant lived experience, stories, perceptions, habits, and routines that may involve human foodborne pathogens exposures in local snail consumption practices. Two theoretical frameworks; soft systems methodology and social practice theory were used to interpret the study findings. In our analysis from this research study area (Buea), several opportunities for human exposure to foodborne pathogens were apparent such as, snail picking in domestic wastes and sewage, the selling of unpackaged live snails, improper snail meat washing and hawking in loosely closed buckets.

In Chapter 3, we examine the faeces of live edible African land snails, sampled within the same population described in Chapter 2, for the presence of Shiga-toxin producing *Escherichia coli* (STEC), *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. Experiments were conducted using high-fidelity DNA polymerase (repliQa Hifi toughmix: Quantabio, MA, USA) in single PCR techniques. The presence of an amplicon of the appropriate size for each PCR assay in each sample was recorded as a positive result. For STEC, a positive result required the detection of both *Stx1* and *Stx2* genes. The results showed that land snails from the study area contain high prevalence (57-86%) of STEC, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. Indeed, at least one

pathogen was detected in every sample examined, highlighting the threat of foodborne disease.

Lastly, in Chapter 4, we examine the transmission dynamics and survival of pathogens in snails. A model system was established and used to explore the proliferation and survival of *E. coli* (STEC/non-STEC) strains. Well-studied STEC and non-STEC strains in the faeces of *E. coli*-inoculated land snails, which had been grown in a laboratory model system, were compared using plate counting method. Colonies were visually enumerated and expressed as a logarithmic function of colony forming units over 30 days of the experiment. In this system, the average rate of decline of non-STEC strain (CSH-62) in the faeces of live snails was significantly ($p<0.05$) faster than STEC strain (ERL 06-2503). Thus, using the non-invasive laboratory-adapted *E. coli* strain, CSH-62 and Shiga-toxigenic pathogenic *E. coli* strain, ERL 06-2503 in this research has demonstrated that the outcome of *E. coli* interactions with land snails may be significantly ($p<0.05$) strain dependent.

In conclusion, our findings revealed that foodborne health risks in local snail practices have, until now, been poorly described. Thus, this research's qualitative and quantitative approaches reveal local practices and perceptions, incidence of individual foodborne pathogens and survival traits of Shigatoxigenic and non-toxigenic *E. coli* strongly indicate that unidentified foodborne diseases could be occurring among snail meat consuming populations. It also suggests fruitful opportunities aimed at improving health outcomes in the handling and/or consumption of snail meat which could be achieved in conjunction with deeper understanding of community food practices and eating habits.

Keywords: edible land snails, nutritious food, snail consumption practices, microbial contamination, foodborne pathogens, qualitative research, molecular techniques, microbial culturing, public health risks

Acknowledgements

"*There isn't time enough to sing of all you've done. But I have eternity to try. With a thousand hallelujahs. I magnify your name. You alone deserve the glory, the honor and the praise Lord Jesus this song is forever yours. A thousand hallelujahs and a thousand more*"

(Brooke Ligertwood, 2022)

God has been faithful in this research journey, and he keeps proving that his WORD is true and amen. May his name be praised forever.

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May our good Lord richly bless you.

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Chapter 1. Introduction

1.1 Background

The 21st century has evolved into a world of uncertain times. While people continue to strive to adapt to the COVID-19 pandemic, there is an urgent need to achieve the United Nations Sustainable Development Goals (SDGs) by 2030. Goals such as "No poverty" (SDG1), and "Zero hunger" (SDG2) remain top priorities in many countries around the world. In 2020, 265 million people in developing countries were estimated to suffer from acute food insecurity (The Lancet Planetary, 2020; WFP, 2020). Many people lack access to sufficient food or food of adequate quality and are thus, pushed into food poverty (The Lancet Planetary, 2020).

In the quest to eradicate poverty and end hunger, the greatly affected local populations need to be educated (SDG4), on 1) the benefits of eating healthy nutritional diets, 2) the risks of foodborne disease if contaminated foods are consumed, and 3) the importance of improving public health through microbial-free food consumption practices. Local and governmental bodies need to be informed on the relationship between community eating habits and food safety risks, and its consequences on the health and wellbeing (SDG3) of its population. Best results could be achieved when food safety risks in domestic and food vending/eating settings are fully understood, communicated, and acted upon.

In this research, we sought to improve health outcomes in snail meat consumption practices. We considered the process of picking[1] *land snails, their preparation and subsequent human consumption as a system of activities (processes) with interacting actors (people).*

[1] The wandering around on foot in search of snails, and subsequent gathering for human consumption

The picking of land snails from the wild for human consumption has been a traditional and ancestral activity in many European and African countries (Adeyeye et al., 2020a; Kaldjob et al., 2019; Mohammed et al., 2014; Ngenwi et al., 2010; Waldhorn, 2020). With the exact period still unclear in the literature, archaeologists estimated that snail picking, and consumption started some 12,000 years ago or some will say, since prehistoric times (Okafor, 2009). The interactions between snails and humans became very prominent during the height of the Roman Empire when it was a common practice to eat snails in the courts of the emperor (Okafor, 2009; Paszkiewicz et al., 2014). Growing up among snail-consuming populations in Cameroon, I have observed and participated in the picking of land snails for sale and domestic consumption, as early as the 1980s when I was born. Many families often narrate how they package dried snail meat and air freight to their loved ones overseas. Given that I was taught by my experienced parents who were also taught by their parents, etc., it thus indicates that the picking of land snails for domestic consumption and exportation has existed in many generations in this research study area, Buea, Cameroon.

During the same 20th century, the overcollection of snails in several regions in Europe (such as, France and Spain), depleted the number of snails in the wild and its tradition collection has declined over time (Waldhorn, 2020). Today, increasing awareness of the nutritional and health benefits of consuming snail meat is promoting the initiation of snail farming systems across Europe (Murphy, 2001; Paszkiewicz et al., 2014). In West and Central Africa, edible land snails are largely abundant in the jungles, and snail picking remains a common practice until date. Since Africans are greatly affected by acute protein shortages, people in the region have been found to eat snails to alleviate these

protein acute shortages (Okafor, 2009). It is also 'big business'[2] and often a relevant source of livelihood for rural dwellers in humid forests and derived savanna zones (Mohammed et al., 2014; Ndah et al., 2017; Ngenwi et al., 2010; Waldhorn, 2020).

However, increasing demand for African snail species has prompted a random collection of edible land snails from locations that could be termed 'unhygienic' (Akpomie, 2013; Mohammed et al., 2014; Nyoagbe et al., 2016; Tanyitiku et al., 2022a). The tradition of searching for snails in the wild and/or within dwellings, especially to obliterate hunger and/or to earn an income (Mohammed et al., 2014; Ngenwi et al., 2010; Nyoagbe et al., 2016; Okafor, 2009), could also expose people to foodborne pathogens and consequently, contribute to foodborne disease burdens in the world and Africa in particular.

Consequently, my research project for the award of a PhD sought to understand foodborne disease risks in snail handling and/or consumption. It was carried out in 1) Buea, Cameroon, and 2) Lincoln University, New Zealand.

1.2 Research problem

Snails are primarily consumers and decomposers at the last trophic level in food webs. They feed on plants, algae, and other producers that make them exhibit numerous roles such as, 1) in transforming organic wastes (decaying plants) into inorganic (nutrient-rich) soil (Okafor, 2009), and 2) as commensals since they inhabit many bacterial communities (Kiran et al., 2012; Pawar et al., 2015). In as much as humans are largely reaping the benefits of having land snails in the ecosystem including snail as a source of food (Adeyeye et al., 2020; Cobbinah et al., 2008; Kaldjob et al., 2019; Ngenwi et al., 2010;

[2] A commercial activity with opportunities for large-scale production, thus leading to significant financial benefits

Nyoagbe et al., 2016; Otchoumou et al., 2010), their disease-causing potentials should not be ignored (Nyoagbe et al., 2016; Tanyitiku et al., 2022a).

Across sub-Saharan Africa, these studies (Adagbada et al., 2011; Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Okafor-Elenwo & Imade, 2019; Okafor & Ogbo, 2019) have highlighted the food safety risks of land snails/snail meat harvested in farms and sold at local markets. However, the microbial contamination of land snails collected from free-living environments (including dwellings) for human consumption, have been significantly ignored (Tanyitiku et al., 2022a; Tanyitiku et al., 2022b). Local snail handling and/or consumption practices that could expose people to foodborne pathogens remain under-researched. Cameroon was chosen as the study area for three reasons: 1) my own lived experience in Cameroon of snail picking to consumption practices; 2) my access to the local population as the main researcher of this thesis; and 3) snail meat is consumed by many of the local inhabitants in Cameroon, and snails are often collected from obviously unhygienic situations. Snail meat is a desirable source of food, and it is richly consumed in homes, ceremonies as well as sold as street food along road junctions, bus stations, motor parks, restaurants, and local markets.

With a specific focus on snail handling and consumption practices, this research was carried out because there were current gaps in food safety knowledge, and limited understanding on *where people get snails for consumption, what people do to get snails, why they do what they do, what they know about what they do vis-à-vis hygiene,* and what *they know about healthy eating habits and food safety at home and on the streets*. In addition, similar to many food products, no information exists on food safety issues that could occur during snail harvesting, handing and consumption in Cameroon. There are no data on the foodborne disease risks of consuming contaminated snail meat. This has limited local and

government initiatives aimed at addressing foodborne disease risks in snail handling and/or consumption. As such, there was an urgent need to identify and document the microbiological hazards that people encounter during snail handling and/or consumption specifically in Cameroon and other snail consuming countries.

1.3 Research objectives

The purpose of this study was to provide insights on the public health risks of consuming contaminated land snails. Figure 1.1 presents a relationship between the three specific objectives of this research.

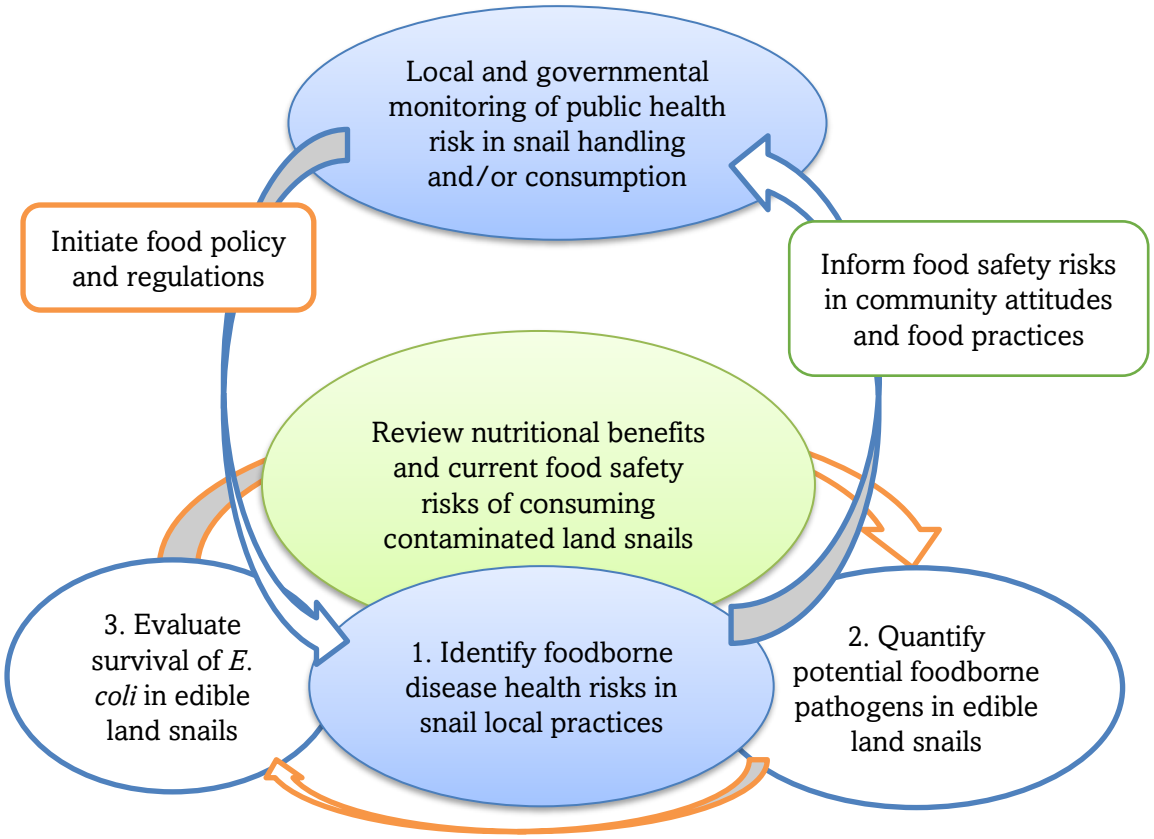


Figure 1.1. Relationship between this research three objectives

From Figure 1.1 above, this research was carried out through three studies:

a. To identify human exposures to foodborne pathogens in local snail practices, a case study involving rural inhabitants of Buea, Cameroon,
b. To examine the presence of potential foodborne pathogens (STEC, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp.) in the faeces of edible land snails, sampled in the people's snail picking locations in Buea, Cameroon, and
c. To develop and establish a laboratory model snail system, to evaluate the survival characteristics of STEC and non-STEC strains in the faeces of edible land snails.

With the application of qualitative (social research) and quantitative (microbiological analyses) approaches, the study findings provide fruitful opportunities to improve food-related health outcomes among snail handlers and consumers.

1.4 Rationale of the study

Microorganisms are found everywhere in nature, in food and non-food products. Some are beneficial, such as in ripened cheeses, pickles, and fermented sausages, and give characteristic flavours and qualities to food (Erkmen & Bozoglu, 2016; Thomas et al., 2017). Others such as moulds, deteriorate and spoil food causing unpleasant odours, flavours, and textural changes. On the contrary, pathogenic microorganisms may not cause visible changes to food, therefore, a food handler or consumer will not know if the bacteria are present (Erkmen & Bozoglu, 2016; ICMSF, 1998; Labbe & Garcia, 2013; Quinlan, 2013; Sofos, 2013). The entry of pathogenic microorganisms (foodborne pathogens) into food is a threat to the safety of food handlers and consumers (Havelaar et al., 2015; Heredia & García, 2018; Labbe & Garcia, 2013; Quinlan, 2013; Sofos, 2013; Spanu et al., 2014; WHO, 2015).

The WHO Foodborne Disease Burden Epidemiology Group (FERG) estimated that the global burden of foodborne illnesses primarily originates from a selection of 31

foodborne hazards which include 32 diseases: 11 diarrhoeal disease agents (one virus, five bacteria, and one protozoa), 10 helminths, and three chemicals (Havelaar et al., 2015; Hoffmann et al., 2017; Labbe & Garcia, 2013; WHO, 2015). In the African regions of the WHO, these 31 foodborne hazards were estimated to cause 1200 -1300 DALYs[3] per 100,000 inhabitants in 2010, compared to 35 - 711 in other regions (Pires et al., 2021).

Foodborne illnesses occur when a pathogen is ingested with food and establishes itself (and usually multiplies) in the human host, or when a toxigenic pathogen establishes itself in a food product and produces a toxin, which is then ingested by the human host (Bintsis, 2017). Depending on the incubation period, that is, the time from food ingestion to the appearance of symptoms, foodborne illnesses are often classified as food infections or food intoxication (Bintsis, 2017). In both cases, its severity is dependent on the health status of the individual and could range from mild (diarrheal, vomiting, dizziness, abdominal pains) to severe cases such as hemolytic uremic syndrome, and sometimes death. Meat borne infections occur as result of the reaction of the body tissues to the entrance of microbial pathogens in the human host during the consumption of meat and meat products (Almashhadany, 2021). Most of these diseases are zoonotic diseases that are transmitted to humans directly or indirectly, and therefore, meat and its products play important roles in the transmission of foodborne pathogens (Bintsis, 2017; Almashhadany, 2021). Indeed, FERG further estimated that about 60% of foodborne illnesses are caused by microbial pathogens such as *Listeria monocytogenes, Staphylococcus aureus , Clostridium botulinum, Clostridium perfringens, Bacillus cereus , Escherichia coli , Salmonella* spp.*, Yersinia enterocolitica* and *Campylobacter jejuni* (Bintsis, 2017; Havelaar et al., 2015; ICSMF, 2005;

[3] Disability Adjusted Life Years (a time-based measure that combines years of life lost due to premature mortality (YLLs) and years of life lost due to time lived in states of less than full health). The WHO estimates that diarrhoeal diseases have a global burden of 99,727,954 DALYs and that 90% of these are the result of illness in lower income and lower middle-income countries (Grace, 2015b)

Kirk et al., 2015), which are principally frequent in bacterial meat borne infections (Almashhadany, 2021).

Furthermore, the World Health Organization's most recent data on global deaths, showed that about 600 million people, that is, one in ten people in the world each year, fall ill after eating contaminated food (Hoffmann et al., 2017; WHO, 2015). Annually, 420,000 people die, including 125,000 children under the age of five years (Hoffmann et al., 2017; WHO, 2015). In many developing countries, foodborne disease outbreaks are underreported or underestimated, and may only be noticed if connected to situations that are of major public health or economic impact (Odeyemi, 2016; Pires et al, 2021), because research, disease surveillance data, and reported incidence of foodborne disease burdens among the African population is limited (Pires et al, 2021; WHO, 2015). This limited availability is caused by various factors, such as, the lack of capacity to generate, compile and analyse data, limited political commitment to strengthen disease surveillance systems, limited understanding of the benefits of studies on disease burdens and more focus on selected notifiable priority diseases[4] (Zandonadi et al., 2020; Pires et al., 2021). Unfortunately, these above-mentioned factors and more, have contributed to uncertainties in foodborne disease estimates in Africa (Pires et al., 2021).

In this research, foodborne disease (FBD) is defined as any disease that results from the ingestion of microbiologically contaminated food (Grace, 2015b). FBD represents one of the most common and important public health problems in the world, since it is one of the major causes of personal distress, preventable deaths, and avoidable economic burden (da Silva Farias et al., 2019). Despite the considerable global burden of FBD, the full extent of unsafe food with respect to its damage to the public is unknown (Blaser,

[4] It should be noted that foodborne diseases are not on the priority list in many developing countries (Odeyemi, 2016; Pires et al., 2021)

2011; da Silva Farias et al., 2019). A public health risk is defined as those microbial exposures that are likely to be harmful to the health of snail meat consuming populations. Health risk factors represent those characteristics, conditions, or behaviours that could increase the people's likelihood of getting sick from foodborne diseases. Several researchers (see Table 1.1) have demonstrated that land snails contain the required food nutrients for a balanced diet, but their interactions with pathogenic microorganisms could be devastating, and human exposure to these foodborne pathogens could contribute to infectious conditions or foodborne diseases after snail handling and/or consumption (such as in foodborne disease outbreaks).

Previous research (Erkmen & Bozoglu, 2016; ICMSF, 1998; ICSMF, 2005; Meffowoet et al., 2020; Van Horn et al., 2012) has indicated that snails could serve as intermediate hosts to many parasites including *Clonorchis sinensis, Eschinastoma ilocanum, Fasciola hepatica, Fasciola gigantica, Fasciolopsis buski, Opisthorchis felineus* and *Paragonimus mestermani*. In addition, ready-to-eat snail meat contaminated with enteric pathogens represents significant food safety risks to handlers and consumers. Studies of microbial pathogens in land snails (an essential meat/meat product) appear to be uncommon (Adagbada et al., 2011; Akpomie, 2013; Barimah, 2013; Cicero et al., 2015; Nyoagbe et al., 2016), and, to our knowledge, absent in Cameroon, an aspect addressed in this study (Tanyitiku et al., 2022a; Tanyitiku et al., 2022b; Tanyitiku et al., 2022c). For example, as presented in Table 1.1, the potential of land snails to cause meat borne infections of significance to public health have received very little attention. As such, to improve public health, information on the food safety of edible land snails/snail meat is required like any other meat product which are handled and/or consumed by humans.

Table 1.1 Gaps in knowledge on health risks in snail handling and/or consumption.

Existing knowledge	**Research Gaps**
Microbiological evaluation of animal meat products including chicken (Nzouankeu et al., 2010) pork (Yannick, 2013), beef (Djoulde et al., 2015) in Cameroon	For Cameroon, no comparable studies in live edible snails collected in natural habitats for human consumption, or in live snails and snail meat sold in local markets, or on ready-to-eat snail meat sold in local markets, streets, roadsides, and motor parks.
Microbiological evaluation of edible land snails in farms and markets in Ghana (Barimah, 2013; Nyoagbe et al., 2016), Nigeria (Adegoke et al. 2010; Obi & Nzeako, 1980), Greece (Cicero et al., 2015; Parlapani et al., 2014) and in Europe (Kiebre-Toe et al., 2003; ICSMF, 2005; Temelli et al., 2006).	No studies on free-living or farm snails in Cameroon and many other nations, foodborne pathogens of public health significance have been largely ignored No studies on the implications of microbial pathogens to helicicultural practices
Snail intestinal microbial communities' analyses to understand the diversity of their microflora (Dar et al., 2017; Jess & Marks, 1989; Kingsley et al., 2009; Onifade & Aiyenuro, 2018; Oyeleke et al., 2012; Kiran et al., 2012).	No studies on the interactions and survival characteristics of foodborne pathogens associated with edible land snails No studies on the log decimal reduction (D-valves) of microbial pathogens No studies have assessed microbial pathogens of public health importance in the guts of snails
Sources of microbial contamination during industrial snail meat processing in Greece (Parlapani et al., 2014) and Bursa-Turkey (Temelli et al., 2006)	No studies in community food consumption settings and in domestic snail meat consumption practices or that reveal how humans are exposed to foodborne pathogens. No studies on human foodborne pathogen exposures and health risks in snail handling and/or consumption
Studies on standard methods/protocols aimed at isolating human enteric viruses in edible land snails (Paszkiewicz et al., 2014)	No studies for bacterial pathogens that are significant to public health

1.5 Research approach

This research approach was guided by the objective of this study: to understand health risk factors in snail handling and/or consumption. With focus on snail meat consumption we, 1) explored and identified those human practices that could lead to foodborne diseases, and 2) examined the presence of foodborne pathogens in the faeces of edible land snails collected from natural habitats and developed in a laboratory model system. Figure 1.2 presents the methodological framework of this research which incorporated three key terms: edible land snails, human activities, and foodborne pathogens.

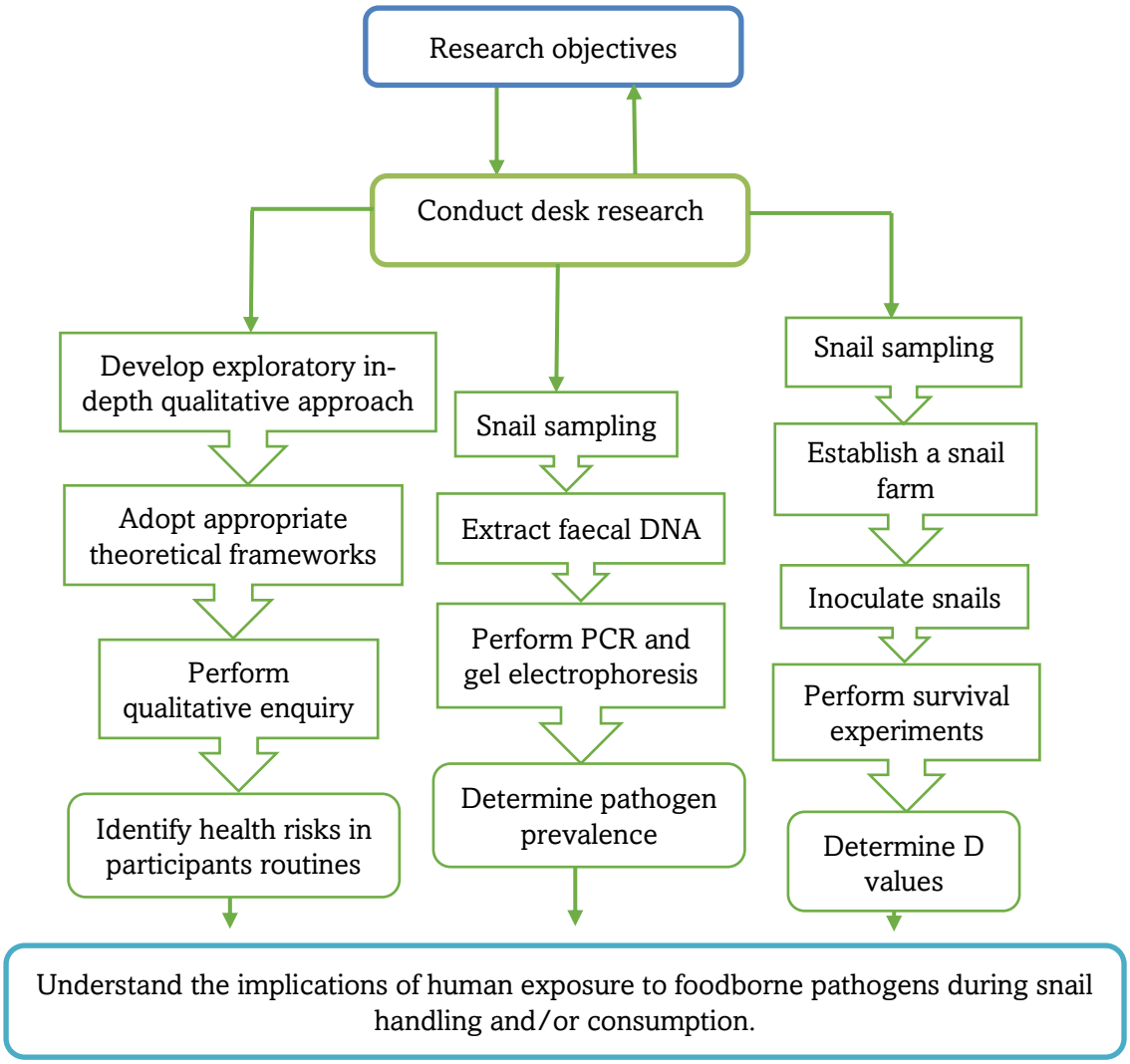


Figure 1.2. Methodological framework

The first part of this research was desk research, as detailed in Appendix A. Here, we carried out a literature search to review previous knowledge with respect to snail as a nutritive food, as well as pathogenic microorganisms associated with land snails and its relevance to foodborne disease outbreaks (Section A.1). An understanding of the nutritional and health benefits of consuming land snails (Section A.3), and current understanding of the significance of the association of microbial pathogens with land snails (Section A.4), provided a rationale for conducting this research (shown in Table1.1). This clearly gave us insights into food safety issues that needed to be addressed if foodborne disease health risks are to be eliminated during snail handling and consumption.

Secondly, a qualitative approach targeted the community eating habits and snail practices in Buea, Cameroon (Chapter 2). We explored snail local practices that could expose local populations to foodborne pathogens. Here, an understanding on how people's routines, activities, perceptions, and behaviors, could lead to public health risks was interpreted. We used in-depth qualitative inquiries to understand the complex social practices (leading to human foodborne pathogen exposures) that existed in a developed systemic model of actors (snail collectors, market sellers, home consumers, street eaters) and activities (snail picking, washing, cooking, vending, eating). Then quantitatively, using molecular detection techniques (DNA extraction, PCR, and gel electrophoresis), live snails were collected from various natural environments (people's habitual snail picking locations), and snail faeces were examined for the presence of five key foodborne pathogens: *STEC*, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp (Chapter 3). The results indicated that unidentified foodborne diseases could be occurring among snail handlers and consumers especially in tropical African regions.

The last part of this research was laboratory-based at Lincoln University, New Zealand. We established a laboratory snail model system and subsequently revealed that non-STEC and STEC strains could survive for at least a month in edible land snails, and this survival was significantly dependent on the strain of *E. coli* (Chapter 4). As estimates of foodborne illnesses can be used to direct food safety policy and interventions (Scallan et al., 2011), this study could inform the implementation of food policies and regulation procedures in snail handling and/or consumption in many nations, who are involved in snail handling and/or consumption. Moreover, the results of this research have established a starting point for future research aimed at eliminating food safety risks in snail meat consumption practices in many sub-Saharan countries, including Cameroon where information on land snails/snail meat foodborne disease risks are limited.

1.6 Interests in these specific foodborne pathogens

The consumption of foods containing foodborne pathogens is responsible for more than two million deaths each year in developing countries (Odeyemi, 2016). In the WHO's African regions, nearly 70% of these foodborne diseases are caused by diarrheal-related microbial pathogens (Odeyemi, 2016; Pires et al., 2021). This research focused on five potential human foodborne pathogens (that is, Shigatoxigenic *E. coli (STEC)*, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp.), which have been documented by FERG as causing significant risks to public health (Havelaar et al., 2015; Hoffmann et al., 2017; Labbe & Garcia, 2013; Pires et al., 2021; WHO, 2015). In this section of the thesis, we have highlighted 1) the morphological characteristics and pathogenicity of these microbial pathogens, 2) the mode of transmission, and 3) their occurrence in selected outbreaks of foodborne diseases.

1.6.1 Escherichia coli

Escherichia coli is made up of a large and diverse group of bacteria that are common members of the intestinal flora of animals, including humans (Bintsis, 2017; Almashhadany, 2021). They are Gram-negative, mobile, or immobile, flagellated, or non-flagellated non-spore forming rods. *E. coli* is characterized as facultative anaerobes and ferment simple sugars such as glucose into lactic, acetic, and formic acids. Its optimum pH for growth is between 6.0 to 8.0, although growth could occur as low as pH 4.3 and as high as pH 10 (Bintsis, 2017). Although most strains of *E. coli* are harmless, some strains possess disease-causing characteristics, including toxins.

The pathogenic variants (pathotypes) of *E. coli* are highly involved in morbidity and mortality worldwide. Based on its different pathogenic mechanisms, pathogenic *E. coli* is classified into six groups: 1) Enteropathogenic *E. coli* (EPEC), 2) Enterohemorrhagic *E. coli* (EHEC, also known as Shiga toxin-producing *E. coli* [STEC] or formerly Verotoxin-producing *E. coli* [VTEC]), 3) Enterotoxigenic *E. coli* (ETEC), 4) Enteroaggregative *E. coli* (EAggEC), 4) Enteroinvasive *E. coli* (EIEC); and 6) Attaching and Effacing *E. coli* (A/EEC) (Bintsis, 2017; Almashhadany, 2021). All the aforementioned groups are of great public health concern as they have lower infectious doses and are transmitted through mediums, such as, food and water (Bintsis, 2017; Almashhadany, 2021).

Mode of transmission: The principal reservoir of pathogenic *E. coli* is the intestinal tract of cattle, although other animals may serve as reservoirs (Bintsis, 2017). Its spread to other parts of the animal (meat) often occurs during slaughter or meat processing (Bintsis, 2017; Almashhadany, 2021). Also, the use of animals' faeces for example, as fertilizers for agricultural crops could contaminate fresh produce and irrigation water (Avery et al., 2004; Kudva et al., 1998). *E. coli* can survive for longer

periods in the environment and can proliferate in vegetables and other food products (Bintsis, 2017; Almashhadany, 2021). Transmission of *E. coli* into human host occurs when we ingest food (such as, lettuce, alfalfa sprouts, salami, and raw (unpasteurized) milk, juice, cider) or water that has previously been contaminated with the faeces of *E. coli*-infected animals (Bintsis, 2017). Infected people, for example, could spread *E. coli* to others if they do not properly wash their hands after using the toilet (Bintsis, 2017; Almashhadany, 2021). STEC infection in particular, could lead to episodes of mild to severe diarrhoea (loose stool), and 5-10% of infections develop into hemolytic uremic syndrome (HUS), a severe complication marked by profuse bleeding, kidney failure and sometimes death (Almashhadany, 2021; Frank et al, 2011).

Occurrence in foodborne diseases: *E. coli* O157: H7 is an STEC strain that emerged as a significant public health threat in 1982 during two foodborne disease outbreaks, associated with undercooked ground meat (Nkane et al., 2016; Scallan et al., 2011). In 1993, an *E. coli* O157: H7 outbreak was associated with hamburgers purchased from a fast food chain and this resulted in more than 500 laboratory-confirmed infections and at least four deaths in the United States (CDC, 1993). Then in 2011, a rare strain of *E. coli* O104: H4 led to the second largest and deadliest outbreak of *E. coli*-associated disease ever recorded in the world (Bintsis, 2017; Frank et al., 2011). For instance, between May 21st and July 22nd, 2011, more than 4,000 illnesses and 50 deaths were reported, and HUS was associated with 800 cases in Germany and 15 other countries (Blaser, 2011; Franck et al., 2011).

Currently, in the United States, *STEC* strain O157: H7 is estimated to cause 63,000 illnesses, 2,100 hospitalizations, and 20 deaths each year (Scallan et al., 2011; CDC 2016a). Indeed, *E. coli* O157: H7 foodborne disease outbreaks have been reported in baby

spinach, leafy greens, romaine lettuce, clover and alfalfa sprouts, Northfork Bison, soy nut butter, ground beef, cake mix, among others (CDC, 2021; CDC, 2022). Today, a wide variety of foods, including fresh produce, serve as a vehicle for the transmission of *E. coli* to humans (Scallan et al., 2011; CDC, 2021; CDC, 2022).

In WHO's African regions, Kenya has recorded several *E. coli* foodborne disease outbreaks. For example, in 2009, a gastroenteritis outbreak was associated with *E. coli* in Madera (Arias-Granada et al., 2021). In 2016, an *E. coli* outbreak in Maru, affected more than 50 children, which led to at least two deaths (Arias-Granada et al., 2021).

1.6.2 Campylobacter

Campylobacters are mobile Gram-negative, comma or s-shaped bacteria. Dozens of *Campylobacter* spp. have been implicated in human diseases worldwide, however, *C. jejuni* and *C. coli* are the most common disease-causing species representing 80-90% and 5-10% of infections respectively (Almashhadany, 2021). *C. jejuni* and *C. coli* are also known to cause bacteraemia in immunocompromised patients (Bintsis, 2017). These species cause illnesses characterized by fever, vomiting, nausea, and abdominal pain typically associated with backache, foul-smelling watery and bloody diarrhoea (Almashhadany, 2021). Campylobacteriosis has an incubation period of between 2 and 11 days or on average 3-5 days.

Mode of transmission: The most well-known source of *Campylobacter* is poultry, although due to various natural habitats of poultry, *Campylobacter* spp. have also been involved in the intestinal flora of healthy domestic and wild animals, including cattle, sheep, goats, pigs, ducks, geese, dogs, cats, rodents, wild birds, and marine mammals. All animals used for food, as well as domestic pets, could inhabit *Campylobacter*. In most cases, campylobacteriosis are associated with consuming food products such as, raw or

undercooked chicken, unpasteurized milk, and contaminated water, or cross-contamination with other foods (Bintsis, 2017; Lahti et al., 2017). *C. jejuni* is often found associated with bodies of water such as troughs, streams, and groundwater (Bintsis, 2017). *Campylobacteriosis* has also been associated with the consumption of raw fruits, vegetables, and unpasteurized fruit juices (Mohammadpour et al., 2018; CDC, 2021).

Occurrence in foodborne diseases: A yearly prevalence of *Campylobacter* foodborne outbreaks linked to fresh produce was reported between 2002 and 2012 in the United States (57%), Japan (8%), and New Zealand (6%) (Mohammadpour et al., 2018). Although few outbreaks of campylobacteriosis have been detected, it is estimated to be the third most common foodborne illness causing an annual incidence of 4.4 and 9.3 per 1000 inhabitants in developed countries such as UK, Australia, New Zealand, and the United States (Bintsis, 2017; Lahti et al., 2017; Moffatt et al., 2016; Mohammadpour et al., 2018). For example, *C. jejuni* is responsible for approximately 850,000 illnesses, 8,500 hospitalizations, and 76 deaths in the United States each year (Bintsis, 2017; Franco & Williams, 2001).

The World Health Organization estimated that approximately 1% of people living in western Europe could be infected with campylobacteriosis each year (Bintsis, 2017). Mohammadpour et al. (2018) further estimated that the worldwide prevalence of *Campylobacter* in vegetables, fruits, and fresh produce was 0.53%, and vegetables such as beans and sprouts had the highest *Campylobacter* prevalence of 11.08%. These non-animal foods contained *Campylobacter* spp. due to the use of previously *Campylobacter*-contaminated irrigation systems (water, or equipment), and during the production or handling of fresh produce (Mohammadpour et al., 2018; CDC, 2021).

1.6.3 Salmonella

Salmonella spp. are non-spore forming, rod-shaped, Gram-negative bacilli of the *Enterobacteriaceae* family (Bacon & Sofos, 2003). They possess peritrichous flagella and are predominantly motile. *Salmonella* spp. are chemotrophs since they obtain their energy from organic sources through oxidation-reduction reactions (Bacon & Sofos, 2003; Cavallaro et al., 2011; CDC, 2012). They are also characterized as facultative anaerobes, that is, capable of generating ATP with available oxygen (aerobic) or through electron acceptors and fermentation (anaerobic), that is, when oxygen is unavailable (Bintsis, 2017).

Salmonella spp. are divided into two species: *S. enterica* and *S. bongori* (FDA, 2017), and approximately eight serovars are significantly involved in food poisoning, that is, S. Typhimurium, S. Infantis, S. Dublin, S. Enteritidis, S. Seftenburg, S. Montevideo, S. Virchow, and S. Newport (Almashhadany, 2021; CDC 2021). Species such as nontyphoidal *Salmonella* can be invasive and cause paratyphoid fever, which requires immediate antibiotics treatment. The presentation of disease symptom (such as, diarrhoea, fever, and abdominal cramps) among people varies considerably with incubation period between 6 hours to 6 days (CDC 2021). Although most healthy people could recover in a few days to a week without specific treatment, the vulnerable including infants, adults 65 years and older, and people with weakened immune systems, may experience severe illnesses that require medical treatment or hospitalization (CDC 2022).

Mode of transmission: Salmonellosis often associated with 1) the consumption of inadequately cooked or thawed poultry, eggs or egg products, unpasteurized milk, and to some extent fruits and vegetables (Bacon & Sofos, 2003; Bintsis, 2017; Mohammadpour et al., 2018; Penteado et al., 2004), 2) cross-contamination of meat and meat products

from infected food handlers, and 3) the presence of small animals and insects such as rats, cockroaches, and flies, in the food environment that act as vectors of disease transmission (Almashhadany, 2021; FSIS, 2017). They are intracellular pathogens, and typically live in the intestines of most livestock, wild animals, and could be frequently shed in the faeces of these live animals (Almashhadany, 2021; O'Grady & Krause, 1999). Specifically in sub-Saharan Africa, typhoidal serotypes can only be transferred from human to human, subsequently causing typhoid and paratyphoid fever (Almashhadany, 2021; CDC 2021). Typhoid fever for example, occurs when *Salmonella* (the typhoidal form) invades the bloodstream and sometimes the entire body while secreting endotoxins (the septic form). Thus, leading to life-threatening hypovolemic and septic shock.

Occurrence in foodborne diseases: The CDC estimated that *salmonellosis* causes approximately 1.35 million infections, leading to 26,500 hospitalizations, and 420 deaths in the United States each year (Scallan et al., 2011; CDC, 2021). *Salmonella* occurrence in foodborne disease outbreaks has been mostly associated with meat and milk products or in raw vegetables with cross-contamination during food processing (Fonteneau et al., 2017; Laufer et al., 2015; Mohammadpour et al., 2018). For example, in 2008, 1,450 salmonellosis cases and two deaths were registered in 43 states in the United States, arising from the consumption of jalapeño and serrano peppers imported from Mexico (Maki, 2009). Recently, *Salmonella* outbreaks have also been traced in facilities producing peanut butter and pastes, and more than 200 US companies used these foodstuffs as raw ingredients in products, such as brownies, cakes, candies, cookies, ice cream, among others (Cavallaro et al., 2011; CDC, 2021; CDC, 2022).

1.6.4 Listeria

Listeria spp. are rod-shaped, Gram-positive, non-spore forming, facultative bacteria. So far, *Listeria monocytogenes* is the only species of *Listeria* that is important for public health (CDC, 2021; FDA, 2017). They are catalase-positive and oxidase-negative and express beta hemolysin, which leads to the destruction of red blood cells in humans. Although *L. monocytogenes* is actively motile at room temperature (20-25^{0}C) by means of its peritrichous flagella (tumbling motility), it does not synthesize flagella at body temperatures (that is, 37^{0}C) (CDC, 2022).

L. monocytogenes causes 20-30% of listeriosis in high-risk individuals including infants, pregnant women, elderly (over 64 years of age), and immuno-compromised persons (Jemmi & Stephen, 2006; EFSA/ECDC, 2016; Bintsis, 2017; Almashhadany, 2021). For example, infections in pregnant women can be devastating to the foetus, resulting in miscarriages, stillbirths, and birth defects (Buchanan et al., 2017; FDA, 2011). They can spread from the intestines to the central nervous system and to the foetal-placental unit during pregnancy. It is the third most common cause of meningitis in newborns, that is, inflammation of the membrane surrounding the spinal cord and brain. It is also responsible for gastroenteritis (inflammation of the mucous membranes of the stomach and intestine), and septicaemia, a systemic spread of bacteria and toxins into blood (Buchanan et al., 2017).

Mode of transmission: *L. monocytogenes* is found in environments such as decaying vegetation, sewage, silage, water, and soil, and they can survive extreme salt concentrations and temperatures, that is, from 0^{0}C to about 45^{0}C (Bintsis, 2017; Almashhadany, 2021). It has been found to associate with food products such as raw or pasteurized milk, ice cream, cheese, hard-boiled eggs, raw or fermented meat, sausages,

raw or cooked poultry, raw or smoked fish, raw vegetables, and whole apples (Angelo et al., 2017; Bintsis, 2017; Almashhadany, 2021). Pregnant mothers are often advised not to eat soft cheeses such as Brie, Camembert, feta and queso blanco fresco, which could easily be contaminated or favour the growth of *Listeria* (CDC, 2021).

Furthermore, *L. monocytogenes* have been isolated in at least 37 domesticated and feral mammalian species, and in at least 17 species of birds, fish, and shellfish (Ferreira et al., 2014; Scallan et al., 2011). They can withstand extreme temperatures and conditions such as, prolonged freezing, drying, and heat. Unlike other foodborne pathogens, *Listeria* multiplies in cold environments, including refrigerators. It can quickly spread in damp buildings, dripping pipes or ceilings, and subsequently onto food. For example, once *Listeria* enter a food-processing factory, it can live for several years, and sometimes a continuous contamination of food products was observed (Jemmi & Stephen, 2006). In addition, the persistence of *L. monocytogenes* persistence in other food-associated environments such as, farms and retail establishments, may also contribute to food contamination and transmission of the pathogen to humans (Ferreira et al., 2014; Scallan et al., 2011).

Occurrence in foodborne diseases: *L. monocytogenes* causes an estimated 1,591 foodborne illnesses, 1,600 hospitalizations and 260 deaths in the United States each year (Scallan et al., 2013; CDC, 2021). Its fatality rate exceeds those of *Salmonella* spp. and *Clostridium botulinum* (CDC, 2021). In 2011 for example, one of the largest and deadliest multi-state outbreaks of listeriosis was felt in the United States where 146 people were affected, leading to 29 deaths and one miscarriage (US Food and Drug Administration [FDA], 2011).

In the European Union, listeriosis followed an upward increase in 2014, where 2,161 confirmed cases and 210 deaths were reported representing a 16% increase compared to 2013 (Bintsis, 2017). Then in 2015, 28 EU member states reported 2,206 confirmed human cases of listeriosis (European Food Safety Authority/European Centers for Disease Prevention and Control [EFSA/ECDC], 2016).

1.6.5 Yersinia

Yersinia spp. are Gram-negative, non-spore-forming, facultative rods. With the exception of *Y. pestis*, all *Yersinia* spp. have peritrichous flagella and are motile at 22-30°C, and non-motile at 37°C (Bacon & Sofos, 2003). Three *Yersinia* spp. are considered pathogenic to humans: 1) *Y. pestis* is the causative agent of plague, 2) *Y. pseudotuberculosis* may cause gastroenteritis in humans when contaminated food or water is ingested or various clinical conditions (enteric disease, abortion and septicemia) in domestic animals and birds, and 3) *Y. enterocolitica* is the most frequent causative agent of yersiniosis - a frequently reported zoonotic gastrointestinal disease after campylobacteriosis and salmonellosis in many developed countries, especially in temperate zones (Rahman et al., 2011).

Specifically, *Y. enterocolitica* are psychrotrophic, and survive in refrigerated foods (at 4^0C) (Longenberger et al., 2014; Bancerz-Kisiel & Szweda, 2015). It grows at temperatures between 0 and 45°C, and optimal growth is observed between 25 to 30°C (Bacon & Sofos, 2003). Also, it can survive alkaline and less acidic conditions (pH 4.0 to 10.0), and optimal growth is observed at pH 7.6 (Bacon & Sofos, 2003). Additionally, *Y. enterocolitica* can grow in the presence of sodium chloride at concentrations as high as 5% (Bacon & Sofos, 2003). It is heat stable, can resist enzymatic degradation, remain stable during prolonged storage, and possess a pH stability similar to that of ETEC thermostable

enterotoxins (Cavallaro et al., 2011). Its pathogenic strains belong to serotypes 1B/O:8, 2/O:5, 2/O:9, 3/O:3, and 4/O:3 (Okwori et al., 2009; Rahman et al, 2011). In Norway, for example, more than 98% of *Y. enterocolitica* infections are caused by serotype O:3, and this is also dominant in Europe, Japan, and North America (MacDonald et al., 2012). In Nigeria, *Y. enterocolitica* 2/O:9 and *Y. pseudotuberculosis* 1/O:1 were found dominant in human and non-human sources (pig, sheep, dog, cowmilk, fish, lettuce) (Okwori et al., 2009).

Mode of transmission: *Y. enterocolitica* is widespread in animals and the environment (sewage, domestic wastes, water, and soil). It can be spread by 1) contact with infected faeces, 2) eating or drinking contaminated food or water, and 3) contact with contaminated soil or infected animals (Almashhadany, 2021; Ministry of Health New Zealand [MHNZ], 2022). It has been isolated from raw milk, some seafoods, tofu, and bean sprouts (Bacon & Sofos, 2003; Bancerz-Kisiel & Szweda, 2015). Its main reservoir is pigs, and eating contaminated pork and pork products remains the most important source of human infection (Bancerz-Kisiel & Szweda, 2015; Lantz et al., 1998). Animals such as wild boars, red deer, roe deer and birds could be vectors of *Y. enterocolitica* (Bancerz-Kisiel & Szweda, 2015).

Symptoms of yersiniosis include watery or bloody diarrhea, fever, vomiting, and right-side abdominal pain (sometimes confused with appendicitis), and may persist for 1-2 weeks in adults and up to four weeks in children (Almashhadany, 2021; Bacon & Sofos, 2003; FDA, 2017; Rahman et al., 2011). This could lead to extraintestinal complications, such as septicaemia, meningitis, Reiter syndrome, myocarditis, glomerulonephritis, thyroiditis, or erythema nodosum (Mossel et al., 1994). However, the enteric forms of yersiniosis are very rare as the bacteria will normally affect the skin, throat, lungs, liver,

and kidneys (Bancerz-Kisiel & Szweda, 2015). Children are infected more often than adults, and the infection is more common in the winter (CDC, 2016b).

Occurrence in foodborne diseases: Yersiniosis is the fourth most common cause of acute bacterial enteritis in many developed countries including Norway (MacDonald et al., 2012). The CDC estimated that *Y. enterocolitica* causes almost 117,000 illnesses, 640 hospitalizations, and 35 deaths in the United States each year (CDC, 2016b). For example, in July 2011, a cluster of *Y. enterocolitica* infections was detected in southwestern Pennsylvania, from 22 people who had previously consumed locally pasteurized dairy products (Cavallaro et al., 2011; Longenberger et al., 2014). A total of 7,017 yersiniosis were reported in the European Union in 2011, corresponding to a 3.5% increase compared to 2010 (Bancerz-Kisiel & Szweda, 2015). Then in 2015, 26 European Union member states reported 7,202 cases of yersiniosis, making it the third frequently reported zoonotic infection (EFSA/ECDC, 2016). In addition, Asian countries such as Japan reported four yersiniosis outbreaks between 2012 and 2013 (Konishi et al., 2016).

Despite significant reports (Bintsis, 2017; Grace. 2015a; Havelaar et al., 2015; ICSMF, 2005; Kirk et al., 2015; Pires et al., 2021) on foodborne diseases across WHO's African regions, outbreaks of foodborne diseases are not well documented. Although many people may be routinely sick, the source of foodborne illnesses is unknown. Indeed, many people in sub-Saharan Africa live in rural communities and are engaged in semi-subsistence agriculture, where foods are mainly produced for home consumption, and/or sold at local markets and in these contexts people are unaware of the benefits of consuming safe food. (Arias-Granada et al., 2021). Rural populations are less aware when they are affected by foodborne diseases and its risk to public health is often neglected (Arias-Granada et al., 2021). However, key similarities in terms of health and living

conditions across regions in Africa, could uniformly inform possible reduction in food safety threats (WHO, 2018; Arias-Granada et al., 2021).

1.7 Outline of the thesis

This thesis is structured in a journal-style format, as highlighted by the 'thesis with publication' guidelines of Lincoln University, New Zealand. It is made up of five chapters including three results chapters.

In **Chapter 1**, a general introduction highlights the importance of 1) understanding the association of foodborne pathogens with edible land snails, and 2) human exposures to these foodborne pathogens during snail handling and consumption. We also highlight the methodological framework used in attaining these research objectives in Buea, Cameroon and Lincoln University, New Zealand.

Chapter 2 explicitly covers qualitative approach and subsequent findings which revealed that human activities in snail consumption practices could lead to outbreaks in foodborne diseases. The methodological challenges in achieving this research finding in Buea (Cameroon) are also discussed.

Chapter 3 examine five potential foodborne pathogens in the faeces of edible land snails sampled in Buea, Cameroon. We then placed emphasis on the public health risks of these identified foodborne pathogens in the faeces of land snails, particularly to snail handling and consuming populations.

The last result chapter (**Chapter 4**) reveals the survival characteristics of two strains of *E. coli* (that is, laboratory-adapted and pathogenic *E. coli* strains) in the faeces of edible land snails. Its practical implications for heliciculture and public health are then discussed.

Finally, **Chapter 5**, is a general discussion and conclusion of the objectives and findings of this thesis.

Chapter 2. Human exposure to foodborne pathogens in snail handling and/or consumption: Buea, Cameroon

*Chapter 2 is a combination of two journal articles: Tanyitiku et al., 2022a (doi:10.1177/16094069221078132) and Tanyitiku et al., 2022c (*doi:10.1186/s13690-022-01009-8*). The findings were presented at the 7th One Health Aotearoa Symposium (Dec. 7-8, 2021), available at* https://onehealth.org.nz/symposium-2021/

2.1 Summary

Background: Snail meat is an important source of nutrition in Cameroon, but the food safety risks are poorly understood. We characterized public health risks from snail meat consumption in Cameroon, by examining local practices that expose snail meat handlers and consumers to foodborne pathogens.

Methods: We used exploratory qualitative approaches, that is, lived experience, face-to-face in-depth interviews, participant observation and a focus group, to explore fifteen key informants' routines and lived experiences, and the perceptions of two health officials on the food safety of snail meat consumption in Cameroon. This information was organized and interpreted using soft systems methodology and social practice theory, which allowed systemic appreciation of local practices.

Results: We distinguished five kinds of actors (snail vendors, market sellers, street vendors, street eaters and home consumers), who performed seven successive practices (picking, selling, cracking, washing, cooking, hawking, and eating). We then identified three worldviews about snails: family support or to reduce poverty, a source of nutrition, and a food choice (taste, preference). Our findings revealed that participants' competences were based on childhood learning and 'inborn' experiences, and materials used in snail activities reflected participants' parentage and 'state of poverty'. Although most of the interviewees highlighted 'unhygienic conditions' when explaining snail

picking locations, the participants believed that washing and cooking should kill all contaminants.

Concluding remarks: Several opportunities for human exposure to foodborne pathogens including snail picking in domestic waste and sewage, the sale of unpackaged live snails, improper snail meat washing, and hawking in loosely closed buckets, were apparent from our analysis. These findings suggest copious opportunities for improving health outcomes among African snail meat handlers and consumers.

2.2 Introduction

The World Health Organizations' first global and regional disease estimates in 2015 revealed 420,000 deaths per year from unsafe food (Havelaar et al., 2015; Pires et al., 2021). These estimates also showed that low-income countries bear the greatest burden, with nearly 70% of deaths originating from diarrheal disease agents (Havelaar et al., 2015; Pires et al., 2021; Havelaar et al., 2022). Due to the lack of evidence on the burdens and benefits of tackling food-related diseases in WHO African regions, food security policies and initiatives have often paid little attention to emerging foodborne diseases (Havelaar et al., 2015; Pires et al., 2021). With few processed and packaged foods, large volumes of raw and fresh foods are traded in informal markets by vendors who possess limited awareness on food safety and hygiene (WHO, 2002, Havelaar et al., 2022). An example of such foods are African land snails, an affordable source of nutrition and livelihood for many inhabitants in Africa (Akpomie, 2013; Cobbinah et al., 2008; Mohammed et al., 2014; Ndah et al., 2017; Nyoagbe et al., 2016).

Locally called "nyamangoro", "Congo meat" or "slow boys", terrestrial snails are considered a desirable source of food and livelihood in Cameroon (Ngenwi et al., 2010). Snail meat is higher in protein (37-51%) when compared to guinea pig (20.3%), poultry (18.3%), fish (18%), cattle (17.5%), sheep (16.4%) and swine (14.5%) (Adeyeye et al., 2020a). As detailed in Appendix I, Section I.3 of this thesis, land snails are low in fats and carbohydrates, contains no cholesterol and are good sources of iron, magnesium, calcium, and zinc (Adeyeye et al., 2020a; Nyoagbe et al., 2016). Indeed, an increase awareness on the nutritional benefits of edible land snails, has increase its demand among local consumers (that is, in restaurants, hotels, street food industry and food festivals) and foreign exporters (USA, France, Australia, South Korea, among others) (Cobbinah et al.,

2008; WHO, 2002). For instance, in Ghana, demand currently outstrips supply (Cobbinah et al., 2008; WHO, 2002). Kaldjob et al. (2019) mentioned that 76.30% of 211 people consume snail meat in Fako division, Cameroon. Internationally, the African snail species fetches about one third the price of European *Helix* species leading to hundreds of millions of US dollars' worth of African snail meat exported annually (Cobbinah et al., 2008).

Furthermore, since protein-energy malnutrition is one of the most devastating problems facing vulnerable groups (children, pregnant women, aged people) in developing countries, readily available and affordable African land snails are recommended as good substitutes for more expensive animal meat proteins (Engmann et al., 2013). In brief, protein-energy malnutrition causes approximately 49% out of 10.7 million deaths of children under the age of five in Africa each year (WHO, 2000). Vitamin A deficiency remain the greatest preventable cause of unnecessary childhood blindness, and the lack of iodine is the greatest cause of brain damage and mental retardation (WHO, 2000). As such, incorporation of snail meat in infant food is particularly encouraged in rural communities, such as in Cameroon and Nigeria, where more than 80% of its population cannot afford conventional meat products (Adeyeye et al., 2020a; Cobbinah et al., 2008; Engmann et al., 2013; Kaldjob et al., 2019).

Despite this significant demand for snail meat, commercial snail farming or heliciculture, hardly exists in Africa. Snails are gathered from the environment including forest, farms, gardens, bushes, backyards, footpaths, roadsides, especially during the wet seasons (Akpomie, 2013; Cobbinah et al., 2008; Mohammed et al., 2014; Ndah et al., 2017; Nyoagbe et al., 2016).

Previous studies (Adagbada et al., 2011; Akpomie, 2013; Nyoagbe et al., 2016) have established terrestrial snails as a credible source of infection based on enumerated

pathogenic microorganisms. Important pathogens include *Staphylococcus aureus, Salmonella* spp., *Campylobacter* spp., *Yersinia* spp., and *Listeria* spp. (WHO, 2002; Temelli et al., 2006; Tanyitiku et al., 2022b). Local snail consumption activities that could lead to foodborne illnesses through handlers and consumers' exposures to potential foodborne pathogens remain under-researched. These local practices from snail gathering, handling to consumption have been largely ignored, rendering the identification of health risk factors and, therefore, targeted interventions difficult. To improve global health among snail meat handlers and consumers, this chapter explores local practices that could lead to food-related illnesses in snail meat consumption. We considered real-world situations in Buea - Cameroon, and specifically:

1. Describe snail meat consumption in Buea as a system of activities,
2. Identify routine practices in snail consumption activities in Buea,
3. Explore participants' perceptions on - handling and consuming snail meat, and on potential health risks in such snail activities, and
4. Propose opportunities to develop interventions to mitigate these health risks.

This study considered the snail meat consumption process as a system of activities (harvesting, preparation, vending, consumption) with interacting actors (snail meat handlers and consumers) performing distinct set of practices with the aim of improving health outcomes of a targeted population in Cameroon. In addition, the chapter provides background on the methodological challenges in the context of Cameroon and COVID-19 pandemic, and a rationale for the choices made to address the challenges.

2.3 Research context

In this section, we described the context of the population under study. Its incorporation in this research was important as it gives the reader insights on current African issues in naturalistic settings and Cameroon in particular.

The study was carried out between June 10, 2019 and August 24, 2021 in Buea, the capital of the southwest region of Cameroon. Buea is located on the eastern slope of mount Cameroon at latitudes 4°12'N and longitudes 9^{0}12'E. It is made up of a tropical rainforest with annual rainfalls between 3000mm to 5000mm throughout the year (Buea Communual Development Plan [BCDP], 2012). Its vegetation is an evergreen tropical ecosystem ranging from thick forest, secondary forests, shrubs, to savannas towards the peak of the mountain, and this has favoured the abundant growth and survival of land snails (Kaldjob et al., 2019; Miegoue et al., 2019; Ndah et al., 2017).

We were interested in four urban spaces (locations) and road junctions: Buea station, Great Soppo, Molyko/Mile 17 axis, Muea, including participants' arable land[5] and homes (see Figure A.3). These densely populated locations in Buea contained a high availability of key informants[6], which we identified as snail collectors, market sellers, roadside street vendors, home consumers and street eaters.

This research context is best described by 1) social and sanitation conditions, 2) pandemic and political conflicts, and 3) economic, cultural, and hygienic contexts.

2.3.1 Social and sanitation conditions

The people of Buea live in houses separated at approximately zero to one hundred meters from each other, with arable land in-between. This is thought to maintain easy

[5] Small areas of land used for vegetable cultivation destined for household consumption or income earning when production exceeds household needs

[6] Key informants are perceived to provide more knowledge about a topic by the nature of their position in a culture, and their information-rich connections (Lokot, 2021)

communication, harmony, social proximity, and security from robbery (Manyi, 2007). With changes in consumption habits, poor community participation and the role of HYSACAM[7], this system of housing has led to unsafe sanitary waste disposal such as open defecation[8] and flying toilets[9] (Assob et al., 2012; Bate, 2020; BCDP, 2012). Household wastes along with human faeces are commonly found between habitations (Field notes, September 20, 2019). Bate (2020), describes the current waste disposal and management situation in Buea as "poor", "pathetic" and "rudimentary orchestrated", which may increase disease prevalence. Figure 2.1 presents similar observations made during this research fieldwork.

[7] A communal company in charge of waste management in Cameroon
[8] Defecating in open spaces such as farms, forests, water bodies
[9] Defecating into a plastic bag while inside the home and throwing the waste unnoticed to the environment

Figure 2.1. Pile of rubbish by the HYSACAM van along the roadside, Buea

2.3.2 Pandemic and political conflicts

Buea has a surface area of 870km^2 spreading between four urban spaces (Buea station, Great Soppo, Muea and Molyko/Mile 17 axis). Due to politics and pandemics, its population is blended with people from neighboring villages seeking refuge, since it is considered more secure and accessible than the rest of the southwest region of Cameroon (Nicholas et al., 2020a). In October 2016 for example, a marginalization crisis started with lawyers and teachers demanding professional reforms. This later lead to armed conflicts termed the Anglophone crisis[10], with separatist fighters calling for secession of English

[10] The Anglophone Crisis, also known as the Ambazonia War, or the Cameroonian Civil War, is an ongoing civil war in the southern Cameroons regions of Cameroon

Cameroon (that is; twenty-percent of the total Cameroonian population), to a formation of an "independent Ambazonia" state (BTI, 2020; Ngange et al., 2019). In the southwest region, this resulted in over 3,000 deaths, with about 200,000 internally displaced and over 100,000 as refugees in Nigeria (BTI, 2020; Ngange et al., 2019). Consequently, Buea's population reduced from 300,000 inhabitants in 2005 to 169,745 inhabitants in 2017 (BCDP, 2012; Nicholas et al., 2020a).

In addition, Cameroon registered its first case of COVID-19 on March 6, 2020, and more than 1,000 people became infected in a month (Nicholas et al., 2020b). Travel restrictions were implemented to prevent a rapid spread, due to an existing Cameroonian culture of tight family relationships with people making several contacts per day and its poor health systems (Nicholas et al., 2020b). This situation proved challenging in obtaining key informants for this research and further fieldwork later in 2020 and 2021.

2.3.3 Economic, cultural and hygienic contexts

Buea is best described by a complex community of urban, semi-urban, rural, and traditional settings. In this rural setting typical in Africa, only a minority of women are educated. It is assumed that a woman will eventually get married thus transferring money that would have been spent in her education into her marital household (Adelakun-Odewale, 2018). In contrast, male education is strongly encouraged because it is believed to be a productive financial investment that secures the family lineage (Adelakun-Odewale, 2018). This limitation in female education has contributed to early polygamous marriages (that is, before eighteen years), and to varying household day-to-day living arrangements, aspirations and expectations (Cislaghi et al., 2020; Wetheridge & Antonowicz, 2014). According to Adelakun-Odewale (2018), early marriages are believed to be another chance into a better life. Wetheridge and Antonowicz (2014) relate that

women experiences through these pathways are of living in rural areas, poverty, traditional practices, illiteracy, and early sexual debuts. With increasing costs of living, many Cameroonians live below the monetary poverty line that is, at less than 931 FCFA[11] (less than 2 USD) per day, with about 16% of households being undernourished (Kaldjob et al., 2019; WFP, 2017). Although the government had aimed at reducing the poverty rate to 28.7% through an average sustained economic growth of 5.5% between 2010 and 2020, the International Monetary Fund (IMF) foresee a growth decrease due to weak business environment among others (WFP, 2017).

With this present situation and confronted with all sorts of hardships as a mother and sometimes as main provider of her household, a woman is forced into diverse profitable ventures, such as, arable farming and street food vending (Assob et al., 2012; WFP, 2017). Arable farming is typically practiced at zero to four kilometers from homes, and involves the cultivation of crops such as grains, nuts, tubers and vegetables (BCDP, 2012; WFP, 2017). Street foods are ready-to-eat foods sold on the streets, markets, or other public places. These activities are not legally recognized or regulated. Due to minimal or no knowledge of hygienic food handling practices, food preparations occur under microbiologically unsafe conditions (Assob et al., 2012; Yongsi, 2014). Assessing the personal hygiene and health status of vendors involved in informal sales of food in Buea, Assob et al. (2012), isolated one or more faeco-orally transmissible parasites from their faeces. Also, the presence of enteric microorganisms in chili pepper sauces served with street food (including snail meat), around the University of Buea campus, Mile 17 Motor Park, Malingo and Muea market have been attributed to poor handling procedures (Nicholas et al., 2020a).

[11] CFA franc is a currency use in west and central African French colonized countries, and is guaranteed by the French treasury

The above conditions constitute key elements in the context of this study, and consequently the status of our research participants.

2.4 Materials and methods

The methods used to attain the above mentioned four objectives in section 2.2 and the challenges encountered (Tanyitiku et al., 2022a), are described in six sections, that is, 1) the type of enquiry and selection of theoretical frameworks, 2) selection of sites for the study, 3) selection and recruitment of, and engagement with participants, 4) design and use of interview instruments, 5) interpretation of qualitative information, and 6) trustworthiness of the study findings.

2.4.1 Type of enquiry and selection of frameworks

A qualitative approach was used in this study. As opposed to quantitative research, which seeks to confirm hypotheses about statistical phenomena, qualitative research provides insights on the "human" side of an issue such as behaviors, beliefs, opinions and relationships among individuals (Mack et al., 2005). We were guided in our choice of approach by our purpose: to understand health risks that could arise from handling and consuming snails infested with pathogenic microorganisms in Buea. To do this, we needed to explore and capture rich and interpretative information from participants (that is, snail vendors, consumers, an epidemiologist, and a veterinary doctor) about human practices.

We were seeking to know how our informants themselves understood their practices, by treating key actors as 'carriers of practices' (Jackson, 2019) and explored routines, lived experiences and opinions of key informants using qualitative research methods. We used lived experiences (Creswell, 2014), participant observation (Mack et al., 2005) and face-to-face in-depth interviews (Creswell, 2014; Mack et al., 2005), to reveal participants' naturally occurring behaviors vis-à-vis snail meat consumption, and relate their narrations or experiences to their actual home routines. Mack et al. (2005) and

Creswell (2014) encourages these combination of qualitative methods as it produces a thick or detailed description of participants' feelings, opinions, experiences and interprets the meanings of their actions. Our field work produced audio recordings of exploratory interviews, field notes and photos as potential information that could be regarded as "unstructured" stories and experiences.

Our approach presented particular challenges in the context of this study. For example, it was not always possible to find participants homes for observations and interviews. Presently in Cameroon, locations are verbally indicated as streets and home addresses are still to be recognized. Basic utilities such as electricity and internet to ease communication is unavailable in certain locations and/or some days/weeks. While in the search of participants' homes, and due to urban construction and misunderstandings, we walked through a wrong path into an uninvited house in three occasions. This led to a postponement of two interviews, and we ended up missing on one of the key informants. In addition, to the greater population in Buea, snail meat is viewed as a source of nutrition, an inherited dish, and a delicacy. Getting key informants to participate on the basis that it might lead to illnesses was regarded as "bizarre" and not part of their beliefs. For example, in some of the interviews, statements as below were recorded.

Do you eat snails around the toilet? "Yes, why not, is it not meat" home consumer

"If snails eat excreta and you eat snails, [...], you have eaten snails and not excreta" snail collector

We needed to be clear in the explanation of our enquiry to participants that it was based on the fact that "maybe" their practices could get them sick and not that they were actually or already sick because they eat snails.

A further methodological choice was the decision to use two theoretical frameworks which focused on a system thinking approach, that is, Soft Systems Methodology (Checkland, 1999; Checkland, 2000), and practices as social systems, that is, Social Practice Theory (Shove et al., 2012), as shown in figure 2.2.

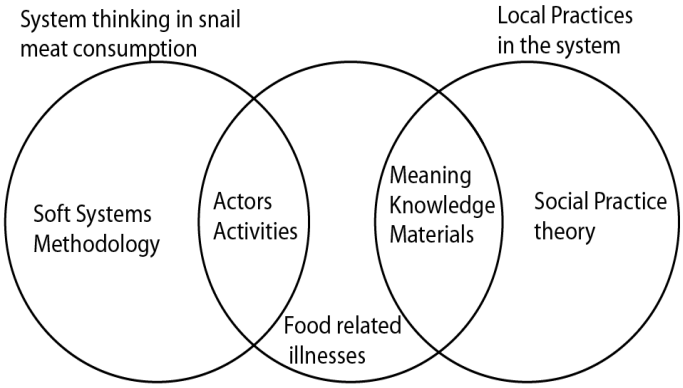


Figure 2.2. Theoretical framework of the qualitative enquiry

Particular aspects of the research drove our choice and specific use of these frameworks: to identify local practices in participants' shared experiences in an undefined food-disease causing problem situation. "Soft" systems thinking permitted an exploration of the erratic and unstructured nature of our obtained information, by moving from the real-world situation to a model system, then back to the real world. According to Augustsson et al. (2019), there are no publications on mapping the use of SSM in healthcare, especially in identifying the type of problems to which SSM has been applied, or the types of interventions and outcomes that have been reported following the use of SSM. However, inspired by the problem-solving approaches of Maqsood et al. (2003), Yadin (2013) among others, Soft Systems Methodology (SSM) was chosen for its capacity to structure and conceptualize complex human situations (Checkland, 2000; Checkland & Poulter, 2006; Proches & Bodhanya, 2015). Maqsood et al. (2003) presents five case studies dealing with confused situations involving humans and organizations, Yadin (2013) analyzed real life examples of students' perceived learning systems by revealing

their specific difficulties. In contrast to hard systems where problems can be well-defined with optimal solutions, SSM attempts to explore and appreciate problems using models of human activities in real-world situations where people act purposefully (Burge, 2015; Checkland & Poulter, 2006; Proches & Bodhanya, 2015). It analyses the problem situation and permit purposeful actions by accommodating the perceptions, judgments and values of different actors in the system (Checkland & Poulter, 2006; Proches & Bodhanya, 2015). Further, we used SSM within the tradition of critical systems thinking (Jackson, 2019), meaning we were not claiming that our observations can be accounted for by a systems analysis; only that viewing practices as if they were expressions of multiple interactions, perspectives and judgements is a sound way to explore complex practices (Jackson, 2019; Ulrich, 2003). SSM in this research was used as a descriptive tool to gain an appreciation of interacting influences, and differing perceptions, judgments, and values.

As outlined by Proches & Bodhanya, (2015), as originally modified from the seven stages of Checkland (1999), we used a four-staged SSM approach, namely, a rich picture[12], root definitions[13], CATWOE[14] and a conceptual model[15]. A rich picture, in SSM, is way of representing the complexity of human situations with multiple interacting relationships. Usually, in SSM, it is done as a literal picture. Root definitions (RD) are a standardized systemic description of a system. An RD describes succinctly an action, how it is performed and its intended purpose. In our case, RDs enabled us to describe systems from differing perspectives. CATWOE, is a SSM acronym used for systemic description of purposeful activities. It identifies customers (those affected by a transformed situation),

[12] A way of representing the 'complexity of human situations' with 'multiple interacting relationships' usually, in SSM, done as a picture (Checkland & Poulter, 2006)
[13] a structural description of a system
[14] A systemic description of purposeful activities by identifying customers, actors, the desired transformations, owners, and operating environment.
[15]to represent a purposeful activity system through a set of logical actions implied by the description of the system, or root definition

actors (those bringing about a transformed situation), the transformation (the envisaged difference that would be made), worldview (assumptions that make the transformation make sense), owners (those with control), and operating environment (givens that may affect the transformation). This enabled us to recognise and describe the effective systems relating to differing worldviews and desired transformations. Conceptual models are used to represent a purposeful activity system through a set of logical interactions implied by the description of the system, or root definition. They make dependencies discussable. In our case, such a model made clear the range of interlocking activities that constitute the social system underlying snail meat consumption. With this, we were able to develop and describe snail meat consumption as a complex human system involving different actors (rural women) with interrelated activities (snail picking, cracking, cooking, and vending). This approach helped us gain insights into the problem situation (that is, possible human pathogenic exposures) in participants' routines.

Our research context led to distinctive ways of applying SSM such as building a rich picture using an Ishikawa diagram. Also known as the cause-and-effect (CED) diagram or the Fishbone diagram, the Ishikawa diagram is a quality control tool that graphically illustrates the relationship between a given outcome and all the factors that influence such outcomes (Suárez Barraza & Rodríguez González, 2018). It has been used in different contexts from manufacturing companies to service organizations such as medical services, supermarkets, banks and restaurants to identify, sort, and display possible root causes of a specific effect, problem or condition (Suárez Barraza & Rodríguez González, 2018). We decided against a conventional SSM rich picture for two main reasons: limited opportunities for a prolonged field work in Buea due to COVID-19 and its effects on travel restrictions, and the fact that our participants did not see their

practices as a health risk-problem situation. To do this, we modified SSM's conventional action-oriented approach which sought to improve already existing problematical situations (Checkland & Poulter, 2006; Proches & Bodhanya, 2015). Based on our "rich picture" development and in-depth interviews, we articulated five root definitions to adequately capture the perspectives of key actors: snail collectors, market sellers, street vendors, home consumers and street eaters. We then chose three transformations (that is, snail meat is a source of income, a source of nutrition and a food choice), to describe using CATWOE. Our choices for developing a conceptual model were to illustrate an ideal systematic set of activities needed by participants (our key actors) to realize these transformations.

Our second theoretical framework, Social Practice Theory (SPT) was chosen for its capacity to uncover the day-to-day practices (Shove et al., 2012), of snail collectors, vendors, and consumers. SPT integrates different elements such as materials, knowledge, emotions, skills, and meaning to people as part of their everyday activities (Reckwitz, 2002). It is based on everyday practices and the way they are typically and habitually performed in a society (Blue et al., 2014). The theory of social practices has been used in various domains including understanding unhealthy public health practices (Blue et al., 2014; Maller, 2015) and in domestic food safety practices (Meah, 2014). For example, Blue et al. (2014) considered tobacco smoking as a social practice that regularly depends on an integration of materials (cigarettes, tobacco crops, factories, transport systems, retail infrastructures, matches and lighters), competence (to know where, when and how to smoke, not only how to light a cigarette and inhale, but how to smoke in the 'correct' fashion for a given social situation) and meaning (understanding smoking as a normal and socially acceptable thing to do, variously associated with relaxation, sociability,

masculinity, glamour and toughness). In addition, from the 4C's (cleaning, cooling, cooking, avoiding cross-contamination) of food safety initiated by the Food Standards Agency, Meah (2014) argues behavior-based approaches in tackling food safety are limited and simple as they focus on the "what" and little on the "how" and "why" which are all implicated in what transpires in a kitchen. Meah (2014), further suggested that given routines contribute to how people handle food, it is appropriate to apply a theoretical framework (for example, SPT), which reflects those routines and the embedded nature of what takes place in a domestic kitchen. Following this logic, we adopted Shove et al. (2012) three elements of materials, meaning and competences, as in Table 2.1.

Table 2.1 Three components of social practice theory

Competences	Skills, know-how and techniques for a practice
Materials	Objects, things, technologies, tangible physical entities; required to carry out a practice
Meaning	Symbolic meaning, ideas, and aspirations of participation in the practice

We considered informants as 'carriers of practices'(Shove et al., 2012), where this implies "they are neither autonomous nor the judgmental dopes who conform to norms. They understand the world and themselves, and use know-how and motivational knowledge, according to the particular practice" (Shove et al., 2012). We focused on these three elements, that is, materials, competence and meaning, in order to discover possible pathogen exposure routes in participants' experiences. It should be noted that practices are social as they are similar for different individuals at different locations and different point in time, for example, in picking snails, selling at the market, cooking, hawking. Individuals are seen as the "carriers of practices" who do not freely choose between

practices based on utility or similar individualistic concepts but are "recruited" to practices according to their background and history (Reckwitz, 2002). The use of SPT was appropriate for our study as we were dealing with human practices, which are bound to change over time. For example, in the description of snail meat preparation, one of our participants acknowledged modifying her method of snail cracking to boiling a few days prior to the interviews on the basis that the previous method was “nasty”. She admitted adopting this new method from her neighbor. According to Shove et al. (2012), *“practices emerge, persist, shift and disappear when connections between these three types [materials, competences, meaning] are made, sustained or broken”.*

In conclusion, SSM provided a way to gain and use a ‘systemic’ understanding of the risks. Rather than simply identifying risk factors, SSM showed how factors and actions relate to one another or influence one another. SPT provided a generic framework for interrogating particular practices. It was used to describe practices considered important for understanding human exposure to pathogen hazards associated with consumption of snail meat. We see the two frameworks as very complementary. Each assumes systemic interaction between multiple elements to result in outcomes. Their distinction can be seen in the unit in focus. For SSM it is the experienced mess (Checkland, 1999), for SPT some identifiable practice or activity is envisaged. In common between the two approaches is an appreciation of human motivation and action being, in part, products of how actors and communities make sense of their situation; for SSM that is attention to worldview, for SPT it is attention to meaning. With this, SSM and SPT were selected as suitable frameworks to understand the local practices of our participants, since the consumption of snail meat is a day-to-day eating habit and/or occupation of the people of Buea, Cameroon.

2.4.2 Selection of field sites for the study

Four main motivations guided our choice of Buea: 1) snail meat consumption is ancestral and culturally embedded, 2) Buea's agro-climatic characteristics favors a productive survival of snails (Maegan, 2011; Miegoue et al., 2019; Ndah et al., 2017; Tanya, 2017; Welleng, 2021), 3) the availability of snail collectors, mobile and stationed snail meat vendors and consumers (Kaldjob et al., 2019; Ndah et al., 2017) and 4) the researcher has a deep knowledge of the locality under study through lived experience. After brainstorming with the researcher's family members, friends, and neighbors, we selected specific fieldwork locations (Mile 17 Motor Park, streets, and markets in Molyko, Buea town, Muea, Bomaka, Mile 16, Sandpit, Small and Great Soppo, participants' homes and farms). We were interested in those locations that incorporated live snails and snail meat in order to observe snail local practices and meet key informants.

Designing the enquiry was particularly challenging in this context as approaching someone on the footpath or road to ask for directions to snail vending sites or clues regarding key informants, could easily lead to suspicions that we were Ambazonians[16]. In the early days of the Anglophone crisis earlier mentioned, separatists created the Ambazonian Freedom Fighters on Facebook and WhatsApp to rally supporters against the Cameroonian government. Through these pages, activists gained momentum to initiate strike actions, impose ghost towns[17] and create fear among the population (Ngange et al., 2019). Enquiring from people we met in public places (streets, bus stops) lead to questions such as '*where do you live*', '*what quarter do you come from*', '*who are your parents*', '*how did you know I sell or eat snails*', which we believed was their strategy to

[16] Ambazonia is an umbrella term associated with a political movement in Cameroon whose goal is to promote Anglophone separatism

[17] On a "ghost town" day, no one is permitted to sell or buy items or move from one point to another

maintain privacy and confidentiality. On the other hand, our honest responses to the above questions were vital as it facilitated the recruitment of participants, and positively contributed to the quantity and quality of information obtained in this research. Another challenge in carrying out research at the selected sites was that the study could only be carried out in the rainy season, that is, from March to October each year. It is a period of snail reproduction and abundant availability compared to the dry season, that is, November to February (Kaldjob et al., 2019; Ndah et al., 2017). During this period, snail collectors could be heard and seen at night actively picking snails, and roadsides are filled with snail hawkers (Kaldjob et al., 2019; Ndah et al., 2017). We needed to walk with the collectors to these sites, which are slippery and muddy due to heavy downpours, and in most cases, sites are only accessible with torches.

> "We use very good and shining torches to pick snails; places are very dark, if you do not use a torch that the light is good, you will not see snails" snail collector

In addition, access to markets on rainy days was difficult due to its nature being muddy mixed with domestic waste, and mostly overcrowded with vendors, buyers, and pickpockets (Field notes, August 15, 2019). This led to a longer observation phase than expected as: a) the picking process was at random and entailed more intense walks given many households are involved in the search of snails, b) markets are not open daily, for example, the opening days of the Muea market is only on Thursdays and Sundays, and c) travel restrictions and ghost town imposed by the above-mentioned Anglophone crisis. One of the ways we managed these contextual challenges, was to use key informants with lived and memorable childhood experiences as recorded below.

"Like if I am out of Buea [...] if I see a pit toilet[18] that is locally open [..], it can be certain and I will just know that snails will be present there" home consumer

Figure 2.3. Pit toilet in Buea, Cameroon

2.4.3 Selection and recruitment of, and engagement with participants

To develop a system understanding of snail meat consumption in Buea, we sought to understand the perceptions of those who possessed childhood experiences and still incorporate snails in their routines. The recruitment criteria required participants must have lived in Buea for at least five years with active participation in snail consumption stages, that is, as snail collectors/pickers, snail local market sellers or snail vendors, snail home-based consumers, snail meat cleaners, snail street hawkers and street consumers. These stages were aimed at capturing experiences from snail harvesting to consumption. The number of participants in this study (seventeen), was based on the logic of small samples outlined by Crouch and McKenzie (2006) for interview-based qualitative research. These authors argued that a sample size of say less than 20 will facilitate the

[18] A pit latrine, also known as pit toilet, is a type of toilet that collects human faeces in a hole in the ground. Urine and faeces enter the pit through a drop hole in the floor (see Figure 2.3)

researcher's close association with each participant, and thus, enhance the validity of fine-grained, in-depth inquiry in naturalistic settings (Crouch & McKenzie, 2006).

The first step in recruiting participants was the integration of a note taker. A note taker according to Mack et al. (2005), should know the research material, the method in use, possess the ability to quickly identify and take down individual quotes that capture the spirit of a given point. We recruited a female geographer who had recently used semi-structured questionnaires to carry out a survey in the southwest region of Cameroon. She had a full knowledge of the qualitative approach in this study, as well as the landscape of the Buea municipality. The researcher and note taker started by visiting markets, road junctions, and streets for observations, identification of key informants[19] and recruitment of participants.

A key to recruiting and engaging participants required sufficient trust and relationship with our participants. To gain trust according to Gehlert and Mozersky (2018), a researcher needs to be honest about her intentions and expectations, consider the cultural settings, highlight the benefits of the study to participants and ensure confidentiality. As previously noted, our enquiry was carried out in a context that included suspicion. Prior to engaging key informants to participate in the study, we made observations and enquiries within a hundred meters from potential participants. When we came across a seller or buyer of snails, we needed to approach them in a polite, honest, and informal manner. In some instances, we started by supporting their businesses, such as, buying snails from them, and while packaging the purchased snails, the researcher got into an informal conversation with her key informants, leading to an explanation of our research interests. This was a strategy to gain the attention of our participants, as

[19] Key informants are perceived to provide more knowledge about a topic by the nature of their position in a culture, and their information-rich connections (Lokot, 2021)

approaching a hawker without purchasing intentions could cause instant reluctance and rejection of participation. Key informants asked questions such as: "*how is that important to me*", "how *can I make more profits from that*", "*are you saying eating snails is bad".* Drawing inspiration from the Italian proverb "*he who enjoys good health is rich, though he knows it not*", the researcher successfully drew their attention to the fact that it was for their family wellbeing and participation was voluntary. Potential participants were also given a written information sheet and consent form, as detailed in B.2 and B.3 respectively.

Consistent with our field observations, similar studies by Miegoue et al. (2019) and Ngenwi et al. (2010), respectively, recorded 57.69% and 60% of women in snail businesses, thus we chose to focus only on women who are also housewives and therefore, in charge of family food preparations. The involvement of women who are termed "vulnerable", initiated another challenge in this study. "Vulnerable" describes those who are disadvantaged or marginalized, and have experienced homelessness, poverty, and unemployment situations (Bashir, 2018; Gehlert & Mozersky, 2018). As highlighted earlier, the socio-economic and cultural status of our participants can render them vulnerable. With this, two participants in this research refused an audio recording of their voices emphasizing that rumors and misinterpretations were common in Buea.

With our enquiry, it was obvious the participants were afraid of gossip, which could arise from family members and friends listening to what they say about snails; their source of income was at stake. During one incident, using facial gestures, one of the participants' daughters contradicted the mother in her presence, regarding her detailed method as outdated and unpleasant, and told her mother to be careful with what she was sharing. There was this fear of losing customers due to the hygiene they practice in their homes and businesses. Nonetheless, prior to and during the interview sessions, participants

invited trusted relatives with formal education or curious friends to explain deeply the purpose of the interviews and the confidentiality agreement of the entire study. According to Mack et al. (2005), the willingness of participants to participate in an interview will depend on how well the participants understand the study, what will be expected from them, and how their privacy will be respected. Knowing the peoples' customs, we regarded it as unethical to tell her family members and friends to leave, as we were strangers at their homes. However, their presence was helpful to us especially as most of our participants have never heard of or participated in research-based studies or face-to-face interviews, and could neither read nor write as elaborated by a recruited epidemiologist:

> "You will see that a majority of the population is still uneducated, so they don't have knowledge on disease prevention, they don't have knowledge on how to manage particular diseases when they are infected or affected by that disease"

Although cultural background determines what is eaten as well as when and how (Oniang'o et al., 2003), this was challenging as the presence of others could affect the participants' ability to share potential information (Bashir, 2018; Gehlert & Mozersky, 2018). To avoid these setbacks, we chose interview settings and time at each participant's conveniences and privacy. Bashir (2018) confirms that the more comfortable participants are in their own personal space, the more they are to disclose information that reveals the nature of their lived experiences. In addition, the researcher created useful discussions among family members, friends present during our visits, and their opinions were stated in the field notes. Shenton (2004) encourages this discussion technique, which allows for individual viewpoints and experiences to be verified against others. Consequently, their

attitudes or behaviors contributed to a rich picture, based on the contributions of the different participants in this research.

Finally, acknowledging that the researcher's educational background could affect participants' responses, or the information obtained, we adopted as strategy to speak the Cameroon Pidgin English (CPE)[20] throughout the fieldwork. This strategy portrayed a sense of belonging to the same community as participants, thus, contributing to an atmosphere of self-confidence and trust within participants.

2.4.4 Design and use of interview instruments

This research used interview instruments: ethical guidelines, a written consent form, information sheet[21] (see Appendix B.2) and an interview protocol[22] (see Appendix B.4), which was designed according to Mack et al. (2005), Creswell (2014) and human ethics at Lincoln University-New Zealand. An approval (approved number: HEC 2019-25) was obtained from the Lincoln University human ethics committee (see Appendix B.1). These instruments addressed issues outlined in this research context, our methodological choices, the expected impact of the study, and how confidentiality was to be ensured. The interview protocol consisted mainly of statements that focused on the three components of SPT, as highlighted in Figure 2.4 and detailed in B.4. We decided on these components as it logically organized and interpreted these research findings.

[20] CPE is a language of communication in public places (markets, streets) as well as in informal settings, adopted during the British colonial period when the Cameroonians were curious to learn British English
[21] A form outlining this research purpose and ethics, to be handed to each participant
[22] A guide used during interviews and in writing down information during our field study

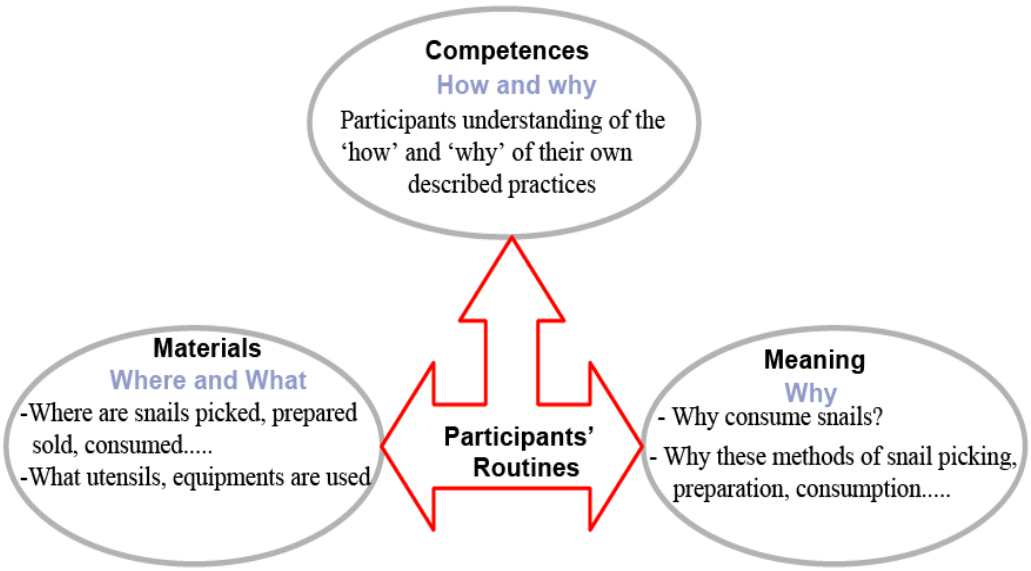


Figure 2.4. Summary of interview protocol

Due to the socio-cultural, economic, and political differences between Cameroon and New Zealand, these interview instruments presented certain challenges during its approval and in obtaining consents from participants. For example, the ethics committee required details on participants' low level of education, how participants' consents were to be obtained due to a lack of a CPE translated version and how participants will withdraw from the study if they possess no telephones or email addresses. This was practically true as although individuals are competent with the capacity to make rational decisions, lack of formal education and/or language barriers presents an inordinate challenge in the comprehension and understanding of informed consent (Gehlert & Mozersky, 2018; Punjwani, 2015). To resolve these contextual challenges, the researcher ensured that each recruited participant had a mobile phone to reach us on the contact numbers provided on the information sheet and had sufficient time (up to a year) to withdraw from the study.

We held brief conversations with individual participants during our first contacts and following their voluntary acceptance to participate in the interview sessions, they

were immediately handed copies of the afore-mentioned information sheet and consent form. Our reasoning being they could seek further explanations at their discretion from trusted friends and families prior to the interview dates. This was challenging in that it created some degree of reluctance to participate as participants needed to put in valuable time to understand the rationale of the study particularly written in the English language. However, on the interview day, the researcher gained participants' interest by conversing in CPE, engaging participants' relatives, and friends to clarify questions or doubts and highlighting the importance of the study findings.

2.4.5 Interpretation of qualitative information

We choose to use two notions: "interpretation" and "information" throughout this research. Information is considered as processed data with specific meaning or ideas, and thus, is an appropriate term for the experience-based narrative material obtained in our study. Here, we sought to interpret participants' personal perceptions, way of life and stories into useful and summarized knowledge. We answered Creswell (2014) qualitative research question: "*what was the lesson learned?* These lessons could be the researcher's personal interpretation, couched in the understanding that the inquirer brings to the study from a personal culture, history, and experiences". Contrasting to "data interpretation" in quantitative research, which draws conclusions from research questions or hypotheses (Creswell, 2014), we followed a step-by-step qualitative interpretative approach, as detailed in Table 2.2.

Firstly, we manually transcribed[23] and hand coded all texts and images. This process was found adequate since audio recordings were in CPE and contained inaudible speeches and pronunciations, thus, rendering automated transcription unsuitable. Next,

[23] a Microsoft word version of our findings

we used inductive and deductive reasoning including SSM and SPT, to organize derived themes. Creswell (2014) noted that "this inductive process illustrates working back and forth between the themes and the database until the researchers have established a comprehensive set of themes. Then, deductively, the researchers look back at their data from the themes to determine if more evidence can support each theme or whether they need to gather more information"

Table 2.2. Six steps in qualitative research interpretation

Steps	**Description**
Step 1 Familiarization with the data	Recordings were played several times and typed in Microsoft office word 2010. Transcripts were read and correlated with field notes and photos
Step 2 Generation of initial codes	Meaningful codes which represent the typed information/transcripts were derived
Step 3 Searching for themes	Initial codes were grouped into eight themes, and drafted on a rich picture
Step 4 Reviewing themes	A rich picture which represents our problem situation was used to construct a conceptual model of interacting activities
Step 5 Defining and naming themes	Five root definitions, three CATWOE of participants worldviews, six local practices and three components of SPT were used as organizing principles at this stage
Step 6 Final write-up	The organized information was interpreted with respect to these research contexts and recommended food safety guidelines

Note. As adapted from Braun and Clarke (2006)

As stated in Step 5, we derived five root definitions (snail collectors, market sellers, street vendors, home consumers and street eaters), three CATWOE (snail is a source of income, a source of nutrition and a food choice), and six local practices (picking, cracking, washing, cooking, selling, and eating). Following the SPT categories, we deduced participants' competences, materials used and symbolic meaning. These different organizing themes gave an interpretation of our study findings that could be readily discussed, understood, and critiqued.

Particular challenges in linking this organized information to public health risks were: 1) snail meat food safety regulations are still to be implemented in Cameroon, 2) we focused on participants who have little or no knowledge on the principles of food safety, and 3) the "local" practices identified in this research have never been questioned or studied or assessed. To resolve these challenges, we sought to interrogate our findings using: *"does each performed practice respect proper food safety procedures"* (Figure 2.5).

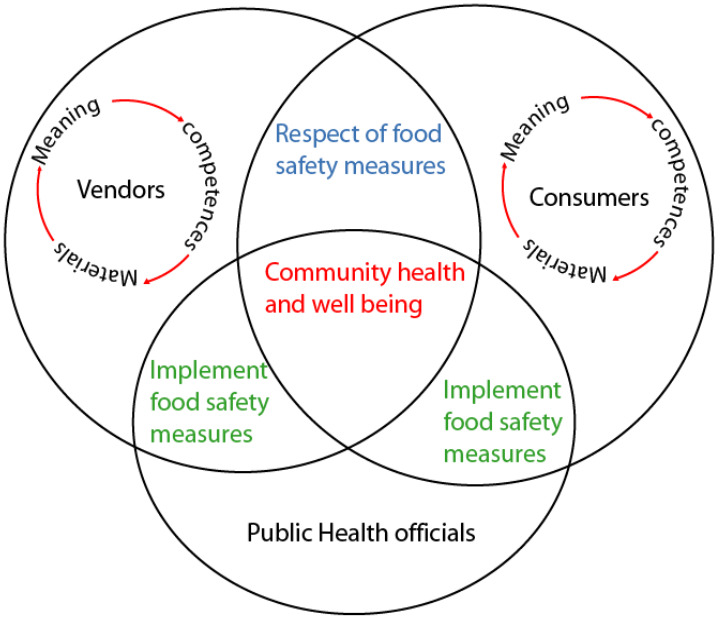


Figure 2.5. Interpreting the qualitative findings

The interpretation of the study findings was informed by two public health officials (an epidemiologist and a veterinary doctor). In Cameroon, epidemiologists assess the root causes of disease prevalence while veterinary doctors/nurses perform regular inspections on meat (beef, pork) in abattoirs and markets. These public health officials revealed current food safety implementation procedures, disease prevalence in Buea, and their stances on the current snail consumption practices as a contributor to foodborne illnesses. Their opinions were correlated with recommended guidelines, such as WHO (2006), for the interpretation of this study findings.

2.4.6 Trustworthiness of the study findings

Qualitative researchers have adopted criteria to "judge" their findings as those of internal validity, reliability, used in quantitative research have been shown to be unsuitable (Korstjens & Moser, 2018; Shenton, 2004). The principal criterion applied is trustworthiness, and it addresses the question: *Can the findings be trusted*? Among the several definitions and criteria of trustworthiness, we principally applied the best-known criteria of Lincoln and Guba (1985), which are credibility (prolonged engagement, persistent observation, triangulation, and member check), transferability, dependability, and confirmability. We encountered certain contextual challenges in ensuring this research was trustworthy.

As earlier mentioned, Buea persistently observed "complete lockdown" days colloquially referred to by residents as "ghost town" (BTI, 2020; Ngange et al., 2019). On such a day and thereafter, fear was common, and inhabitants were forced to engage mostly in indoor activities. On several occasions, we needed to re-schedule field observations and face-to-face interviews. This greatly affected our initial strategies to ensure prolong engagement and persistent observation. However, triangulation, member checking and clarifying the researchers' bias were instrumental in this research.

Firstly, we used multiple sources of information (variant field sites, grey literature) and information collection techniques (interviews, observations) as a triangulation strategy. Interview sessions were between twenty-five to sixty minutes per participant with field observations repeated multiple times on the same day and/or on different days. The use of an interview protocol and the inclusion of a note taker permitted us to observe while recording, and at the same time, take down notes and images. Also, with limited documents from online searches, we sought information from Youtube and closed groups

on Facebook. We were interested in uploaded videos, statements and comments on people experiences as snail consumers and vendors. We watched two videos of snail meat preparation from Buea (SCOLA, 2014; China Global Television Network [CGTN] Africa, 2020), and several others from Nigeria and Ghana (Grandma Gift, 2018; Edith channel, 2020). According to Creswell (2014), "If themes are established based on converging several sources of data or perspectives from participants, then this process can be claimed as adding to the validity of the study". Therefore, these findings are to be trusted.

Secondly, member check is feedback data, interpretations and conclusions by participants from whom the data were originally obtained (Creswell, 2014; Korstjens & Moser, 2018). Travel restrictions enforced by COVID-19 came at a time when the principal researcher of this study was in Christchurch-New Zealand. To get to our participants in Buea-Cameroon, we modified our member check strategy of a focus group. Here, the note taker conducted discussion sessions with participants using a video pre-recorded by the principal researcher describing our major findings. Prior to watching the video, participants were briefed about its content, and the video was paused at regular intervals to clarify participants' doubts, take down comments and opinions of the study findings. These comments were used to enrich and confirm our findings.

Finally, a clarification of the researchers' bias further strengthens our findings. Referred to as reflexivity, the researchers reflect on how their role and personal background, culture and experiences can shape the interpretation of the findings (Creswell, 2014). As a native of Buea, the principal researcher's early childhood was in Bomaka and Muea. She still recounts memories of snail picking with her siblings in the early hours of 4.00am.

> "We will crack the shells with stones, separate the foot from the internal organs with our hands, and our mother will wash and prepare later in the day. Knowing that the quantity of snail meat prepared was dependent on the amount of snails picked, there was this zeal to search for snails in the dark"

Certain challenges witnessed at this stage were to convince participants to narrate their own experiences. To some participants, the information we sought to understand was simple and direct, not requiring all the explanations and procedures recommended for qualitative research. To others, it was "a waste of their time" since they knew that the researcher was a native of Buea who should obviously know how snails are picked up until consumed. According to Creswell (2014), a researcher needs to have a continuous internal dialogue and critical self-evaluation with explicit recognition that their presence may affect the research process and outcomes. With this in mind, we recognized participants in Buea as experts and the principal researcher as a learner.

These abovementioned methodological decisions embed trust in our study findings as it revealed participants' routines and lived experiences in snail meat consumption.

2.5 Results

To understand health risks among snail meat handlers and consumers in Buea, we needed to pinpoint: 1) a systemic model of key activities, 2) local practices in snail meat consumption, and 3) participants' perceptions on snails as food and on potential health risks.

2.5.1 Systemic model of key activities

To produce a systemic model of key activities, we followed SSM (cf. Section 2.4.1) in picturing the problem as a complex, 'messy' situation with many contributing and interacting factors, and then identified some specific human activities to model.

2.5.1..1 Context and complexity

Information obtained from participant experiences, in-depth interviews, observations, and grey literature was developed into a rich picture using an Ishikawa diagram (Figure 2.6). Ishikawa diagrams are an approach which thoroughly analyzes situations to identify the possible causes of a problem (Luca, 2016). Using this approach permitted us to illustrate the complex potential contributors of health risks in snail consumption activities by demonstrating the possible role of eight kinds of inputs into potential health outcomes from human exposures. With this in mind, we then focused on 'high salience' human activities in order to better understand these health risks.

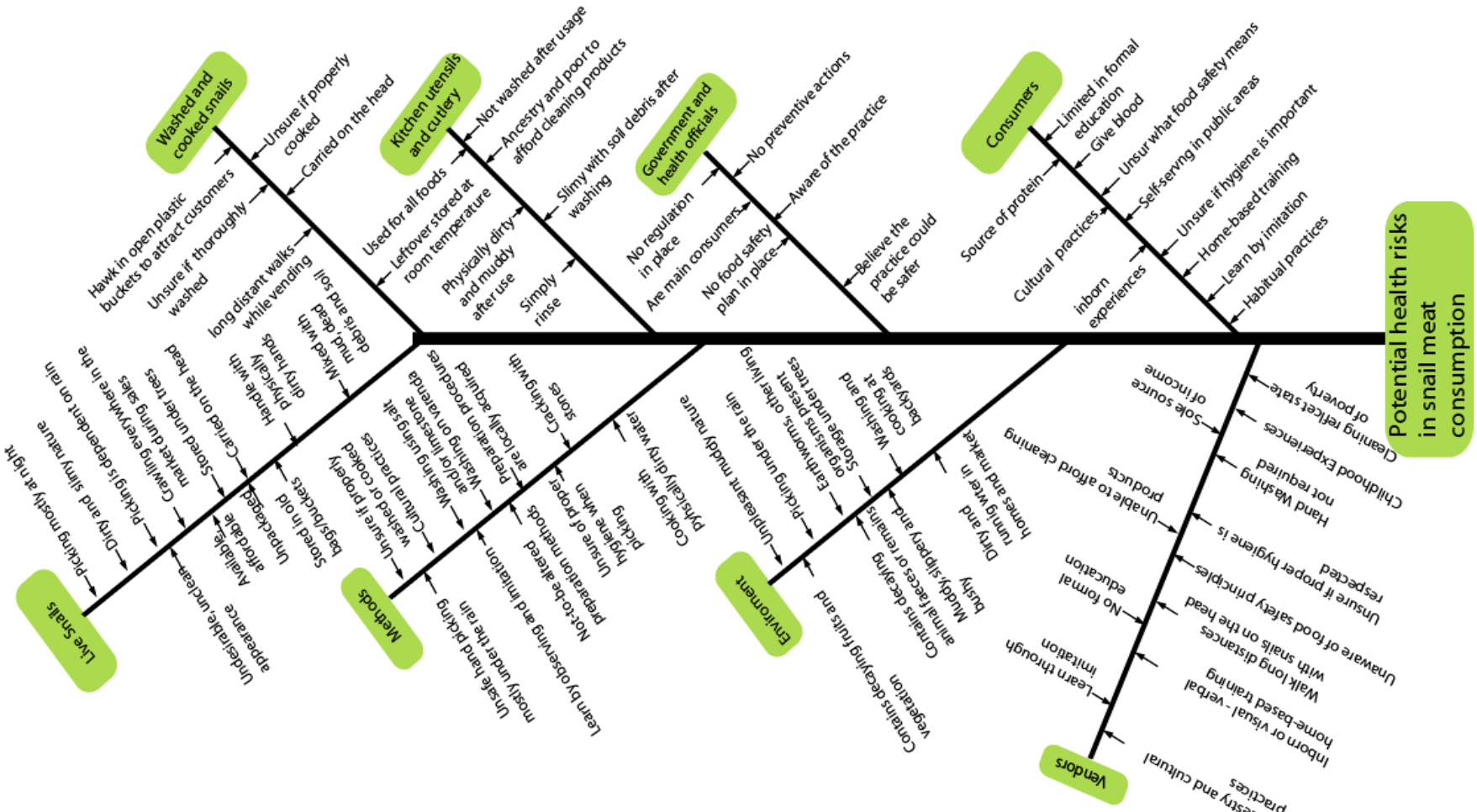


Figure 2.6. Ishikawa diagram permitting brainstorming of potential health risks

2.5.1..2 Identifying and modelling human activities

To map key human activities in snail meat consumption, based on methods described in Section 2.4, we developed a systemic model in Figure 2.7, and then in Figure 2.8, highlighted the connections (that is, common words in addressing each snail activity), that existed among participant excerpts. For example,

> "We pick and wash every Saturdays and sell on Sundays in the market. I wash snails every weekend…" (Ms)
>
> "……picking, washing**,** and selling snails have safe my family from many situations [...] from hunger" (Sv)

From these common ways of speaking, we distinguished seven interrelated local practices performed in a succeeding order (Figures 2.8 & 2.9). For instance, picking, cracking, and washing occurred before cooking while hawking and/or eating thereafter. To reveal different snail activities in terms of our diverse research participants (actors), their purpose of acting or performing each local practice, we used root definitions (Table 2.3). We came up with five statements using the PQR formula, that is, do P-what, by Q-how, in order to achieve R-why (Proches & Bodhanya, 2015). 'To do P' refers to the transformation. 'By Q' specifies the activities that are needed to do P. 'In order to achieve R' refers to the worldview that makes the transformation meaningful (Christis, 2005). We then used SSM mnemonic CATWOE to elaborate on these actors and activities (Table 2.4). Snail consumption activities are defined by a transformation process (T) and worldviews (W) which required actors, women (A) to do the activities which make up T. It will affect these women and street eaters (C) who are beneficiaries or victims (customers of the system). These activities embrace constraints from its environment (E)

and the whole transformation can be seen as 'owned' (O) by these women; vendors, consumers, or health officials in that they have the power to change or stop the system.

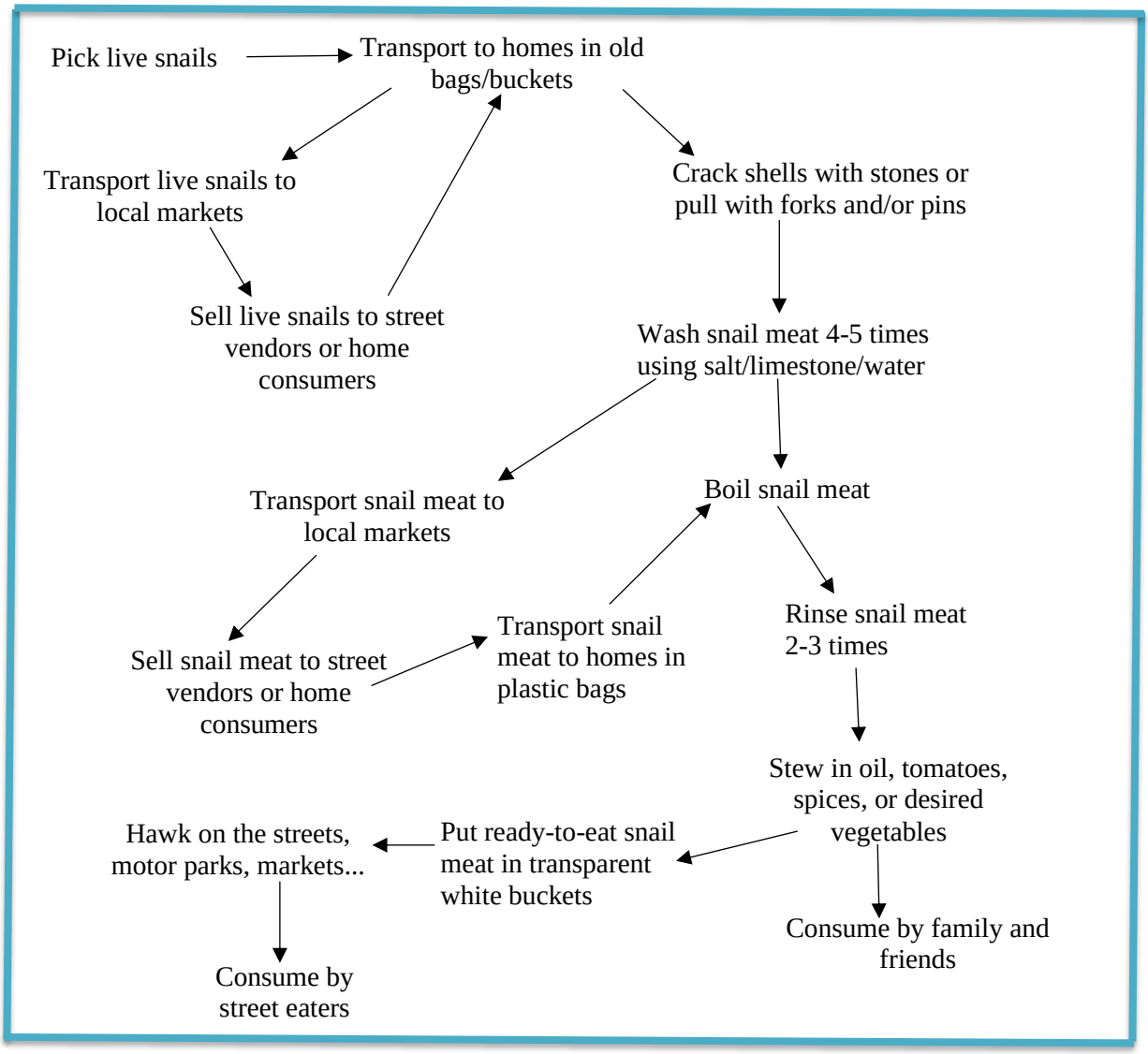


Figure 2.7. A model of snail consumption activities

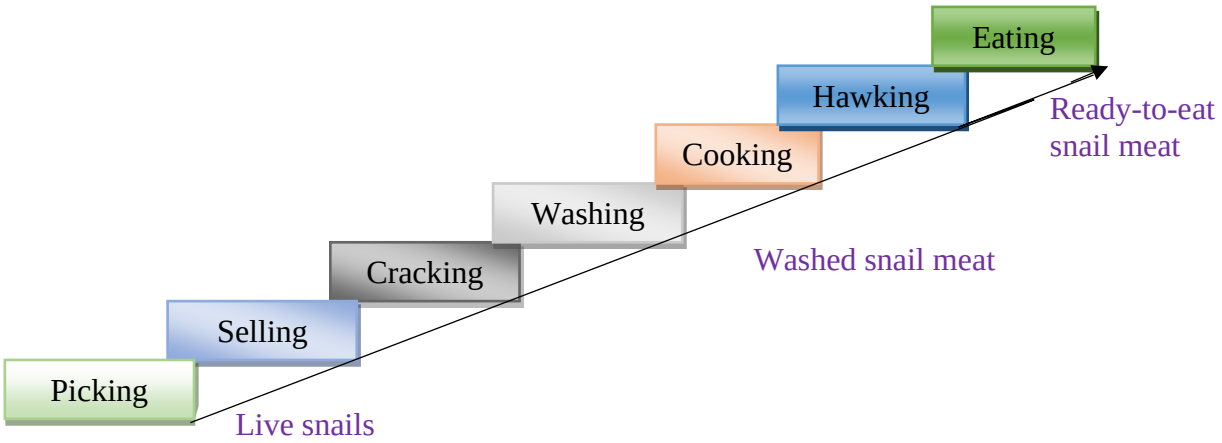


Figure 2.8. Sequential local practices from live snails to ready-to-eat snail meat

Figure 2.9. Snail preparation stages as observed in this research

Stewed snail meat with chili pepper

Snail meat in eru (*Gnetum africanum)* and water fufu (fermented *Manihot esculenta*)

Figure 2.10. Typical snail meat diets consumed in Cameroon

Table 2.3. Actors in snail consumption activities

Actors		Root definition
1	Snail collectors (Sc)	Collectors who earn money by picking live snails in order to support family or reduce poverty
2	Market sellers (Ms)	Sellers who earn money by selling[24] live snails or washed snail meat in order to support family or reduce poverty
3	Street vendors (Sv)	Hawkers who earn money by hawking[25] ready-to-eat snail meat in order to support family or reduce poverty
4	Home consumers (Hc)	Households who increase nutrient intake by eating snail meat in order to reduce malnutrition
5	Street eaters (Se)	Road users (traders, students, travelers….) who satisfy tastes by eating ready-to-eat snail meat in order to respect regular eating behaviors

Table 2.4. CATWOE of the model system

CATWOE	**Description**		
	Support family or reduce poverty	**Source of nutrition**	**Tastes, preference**
Customer	Sc, Ms, Sv	Hc, Ms	Sv, Se
Actor	Sc, Ms, Sv	Hc	Se
Transformation	Sc, Ms pick and sell live snails, Sv sell ready-to-eat snail meat to earn money	Hc pick or buy live snails, cook and eat snail meat to increase protein and calcium intake	Se buy and eat ready-to-eat snail meat to satisfy tastes
Worldview	Readily available, little or no investment, no skills required	Source of calcium and blood, cheap, affordable, cultural dish	Delicious, satisfying, tasteful, affordable
Owner	Sc, Ms, Sv, Hc, Se, health officials	Ms Hc, Health officials	Sv, Se, Health officials
Environment	Weather and poverty-driven, increase family needs	Diet-conscious, cultural upbringing, poverty, beliefs	Habits, lifestyle, irresistible tastes

[24] Selling indicates the point of sale is static, most commonly at a roadside location.
[25] hawking identifies the point of sale as mobile, whereby goods are transported in a bucket on the seller's head around the locale.

2.5.2 Understanding local practices and perceptions

Having identified and modelled human activities that make up the system of snails as food in Buea, we now describe the competencies, materials and meaning-making that that influence local practices (and therefore influence the potential for exposure to foodborne pathogens). For this we used SPT. Table 2.5 presents evidence from participants in Buea on social practice around snails as food and associated hygiene.

In terms of competencies: competence is 'inborn', 'from childhood', that is, 'the way of seeing things being done and imitate'. It is locally acquired through family and community. Hygiene is seen as each household's 'state of cleanliness'. It is based on 'this is the way I was taught'. For instance, washing and cooking involve inherited visual and verbal 'instructions' initiated from one generation to the next. Preventing diseases or staying healthy is understood to depend on washing and cooking practices.

In terms of materials: materials reflect participants' 'inborn' experiences, parentage and 'state of poverty'; for example, picking at night with torches, transporting live snails in old bags and buckets, pulling snail meat with stainless-steel table forks or 'pins' or cracking snail shells with stones, cooking by visual observation. Handpicking involves poor or uncontrolled hygiene. Live snails are physically dirty, 'unhygienic' containing soil debris, food wastes. Local practices are constrained by the very simple technologies available. For instance, selling live snails in old bags/buckets, washing in two silvery or plastic basins on the veranda, cooking on three-stone fires in front or behind the house and hawking in loosely closed plastic buckets.

In terms of meaning: snails support family economies - as a source of nutrition, a source of income and a food choice. Although eating snail meat is seen as delightful, snail picking to cooking are ‘unpleasant’ and ‘nasty’ activities.

Table 2.5. Constraints and drivers that influence snail local practices

Element		**Snails as food**	**Hygiene**
Competence		*What competence influences practice? How do people get this knowledge?*	*What do participants know about hygiene when handling and eating snails? How do people learn?*
	Participants' evidence	Hc: "We have been eating since we were small, so it is but normal, we just know that you want to pick you just go there" [pointing at the back of her house] Hc: It is but obvious, we are growing in a society where we eat snails, we have been eating since we were small, [..], so it is not like there is a particular skill that we have been taught, no, we just know [..]" Sv: "I think for those who have never seen how they are washing it anywhere, they don't need any expert [..] but if you are somebody who have seen where they are washing, just follow what the people do, [..] no you don't need any training Researcher: '*If you are teaching your daughter to do what you do, what would you teach her?*' Sc: "I will ask her to go out, look for cold or hidden places, under the grasses where there is no or less sunlight, she will find snails"	Hc: "We don't really pay much attention to hygienic conditions, besides when you go and pick in very rotten things, [..] people [snail collectors] go to the bush, excrete there and keep it for snails to come and they will pick in the evening, it is funny, but it is the truth" Sc: "To protect yourself from food poisoning, snails need to be washed thoroughly, [..] some people hurry [..] take your time and make something good, for yourself and family" Hc: "For many years, since they gave birth to me, my mother showed me how to wash and how to cook snails to eat in the house" Hc: "You can learn from anyone who does it. I was not trained in a school. She just needs to watch from beginning to end"
	Summary finding	Competence is 'inborn', 'from childhood', that is, '*the way of seeing things being done and imitate'*. It is locally acquired through family and community.	Hygiene is each household 'state of cleanliness'. It is based on '*this is the way I was taught*'. For instance, washing and cooking involve inherited visual and verbal 'instructions' initiated from one

			generation to the next. Preventing diseases or staying healthy depends on washing and cooking practices
Materials		*How do materials influence the picking, handling, and consumption of snails?*	*What technologies and methods influence how people practice hygiene when handling or consuming snails?*
	Participants' evidence	Sc: "we use very good and shining torches to pick snails; places are very dark, if you do not use a torch that the light is good, you will not see snails" Sv: "…I think it is a nasty method, imagine taking this grinding stone, [..] the one we use to grind pepper, you make crack, those shape particles [snail shells] are piercing your hand and the meat" Researcher: *do you know the time and temperature you cook your snail meat?* Hc: 'I do not have a thermometer, when I see it ready, I will know by looking at it, [..] you do not need to look at the time as well, a change in colour is enough'	Hc: "[..], in moist areas, around the pit toilet, I will send her [her daughter] there to go and pick it [snails]" Hc: "It [snails] is too slimy, so you must wash it outside because you have to throw the slime" Two street vendors mentioned they will prepare on three-stone fires because '*firewood is cheaper than gas'*, and some home consumers preferred firewood due to cultural upbringing
	Summary finding	Materials reflect participants' 'inborn' experiences, parentage and '*state of poverty*'. For example, picking at night with torches, transporting live snails in old bags and buckets, pulling snail meat with stainless-steel table forks or '*pins'* or cracking snail shells with stones, cooking by visual observation.	Handpicking involves poor or uncontrolled hygiene. Live snails are physically dirty, 'unhygienic' containing soil debris, food wastes. Local practices are constrained by the very simple technologies available. For instance, selling live snails in old bags/buckets, washing in two silvery or plastic basins on the veranda, cooking on three-stone fires in front or behind the house and hawking in loosely closed plastic buckets.

Meaning		*What meaning influences people to handle and eat snails?*	*What do people believe about hygiene in relation to snails?*
	Participants' evidence	Hc: "…. yes, it is really delicious when you prepare it and if you know how to prepare it, so it is something that we love eating, so we like to pick and cook it" Se: "snails are nutritive[..]contains calcium, give us blood"	Hc: "I have never thought of that [hygiene] because even the ones that we buy from the market, hmm at times we do not even know where they pick them, so when you pick there [pointing at a pit latrine], it is not actually the excreta but the meat you are picking, so it is good" Sc: "You must pay attention to the sticky liquid, [..] you need courage to touch snails as it irritates many people" Epidemiologist: "…. once you expose yourself to a dirty environment, like picking from unhygienic conditions means they [Sc, Hc, Ms] are already exposing themselves to diseases"
	Summary finding	Snails support family, it is a source of nutrition and a food choice. Two vendors mentioned: '*we need money*', '*it is a moneymaking activity*'.	Although eating snail meat is delightful, snail picking to cooking are 'unpleasant' and 'nasty' activities. For instance, most participants expressed 'hmm' when snail picking and washing were mentioned

2.6 Discussion

Our purpose was to understand health risks in snail handling and consumption in rural settings particularly in Buea. To our knowledge, we report on yet-to-be documented snail meat disease-causing real-world situations. We fill-in the knowledge gap existing between 1) already enumerated microbial pathogens associated with snails in farms as well as those sold in local markets (Adagbada et al., 2011; Akpomie, 2013; Nyoagbe et al., 2016; Tanyitiku et al., 2022b) and 2) the ways in which local people are routinely exposed to foodborne pathogens during snail handling and consumption.

On rainy nights between 9pm to 5am, people often use torches and collect snails in backyards and dwellings (Tanyitiku et al., 2022a). Gatherers may spend up to twenty hours a week in search for snails in both protected and unprotected areas (Ndah et al., 2017; Ngenwi et al., 2010). During the day, snails can be collected in dark and damp areas, such as under the clearing of decaying vegetation, herbaceous plants, and shrubs (Mohammed et al., 2014; Nyoagbe et al., 2016). Snails are picked by hand when actively feeding or moving around and placed in old bags/buckets for transportation (Cobbinah et al., 2008). In Ghana and Cameroon, Nyoagbe et al. (2016) and Kaldjob et al. (2019), respectively, reported that consumers will prefer free-living snails with 'better taste' to commercially farmed snails (Kaldjob et al., 2019; Nyoagbe et al., 2016).

Few studies have used qualitative approaches to raise food safety concerns in snail handling processes. For instance, Nyoagbe et al. (2016) in a survey mentioned snails sold in Ghana were unpackaged, in an unhygienic state, and slime, shelling and dirt were snail preparation problems. Temelli et al. (2006) identified personnel hands and equipment used as secondary snail meat contamination sources during the large-scaled processing of frozen snail meat. Similarly, Novakovj and Grujj (2017) highlighted the importance of

hand hygiene, hand washing and disinfection in snail meat industrial processing. We distinguish our findings from the above research as it sought to understand human exposures to food safety hazards by seeing through the eyes of key informants and provide foodborne disease-related evidence in snail meat practices in traditional settings. Our use of SSM provided a rationale for where to look in a complex social system for risky activities. SPT provided a powerful lens for looking more closely at practices that seem important points of exposure to pathogen hazards (as identified by SSM). Our use of these two frameworks provides a strong lead for the design of future interventions: SSM offers a rationale for which activities to target to improve health outcomes, while SPT offers a more fine-grained rationale for how to intervene for social change in chosen activities, described by SSM.

Our findings showed that snails are seen as financial support for families, a source of food to low-income inhabitants, and are preferred to other foods in terms of taste. In addition, to reduce protein malnutrition, the African giant land snail has been recommended as a good substitute among the vulnerable group including children, pregnant women, aged and adolescents in Nigeria (WHO, 2002) as well as in our research settings. Snail collectors picked snails and sell to market sellers who then sell to street vendors or home consumers. While home consumers prepared the meat and eat at home with family, street vendors cook and sell ready-to-eat snail meat to street eaters in public areas.

In the quest to support family, solve undernourishment, and protein deficiency challenges, our findings revealed snail meat handlers and consumers become undoubtedly exposed to foodborne pathogens, which could lead to adverse food-related illnesses, especially to the above-mentioned vulnerable groups. Competences in carrying

out snail activities are locally acquired through family and community and hygiene is based on '*this is the way I was taught*'. We identified potential human exposures to foodborne pathogens, for instance, handpicking in unhygienic habitats (domestic wastes, decaying vegetation, arable land, footpaths, backyards…), the materials used in picking, washing, cooking and consumption (that is, old bags, open buckets, lack of constructed kitchens - cooking in front or at the back of the house on three stone fires, physically dirty and improperly washed kitchen utensils and cutlery…), street hawking in loosely closed buckets and self-serving on roadsides, road junctions, local markets and motor parks. Furthermore, it should be noted that snails and microorganisms are closely associated with habitats characterized as 'filth', 'sewage' and 'rotten materials'(Akpomie, 2013; Nyoagbe et al., 2016). In our study area of Buea, the current waste management has been described as 'poor', 'pathetic', and 'rudimentary orchestrated' which could increase disease prevalence (Bate, 2020). As such, local practices in snail picking to consumption as well as its improper handling could lead to potential public health risks particularly in Buea and other sub-Saharan countries with comparable snail handling and consumption practices.

Additionally, based on our findings, we initiated a start-up point for further research in an undefined and under-researched health risks problem situation using an innovative combination of in-depth interviews, participant observation, lived experience and a focus group. We then conducted a study in Chapter 3, Tanyitiku et al., (2022b), to determine the prevalence of key enteric pathogens in snails collected from natural habitats, which will assist in a more accurate assessment of risks of infection to local communities consuming snail meat. Our use of soft systems framework and social practice theory provides a strong lead for the design of future interventions which could be achieved by

a) an improved microbial-free systems of snail farming or heliciculture among the local people in sub-Saharan Africa, b) prior to consumption, a starvation (purging) of live snails, which may eliminate ingested excrements and contaminants in snails, and finally c) an initiation of governmental food safety regulation and policy that target the local people cultural snail meat consumption practices. To reduce health risks from consuming snail meat containing foodborne pathogens, changes will need to be engaged with deeply embedded locally acquired competences and worldviews and take account of the material and meaning circumstances that configure key local snail-meat practices.

2.7 Conclusion

Snail meat is a source of food and livelihood to rural inhabitants in the tropical rainforest. With underdeveloped snail farming practices, African giant land snails are typically gathered from natural habitats including forest, farms, footpaths, and decaying vegetation. Situating our study in sub-Saharan Africa and particularly in Buea - Cameroon, our purpose was to understand potential health risks in local snail meat picking, handling, and consumption activities. This was important given the high incidence of foodborne pathogens we detected in snails sourced from this region (Tanyitiku et al., 2022b). We revealed snail handlers and consumers are substantially exposed to foodborne pathogens, thus indicating local snail consumption practices as risks significant to public health. Snail picking occurred in locations with poor or uncontrolled hygiene. Snail meat washing, cooking, and selling took place in uncertain and possible food-contaminated cultural settings that reflected participants' parentage and 'state of poverty'. This research suggests fruitful opportunities for designing and testing interventions aimed at improving health outcomes for families and communities involve in the handling and consumption of edible terrestrial snails.

Chapter 3. Prevalence of foodborne pathogens in edible land snails: Buea, Cameroon

Chapter 3 has been published as Tanyitiku et al., 2022b (doi:10.3201/eid2808.220722). It was also presented at the 1) Young Scientist Programme (YSP) of the Federation of Asian and Oceanian Biochemists and Molecular Biologists (FAOBMB), Nov. 19-21, 2021, and 2) 16th International Congress of FAOBMB (Nov. 22-25, 2021)

3.1 Summary

Background: African land snails serve as a source of food for many people in tropical countries. Snails are rarely farmed, but collected from environments with little, or poor sanitation. These snail consumption practices could expose people to foodborne diseases. This study aimed to understand potential health risks from snail meat consumed in Cameroon.

Methods: We used PCR techniques to detect key foodborne pathogens in the faeces of snails sampled in three locations: within people's homes, arable land, and local markets.

Results: The results showed that every edible snail examined contained at least one pathogen examined. Overall, the prevalence of Shiga-toxin producing *Escherichia coli* *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. ranged from 57-86% in the samples examined.

Concluding remarks: Our results indicate that snail meat is a significant vector for enteric diseases in populations where such meat is an important source of nutrition.

3.2 Introduction

African land snails serve as a source of food for many people, especially in sub-Saharan Africa (Adegoke et al., 2010; Adeyeye et al., 2020a; Mohammed et al., 2014; Nyoagbe et al., 2016). The important species consumed are *Achatina achatina, Achatina fulica*, and *Archachatina marginata*. Snail meat is tender and chewy, with a unique floral and mushroom-like flavour. It contains 37-51% protein, which is higher than the protein content (protein per mass of skeletal muscle) in guinea pig (20.3%), poultry (18.3%), fish (18%), cattle (17.5%), sheep (16.4%) and swine (14.5%) (Adeyeye et al., 2020a).

In rural settings, commercial snail farming is uncommon. Rural dwellers may spend up to twenty hours a week in search of edible snails in environments which include marshes, decaying vegetation, domestic wastes, roadsides, footpaths, and bushes (Akpomie et al., 2019; Mohammed et al., 2014; Ndah et al., 2017; Nyoagbe et al., 2016). From surveys conducted by Nyoagbe et al. (2016) in Ghana and Kaldjob et al. (2019) in Cameroon, it was reported that consumers will prefer free-living snails of 'better taste' to commercially farmed snails. Kaldjob et al. (2019) further highlighted that 66.82% of 211 respondents purchased snails at local markets, 9.48% collected snails in farms, and 23.69% acquired snails in both farms and local markets. These local practices of collecting, handling, and consuming African land snails could expose snail meat handlers and consumers to foodborne illnesses. However, to our knowledge, such risks have never been quantified.

Previous research (Adagbada et al., 2011; Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Okafor-Elenwo & Imade, 2019; Okafor & Ogbo, 2019) has raised concerns about food safety due to significant microorganisms isolated in edible land snails. For example, Akpomie (2013) detected $2.0x10^3$-$2.8x10^4$CFU/ml of

Escherichia spp., *Salmonella* spp., *Pseudomonas* spp., *Shigella* spp. and *Bacillus* spp. in the visceral mass and effluents of African land snails. Okafor-Elenwo & Imade, (2019) estimated that 1,750 per 10,000 potential snail meat consumers above the age of three years were most likely to fall ill after consuming edible terrestrial snails in Nigeria.

Although these studies (Adagbada et al., 2011; Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Okafor-Elenwo & Imade, 2019; Okafor & Ogbo, 2019) have highlighted the close association of edible snails with pathogenic microorganisms, potential health risks that could contribute to the burden of foodborne diseases in Africa have been significantly overlooked. In Cameroon, there is no data on the epidemiology and prevalence of foodborne pathogens in snail meat consumption. Given that many patients do not have access to medical laboratories (Tchientcheu et al., 2021), the potential of snail meat consumption practices to cause enteric diseases to the local population is unknown. This has hindered subsequent interventions to target foodborne health risk factors. The purpose of this paper was to assess the prevalence of potential foodborne pathogens in African land snails consumed in Cameroon. This research examined the faeces of edible snails collected in three locations:

1. Within people's homes - 0-1km between dwellings in Buea, Cameroon
2. Arable land - small areas of land used for vegetable cultivation destined for household consumption or income earning when production exceeds household needs, and
3. Local markets - unsheltered settings where people gather on a weekly basis to sell and purchase food items (such as live animals, meat and fish products, fresh fruits, and vegetables) and durable goods, such as fabrics and electronics.

3.3 Materials and methods

3.3.1 Sampling strategy of live snails

Live snail sampling was carried out in Buea, Cameroon, between June and October 2019. Buea is situated between longitude 9°4 '30' to 9°22'30' east and latitude 4°3 '00' to 4°16'30' north (Tanyitiku et al., 2022a). It is at the slope of mount Cameroon and has a population of 300,000 inhabitants. Buea is made up of a tropical rainforest with annual rainfalls between 3000-5000mm throughout the year (BCDP, 2012; Tanyitiku et al., 2022a). These climatic conditions have favored the abundant survival of snails (BCDP, 2012; Kaldjob et al., 2019; Miegoue et al., 2019; Ndah et al., 2017; Ngenwi et al., 2010). Due to the increasing demand in domestic and international consumption of African snail meat, live snail collectors, mobile and stationed snail meat vendors and consumers are becoming more and more noticeable in Buea (Tanyitiku et al., 2022a).

We sampled live snails within people's homes between 9pm and 5am on rainy nights, and in arable land, during the day. In Buea, live snails could be found actively moving around at night (Tanyitiku et al., 2022a). During the day, live snails are usually present underneath decaying vegetations in farmlands and plantations (Tanyitiku et al., 2022a). Samples from local markets were purchased weekly from snail vendors. These sampling locations and time frames were based on previous findings arising from field observations and face-to-face inquiries with the local people of Cameroon (Kaldjob et al., 2019; Tanyitiku et al., 2022a). As detailed in Chapter 2, we were also interested in these locations as it represented the people's snail collection location in Buea, Cameroon. Here, our target of these three different sampling locations (within people's homes, arable land, and local markets) emerged from the people's response to questions such as '*where do you get the snails you eat or sell at the market? How do you get the snails you eat or sell? how*

do you know snails are present there?', if you are to teach your daughter on how to get snails, what will you teach her?" (Tanyitiku et al., 2022a). It should be noted that participants mainly included women who: a) have lived in Buea for at least five years, b) possessed childhood experiences from snail collection to consumption activities, and c) still actively incorporate snails in their routines, that is, as a snail meat handler and/or consumer (Tanyitiku et al., 2022a).

Fecal samples from African live snails were taken as follows. Live snails were collected weekly from the location studied (within people's homes, markets, and arable land), and stored at room temperature in the laboratory in 2L sterile Sistema containers (Sistema Plastics, NZ). The feces of 6-12 edible snails per sample, were aseptically collected within 12-18 hours, pooled, and placed in 15 ml sterile Eppendorf tubes (Figure 3.1). The samples were then stored at -80^{0}C prior to DNA extraction using the manufacturer guidelines of Presto™ stool gDNA extraction kit (see Section 3.3.2.1). Total DNA extracts were then stored at 4^{0}C prior to air freighting to Lincoln University, New Zealand for PCR analysis of the specific pathogens (see Section 3.3.2.2).

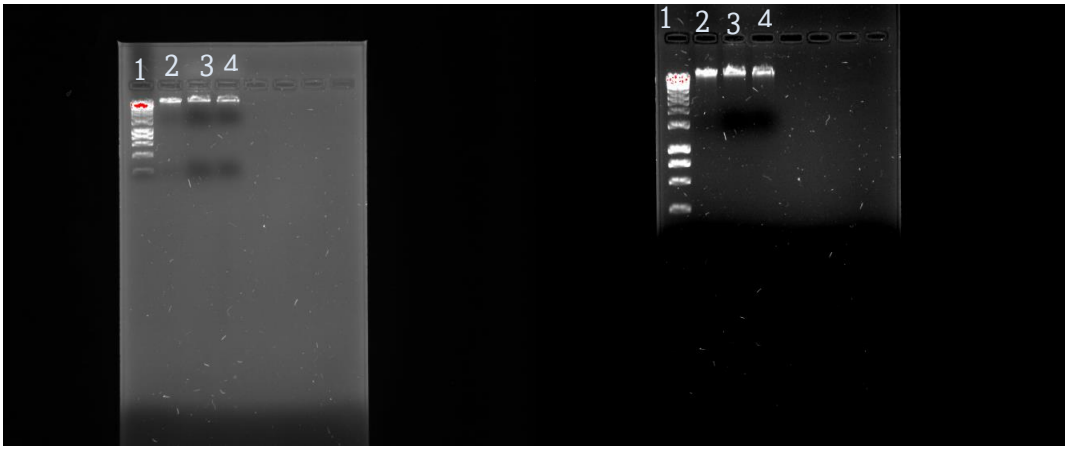
Figure 3.1. Sampling of snail faeces for DNA extraction

3.3.2 Microbiological analysis of snail faecal samples

3.3.2..1 DNA extraction

The manufacturers guidelines of the Presto™ stool gDNA extraction kit were followed. 200mg of snail feces was centrifuged at 8000g for 2mins in 800µl ST1 buffer solution and incubated at 70°C for 5mins. 500µl of supernatant was placed in a 1.7ml microcentrifuge tube containing 150µl of ST2 buffer, briefly vortex, and incubated at ± 4^0C for 5 mins. The mixture was centrifuged at 16000 g for 3 mins and a clear supernatant of 500µl was transferred to the inhibitor removal column. It was then centrifuged at 16000 g for 1 min and the column was discarded. 800µl of ST3 buffer was added to the flow through and then to a new GD column and centrifuged at 1600 g for 30 secs. This process was repeated three times to completely wash the bounded DNA. 100µl of preheated 10 mM Tris-HCl, 1mM EDTA, pH8.0 was added at the center of the dry GD column, centrifuge at 16000g for 2mins to obtain the eluted DNA.

It should be noted that prior to the use of the Presto™ stool gDNA extraction kit on African land snails (Figure 3.1), preliminary trials, as shown in Figure 3.2, using the faeces of garden snails (*Helix* spp.), harvested in Christchurch, NZ (Figure 4.3), were performed and satisfactory results were validated in-house.



A. Imaging before PCR B. Imaging after PCR

Note. Lane 1: 1kb plus DNA ladder, Lane 2: *E. coli* NZRM 4396 (0178:H7, stx1 positive), Lane 3: 1:1 dilution snail faecal DNA, Lane 4: 1:50 dilution snail faecal DNA

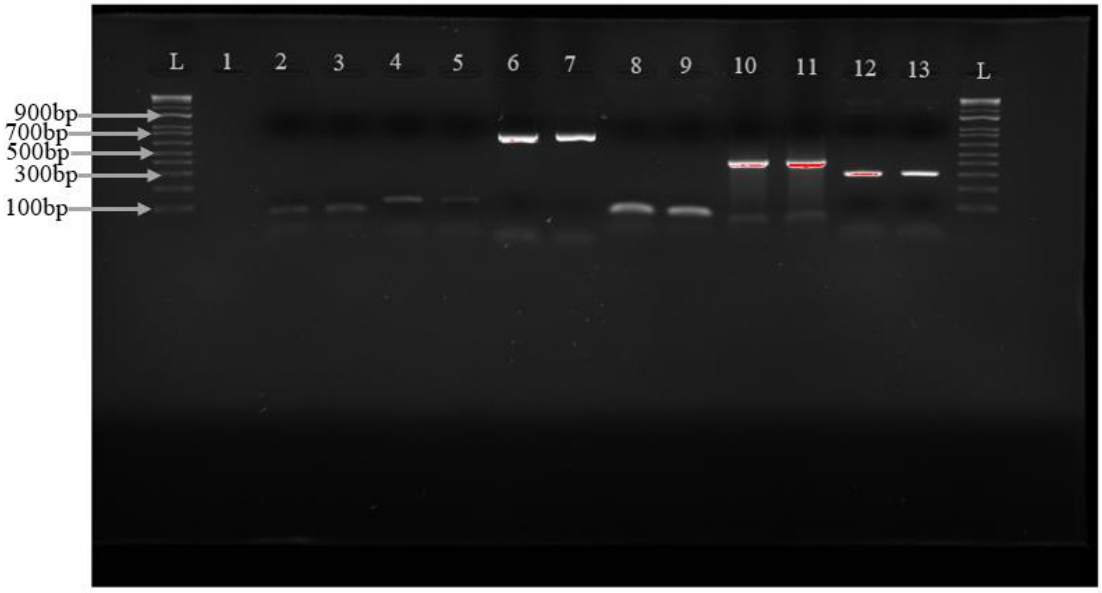
Figure 3.2. Validation of Presto™ stool gDNA extraction kit

3.3.2..2 PCR amplification

The presence of Shiga-toxin producing *Escherichia coli* (STEC), *Campylobacter* spp., *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. was examined in snail fecal DNA extracts using previously described PCR methods (Chakravorty et al., 2007; Kawase et al., 2016; Lantz et al., 1998; Linton et al., 1997; Waage et al., 1999). Table A.6 presents the PCR primers and optimal conditions, with the principle of PCR application guided by Fawley & Wilcox, (2005) and Kurkela & Brown, (2009). The PCR reactions with the aforementioned cycling conditions were performed on a 96-well GenePro thermocycler (BIOER technology, England). Each reaction mixture was prepared in a volume of 20 µl consisting of 2 µl of a 1 in 100 diluted DNA extract, 6µl of distilled water, 1µl each of forward and reverse primers (100μM prepared working solution), and 10µl of Quantabio repliQa Hifi toughmix, that includes 2x reaction buffer containing optimized concentrations of $MgCl_2$, dNTP's and proprietarily formulated HiFi polymerase, hot start antibodies and ToughMix chemistry (repliQa Hifi toughmix: Quantabio, MA, USA).

These protocols were adapted for use with a high-fidelity DNA polymerase (repliQa Hifi toughmix: Quantabio, MA, USA), and validated in-house using authenticated

reference strains as positive and negative controls. The isolates *Escherichia coli* NZRM 4396 (0178:H7, stx1 positive), *E. coli* NZRM 4397 (0171:H2, stx2 positive), *Listeria monocytogenes* NZRM 44, *Campylobacter jejuni* NZRM 2397, *Salmonella Enterica* serovar Menston NZRM 383 and *Yersinia enterocolitica* NZRM 2603 were used to evaluate the different cycling protocols. Appropriate positive and negative controls from reference strains were included in each run. The 16S rRNA gene (Table A.6) served as the positive control while *Pseudomonas marincola* LU P2 served as a negative control for all experiments. The specific bands of each bacterial isolate obtained under optimal conditions are presented in figure 3.3.



Note. Lane L: 1kb plus DNA, ladder 1: *Pseudomonas marincola* isolate (*Salmonella* spp. assay, negative control for all assays), lane 2/3: *E. coli* Stx1, lane 4/5: *E. coli* Stx2 gene; lane 6/7: *Campylobacter jejuni*; lane 8/9: *Listeria monocytogenes*; lane 10/11: *Salmonella Enterica* serovar *Menston*; lane 12/13: *Yersinia enterocolitica*.

Figure 3.3. Specific DNA bands of interest at optimized PCR conditions.

3.3.2..3 Gel electrophoresis

All PCR products were detected by electrophoresis. Each electrophoretic setup was composed of 0.8% agarose gel stained with 2µl SYBR Safe (Invitrogen, MA, USA). A 0.5M TBE (Tris-borate EDTA, pH 8.0) was used as the running buffer. Each well was loaded with 2µl of PCR product after mixing with few drops of 6X 30% glycerol. An Invitrogen 1kb plus DNA ladder (Thermofisher scientific, USA) served as the molecular-weight size marker. Electric power was supplied to the set up at 100V for 40minutes. Electrophoresed gels were visualized using a UV-fluorescence Bio-Rad imaging system (Bio-Rad laboratories, USA).

3.3.3 Interpretation of laboratory analysis

The presence of an amplicon of the appropriate size for each PCR assay to detect *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. in a sample was recorded as a positive result. For Shiga-toxin producing *Escherichia coli* (STEC), a positive result required the detection of both Stx1 and Stx2 genes. These criteria determined the prevalence of each pathogen in each sampling location (people's homes, markets, arable land: Figure 3.4). We also calculated the pathogen prevalence in the sampling points within the three sampling locations to reveal the distribution of each pathogen within the three sampling locations.

3.4 Results

The results of this research are summarized in Figures 3.4. The overall pathogen prevalence among the samples examined was high, ranging from 57 to 86 % (Table 3.1). At least one pathogen was detected in every sample examined, with the majority containing multiple pathogens.

Table 3.1. Frequency of pathogen occurrence in African land snails

Pathogen	STEC	*Campylobacter* spp.	*Salmonella* spp.	*Listeria* spp.	*Yersinia* spp.
Frequency (%)	57	75	69	86	71

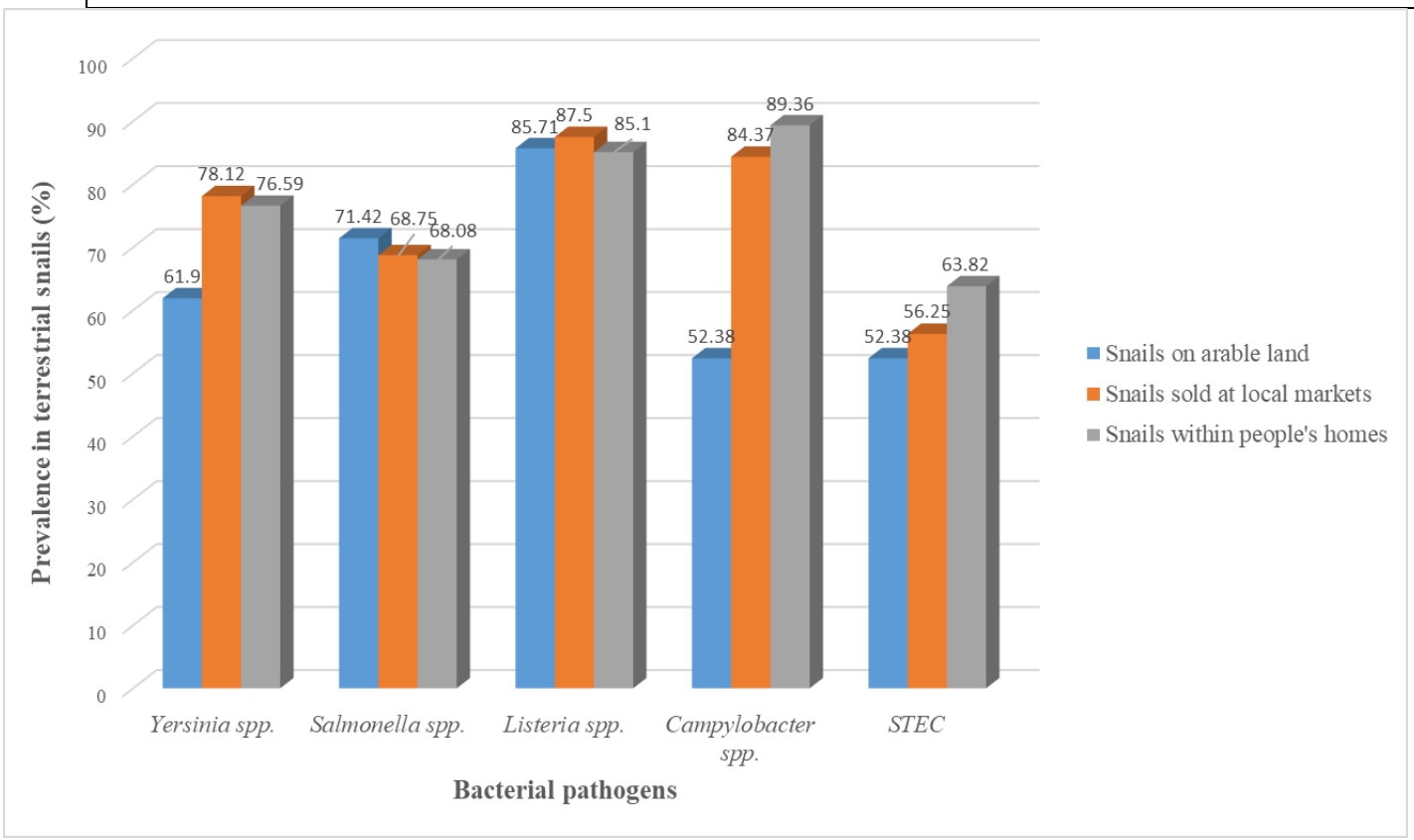


Figure 3.4. Overall prevalence of foodborne pathogens in each sampling location, Buea, Cameroon

From Figure 3.4, the pathogen prevalence among the samples examined contained STEC ranging from 52.38-63.82%, *Campylobacter* spp. ranging from 52.38-

89.36%. *Salmonella* spp. ranging between 68.08-71.42%, *Listeria* spp. ranging from 85.10-87.50%, and *Yersinia* spp. ranging from 61.90-78.12%.

Figure 3.5 presents the distribution of these microbial pathogens in different dwellings (within people's homes) in Buea. The faeces of African land snails collected within people's homes were highest in *Campylobacter* spp. (89.36%) and lowest in *STEC* (63.82%). In terms of distribution, Great Soppo recorded 100% in *STEC*, *Campylobacter* spp., and *Salmonella* spp. GRA and Lower Muea contained 100% in *Listeria* spp. and *Campylobacter* spp., respectively. Clerks' quarters recorded 85.70% in *Yersinia* spp.

Figure 3.6 presents the pathogen distribution in the different sampling markets in Buea. African land snails sampled in local markets were highest in *Listeria* spp. (87.50%) and lowest in STEC (56.25%). Snails in Great soppo market recorded 100% *Listeria* spp. and *Yersinia* spp. Muea also recorded 100% in *Yersinia* spp. Molyko and Mile 16/17 markets both contained 100% *Salmonella* spp. Snails sold at the Molyko market were highest in *Campylobacter* spp. (88.80%) and those sold in the Great soppo market were highest in STEC (83.30%).

Lastly, the distribution of the pathogens in different farmlands within the Buea suburbs are presented in Figure 3.7, *Listeria* spp. was highest (85.71%) and *Campylobacter* spp. and STEC were both lowest (52.38%). Edible snails sampled in Mile 18 were highest in *Listeria* spp. and *Salmonella* spp. at 100% pathogen prevalence. Snails in Mile 16 were the highest (83.36%) in *Yersinia* spp. and those collected in Bokwango were the highest in STEC (63.60%) and *Campylobacter* spp. (55.50%).

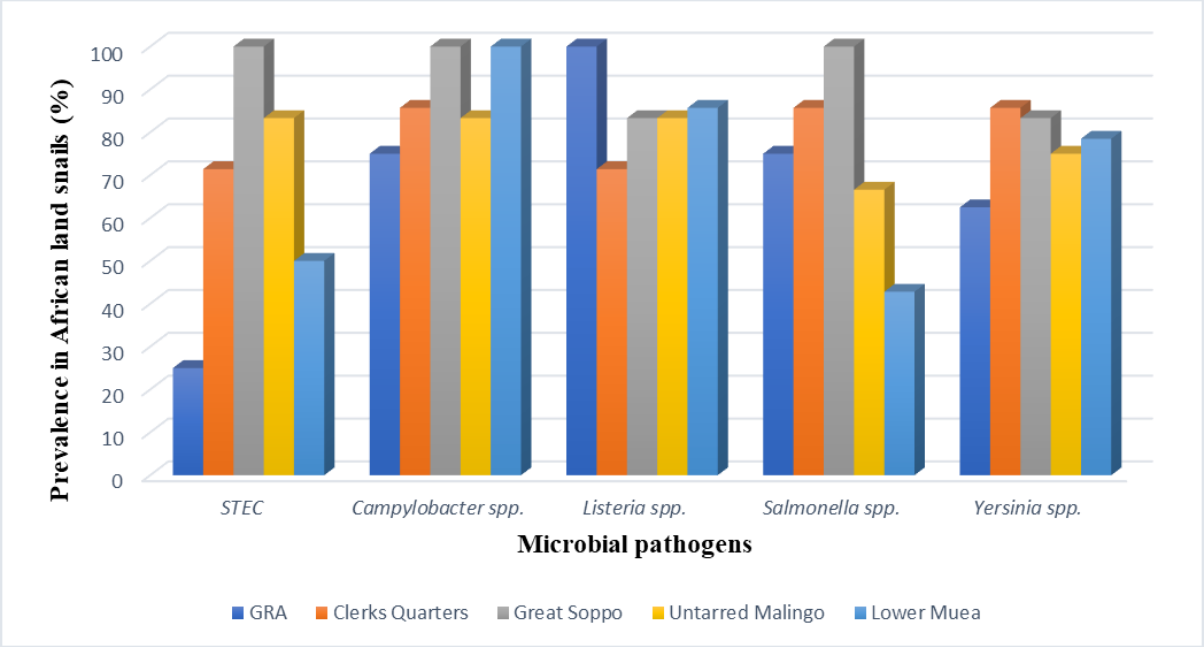


Figure 3.5. Prevalence of foodborne pathogens in snails sampled within people's homes

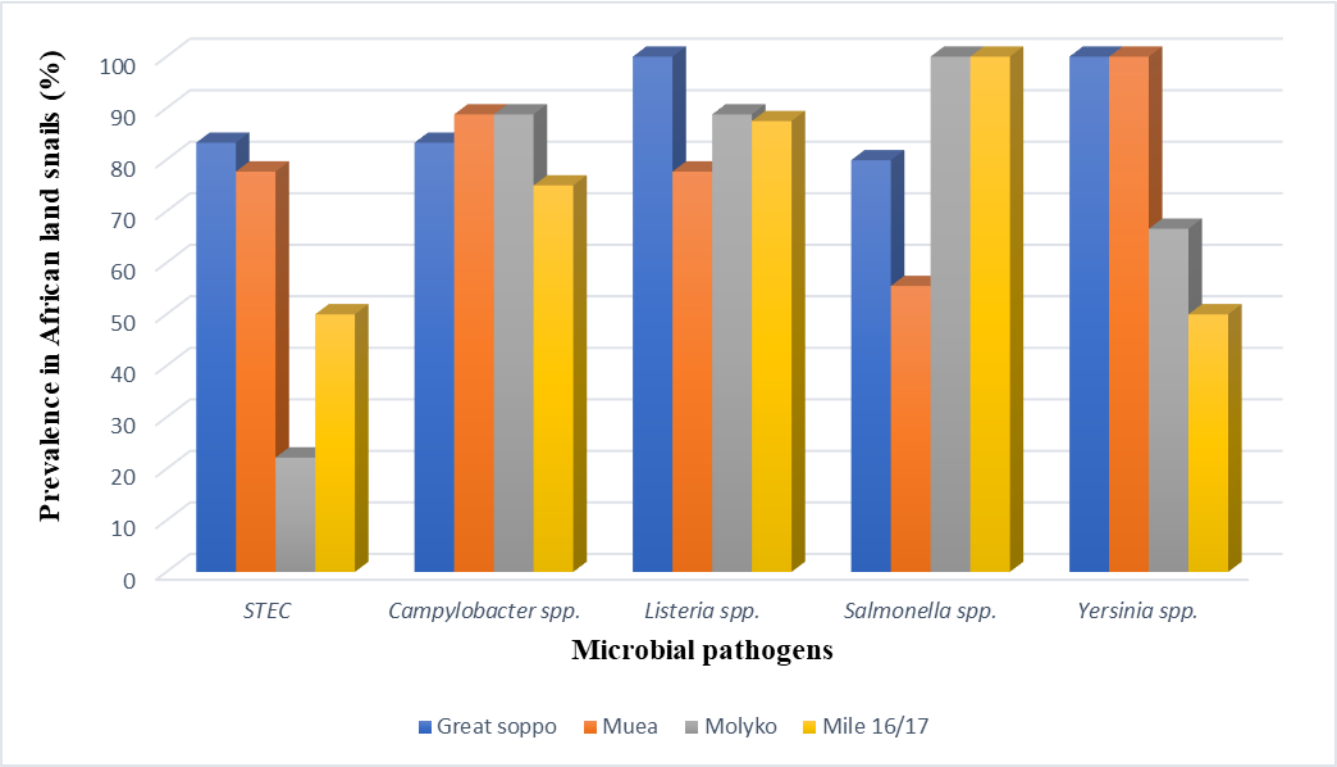


Figure 3.6. Prevalence of foodborne pathogens in snails sampled in local markets

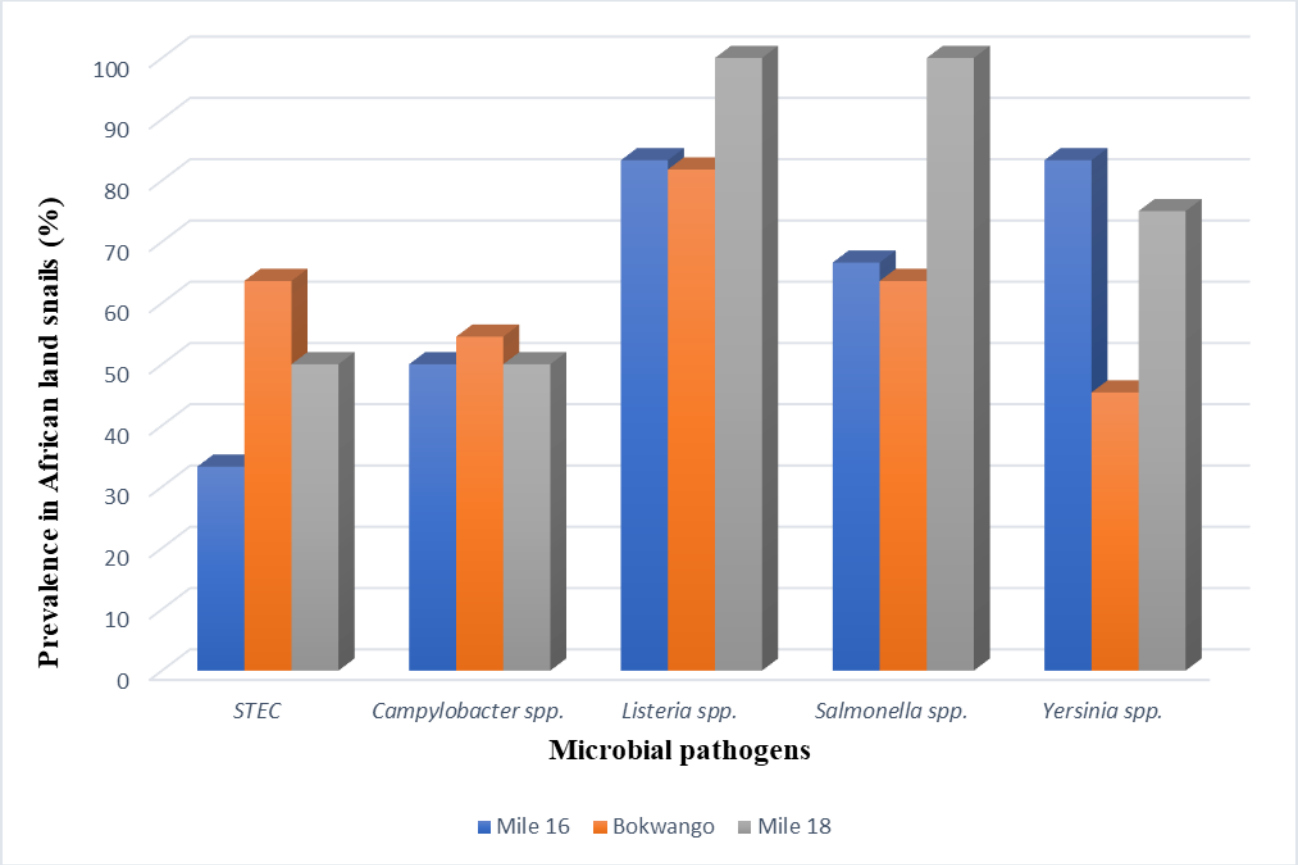


Figure 3.7. Prevalence of foodborne pathogens in snails sampled in arable land

3.5 Discussion

This study aimed to assess the prevalence of potential foodborne pathogens in African land snails consumed in Cameroon. Although details on the consumption of snail meat is not available in Cameroon, live snails are sold in almost every local market in the country (Meffowoet et al., 2020). Similar with other countries in sub-Saharan Africa, an increase in demand has prompted the random collection of edible snails from locations which could be termed 'unhygienic'(Akpomie, 2013; Mohammed et al., 2014; Nyoagbe et al., 2016; Tanyitiku et al., 2022a). This study provides tangible evidence, for the first time, on the public health risks in the handling and/or consumption of raw or undercooked edible snails collected from natural habitats.

All edible snails contained at least one of the microbial pathogens examined in this research. Similar pathogenic microorganisms have been isolated in edible snails consumed in Nigeria (Adagbada et al., 2011; Adegoke et al., 2010; Adeyeye et al., 2020; Akpomie, 2013; Okafor & Ogbo, 2019) and Ghana (Barimah, 2013; Nyoagbe et al., 2016). Adagbada et al. (2011) detected *Salmonella* spp. (6.6%), *E. coli* (17.7%) and *Yersinia* spp. (2.2%) in the visceral fluids of giant land snails sampled in markets across Cross river and Akwa Ibom states, Nigeria. Onifade & Aiyenuro (2018) isolated *E. coli* (8.7%) and *Salmonella* spp. (13.82%) in the intestines of *Achatina achatina* sold in Ondo state, Nigeria. Descriptors of STEC and *Campylobacter* spp. in edible African land snails are, to our knowledge, nonexistent in the literature.

The presence of higher levels of *Listeria* spp. in African land snails collected on arable land, as well as those purchased in local markets, is of particular relevance to pregnant women and their newborns, adults 65 years or older, and people with weakened immune systems (CDC, 2021). Higher prevalence of *Campylobacter* spp. (75.37%) and

Listeria spp. (86.10%) may reflect the common practice of free-range poultry farming in Buea and the direct contact of snails with the soil and decaying vegetation (Barimah, 2013; Nyoagbe et al., 2016). Edible snails could easily have ingested these pathogenic bacteria while feeding. Similar microbial pathogens have also been isolated in livestock in Cameroon. Nzouankeu et al. (2010) isolated 90% *Campylobacter* spp. (68.9% *C. coli* and 31.1% *C. jejuni*), 11.3% enteropathogenic *E. coli* (EPEC), *Salmonella Enteritidis* (45.6%) and *Salmonella Hadar* (28.1%) in chickens collected from eight retail markets in Yaounde. Ngogang et al. (2020) isolated *E. coli* (59.1%) and *Salmonella* spp. (15.5%) in chicken litters sampled from 26 farms in urban area settings. Akomoneh et al. (2020) reported that 56 (10.9%) *E. coli* O157 was detected in 512 samples of cattle slaughtered in Buea. The presence of the virulence genes stx2, eaeA, and hylA was demonstrated in 96.4% (54/56) of the isolates and stx1 in 40 (71.4%) of the 54 isolates (Akomoneh et al., 2020).

In addition, infections with microbial pathogens such as *STEC* can cause abdominal cramps, bloody diarrhoea, vomiting, and fever. In some cases, patients may develop haemolytic uraemic syndrome, characterized by haemolytic anaemia, thrombocytopenia (decrease in blood platelets) and kidney failure (CDC, 2021). Although foodborne diseases remain unrelated to food commodities in Cameroon, the above clinical cases have been documented. Tchientcheu et al. (2021) reported that non-pathogenic *E. coli* represented more than a third (33.89%) of stool samples from patients in several hospitals in Douala. Tchente et al. (2019) reported the presence of 48.6% *E. coli* in the urine of asymptomatic pregnant women attending antenatal clinics. Other important microorganisms isolated included *Klebsiella pneumoniae* (22.26%), *Enterobacter aerogenes* (11.96%), and *Salmonella spp.* (10.30%) and *Yersinia enterocolitica* (4.65%) (Tchientcheu et al., 2021).

Moreover, in developed countries such as the United States, the pathogens isolated in this study have been associated with several foodborne outbreaks. The CDC estimated that *Salmonella* causes about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States each year (CDC, 2021). *Campylobacter* causes an estimated 1.5 million illnesses and an estimated 1,600 people get listeriosis with about 260 deaths each year (CDC, 2021). Even though previous studies (Barimah, 2013; Tanyitiku et al., 2022a) highlighted that the local people believed their practices of snail washing with aluminum sulfate (alum) or salt/lime in addition to boiling and then stewing could kill all microorganisms. Akpomie et al. (2019) isolated significant bacteria in snail meat following boiling, frying, smoke and oven drying in Nigeria. Walczak and Czerwińska (2013) observed a significant growth of *Bacillus subtilis, Escherichia coli, Staphylococcus epidermidis,* and *Enterobacter gergoviae* when edible snails (*Helix pomatia*) were subjected to different temperature conditions: immersion in water at 100°C for 30 seconds and subsequent boiling for 5 minutes.

Thus, our results strongly suggests that foodborne outbreaks are occurring but unidentified in Cameroon, and likely other sub-Saharan African snail-consuming countries. It clearly indicates a pressing need for interventions to improve public health, for which best results may be obtained in conjunction with a deeper understanding of community attitudes and food practices (Kaldjob et al., 2019; Tanyitiku et al., 2022a).

3.6 Conclusion

The presence of potential foodborne pathogens in African land snails collected from locations which include within people's homes, arable land, and local markets, indicates snail meat consumption practices present a significant threat to public health in snail meat consuming countries, in the rainforest belt of Africa. The high prevalence of STEC, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. in edible snails strongly suggests that foodborne outbreaks are unidentified in Cameroon, and several other sub-Saharan African land snail-consuming countries. Consequently, outbreaks of foodborne diseases are likely occurring among the local population.

Chapter 4. Survival of *Escherichia coli* in edible land snails: implications for heliciculture and public health

Chapter 4 is based on the manuscript Tanyitiku et al. (in-review). This paper was presented at Lincoln University Postgraduate conference (Nov. 1-3, 2021)

4.1 Summary

Background: Land snails are a delicacy in many cuisines in Europe and sub-Saharan Africa. However, the interaction of microbial pathogens with land snails could lead to public health threats in snail handling and/or consumption. This study examines the survival of *Escherichia coli* in edible land snails in a model system.

Methods: Well-studied Shigatoxigenic (STEC) and non-STEC strains were compared. Mature *Helix* spp. were experimentally fed with *E. coli*-inoculated oats for 48 h. The snail faeces after inoculation were periodically sampled and cultured for a 30-day period and subjected to microbiological analyses.

Results: The average rate of decline of the non-*STEC* strain CSH-62 in the faeces of live snails was significantly ($p<0.05$) faster than that of *STEC* ERL 06-2503. In addition, the viable population of *E. coli* ERL 06-2503 significantly ($p<0.05$) persisted for a longer time in the intestine of land snails than *E. coli* CSH-62. The results showed that the viable population of *E. coli* demonstrated first-order kinetics and their survival (CFU/ml) appeared significantly ($p<0.05$) dependent on the *E. coli* pathotype.

Concluding remarks: The continuous enumeration of *E. coli* in snail faeces indicated that land snails could serve as a mode of transmission of microbial pathogens to susceptible hosts, including humans.

4.2 Introduction

Humans consumed snails as early as 10.5 thousand years ago (Paszkiewicz et al., 2014). Land snails were very prominent during the era of the Roman Empire, where it was a common aphrodisiac practice to eat snail meat in the courts of the emperor (Okafor, 2009; Paszkiewicz et al., 2014). Today, edible land snails are defined by regulation (EC) No. 853/2004, as terrestrial gastropods of the *Helix* and *Achatinidae* family (APHA, 2020). Although *Helix* spp. *(Helix pomatia Linné, Helix aspersa Muller, Helix lucorum)* remain a delicacy in many European cuisines, species of the *Achatinidae* family, specifically *Achatina achatina, Achatina fulica,* and *Archachatina marginata,* are popularly consumed in the tropical and savannah zones of Africa (Akpomie, 2013; APHA, 2020; Barimah, 2013; Cobbinah et al., 2008; Mohammed et al., 2014; Nyoagbe et al., 2016). Its global consumption is currently at 450-500,000 tons per year. Snail meat consumption is in increasing demand due to its ready availability, high-quality protein, low fat, high calcium, magnesium, and iron content (Kocatepe & Çelik, 2017; Paszkiewicz et al., 2014; Pissia et al., 2021).

Snail-consuming populations have often picked edible land snails from locations including swamps, ditches, forests, gardens, footpaths, farmlands, domestic waste, stones, and logs of wood (Mohammed et al., 2014; Ngenwi et al., 2010; Parlapani et al., 2014; Tanyitiku et al., 2022b; Waldhorn, 2020). Paszkiewicz et al. (2014) highlighted that only 55,000 tons (13.8 %) of the total snails consumed in the world each year are obtained from snail breeding farms. France, for example, which is famous for its cuisine of *Helix* spp. (escargots), consume 44,000 tons per year and only approximately 1% are obtained through snail farming (Kiebre-Toe et al., 2003). Moreover, significant disease-causing pathogenic microorganisms including pathogenic *Escherichia coli (STEC), Campylobacter*

spp. *Salmonella* spp. *Listeria* spp. *Yersinia* spp., among others, have been detected in our studies and others in edible land snails in both snail farms and free-living environments (Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nwuzo et al., 2016; Nyoagbe et al., 2016; Parlapani et al., 2014; Tanyitiku et al., 2022a; Tanyitiku et al., 2022b; Temelli̇ et al., 2006). Notably, 57% of snails sampled in Cameroon were found to harbour Shiga toxin-producing *E. coli* (STEC) (Tanyitiku et al., 2022b).

Among Gram-negative bacteria, Shigatoxigenic *E. coli* is a leading cause of foodborne illnesses. At least 130 of the 151 serogroups of STEC have been reported to be associated with human illnesses around the world (Capps et al., 2021). They are responsible for mild enteritis to hemorrhagic colitis, which in some cases leads to complications, including hemolytic uremic syndrome (HUS) and even death (Capps et al., 2021). As a mode of transmission of *E. coli* to humans, some studies have reported the survival of *E. coli* in the faeces of cattle (Avery et al., 2004; Capps et al., 2021; Kudva et al., 1998), sheep (Avery et al., 2004), pigs (Avery et al., 2004), Canada goose (Moriarty et al., 2012), rats, and pigeons (Cízek et al., 2000). To our knowledge, there are no existing data on the survival characteristics of microbial pathogens in edible land snails.

Considering foodborne disease risks that could arise in snail handling and/or consumption, the purpose of this current study was to provide evidence on the survival of *E. coli* in the faeces of edible land snails. We also highlight the public health risks of *E. coli* in the faeces of edible land snails and its implications for helicicultural practices.

4.3 Materials and methods

4.3.1 E. coli isolates and inoculum pre-preparation

Two *E. coli* strains were used in this study. *E. coli* ERL 06-2503 is a Shiga-toxigenic variant that was found to bind strongly to the bovine intestines in *ex-vivo* experiments (Brandt & Paulin, 2012). It was obtained from a stock culture collection of the Enteric Reference laboratory, a department of the Institute of Environmental Science and Research (ESR, NZ). Another strain, *E. coli* CSH-62 is a non-invasive, non-Shigatoxigenic laboratory-adapted strain, which has often been used in transformation experiments (Nakane et al., 2016; Thorne & Farrar, 1973).

Before the inoculation of experimental snails, described in Section 4.3.3, each strain of *E. coli* was recovered from a 40% glycerol stock solution that had been stored at -80^{0}C. A loopful of each strain of *E. coli* was individually streaked on Tryptone Bile Glucuronic, TBX agar (Oxoid, England) plates and cultured at 37^{0}C for 20h. Serial dilutions of 10^{-1} to 10^{-6} of a single colony in cultured TBX plates were again subcultured in TBX. The presence of *stx1* and *stx2* genes for pathogenic *E. coli* ERL 06-2503 in the cultured plates were verified using a high-fidelity DNA polymerase (repliQa Hifi toughmix: Quantabio, MA, USA), as described in previous PCR protocols (Tanyitiku et al., 2022b). Each single colony on the 10^{-6} TBX plates was aseptically transferred into 1ml of distilled water and briefly vortexed to obtain the bacterial suspension for inoculation experiments.

4.3.2 Preparation of experimental snails

The snails were prepared for inoculation experiments based on established methods for laboratory snail farming (Garcia et al., 2006) and outdoor snail farming or heliciculture (Cobbinah et al., 2008; Murphy, 2001; Thompson & Cheney, 1996), shown

in Figures 4.1 to 4.4. In brief, 50 mature land snails (*Helix aspersa*) of reproductive age were collected from vegetable farms in Christchurch, New Zealand, during the summer months of November to January 2019. Snail species were identified by their morphological characteristics; for example, the shells were brown with 4 to 5 whorls and measured 30 to 45mm (Paszkiewicz et al., 2014; Thompson & Cheney, 1996).

Snails were transported to Lincoln University, New Zealand, maintained in optimal growth conditions (20±2^{0}C, 14h light, 10h darkness, 80% humidity), and fed with vegetables (cabbage, lettuce) and eggshells (a calcium carbonate supplement) until egg laying commenced (Figure 4.3) (Garcia et al., 2006; Murphy, 2001). Aseptically, egg batches were embedded into 5mm thick pre-autoclaved organic soil (Garden box, Christchurch, NZ), kept moist with sterile distilled water, and contained in 2-liter perforated Sistema containers (Sistema Plastics, NZ) pre-treated with 70% w/v ethanol and then exposure to ultraviolet light (intensity: 2.1mJ/cm^2) for ≥2h. Within 18-21days, juvenile snails emerged from the organic soil and the snails were raised till maturity, that is, 18 months (Figure 4.4).

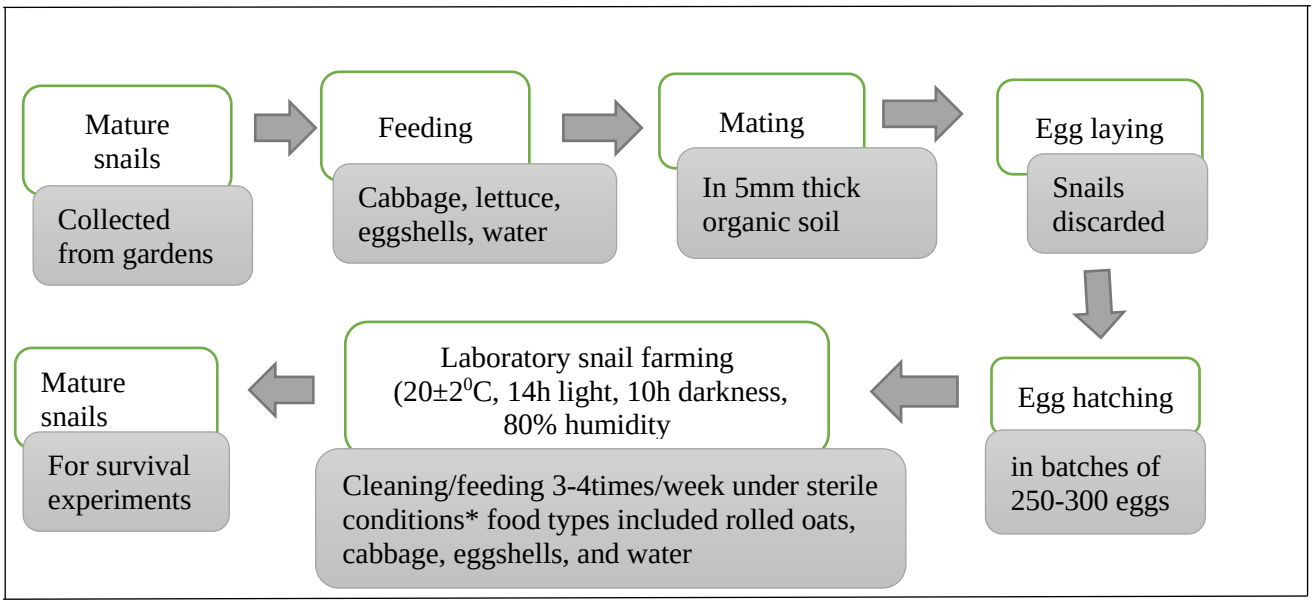


Figure 4.1. Preparation of snails for bacterial inoculation

Note. *Regular sterilization (121^{0}C/2 h) of all food types, materials and equipment used.

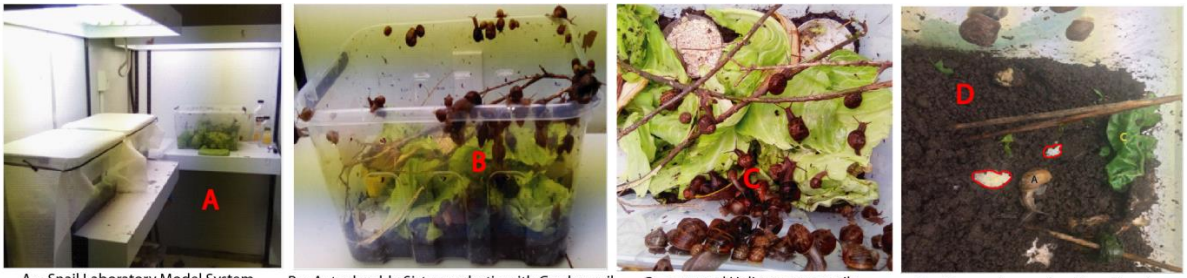


Figure 4.2. Snail farming prior to egg laying

Figure 4.3 Egg laying of *Helix* spp. for the laboratory model system

Juvenile snails at 10 weeks Snail farming in 2-litre perforated Sistema containers

Figure 4.4. Farming of experimental snails

4.3.3 Inoculation of experimental snails

All inoculation experiments were conducted in a biological safety hood. Bacteria suspensions (1ml), prepared in section 4.3.1, were transferred into 15ml Eppendorf tubes containing 1g of moist rolled oats each. The rolled oats were rehydrated according to the manufacturer's instructions (Harraways, NZ). The inoculant concentration in the rolled oats was such that when initially examined on TBX agar at 37^0C for 20 h, the bacterial colonies were approximately 10^6 CFU/mL. A total of 40 tubes were prepared, thoroughly mixed, and poured into a sterile 2-liter perforated Sistema container (Sistema Plastics, NZ). 40 mature land snails, previously purged in rolled oats for 36 h and then starved for 48 h (Garcia et al., 2006), were then introduced into the mixture of bacteria-rolled oats for 48 h (Figure 4.5). In principle, this implied that 1g of inoculated oats corresponded to 1 land snail. Oats used throughout the study had been previously screened for the presence of *E. coli* (using methods described by Silva et al. 2018), modified by the use of 5g samples in the protocol and tested negative for this organism.

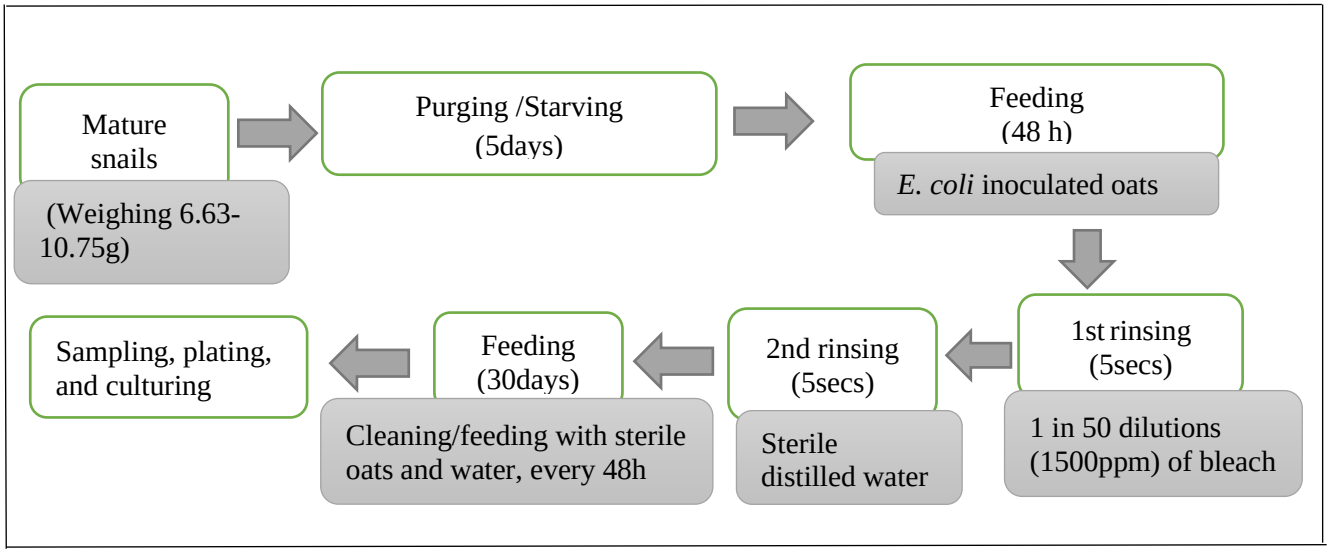


Figure 4.5. Inoculation of snails for *E. coli* survival experiments

After 48 h of exposure and feeding on inoculated oats, the snails were rinsed for ca. 5s with 15 ml of 5% sodium hypochlorite (Janola premium bleach, Pental Products Pty Ltd, AUS), and then 15 ml of sterile distilled water, to achieve surface decontamination. Preliminary experiments with snails intentionally contaminated with *E. coli* demonstrated that this protocol was effective in snail surface decontamination when rinse water was cultured on Nutrient agar no. 2 (Oxoid, UK) at 37^0C for 18 h. Snails remained viable after this treatment. Other preliminary trials laboratory grade disinfectants are detailed in Appendix D.1.

4.3.4 E. coli survival experiments

After the brief rinsing with 5% sodium hypochlorite and distilled water, the 40 snails from each *E. coli* strain inoculation, were used as the starting samples for the 30-day survival experiments (Figure 4.5). Snails were then fed with 40 g of sterile moist rolled oats and periodically every 48 h, their faeces were collected at the point of cleaning and feeding with fresh rolled oats. The enumeration of *E. coli* in snail faeces was conducted based on Silva et al. (2018), with slight modifications. 5 g of snail faeces were thoroughly

vortexed in 45ml of Phosphate buffered saline, PBS (10^{-1} dilution) and the suspension was allowed to stand for 30mins. Subsequent dilutions (10^{-2} to 10^{-6}) of the sample were prepared and 1ml each of three appropriate dilutions (10^{-1}, 10^{-2}, 10^{-4}) were inoculated into Durham tubes containing 2ml *E. coli* (EC) broth. The samples were then incubated at 44.5±2^{0}C/24h in a shaker incubator (ThermoScientific, NZ), rotating at 120rpm.

Using a spread plate method, 50µl of serial dilutions (10^{-1} to 10^{-6}) of EC broth samples were plated on TBX agar. The plates were incubated at 37^{0}C for 20 h, and 30-250 bacteria colonies were visually counted using a 6X LED illuminated linen tester (Magnifiers, NZ). As shown in Figure A.5, the surviving colonies of *E. coli* were confirmed as blue-green colonies in TBX agar plates (Vergine et al., 2016). Further *E. coli* confirmation tests for inconclusive colonies were performed using previously described PCR protocols (Tanyitiku et al., 2022b). The same procedure was used to screen for *E. coli* in rolled oats prior to feeding inoculated snails during the entire 30-day survival experiments.

It should be noted that 30 days were found adequate for survival experiments for significant sample collection for microbiological analyses, given that from repeated trials beyond 30 days, snails barely survived under the aforementioned post inoculated laboratory feeding and growth conditions.

4.3.5 Statistical analysis

All experiments were carried out in triplicate. Values of CFU/g for each timepoint were expressed as mean ± standard deviation of the three separate survival experiments, with mean values used as the basis for statistical analysis. A commercial spreadsheet software (Microsoft 2010) was used to perform all calculations. R programming (version 4.0.3) was used in the plotting of the best fit graphs and in performing statistical analyses,

that is, Analysis of Variance (ANOVA), student t-tests. The mean number of CFU/g from each sampling time was calculated and represented as logarithmic transformed data; that is, each calculated CFU/g was replaced with the transformed value $\log_{10}(x)$, where x=CFU/g. The normal distribution of each logarithmic transformed data set was graphically verified (with a confidence level of 95%). Linear regression analysis was used to determine the best relationship between the two *E. coli* strains. The D-values (the time required to reduce the *E. coli* population by 1 log cycle) were also determined by taking the negative reciprocal of the slope from the linear regression equations (Edelson-Mammel & Buchanan, 2004).

4.4 Results

The number of CFU/g for each strain decreased over the examination period (Table A.7). The average rate of decline of the laboratory-adapted *E. coli* strain CSH-62 in the faeces of live snails was significantly ($p<0.05$) faster than the pathogenic (Shiga-toxigenic) *E. coli* ERL 06-2503. Furthermore, the viable population of the Shiga-toxigenic *E. coli* strain ERL 06-2503 persisted significantly ($p<0.05$) for a longer time in the intestinal tract of land snails than that of the laboratory-adapted *E. coli* CSH-62. These trends are clearly seen in the graph using the "best fit" algorithm in the "R" software environment (Figure 4.6).

Strain ERL 06-2503 showed a relatively rapid decrease to 6.78 $\log_{10}$CFUmL^{-1} within the first two days but was constant for the next 14 days before a marked decrease at day 28: in contrast, *E. coli* strain CSH-62 decreased steadily throughout the experiment (Figure 4.6). For both strains, viable bacteria remained detectable up to the end of the sampling period, that is, approximately 165,000 CFU/g for *E. coli* CSH-62 and 403,000 CFU/g for *E. coli* ERL 06-2503. The mean decline in the viable population demonstrated first-order kinetics. With no clear evidence of shoulders or tails, this permitted the calculation of the decimal reduction times (D-values) from the linear parts of the survival curves (Avery et al., 2004; Edelson-Mammel & Buchanan, 2004), as shown in Figures A.6 & A.7.

E. coli CSH-62 displayed three timepoints at which a 1-log decrease in its viable population was evident (Table 4.1). In the first two days, a 0.95$\log_{10}$CFUg^{-1} decrease in viable counts was observed. The calculated D value (time for a 1 log decrease in *E. coli*) for this initial time period (0 to 2 days) was 1.59 days. An additional decline of 1.28$\log_{10}$CFUg^{-1} in viable bacteria occurred during the next 18 days. The calculated D-

value for this second phase (day 2 to 18) was 14.64 days. Finally, the third decrease in the viable count of 1.07$log_{10}CFUg^{-1}$ for the remaining 30 days of the study occurred between day 18 and day 26. Its D-value was 25.39 days.

However, corresponding analysis of the results for STEC strain ERL 06-2503 revealed only two timepoints at which a 1-log decrease was seen (Table 4.1). An initial decrease in viable counts of 1.11$log_{10}CFUg^{-1}$ was observed on day 2 after inoculation. The calculated D-value for day 0 to 2 was 1.38 days. The second decrease of 1.03$log_{10}CFUg^{-1}$ in viable count was observed on day 22. The calculated D-value (day 2 to 22) was 18.08 days. From day 22 to 30, we obtained a D-value of 28.96 days, of which a further 1.03$log_{10}CFUg^{-1}$ of viable bacteria was observed on day 30 (the last day of sampling).

Table 4.1. Calculated D-values of *E. coli* strains examined within the 30-day period at timepoints showing a ≥ 1-log decrease in colony forming units.

***E. coli* isolates**	**Day of ~1log cycle**	**$log_{10}CFUg^{-1}$**	**D-value (days)**
E. coli CSH-62	2	6.952	1.59
	18	5.675	14.62
	26	4.604	25.36
E. coli ERL 06-2503	2	6.795	1.38
	22	5.765	18.08

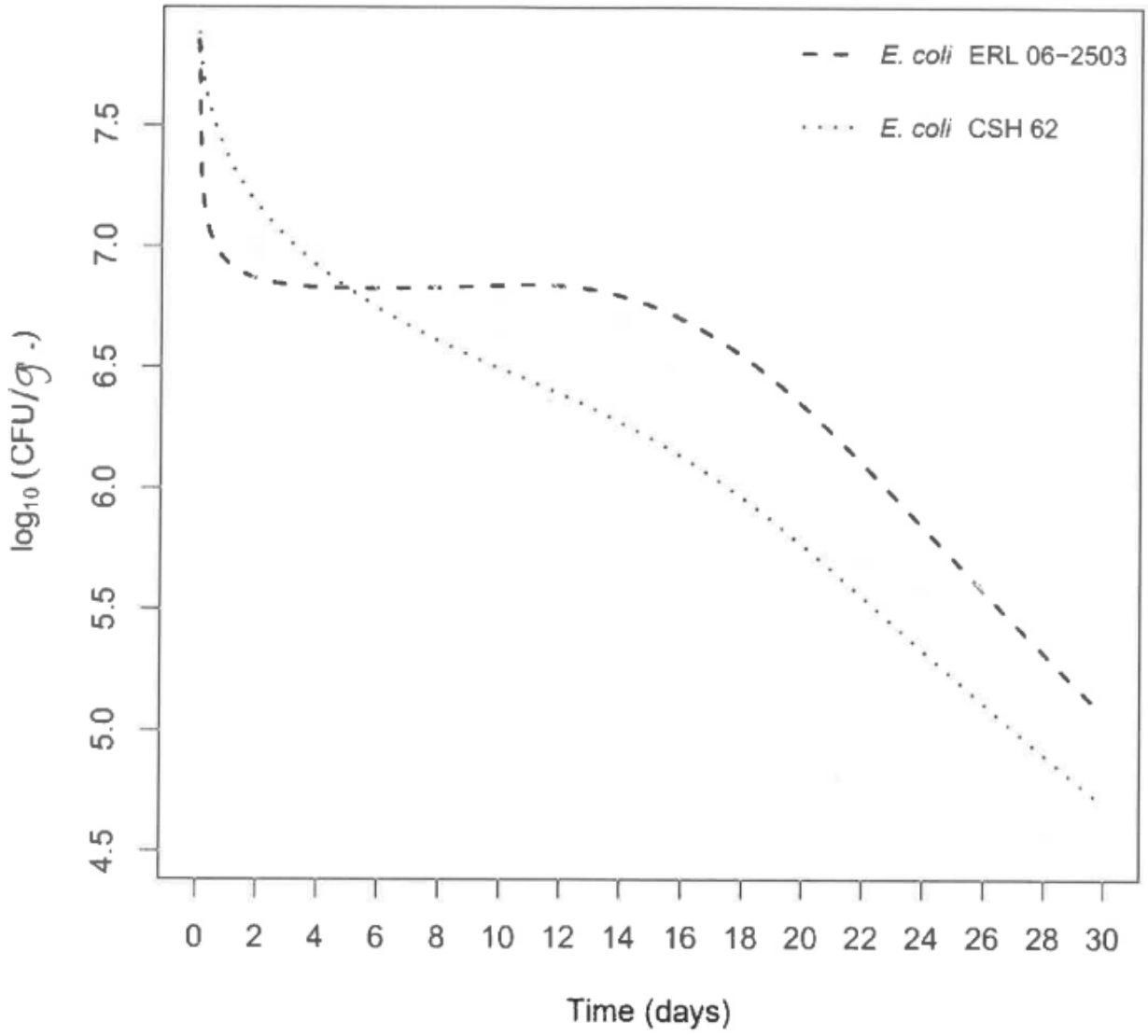


Figure 4.6. Survival of *E. coli* in the faeces of live snails

4.5 Discussion

Snail farmers all over the world are experimenting with different types of snail production systems; the challenges in adapting well-defined snail farming systems are due to the ability of snails to adversely respond to changes in climatic conditions (Cobbinah et al., 2008; Murphy, 2001), adverse situations that were also experienced in this current study. Contrary to outdoor settings where snails are left to breed, reproduce, and grow virtually unattended until harvesting, indoor snail farming require the farmer to control conditions such as feeding, cleaning, protection from the sun, wind, and hail (Cobbinah et al., 2008; Garcia et al., 2006; Murphy, 2001). In both farming systems, it is well known that land snails, such as African giant snails, are hosts to many parasites (Meffowoet et al., 2020). Their ability to host a diverse population of bacterial communities (including *Lactococcus, Kurthia, Citrobacter, Actinobacteria, Enterococcus)*, have gained particular attention in recent years (Okafor, 2009; Oyeleke et al., 2012; Pawar et al., 2012). In Chapter 2 of this thesis (Tanyitiku et al., 2022b), at least one enteric pathogen was detected in every snail sample examined, with STEC found in 57% of the samples examined. In this research, we studied *E. coli* interactions with land snails, *Helix* spp. We have provided evidence that this species can survive in edible land snails for at least a month, and that pathogenic variants such as STEC may survive for longer.

Land snails are known to occupy various environments and are susceptible to *E. coli* contamination due to 1) their free-living nature, 2) their close contact with the soil and 3) their random feeding patterns (Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Tanyitiku et al., 2022b). Previous research has also detected different strains of pathogenic *E. coli* in the faeces of healthy animals that were raised in indoor and grassland systems (Avery et al., 2004; Kudva et al., 1998). For example, to establish the transmission

pattern of *E. coli* O157: H7 between cattle groups, Scott et al. (2006) reported that a total of 8.7% (6/69) of cattle shed *E. coli* O157: H7 in the first month, with faecal shedding increasing to 52% (36/69) in the third month after infection. Kudva et al. (1998) recovered *E. coli* O157: H7 in the range of $< 10^2$ to $< 10^6$ CFU/g in the manure of inoculated sheep, further demonstrating that *E. coli* could survive for 21 months in bovine faeces, despite fluctuating environmental conditions. Furthermore, Avery et al. (2004) demonstrated that *E. coli* from the faeces of indoor cattle, sheep and pigs can survive on grass for at least 5-6 months. Under such conditions, host animals are continually re-exposed to strains from the environment and possess body temperatures favourable to *E. coli* multiplication. Snails are poikilothermic and, in our experiments, exposed only once to the inoculant. Despite these conditions, both pathogenic and laboratory-adapted strains of *E. coli* were detected for 30 days. This suggests that the faeces of infected snails in the environment could act as a significant reservoir for the transmission of *E. coli* to humans and/or animals. This present study has provided the extent to which snail handlers and subsequently consumers, are exposed to foodborne pathogens, most especially in helicicultural practices.

Similarly, the presence of *E. coli* in the faeces of snails further revealed that snails as intermediate hosts and/or the consumption of raw or undercooked snail meat may not necessarily be the only route of foodborne illnesses associated with land snails. For example, snails are known to leave their slime and faeces on locally produced green leafy vegetables in gardens, which is an example of this research initial snail harvesting location. Humans could directly ingest these bacterial pathogens from the faeces of snails present in their daily gardening. For example, in 2021, *E. coli* O157: H7 foodborne outbreaks were reported in baby spinach, leafy greens, and several unknown food sources

in the United States (CDC, 2021). Mukherjee et al. (2006) reported a sporadic case of *E. coli* O157 infection of a child who acquired *E. coli* O157: H7 from garden soil that had been previously fertilized with manure from cattle in Minnesota, USA. Through PCR and pulsed-field gel electrophoresis techniques, it was demonstrated that *E. coli* O157:H7 strains could survive on manure-amended soil for more than two months (Mukherjee et al., 2006).

Using the non-invasive laboratory-adapted *E. coli* strain CSH-62 and Shiga-toxigenic pathogenic *E. coli* strain ERL 06-2503 in this research have demonstrated that the outcome of *E. coli* interactions with land snails may be significantly ($p<0.05$) strain dependent. The STEC strain used was specifically chosen for its ability to robustly colonise an *ex vivo* bovine intestinal model; more robustly, in fact, than other strains studied (Brandt & Paulin, 2012). Strain variance was strongly evidenced in our study. Strain CSH-62 demonstrated an approximate $3\log_{10}CFUg^{-1}$ decline in its viable population, while the pathogenic *E. coli* strain ERL 06-2503, demonstrated $2\log_{10}CFUg^{-1}$ reduction in its viable population. Similar results have been reported by Alsam et al. (2006), where invasive *E. coli* K1 had the ability to penetrate and remain viable in the free-living protozoan, *Acanthamoeba*, while non-invasive *E. coli* K-12 was progressively killed. Although it is unclear how both *E. coli* strains maintained a longer survival in land snails, their continued persistence in edible land snails represents significant risks to public health. Previous recommendations by Garcia et al. (2006) and Cerezal Mezquita et al. (2009) indicate that the purging/starvation of land snails for 3 to 5 days prior to human consumption, will accelerate the process of ridding the snail digestive system of soil, food stuffs and microorganisms. However, this is contrary to our findings, as both pathogenic and laboratory-adapted *E. coli* strains were actively viable beyond day 5 after inoculation,

and during the entire period whereby the sole nutrition source were soaked oats, a commonly used purging agent. Our results provide evidence that land snails may host both pathogenic and non-pathogenic *E. coli* for prolonged periods (30 days), which may be transmitted to susceptible hosts, including humans. More research is required to understand the fate of *E. coli* after this experimental research period; the survival mechanisms of the different *E. coli* serotypes in the guts of edible land snails; and the spectrum of survival behaviours from additional strains and pathovars of importance to human health.

4.6 Conclusion

This study revealed that upon ingestion and continuous shedding in the faeces of snails, *E. coli* could survive in edible land snails for more than a month. Although the results indicate that the viable populations of *E. coli* declined significantly within the 30-day experimental period, the ability of *E. coli* to survive throughout the study under controlled laboratory conditions precluding reinfection is of great significance for helicicultural practices and public health, especially in regions where the carriage of pathogenic Shigatoxigenic strains is so prevalent (Tanyitiku et al., 2022b), and where snails are frequently consumed as a source of nutrition.

Chapter 5. General discussion and conclusion

5.1 Introduction

The purpose of this thesis was to understand foodborne disease risks in snail handling and/or consumption. From my lived experience, and desk research, this research established that the food safety risks of snail practices were largely unquantified, and the potential health risks that snail consumption practices could contribute to the burden of foodborne diseases in Africa had been significantly overlooked. To fully understand the public health risks in the handling and consumption of land snails and how these risks could be mitigated, set out to 1) identify snail handling and consumption practices, 2) understand how people (especially in Africa) are exposed to these microbial pathogens in their snail handling and consumption routines, 3) assess the prevalence of selected pathogens in locally sourced edible land snails, and 4) study the interaction of microbial pathogens with land snails in a laboratory model system.

To achieve these research objectives, our research approach 1) explored human practices that could lead to foodborne pathogen exposures, (Chapter 2), 2), examined the presence of Shiga-toxin producing *E. coli, Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. in the faeces of edible land snails, in Chapter 3, and 3) evaluated the survival characteristics of non-STEC and STEC strains in snail faeces within 30 days in a laboratory model system (Chapter 4). The results obtained in this research with respect to its practical implications to snail consuming populations and snail-importing countries could then inform opportunities for interventions in snail handling and consumption practices.

5.2 Foodborne pathogens in edible land snails: practical implications for snail handling and consuming communities

As mentioned earlier in this research, land snails are abundantly available in jungles (natural habitats) especially during the rainy seasons (Ngenwi et al., 2010; Ndah et al., 2017; Tanyitiku et al. 2022a). Specifically in sub-Saharan Africa, a constant increase in snail consumption in local and international markets, has led to its collection from 'anywhere' including, household waste, homes, roadsides, and farms (Tanyitiku et al. 2022a). In Ghana, several authors including Barimah (2013), Mohammed et al. (2014), and Nyoagbe et al. (2016), highlighted that snails were also collected along footpaths and bushes, and sold to consumers under unhygienic conditions. Such snail picking locations, including pit latrines, were revealed in my own fieldwork in Buea, Cameroon. Details of snail consumption practices in Buea, Cameroon, are presented in Chapter 2 of this thesis. The handling and/or consumption of contaminated land snails/snail meat could lead to various food-related infections in humans (Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Akpomie et al., 2019; Okafor & Ogbo, 2019; Tanyitiku et al., 2022b). To eliminate foodborne disease risks in snail consumption practices, edible land snails, like other food products, should not contain pathogenic microorganisms or be handled and prepared in a way that avoids human exposure.

To understand these foodborne disease risks, we employed a methodological approach of 1) observations and seeing through the eyes of people (key informants) who are involved in everyday snail local practices (Chapter 2) and then, 2) quantify foodborne pathogens in land snails (Chapters 3 & 4). Through participants stories, ideas, narrations, beliefs, and opinions, considered as unstructured information, we were able to identify potential points at which snail-consuming populations, especially in Cameroon, are

routinely exposed to foodborne pathogens. For example, in Chapter 2, we highlighted the various sequential human practices and possible foodborne pathogenic exposures in snail picking, the transportation and sale of unpackaged live snails at the local markets, improper snail meat washing and preparation, and hawking in loosely closed buckets along the streets, road junctions and motor parks in Buea (Tanyitiku et al., 2022a; Tanyitiku et al., 2022c). This approach has revealed fruitful insights on 1) how snail handlers and consumers can be exposed to foodborne pathogens in domestic and local business settings and, 2) what needs to be done to mitigate these foodborne disease health risks.

Specifically, the findings in Chapter 2 showed that the practice of selling land snails/snail meat at rural/local vending points, such as, local markets, road junctions, roadsides, and streets, can serve as a mode of transmission of foodborne pathogens among snail-consuming populations. In developed countries, there are strict regulations governing the sales and supply of safe and saleable food, and food products are often packaged and sold in supermarkets. Conversely, in developing countries, local markets and the street food industry are greatly involved in the sale and consumption of foods. Local food markets incorporate live or slaughtered animals, fresh and raw food produce, the street food industry trades large proportions of usually unpackaged ready-to-eat foods, these settings are characterized by informal overcrowded environments with little or no infrastructure (Mensah et al., 2002), and vending activities are affected by weather conditions, that is, wet and muddy in the rainy seasons or dusty in the dry seasons. Such markets provide affordable food to many low-income and poor families and are an important source of livelihood to millions of urban and rural dwellers. Low-income groups

depend on these foods due to their relatively low prices and convenience, irrespective of food quality, safety, or personal and environmental hygiene (Mensah et al., 2012).

This research incorporated these above-mentioned food consumption and vending contexts to highlight 1) microbial pathogens associated with land snails, particularly the African giant land snails, and 2) how people could get sick in their snail handling and/or eating routines. Many factors could contribute to human exposure to foodborne pathogens during snail handling and consumption. Firstly, homes could serve as a key contributor to foodborne disease outbreaks due to snail picking locations (as elaborated in Chapters 2 & 3), cross-contamination through snail faeces into raw or prepared food (readily achieved due to the high pathogen prevalence, cf. Chapter 3, and as detailed with experimental snails in Chapter 4), lack of food safety awareness, and poor food safety and personal hygiene (Chapter 2). Some households stored live snails in tree trunks and decaying vegetation, used physically uncleaned cooking utensils, prepared food with unwashed hands, and stored both raw and cooked food together (Field notes, July 11, 2019). These rural, ancestral and parental practices, combined with 'poverty', could further increase cross-contamination and disease re-infections, thus, leading to continuous transmission of foodborne pathogens.

Furthermore, the greatest challenge in handling and consuming food in traditional settings is inadequate knowledge of food safety practices (Mensah et al., 2012; Mensah et al., 2002; Assob et al., 2012; Mwamakamba et al., 2012). For example, in Chapter 2 of this thesis, we detailed that some participants were unaware if their own snail practices (that is, picking, selling, cracking, washing, cooking, hawking, and eating) could make them sick. An epidemiologist, who served as one of the participants in this research, related the people's low level of education to these limitations in food safety awareness

(Field notes, September 20, 2019). In accordance with these research findings, Mensah et al. (2002) also highlights the inappropriate washing of hands, utensils and dishes with water contained in bowls or buckets since running water and washing facilities are limited in Ghana. Further in Chapter 2, live snails were sold unwashed and physically dirty in local markets, motor parks and road junctions. Unpackaged ready-to-eat snail meat and food in general was sold in 1) open or loosely closed buckets, 2) with limited protection from flies and rodents, and 3) with refrigeration usually unavailable (Tanyitiku et al., 2022a). Microbial contamination could easily arise from the raw materials used, the place of preparation, the utensils used in cooking and serving, and the personal hygiene of vendors (Mensah et al., 2002; Assob et al., 2012; Mensah et al., 2012). In addition, our findings further suggests that in this research study area, Buea, the serving of contaminated snail meat by infected food handlers could lead to a continuous transmission of foodborne pathogens. These vending settings could make people vulnerable to foodborne illnesses (Assob et al., 2012) and thus increase the prevalence of foodborne diseases. Earlier in this research (Chapter 1, Section 1.6), we highlighted cases of foodborne disease outbreaks where kitchen staffs and food handlers have served as sources of disease transmission in more developed and food safety-conscious settings. In an era of the COVID-19 pandemic where additional measures for crowd control, physical distancing, hand washing, sanitization and education on respiratory hygiene (for example, the use of face masks) are essential, local market settings require immediate personnel and food safety regulations (WHO, 2021). Unfortunately, efficient food safety regulations hardly exist in these typical African markets and food consumption settings.

In Chapter 3, we demonstrated that land snails sampled from various natural habitats contained 57-86% of STEC, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and

Yersinia spp. (Tanyitiku et al., 2022b). These results indicated that snail harvesting or commonly termed 'picking' in natural habitats is an unsafe practice that could lead to outbreaks of foodborne diseases. Previous studies (Adagbada et al., 2011; Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Okafor-Elenwo & Imade, 2019; Okafor & Ogbo, 2019) had highlighted the close association of edible snails with pathogenic microorganisms. In general, most of these authors (Kiebre-Toe et al., 2003; Adagbada et al., 2011; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016) had enumerated mainly microbial pathogens of the *Enterobacteriaceae* family, including, *Bacillus cereus, Staphylococcus aureus, E. coli* (coliforms), *Salmonella, Klebsiella pneumonae,* and *Pseudomonas*. They (Adagbada et al., 2011; Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Okafor & Ogbo, 2019) had questioned the food safety of snails sampled in snail farms as well as those displayed for sale in local markets.

What we had learned from previous publications was that edible land snails and microbial pathogens could dwell in the same environment within an ecosystem. For example, microorganisms can exhibit commensalic or parasitic relationships with land snails (Pawar et al. 2012; Pawer et al. 2015; Dar et al., 2017; Meffowoet et al., 2020), and pathogenic microorganisms are usually found in the edible portions (foot or snail meat), environmental air, soil, and litters in snail breeding farms (Adagbada et al., 2011; Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Okafor & Ogbo, 2019). However, these studies have largely ignored 1) the most frequent foodborne pathogens in foodborne disease outbreaks, for example, STEC and *Campylobacter* spp. (Bintsis, 2017; CDC, 2021); 2) how humans are exposed to these microbial pathogens, and 3) if land snails could actually ingest these microbial pathogens and serve as a mode of transmission to humans, which were all relevant to address risks to public health in this

research. Through our approach of qualitative enquiry, snail sampling and highly specific molecular and plate counting techniques, fruitful opportunities to identify outbreaks of foodborne diseases and thus, to improve health outcomes precisely in rural communities in Africa were identified especially where ancestral and community eating habits are practiced.

Finally, even though reports on foodborne diseases from several developed countries show that outbreaks could be properly managed, people in developing countries are routinely exposed to unsafe food through 1) poor access to clean water to inadequately washed food items, 2) unsafe transportation and/or inadequate storage of foods, 3) inadequate knowledge of safe food processing and handling practices, and 4) compromised immune responses to foodborne infections (WHO, 2015). In addition, less developed countries are limited in their capacity to enforce effective food safety measures, including efficient foodborne disease surveillance and monitoring systems, food safety regulations and functioning inspection systems, food safety education programs, and community attitudes (WHO, 2015). The high prevalence of diarrhoeal diseases in many developing countries points to an urgent need to prioritize foodborne disease prevention and control in development and national health plans. Therefore, it is important that local and national government step in to educate food handlers and consumers, especially in snail meat consumption, about the need to ensure food safety and personal hygiene, since our findings showed that there is a strong correlation between what people do to keep their family safe (in Chapter 2) and the ecological/environmental contamination of land snails (in Chapters 3 & 4).

5.3 Foodborne pathogens in edible land snails: practical implications for snail importing countries

Due to regulations on agricultural management and pesticide control, live snails are prohibited from international trade in many developed countries (APHA, 2020), although snails are traded as dried, frozen, or canned. Snail meat is a widely traded commodity with a share export of 80%, observed between 2007 and 2016 (Indexbox, 2022). With nearly 69% of global consumption in the world each year, the highest snail meat importers are Spain (16,500 tons), Morocco (6,000 tons), France (5,300 tons) and Italy (2,100 tons) (Food Dive, 2018; Indexbox, 2022). Even though there is little information on the frequency of consumption of land snails in Africa, these authors (Barimah, 2013; Kaldjob et al., 2019; Meffowoet et al., 2020; Mohammed et al., 2014; Nyoagbe et al., 2016), reported that live snails are sold in almost every local market. Comparable to the European *Helix* spp., the African snail species (*Achatina* spp.) are the most traded at international markets (Cobbinah et al., 2008). For example, in Nigeria, Olaoluwa (2017), reported that business owners frequently buy snails in local markets and process (oven or sun dry) for international markets in Europe, Asia, and North America.

Despite this handling and consumption of land snails, no information exists on human food-related diseases or outbreaks of foodborne disease that could be linked to snail handling and/or consumption. In addition, food safety regulations specific to the trading and consumption of snail meat are unavailable in many developed and developing countries. In some countries, these food safety regulations have been found to be limited. For example, in the European Union, land snails for human consumption are defined by Annex 1 of Regulation (EC) No. 853/2004, as terrestrial gastropods of the *Helix* and *Achatina* species (APHA, 2020). The food safety of land snails is guided by Section XI,

Annex III of Regulation (EC) No. 853/2004 which sets out the requirements for the killing and preparation of snails for human consumption (APHA, 2020). However, the afore-mentioned regulation covers principally the processing and packaging of snail meat and "*does not apply to primary production of snails, the retail sale of snails or to the supply of snails from one retail establishment to another retail establishment when that supply is on a local, marginal and restricted basis*" (APHA, 2020. p.2). Here, "*primary production is defined in Article 3.17 of Regulation (EC) No. 178/2002 as: the production, rearing or growing of primary products including harvesting, milking, and farm animal production prior to slaughter. It also includes hunting and fishing and the harvesting of wild products*" (APHA, 2020. p.2). Furthermore, although these authors (Cobbinah et al., 2008; Olaoluwa, 2017) have documented the exportation of African snails into Europe, United States and Asia, countries in Africa are not included in the list of snail-importing countries into the European Union (APHA, 2020).

This research has indicated that the transportation of improperly processed snail meat, containing foodborne pathogens, could lead to foodborne diseases probably across international borders. Although no association has been made to date between snail meat and foodborne disease outbreaks, the microbial pathogens examined in this research are currently linked to several meat products causing foodborne diseases in many developed and developing countries. For example, in the United States, a *Listeria* outbreak resulted to three hospitalizations and one death in 2021, and this was linked to fully cooked chicken supplied by Tyson Food Inc. (CDC, 2021). In the same year, a *Salmonella* outbreak was recorded in whole red, white, and yellow onions imported from Chihuahua state, Mexico, and *E. coli* outbreaks occurred in unknown food sources that resulted in 11 hospitalizations and one death (CDC, 2021). These foodborne pathogens live in the

intestines of many farm animals, as shown in Chapter 4 with edible land snails, and infections usually occur when people eat raw or undercooked foods.

Further in this thesis, precisely in Chapters 2 & 3, our findings have shown that the primary production of snails, such as heliciculture or the collection of snails in environments (containing animal droppings, sewage, decaying food materials, contaminated water, and decaying plant and animal remains) could lead to foodborne disease transmission to humans (Tanyitiku et al., 2022a; Tanyitiku et al., 2022b). Chapter 4 further revealed that foodborne pathogens (specifically STEC) could live in the intestines of land snails (either in natural habitats or on snail farms) for up to a month. Humans could consume these contaminated snails or come in contact with snail faecal droppings. As such, the presence of microbial pathogens in the intestine and faeces of land snails could pose a significant risk to heliciculture, to the environment and eventually public health; potential disease-causing situations requiring urgent inclusion into food safety implementation programmes.

5.4 Study limitations and future investigations

We have shown that snails harbour microbial pathogens and serve as a mode of transmission to humans during handling and/or consumption. As demonstrated from our results, foodborne infections may be occurring among snail-consuming populations, although they are still unidentified. From desk research, 1) many countries lack data on the risks of foodborne diseases in snail consumption practices, 2) no studies had examined the food safety risks of local snail practices from the 'human side' of pathogen exposure, and 3) no studies have associated snail meat with the prevalent outbreaks of foodborne diseases. Since our methodological approach was novel and with limited

previous research on the food safety of edible land snails, in order to relate and complement this study findings:

1. Further in-depth analysis to understand the socio-cultural determinants of snail meat handling and consumption practices with respect to changing the local people's food safety and hygienic practices is required.
2. This study findings were based on in-depth narrations, stories, opinions, perceptions, and observations that was limited in mathematically/statistically quantify health risks in snail consumption practices. As such, keeping in mind that this research was exploratory as we sought to find previously unknown relationships of human exposures to foodborne pathogens during snail meat consumption, a complementary quantitative approach needs to be carried out covering a wider sample population (participating group) to confirm the generated hypothesis that humans are exposed to these foodborne pathogens. For example, a) the quantitative extent to which humans are exposed to foodborne disease risks, and b) if human exposure to foodborne pathogens was dependent on participant's level of education (specifically food safety). As such, a quantitative approach will richly complement our work to inform food safety strategies to eliminate snail consumption health risks in Cameroon and other snail-consumption populations.
3. In this research, due to the available resources and timeframe, we were limited to one hundred snail samples to detect five potential foodborne pathogens, that is, STEC, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. Further sampling and other foodborne related pathogens need to be analyzed to establish a comprehensive epidemiology and public health threats of foodborne pathogens in snail consumption practices.

4. Further research on the enumeration of non-STEC and STEC in the faeces of land snails after 30 days of this research experimental period will be necessary to confirm the fate of *E. coli* in the intestines of land snails. It should be noted that 30 days for this research was adopted due to our inability to maintain the snail model beyond 30 days. Furthermore, our study timeframe and resources did not permit similar investigations of the other pathogens identified in Cameroonian snails. As such, future research and strategies are required to systematically understand the mechanisms of interactions of microbial pathogens (in this study, *E. coli*) in the faeces of land snails.
5. Snail food safety risks and possible outbreaks of foodborne diseases in other snail-consuming regions need to be assessed, closely monitored, and documented.
6. The D values of non-STEC and STEC calculated in this research (where other parameters such as, Z and F values were required), should be extended to other food-related microbial pathogens associated with land snails, and
7. Lastly, to ascertain the local people believe on proper snail meat preparation and further conclude on snail-foodborne pathogen transmission to humans, an assessment of the microbiological quality of ready-to-eat snail meat is required.

However, this current research has served as a starting point for guiding future investigations and, for educating consumers, manufacturers, local and national government on the food safety risks in local snail consumption practices.

5.5 Concluding remarks and recommendations

The purpose of this thesis was to improve health outcomes through an understanding of foodborne disease risks in snail handling and/or consumption. This research revealed that the association of land snails with pathogenic microorganisms could be a risk to public health. Prior to this research, no studies had reported on human snail practices as a contributor to foodborne diseases. It was unknown if snail handlers and consumers could suffer from foodborne diseases through their routine snail practices. An understanding of human practices that could lead to pathogen exposure in combination with an examination of snail faeces for potential foodborne pathogens, permitted us to richly comprehend the public health risks in snail handling and consumption. In addition, the continuous shedding of *E. coli* (STEC and non-STEC) in the faeces of edible land snails within one month in a snail model system further indicated that *E. coli*-infected snails could be a mode of transmission of foodborne pathogens to susceptible hosts, including humans.

While it is nutritionally important to consume snail meat in Cameroon, it is also necessary to ensure that health risks in snail handling and consumption, as highlighted in this research, are eliminated. This study revealed that low-income countries in Africa, who are greatly involved in the consumption and trade of snail meat, are at the highest risk of such food-related diseases. However, little is known on foodborne disease risks in general, since foodborne infections are poorly quantified in Africa (Pires et al., 2021). For example, if a laboratory diagnosis is made, there is often no way to identify if the acquired foodborne pathogen was obtained from food, water, the environment, or another person, and documentation is poorly made except in rare cases of emergencies (Grace, 2015a). With respect to snail meat consumption, inadequate information on a) microbial

contamination of land snails and b) snail meat food safety regulation, may put the health of snail consuming populations at risk. We showed that foodborne disease outbreaks could be occurring among snail-consuming populations especially in Africa. With this, we have revealed several opportunities to improve health outcomes in snail handling and/or consumption. For example, this research has provided fruitful insights to 1) improve hygienic standards in snail practices, 2) raise awareness on possible foodborne disease outbreaks in snail meat consumption, and 3) will serve as a guide for national and international decision-making on food safety issues and policies with respect to snail handling and consumption.

To mitigate the foodborne disease risks highlighted in this thesis, live snails accepted for home preparation or industrial processing (both locally and internationally) should be free from microbiological hazards, dirt, and organic wastes. Proper hygiene needs to be employed to decrease the probability of foodborne pathogen cross-contamination into food, thereby reducing the possibility of food poisoning. Local populations need to be educated on the importance of keeping themselves and their families safe from foodborne infections, such as, on cost-effective hand washing and cleaning procedures, hygienic handling and preparation of land snails, proper storage, packaging, and refrigeration of land snails/snail meat during sales and home consumption. There is an urgent need to 1) understand and document food safety risks in snail handling and consumption practices, 2) implement food safety regulations in snail handling and/or consumption, 3) document food-related complaints or infections from snail handlers and consumers, and 4) inform local, national, and international government on fruitful opportunities, aimed at eliminating public health risks in snail handling and consumption. Documented food safety procedures, from the farm-to-table, are needed to

minimise the risks of food-related illnesses among snail handlers and consumers. Finally, snail food safety regulations and disease surveillance systems need to be implemented in a world where snail meat is increasingly becoming a delicacy and routine source of nutrition.

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Appendix A. Desk research

A.1 Summary

The purpose of this review was to conduct a systematic search of pathogenic microorganisms associated with edible land snails. Specifically, we aimed to identify previous studies that have emphasized on the food safety risks of consuming contaminated snail meat and, are in support to the fact that foodborne diseases outbreaks could occur following the handling and consumption of contaminated land snails. We also emphasized the nutritional importance of consuming healthy snail meat diets.

We conducted a literature search in seven published electronic databases including Scopus, Web of Science, PubMed, PubMed Central, ScienceDirect, Directory of Open Access Journals and Journal Storage. This was achieved by introducing search terms such as edible terrestrial snails, edible land snails, land snails as food, pathogenic microorganisms, foodborne pathogens, food safety of snails, human health risks, and public health risks. The obtained published information was analysed independently by three reviewers using pre-determined inclusion and exclusion criteria.

The results indicated that snail meat could almost be considered as a complete food with fat, proteins, water contents ranging from 0.5-0.8 %, 10.0-85.1%, 4.8-84.4 %, respectively. The calorific value of snail meat is 68cal/100g which is lower when compared to other meats (beef (163cal/100g), chicken (120cal/100g) and fish (70cal/100g). Despite several studies (Adagbada et al., 2011; Akpomie, 2013; Nyoagbe et al., 2016) on land snail association with microbial pathogens, we did not find any outbreaks reported of foodborne diseases among snail-consuming populations. This suggest that human exposure to foodborne pathogens in snail food systems and the

potential contributions of snail consumption to foodborne disease risks have been ignored or poorly quantified. In countries where public health surveillance systems are poorly developed (WHO, 2015a), this lack of data is hardly surprising.

A.2 Search strategy

A comprehensive scientific search was carried out in seven electronic global databases, that is, Scopus, Web of Science, PubMed, PubMed Central, ScienceDirect, Directory of Open Access Journals and Journal Storage. We search for English or French-language papers from publication dates up to and including December 2021. The search strategy was performed as highlighted by Mohammadpour et al. (2018). Here, we typed key words or phrases from a thesaurus, such as "edible terrestrial snails, edible land snails, snails as food, snail meat", "snail farming, snail gathering, snail processing, snail cooking, snail consumption", and "pathogenic microorganisms, microbial pathogens, foodborne pathogens, snail food safety, human health risks, human exposures, public health risks". Plurals and all synonyms were included and search for additional information. Research articles, literature reviews and methodological papers containing any of the above-mentioned keywords in their titles and/or abstracts were downloaded and considered in the review process. Reference lists of identified papers were also searched until no more articles emerged from the literature. In total, 2435 articles were retrieved in the literature search.

Two reviewers, MNT and ICNP, jointly screened all papers and excluded 2176 papers based on 1) full texts not available in the database, 2) no English or French version of the full text, 3) full texts not related to land snails, 4) duplicates, and 5) article not related to human consumption of snail meat. According to Mohammadpour et al. (2018), the reliability of the review process was subsequently improved as both reviewers

independently selected the remaining 259 articles using the inclusion and exclusion criteria in Table A.1. Another reviewer (BSS), was independently recruited to debrief, review, and subsequently correlate all included and excluded papers, and through brainstorming, 235 papers were rejected for inclusion in the findings. Any question of doubts and data bias was discussed among the three reviewers, and additional papers that fail the initial inclusion criteria emerged from the discussion sessions, leading to a total of 28 included papers. Discrepancies were resolved by consensus.

Table A.1. Criteria for inclusion and exclusion

The articles that met the inclusion criteria included:

- ✓ Investigations on edible land snails as a source of food for humans
- ✓ Investigations highlighting household and/or industrial processing and preservation of snail meat for human consumption.
- ✓ Manuals and protocols on snail farming/heliciculture destined for human consumption
- ✓ Investigations associating pathogenic microorganism with land snails
- ✓ Investigations relating land snails to foodborne diseases, health risks, and public health

Excluded papers:

- ✓ Investigations on snails other than land snails (for example, non-terrestrial, aquatic/water, mountain, or sea snails).
- ✓ Investigations on non-edible land or mountain snails
- ✓ Investigations on microorganisms other than pathogenic microorganisms
- ✓ Investigations on commensals, parasitic (for example, rat lungworm) non-pathogenic microorganisms associated with land snails
- ✓ Investigations on the archaeology of snails
- ✓ Investigations not involving edible parts of land snails
- ✓ Studies on agricultural management and extinction of snails as pesticides
- ✓ Other uses of snails (medicinal, cosmetics, animal feed, fishing bait….), slime in traditional medicine and cosmetics, shells as ceremonial and chieftaincy decorations.
- ✓ Articles reported in languages other than English or French
- ✓ Citations without abstracts and/or full texts, duplicate studies
- ✓ Non-peer reviewed information, grey literature
- ✓ Papers reporting the same or duplicated nutritional values of land snails and snail meat
- ✓ Articles reporting the same pathogenic microorganisms in the same region/countries within similar period of snail sampling, e.g., several studies sampled land snails from Akwa-Ibom local government area (Nigeria) in 2013, for microbiological analyses.

A.3 Data extraction and analysis

The two main reviewers, MNT and ICNP, downloaded and saved all 28 included papers. Simultaneously, all citations were exported to the Endnote reference manager to

avoid typographical errors. Endnote reference manager also allowed for the alphabetical organisation of the electronic information and the identification and removal of further duplicates. The data was analyzed using narrative and thematic analysis, as described by Braun and Clarke (2006) using six steps:

Step 1: For each article, the content was richly screened and documented for type of studies, and the rationale for characterizing the edible parts of land snails, including 1) nutritional benefits or 2) food safety or 3) both nutritional and health risks.

Step 2: To assess the internal and external validity of the included papers, specific information on the study design and methods used to obtain the study findings were extracted and documented in a separate assessment sheet.

Step 3: We then extracted information on the author(s) names and year of publication. For each paper, we recorded a) the region/country of snail sampling, b) the results of proximate/mineral analyses, c) key microbial pathogens isolated from land snails, and d) the study findings or main message. In addition to bacterial pathogens, some papers highlighted microorganisms of the kingdom fungi, for example, yeasts, and moulds. For these papers, we included only those bacterial pathogens that are associated with foodborne diseases and thus, relevant to public health.

Step 4: For each paper, the evidence and significance of either nutritional benefit and/or public health was highlighted and assessed. The findings were then tabulated in three summary tables, including the proximate analysis, the mineral composition of land snails, and the microbial pathogens associated with edible land snails.

Step 5: The findings were interpreted with respect to what is nutritionally gained from the consumption of land snails, and the documented health risks of snail food practices vis-à-vis proper food safety practices.

Step 6: The potential health implications of consuming contaminated snail meat were highlighted from the findings of the searched information.

A.4 Nutritional benefits of land snails

Snails are gastropods and belong to the taxon Mollusca in the animal kingdom. Mollusca is derived from the Latin word 'mollis', which means soft. During the fourth century, Aristotle first used the term 'mollis', for cuttlefish and other soft flesh animals, and by 1758, Swedish naturalist Carl Linnaeus expanded the use of the term "Mollusca" to include land snails and slugs. Snails are mainly characterized as conspicuous invertebrates having coiled calcareous shells that distinguish them from very small or shell-less slugs (Pissia et al., 2021). With more than 62,000 described living species, snails comprise about 80% of living molluscs. They are second to insects in terms of total number of species, and 150,000 of their species are placed in about 13,000 named genera.

Previous studies (Cereza Mezquita et al., 2007; Adagbada et al., 2011; Adegoke et al., 2010; Akpomie, 2013; Barimah, 2013; Nyoagbe et al., 2016; Okafor-Elenwo & Imade, 2019; Okafor & Ogbo, 2019) have documented that snail meat contains meat proteins that are vital for the human body (Tables A.2 and A.3). These proteins which are broken down for the repair and building of body tissues during digestion, are greater than those in oysters and chicken eggs (Cereza Mezquita et al., 2007). The human body uses 97% of meat protein and most animal products provide essential amino acids that cannot be synthesized by the human body (Strazdiņa et al., 2013). For example, if a diet lacks any of these essential amino acids (tryptophan, lysine, methionine), protein synthesis will be retarded. Adeyeye & Afolabi (2004) reported that African land snails contain essential amino acids ranging from 45.0-361mg/g crude protein (with histidine) and 4.3-331mg/g

crude protein (without histidine). Specifically, snail meat contains dominantly the essential amino acid, lysine (5.7-8.3 g/100g protein), and glutamic acid (11.1-14.1 g/100g protein) as the dominant non-essential amino acid (Adeyeye & Afolabi, 2004; Pissia et al., 2021). As such, lysine-deficient plant-based foods could be complemented with snail meat.

Land snails are relatively low in cholesterol, fat, and carbohydrates. These low values are justified by the fact that land snails are extremely slow in movement, and thus little or no carbohydrates are stored in the form of glycogen, which in most cases, is eventually converted into fat (Engmann et al., 2013). Snail meat does not contain lauric acid, which is responsible for increasing blood cholesterol levels (Sando et al., 2012). The fat content of land snails lies between 0.96-1.36% and this is considerably lower than in chicken eggs (9.6%), mutton (21.4%), and duck (23.0) (Cereza Mezquita et al., 2007; Taiye et al., 2019; Adeyeye et al., 2020a; Pissia et al., 2021). Moreover, the fat content of snail meat is primarily composed of omega fatty acids which are believed to be beneficial in hindering the development of certain human cancers, thus increasing life expectancy (Sando et al., 2012). These fatty acids serve as a good antidote for hypertensive patients and to those that have fat related diseases (Taiye, Abiodun, & Babatunde, 2019).

The lipid fractions of land snails consist mainly of polyunsaturated fatty acids (57%), saturated fatty acids (23.25%), and monounsaturated fatty acids (15.5%) (Adegoke et al., 2010b; Pissia et al., 2021). These polyunsaturated fatty acids consist of linoleic and eicosadienoic acids, that is, n-3 to n-6 (more n-3 and less n-6). They play a protective role in cardiovascular and inflammatory diseases, lupus, diabetes, psoriasis, obesity, Crohn's disease, rheumatoid arthritis, and cystic fibrosis (Engmann et al., 2013; Ghosh et al., 2016; Pissia et al., 2021). Also, they induce a decrease in blood triglycerides and blood pressure,

and regulate the activity of proteokinase, which slows metastatic tumors (Sando et al., 2012). On the other hand, saturated fatty acids are undesirable due to their linkages with atherosclerotic disorders (Engmann et al., 2013; Ghosh et al., 2016; Pissia et al., 2021). Therefore, land snails are more beneficial in human diets than many conventional meat products, which contain excessive amounts of saturated fatty acids (Ghosh et al., 2016; Pissia et al., 2021).

Furthermore, Table A.3 presents the mineral composition of land snails. Snail meat is made up of 30% minerals, and consists mainly of calcium, magnesium, zinc, copper, manganese, nickel, cobalt, aluminium, sulphur, and iodine (Sando et al., 2012). Calcium is essential for normal blood clotting. It is the most abundant mineral in snails at 650-700 mg/100g, which is comparable to those in fatty cheeses, and its value is ten times more than those in fish, beef, and poultry, (Engmann et al., 2013; Sando et al., 2012; Pissia et al., 2021). Therefore, 100 g of fresh snail meat (approximately 20 edible portions) will meet 60% of the daily requirement for body calcium (Sando et al., 2012).

The iron content ranges between 2.7 and 3.5 mg/100g, while chicken egg, mutton and duck contain 1.6, 2.0 and 1.08 mg/100g respectively (Taiye et al., 2019). *A. fulica* contains (55-56g/100g), *A. marginata* (40g/100g), and *A. achatina* (41.11g/100g), which is beneficial in treating anaemia in humans (Adeyeye et al., 2020a; Engmann et al., 2013). The consumption of 100g of dried snail meat could provide the daily requirement of zinc (RDA for adults is 15mg) in the human body (Engmann et al., 2013). Snail meat contain copper at 9.72-16.15 mg/100g, and this high copper content is linked to the special physiology of gastropods and snails in particular (Sando et al., 2012). These copper values are higher than the daily recommended requirement for adults (2.0-3.0 mg/day), children (1.0-2.0 mg/day), and babies (0.5-1.0 mg/day) (Sando et al., 2012). Snail meat is classified

in the first position among animal food products due to its haemocyanin content (Engmann et al., 2013; Ghosh et al., 2016). Haemocyanin is a protein containing copper that is essential for the transportation of oxygen into blood, iron metabolism, neuropeptide activation, and in the synthesis connective tissues.

The presence of phosphorus in snail meat reduces hypophosphatemia and provides anti-rheumatic effects. These effects are often characterized as loss of appetite, anaemia, muscle weakness, bone pain, rickets (in children), osteomalacia (in adults), increased susceptibility to infection, difficulty walking, numbness, and tingling of the extremities (Engmann et al., 2013; Ghosh et al., 2016). Potassium profoundly affects the excitability of nervous tissues (Ghosh et al., 2016). It influences the contractility of the smooth, skeletal, and cardiac muscles, and these mechanisms are used locally to facilitate the stages of labour and, thus reduce blood lose during delivery (Engmann et al., 2013; Taiye et al., 2019).

However, it should be noted that these aforementioned minerals are interrelated and do not play independent self-sufficient roles in the human body. For instance, calcium and phosphorus are involved in the formation of bones and teeth. Iron and zinc reduce the morbidity of diarrhoeal diseases and pneumonia (Engmann et al., 2012; Engmann et al., 2013).

Furthermore, several studies (Edidiong et al., 2016; Ademolu., 2004; Tchakounte et al., 2019) have reported that the nutritional composition of land snails depends on its free-living environments and diet. For instance, comparing with commercially farmed snails, Edidiong et al. (2016) reported a higher proximate composition of moisture (69.7%), ash (9.39%), crude fibre (6.11%), protein (60.56%), lipid (5.67%) and carbohydrate (18.25%) in snails collected from shellfish producing areas in Akwa Ibom

state, Nigeria. Similarly, Ademolu (2004) reported that *A. marginata* increased in crude protein (87.94%) when nitrogen-sourced diets (soybean, fishmeal, poultry droppings, and urea) are used. Tchakounte et al. (2019) recorded a significant increase in crude protein: 45.85%, 50.52%, 55.22% and 57.30% when calcium levels of *A. marginata* increased from 12% 14%, 16% to 18%, respectively.

Table A.2. Proximate composition of the most consumed land snails

Land snails	Moisture (%)	Protein (%)	Carbohydrates (%)	Fats (%)	Fibre (%)	Ash (%)	Authors
Achatina	4.88±0.01	62.56±1.23	27.29±1.21	2.27±0.16	0.03±0.01	3±0.01	Nkansah et al. (2021)
Fulica	73.37±1.91	19.49±2.41	6.00±1.93	4.63±1.62	0.42±0.42	2.98±1.06	Nnamonu et al. (2021)
	83.80	83.13	NA	8.70	NA	8.90	Offiong et al. (2013)
	79.28	10.08	NA	1.61	NA	1.78	Babalola & Akinsoyinu (2009)
Achatina	73.72±3.28	20.03±3.50	4.42±1.66	3.85±1.09	0.63±0.57	3.47±1.14	Nnamonu et al. (2021)
achatina	6.1±0.01	71.66±1.24	13.69±0.15	5.06±0.14	1.21±0.03	3.49±0.01	Nkansah et al. (2021)
	84.44	15.63	NA	2.20	0.14	4.08	Mumeen & Nwandu (2021)
	83.30	65.65	NA	11.90	NA	4.00	Offiong et al. (2013)
	75.28	17.20	NA	2.21	NA	2.33	Babalola & Akinsoyinu (2009)
	77.54±0.02	19.27±0.29	0.42±0.30	1.43±0.01	-	1.34±0.02	Fagbuaro et al. (2006)
Archachatina	5.2±0.05	85.12±2.14	2.25±0.11	4.37±0.06	1.32±0.15	3.06±0.02	Nkansah et al. (2021)
marginata	73.69	19.53	NA	2.44	NA	2.56	Babalola & Akinsoyinu (2009)
	73.14	12.85	NA	2.57	0.25	7.41	Mumeen & Nwandu (2021)

	73.65±2.43	18.83±2.65	6.13±2.68	4.40±2.37	0.36±0.49	2.90±1.05	Nnamonu et al. (2021)
	80.90	78.75	NA	7.65	NA	6.50	Offiong et al. (2013)

Note, values are expressed on the basis of dry matter and, as mean ± standard deviation, NA indicates not available

Table A.3. Mineral composition of the most consumed land snails

Land snails	Na	Ca	K	Mg	P	Zn	Fe	Mn	Cu	Authors
A. fulica	73.38±0.27	402.29±5.18	111.02±0.3	301.2±0.33	61.29±11.34	5.81±0.1	26.64±0.26	1.29	3.83±0.08	Nkansah et al. (2021)
	80.28	116.43	NA	64.19	103.34	0.50	55.56	18.34	16.15	Offiong et al. (2013)
A. achatina	79.00	1112.86	NA	63.35	100.00	0.79	41.11	14.45	9.72	Offiong et al. (2013)
	58.090±.12	656.9±5.46	114.65±0.44	304.62±0.19	241.9±11.3	6.28±0.1	5.75±0.05	0.17±0.03	0.73±0.02	Nkansah et al. (2021)
	NA	316.67	NA	12.00	176.33	1.19	0.30	NA	0.57	Fagbuaro et al. (2006)
A. marginata	67.64±0.19	701.79±4.32	111.43±0.46	308.7±0.42	268.53±5.34	8.41±0.12	6.33±0.05	0.73±0.04	0.98±0.03	Nkansah et al. (2021)
	NA	316.67	NA	24.00	166.00	1.24	0.35	NA	0.61	Fagbuaro et al. (2006)
	81.80	126.43	NA	1611.15	103.34	0.46	40.00	35.56	11.72	Offiong et al. (2013)

Note. values are expressed in mg/100g and mean ± standard deviation. NA: Not Available

A.5 Foodborne disease risks in snail consumption practices

The risk of foodborne disease in snail consumption practices could be highlighted in 1) snail gathering and transportation, 2) snail meat handling, preparation, and vending, and 3) snail meat preservation.

A.5.1 Gathering and transport of land snails

Snail gathering in the rainforest belt varies with a) gathering locations, (for example, bushes, forests, farmlands, and footpaths), and b) weather conditions, that is, after heavy downpours at night (Adeyeye et al., 2020a; Cobbinah et al., 2008; Mohammed et al., 2014). In Table A.4, previous studies have enumerated significant microbial pathogens from edible portions of land snails. For example, Akpomie (2013) detected $2.0x10^3$-$2.8x10^4$CFU/ml of *Escherichia spp., Salmonella spp., Pseudomonas spp., Shigella spp.,* and *Bacillus spp.* in the visceral mass and effluents of African land snails. Nyoagbe et al. (2016) isolated Sal*monella spp. (*2.91±3.19-7.39±0.45CFU/g), *Staphylococcus spp. (*7.68±1.40-2.66±2.99CFU/g), *Bacillus spp.* (4.90±1.07-1.53±1.68CFU/g), and *Pseudomonas spp. (*5.66±0.14-3.97±0.74CFU/g).

Table A.4. Microbial pathogens in edible portions of land snails

Author (s)	Country/ sampling location (s)	Isolated pathogens	Main message/findings
Nwuzo et al. (2016)	Nigeria/Nwofe, Eke-Aba, and Iboko markets in Abakaliki	*E. coli, Pseudomonas* spp., *Shigella* spp., *Enterobacter* spp., *Salmonella* spp. and *Klebsiella* spp.	Snail contamination occurs during sales due to poor handling in open markets, thus adequate preparation is needed to avoid epidemic threats.
Parlapani et al. (2014)	Greece/wild snails from Thessaloniki and farmed snails from Larissa (Thessaly) and Tripoli (Peloponnesus).	*Enterobacteriaceae, E. coli*/coliforms, *Enterococcus* spp., and *Salmonella* spp.	Lower populations of *E. coli*/coliforms, *Enterococcus* spp., and especially the absence of *Salmonella* spp. in farmed snails showed that controlled conditions could decrease the presence of pathogens in snails and, thus significantly contribute to food safety and public health
Daminabo et al. (2020)	Nigeria/Creek Road, Mile 1, Rumuokoro markets in Port Harcourt	*Vibrio spp., Bacillus* spp., *Staphylococcus* spp., *Shigella* spp., *Pseudomonas* spp., *Enterobacter* spp., *E. coli, Micrococcus* spp., *Acinetobacter* spp., *Klebsiella* spp., *Listeria* spp. *Salmonella* spp. *(S. arizonae, S. gallinarum,* and *S. typhi*)	High levels of microbial pathogens indicated that caution must be taken in the handling and processing of snails. *Salmonella* spp. were susceptible to Ofloxacin and Ciprofloxacin and strongly resistance to Ceftazidime and Gentamicin.
Nyoagbe et al. (2016)	Ghana/markets and farms in Greater Accra	*Salmonella* spp., *Staphylococcus* spp., *Bacillus* spp. and *Pseudomonas* spp.	Deshelling, removal of slime, and unpacking are food safety problems associated with snail meat

Akpomie (2013)	Nigeria/Five snail farms in Warri, Sapele and Delta	*Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Klebsiella* spp., *E. coli.*	Microbial counts may increase under unhygienic conditions, leading to health risks. All microorganisms were resistant to chloramphenicol and ampicillin.
Ebenso et al. (2012)	Nigeria/markets and road junctions in Cross river and Akwa Ibom	*Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Vibrio* spp., *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella* spp., *Staphylococcus* spp., *Yersinia* spp.	Bacterial isolates showed multidrug resistance to antimicrobials, thus proper processing of snails is required to ensure food safety
Kirkan et al. (2006)	Turkey/snail meat producing plant	*Listeria monocytogenes*	18 out of 30 samples showed positive PCR reactions, which indicated that *L. monocytogenes* were present in the rural and plant environment.
Adegoke et al. (2010)	Nigeria/Uyo, Itam, Akpan, Andem markets in Uyo, Akwa Ibom	*Bacillus subtilis, Staphylococcus aureus, Lactobacillus* spp., *E. coli, Micrococcus luteus, Bacillus cereus*	Although nutritionally rich, snails are reservoirs of pathogenic microorganisms of significance to public health
Barimah (2013)	Ghana/nine markets in Eastern, Volta, Ashanti, and Greater Accra	*Salmonella* spp., *Klebsiella* spp., *Shigella* spp., *Yersinia* spp. and *Clostridium tetani*	The presence of pathogenic and non-pathogenic microorganism in snails showed that eating improperly cooked snails is unsafe
Adagbada et al. (2011)	Nigeria/Odukpani and Itam junctions, Watt and Uyo markets in Cross	*Salmonella* spp., *Shigella* spp., *Aeromonas*, *Vibrio* spp., *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella*	Bacterial isolates showed multidrug resistance to most antimicrobials significant contributors to gastro-

	river and Akwa Ibom	spp., *Staphylococcus aureus* spp., *Proteus* spp., *E. coli* and *Yersinia* spp.	intestinal infections. Prior to eating, proper cooking of snails is required
Kiebre-Toe et al. (2003)	France/Gonsans in Doubs, Cornillé-les-Caves in Maine and Loire, and Chamberry in Savoie	*Enterobacteriaceae, Aeromonadaceae Enterobacteries (Escherichia, Klebsiella* and *Pantoea* genus), A*eromonads (A. caviae* and *A. hydrophila*).	*Pseudomonads* and other non-fermentative Gram-negative bacteria were inconsistently isolated but, later disappeared at the hibernation stage.
Okafor & Ogbo, (2019)	Nigeria/markets - Ogbete, Enugu, Abakaliki, Ebonyi and Nkwo Igboukwu, Anambra	*Citrobacter, Shigella, E. coli, Staphylococcus, Aeromonas,* and *B. cereus*	There is an urgent need to implement strategies that will reduce bacterial pathogens in edible snails
Temelli̇ et al. (2006)	Turkey/snail processing plant, Bursa)	Total aerobic mesophilic bacteria, coliforms, *E. coli, Enterobacteriaceae*, *Staphylococci, Salmonella* spp., *Listeria* spp.	Raw materials (snails), air and the environment were the main sources of contamination. Sufficient sanitary applications, including proper hygiene, were strongly recommended.

A.5.2 Snail handling and consumption

Freshly collected African land snails are often put in old sacks or buckets and transported to homes on the head or in wheelbarrows. To maintain snail humidity, live snails are then stored underneath tree trunks till preparation or subsequently transported to local markets (Cobbinah et al., 2008). At local markets, snails are sold to consumers or retailers as alive, washed, smoked dried, or as street food. In Ghana, Barimah (2013) mentioned that live snails were sold in open bags or wooden boxes in nine markets in Greater Accra, Eastern, Volta and Ashanti. Snails are mobile and can be seen moving in open vending materials, bench tops, and on the soil (Barimah, 2013; Nyoagbe et al., 2016).

In addition, vendors strive to restrict live snails from escaping, and it is common for vendors to pick fallen snails on untidy soils/floors and add them to the lots displayed for sale (Barimah, 2013). A survey conducted by Nyoagbe et al. (2016) reported that participants purchased unpackaged and unhygienic snails at local markets in Greater Accra, Ghana, and describing snail vending activities, Barimah, (2013) also mentioned all vendors sold in relatively ‘dirty’ environments.

'Dirty referred to the proximity of snail vendors to piles of rubbish [...] flies and muddy water' (Barimah, 2013).

Similar observations were made in our own field work (Tanyitiku et al., 2022a) in Buea, Cameroon, as shown in Figures A.1 and A.2.

Figure A.1. Local market vending setting, Cameroon

Figure A.2. Snail vending at the local market, Buea, Cameroon

Few studies (Barimah, 2013; Nyoagbe et al., 2016; Akpomie et al., 2019; Adeyeye et al., 2020b) have microbiologically assessed the vending, handling, and preparation of African land snails. Our own work did qualitatively explore human exposures to foodborne

pathogens during snail handling and consumption (Tanyitiku et al., 2022b). In these previous studies, *Salmonella* is a frequent contaminant and up to 69% have been isolated in *Achatina* spp., Cameroon (Tanyitiku et al., 2022b), 62% in *A. achatina,* Nigeria (ICSMF, 2005; Obi & Nzeako, 1980), 43% in *H. aspersum,* Morocco (ICSMF, 2005), 27% in *Ampullaria* spp. in Canada and Florida (ICSMF, 2005). *Salmonella* contamination of snails occurs predominantly on the surfaces of the shells and within the meat, however, the pathogens may also penetrate the intestines of snails (ICSMF, 2005).

The cooking processes of snail meat have also been evaluated. For example, Akpomie et al. (2019) microbiologically analysed dressed snails (prepared using boiling, frying, smoke drying and oven-drying) sold in Abraka main market, Nigeria. The isolated bacteria included *Bacillus* spp., *E. coli, Proteus* spp., *Vibrio* spp., *Salmonella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Shigella* spp., *Klebsiella* spp., *Streptococcus* spp. and *Citrobacter* spp. (Akpomie et al., 2019). Oranusi and Nubi (2016) revealed that ready-to-eat snail meat that was sold at travel stop-over terminals along the Lagos-Shagamu expressway, Nigeria contained, *Bacillus* spp. (31%), *Staphylococcus aureus* (18%), *Klebsiella* spp. (13%), *E. coli* (6%), and *Salmonella* spp. (2%). Therefore, these results indicated that the local population could be eating snails that are highly contaminated with foodborne pathogens that are of great danger to public health.

A.5.3 Snail meat processing and preservation

Although limited information exists on the processing and commercialization of snail meat, they are widely sold in local and international markets. In developing countries, African land snails are mainly sold when alive and active. However, unfavourable environmental conditions such as decrease in humidity, and increase in surrounding temperatures could lead to the death of snails, and spoilage can occur within 48 hours (Emelue et al., 2013; Engmann et al., 2012). Due to these setbacks, several

temperature-dependent drying methods (for example, oven drying, sun drying) have been employed to preserve and extend the shelf life of snail meat (Emelue et al., 2013; Engmann et al., 2012). In sun drying, for example, freshly washed snail meat is attached on long sticks of approximately 1m. Depending on the weather conditions, the long sticks containing snail meat are exposed to sunlight for approximately five days (Engmann et al., 2012). The sticks are then exposed to mild heat to obtain a smoke-flavoured dried snail meat. They could be placed in bags or buckets until consumption or for later transportation to local markets. At local markets, dried snail meat is not packaged, but displayed on open trays during sales (Nyoagbe et al., 2016). The leftovers at the end of each day are packed in the same wooden boxes or buckets, transported home and stored while awaiting the next market day (Engmann et al., 2012; Tettey et al., 1997). These vending and preservation activities not only reduce the sensorial and nutritional properties of snail meat, but also expose the meat to environmental and microbiological contamination (Emelue et al., 2013; Engmann et al., 2012; Tettey et al., 1997).

Furthermore, developed countries such as Spain, Turkey, Argentina, Brazil, Peru, France, Bulgaria, Romania, Hungary, Albania, Lithuania, Latvia, and Estonia are among the main producers of snails (Cerezal Mezquita et al., 2007). Kocaman Balıkçılık for example, is one of the largest processors and exporters of snail meat in Turkey (Cerezal Mezquita et al., 2007). The first step in the industrial processing of snails is purging (Murphy, 2001; Cerezal Mezquita et al., 2007). Purging is said to be essential as it aims to eliminate poisonous plants and microorganisms from the intestines of snails (Murphy, 2001). During this period, snails are maintained at ambient temperature or refrigerated, and subjected to a strict diet of corn flour, damp bread or oats for two days (Cerezal Mezquita et al., 2007). Other methods include a fasting of 3 to 5 days, and then immersion in water containing salt and vinegar to eliminate slime, a naturally produced mucus

secreted by snails, and used in locomotion, defense, water retention and cosmetics (Murphy, 2001; Cerezal Mezquita et al., 2007).

Temelli et al. (2006) detailed that the industrial processing of frozen snail meat included, 1) receiving (live snails), 2) washing with potable water, 3) steaming (in steam tunnels at 105^0C for 3 mins), 4) automatic shelled calibration (through sieves with predetermined pore sizes), 5) removal of meat from shell, 6) first boiling in water (at 100^0C for 1 min.), 7) gutting, 8) automatic meat calibration (through sieves with predetermined pore size), 9) second boiling in water (at 100^0C for 3.5 mins meat internal temperature 85^0C), 10) hand packaging and weighing, 11) freezing (at 20^0C), labelling, frozen storage (at-20^0C), and distribution (at -20^0C). Snails are exported frozen or canned, consisting of 10-12 snail meat per can and that could last for approximately 24 months.

The microbial food safety of both locally smoked and industrially processed snail meat is under-researched. Only three studies (Tettey et al., 1997; Temelli et al., 2006; Adeyeye et al., 2020b) on snail meat processing/preservation were identified in the literature. Firstly, Tettey et al. (1997) revealed that unhygienic practices, poor handling, and the sale of unpackaged dried snail meat were sources of microbial contamination of preserved snail meat in Ghana (Tettey et al., 1997). Secondly, Adeyeye et al. (2020b) highlighted that poor hygiene was a major contributor to the presence of *E. coli, S. paratyphi, L. monocytogenes* and *C. jejuni* in cured (that is, treated with 3% salt) and smoked snail meat. Finally, Temelli et al. (2006) revealed that personnel hands and equipment used were secondary sources in the contamination of snail meat. However, with respect to the above-mentioned processing steps, Temelli et al. (2006) further highlighted that secondary boiling and freezing could improve the microbiological quality of snail meat.

A.6 Foodborne disease risks of animal-related products in Cameroon

Although we have emphasized the lack of information of the food safety risks of land snails consumed in Cameroon, previous research (Assob et al., 2012; Nicholas et al., 2020a; Yongsi, 2014; Yongsi, 2018) has raised food safety concerns on food handling practices in Cameroon. In our study area, Buea, Assob et al. (2012) revealed that the faeces of 56.7% of 150 randomly selected street-food vendors contained *Entamoeba coli* (14.0%), *Entamoeba histolytica* (12.67%), *Ascaris lumbricoides* (11.33%), and *Ankylostoma duodenalis* (10.67%). Poor personal hygiene, poor sanitary and the lack of toilet facilities among others, were factors promoting the spread of faecal-oral infections in the southwest region of Cameroon (Assob et al., 2012). Moreover, the food safety risks of other animal food-related products have been highlighted, as presented in Table A.5.

Table A.5. Microbial contamination of comparable meat products, Cameroon

Authors	**Animal food product/location**	**Main findings**	**Main message**
Akomoneh et al. (2020)	Slaughtered cattle/Buea	56 (10.9%) of the 512 samples contained *E. coli* O157. 96.4% (54/56) of virulence genes *stx2, eaeA and hylA*, and 40/54 (71.4%) of *stx1* was detected	Infected cattle with pathogenic *E. coli* O157 could be a potential source of human infections
Yannick et al. (2013)	Street-vended pork/Bamenda	81.8% *S. aureus*, 72.7% *K. pneumoniae*, 54.4% *E. coli*, 45.4% *Salmonella* spp., 27% *P. vulgaris*, and 9% *Shigella* spp.	Results indicate public health hazards and gives warning signal for possible occurrences of foodborne intoxication
Djoulde et al. (2015)	Street-vended beef, pork, chicken/Soudano-sahelian regions	From 200 samples collected, 60(30%) *E. coli,* 46(23%) *Bacillus cereus*, 38(19%) *Staphylococcus aureus*,30(15%) *Salmonella* spp. and 10(5%) yeast and moulds	Although foodborne pathogens were within acceptable limits, meat quality measured need to be improved

Nzouankeu et al. (2010)	Marketed chicken/Yaounde	From 150 chickens: 90% *Campylobacter* (68.9% *C. coli* and 31.1% *C. jejuni*), 11.3% EPEC From 142 chickens: 55.3% contained *Campylobacter, E. coli*, and *Salmonella*	Chicken is a potential risk of infection for consumers and highlights the necessity of public awareness for food safety

Appendix B. Ethics application

B.1 Ethics approval

Research Management Office

T 64 3 423 0817
PO Box 85084, Lincoln University
Lincoln 7647, Christchurch
New Zealand
www.lincoln.ac.nz

7 June 2019

Application No: 2019-25

Title: Understanding the know-how and practices in the snail meat food supply chain among the rural inhabitants of Buea-Cameroon

Applicant: M Tanyitiku

The Lincoln University Human Ethics Committee has reviewed the above noted application.

Thank you for your response to the questions which were forwarded to you on the Committee's behalf.

I am satisfied on the Committee's behalf that the issues of concern have been satisfactorily addressed. I am pleased to give final approval to your project.

Please note that this approval is valid for three years from today's date at which time you will need to reapply for renewal.

Once your field work has finished can you please advise the Human Ethics Secretary, Alison Hind, and confirm that you have complied with the terms of the ethical approval.

May I, on behalf of the Committee, wish you success in your research.

Yours sincerely

Grant Tavinor

Chair, Human Ethics Committee

B.2 Information sheet

Lincoln University

Faculty of Agriculture and Life Sciences

Department of Food, Wine and Molecular Biosciences

Research Information Sheet

Introduction and invitation

We would like to invite you to participate in a project entitled 'Understanding the know-how and practices in the snail meat food supply chain among the rural inhabitants of Buea-Cameroon'. This research have been reviewed and approved by the Lincoln University Human Ethics Committee.

Aim of the project

This project aims to understand the step-by-step process of farming/gathering, handling, preparation/cooking and selling to the consumption of snails in Cameroon.

It is funded by the New Zealand Aid programme from October 2018 to March 2022 and is part of a PhD thesis on potential foodborne pathogens that may be transmitted through snails to humans, as well as if snails are not properly handled or cooked prior to consumption. The ideas gathered from this study will lead to proposition of food safety procedures to improve the snail consumption practices in Cameroon.

Your participation

This project sought participants as you who have been involved in the food sector either in harvesting, cooking, hawking or consuming snails or as an inspector of meat products for some years now. You have been selected for participation based on our observations in different occasions, and convinced by your practical experiences and contributions you have made in the meat food supply chain in Cameroon.

The interview will be for approximately one hour (01 hour) where you will be asked to share with us your experiences and day to day activities as a snail meat handler or consumer or inspector of meat. You should note that there is no correct or required answer as we want to understand what you do on a daily basis probably for future ameliorations. This will take place at your home or office at any time during the day which is convenient for you. After the one-hour interview, We will thank you for your participation and will take our leave.

Prior to the interview, your consent for an audio recording and note taking will be approved by you. We will use the recording for analysing the results of our discussion and for my future references only. You are volunteering to participate and you have the right not to answer any question or decline at any point during the interview if you do not longer wishes to continue with the interview.

Confidentiality of our discussion

My supervisors and I, and if required, the human ethics committee of Lincoln University will have access to the audio recording, notes and data from this interview and this cannot be linked to you in any form, as your identity is not required in our discussion. No information will be revealed to a third party and the data will be stored in a locked cupboard during data analysis and interpretation of which I and I alone will have access. This will be destroyed by December 2021. The results will be available in two years after the completion of the thesis writing and your anonymity will be maintained in any publications with the reveal of no identity. You have up to September 2020, which is one year from today to withdraw your answers by email or telephone. In your email or telephone call, you will have to mention the date and time in which the interview held for traceability purposes and your answers and recordings will be destroyed immediately.

My contact details are;

Tanyitiku Mary Nkongho; tel. +64 221236924, email: mary.tanyitiku@lincolnuni.ac.nz

Supervisors:

Pr. Stephen On; email: stephen.on@lincoln.ac.nz

Dr. Jon Sullivan; email: jon.sullivan@lincoln.ac.nz

Mr. Graeme Nicholas; email: graeme@nicholas.net.nz

B.3 Consent form

Consent Form

Name of project

A study on the microbiological contamination of snails in the snail meat food supply chain in the South West Region of Cameroon

I have read and understood the description of the above-named project. On this basis I agree to participate in the project, and I consent to publication of the results of the project with the understanding that anonymity will be preserved. I understand also that I may withdraw from the project, including withdrawal of any information I have provided, up to *September 2020.*

☐ I consent to having an audio recording made of my interview.

☐ I do not consent to having an audio recording made of my interview but agree to notes being made.

Name: _____

Signed: _____ Date: _____

B.4 Interview protocol

For the rural women:

Introduction

….

Description of interviewee

Role - involvement with snail meat
Extent and length of their involvement
Region of origin, age range, educational level …
Frequency and pattern of consumption
Source of snails

Competence

To understand the skills, knowledge, experiences that may shape or influence the practices of the interviewee.

- What are the most important things you need to know for your ….? [farming, harvesting, handling, selling, cooking, … snails]
- What are some of the most important skills (or methods, or techniques) you need to do your … [farming, harvesting, handling, selling, cooking, … snails]?
- How did you learn to ….? [farm, harvest, handle, sell, cook, … snails]
- What are some experiences in life that prepared you or equipped you for this role with snails?
- If you are teaching your daughter to do what you do, what would you need to teach her?

Materials

To understand how location, tools, regulations, environment, physical and biological realities may shape or influence the practices of the interviewee.

- Tell about the practical process of [farming, harvesting, handling, selling, cooking, … snails].
- Which settings / locations / environments do you think are important for [farming, harvesting, handling, selling, cooking, … snails]?
- What do you need for [farming, harvesting, handling, selling, cooking, … snails]?
- What are some of the barriers you face [farming, harvesting, handling, selling, cooking, … snails]?
- How do you reduce the risk of disease (you or others getting sick) [farming, harvesting, handling, selling, cooking, … snails]?

Meaning

To understand what is important or meaningful to the interviewee, and why it is important or meaningful. This may include economic, social, cultural, personal, or religious factors.

- What made her interested in [farming, harvesting, handling, selling, cooking, … snails]?
- Why does she [farm, harvest, handle, sell, cook, … snails]?
- How important is [farming, harvesting, handling, selling, cooking, … snails] to your income or the quality of life for your family?
- Tell me how you think about hygiene and keeping your [self, customers, family] safe and healthy.
- What is the most important thing to you about [farming, harvesting, handling, selling, cooking, … snails]?
- What would make [farming, harvesting, handling, selling, cooking, … snails] better - in your opinion?

For the public health officials:

Introduction

……

Description of interviewee

- Role in the inspection of meat
- Extend and length of involvement (years of experiences)
- Region of origin (to appreciate familiarity with snails), age range, educational level
- Frequency of involvement in meat inspection
- Types of meat products you inspect
- Locations in which meat inspection are done
- Are you also involved in the inspection of cooked meat, if no, who are involve in the inspection of cooked meat (to confirm that cooked meat are not also inspected in Cameroon)

Competences

- What are the most important things to be considered during the inspection of meat products?
- What are the qualification and skills required for someone to be qualified to handle meat products for consumption?
- How do you know these individuals has the potential skills to handle meat products for consumption?
- Do these individuals despise the abovementioned qualification and skills, required some other experiences (years of meat handling for example) before they could handle or sell meat in the market?

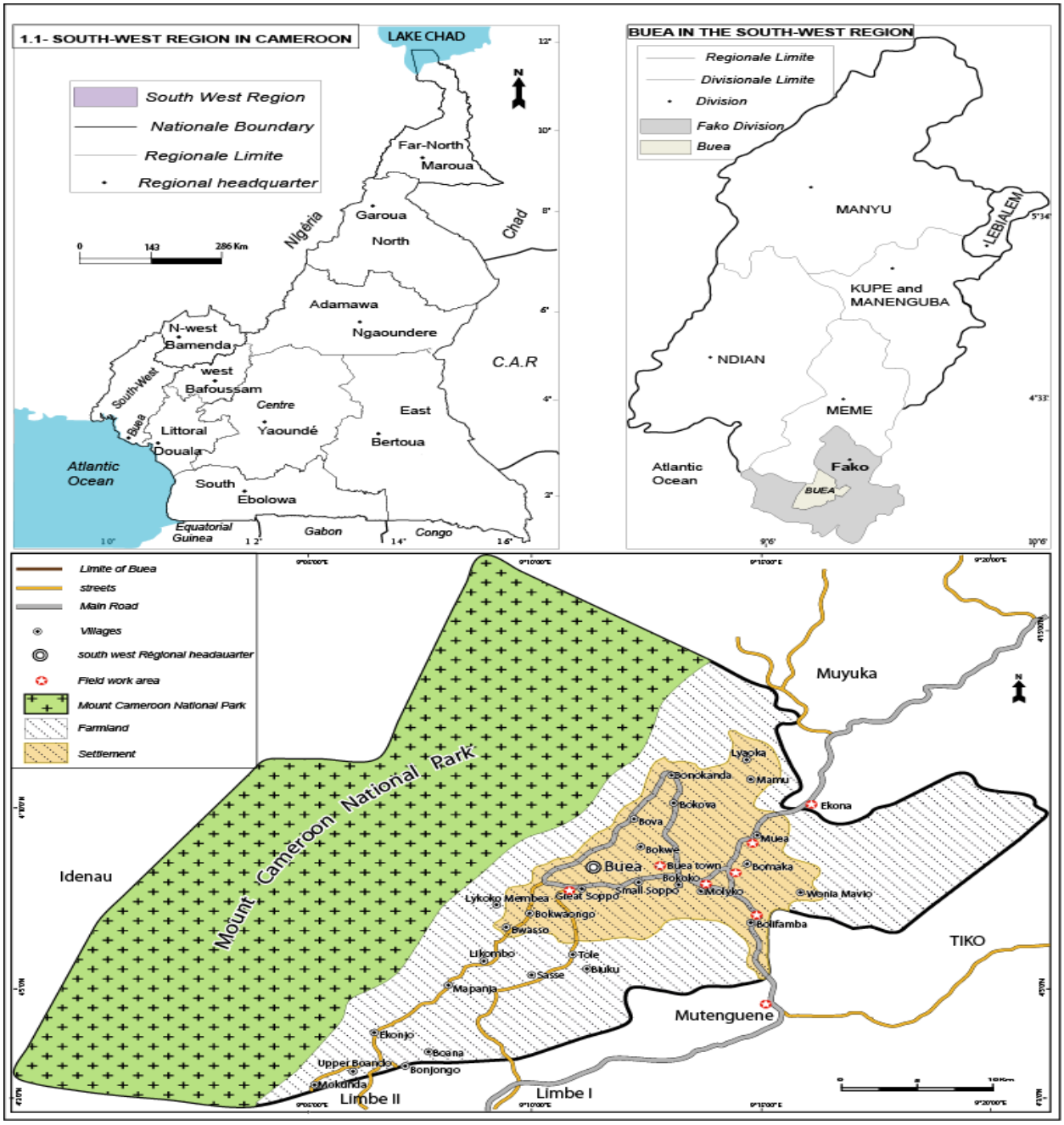
Materiality

- Tell us above the practical process of meat harvesting, slaughtering, preparation, and vending
- Which areas or places do the meat usually move from when it leaves the farm to when it reaches the market to be sold to consumers?
- What are the limitations/challenges of inspecting meat products?
- How are snail meat inspected, if no, why are snail meat not inspected
- What are the challenges if snail meat was to be inspected?

Meaning

- Why are meat products examined?
- How is the inspection of meat important?
- In the inspection process, how is the rate of transmission of diseases reduced or regulated
- How often are meat products rejected after inspection and what are the major causes of rejection
- What are the characteristics of meat to be considered as good quality and safe for consumption?
- How will you rate the quality of meat with respect to the hygienic status of the meat handlers?

B.5 Localization of Buea, Cameroon



Source: Projection: WGS84 / UTM 32N / Data: NIC, 2018

Figure A.3. Localization of the study area, Buea, Cameroon

Appendix C. PCR results/cycling conditions

C.1 PCR results

4a: Within people's homes

Location	*STX1*	*STX2*	*Campylobacter*	*Listeria*	*Salmonella*	*Yersinia*	*16S*	*Pseudomonas*
GRA	0	0	0	1	1	1	1	0
	0	0	1	1	1	1	1	0
	0	1	0	1	0	0	1	0
	0	1	1	1	1	0	1	0
	1	1	0	1	1	1	1	0
	0	0	1	1	1	1	1	0
	0	0	1	1	0	0	1	0
	0	0	1	1	1	1	1	0
Clerks	0	1	0	1	1	1	1	0
Quarters	0	0	1	1	1	1	1	0
	0	0	1	1	1	1	1	0
	1	0	0	0	1	1	1	0
	1	0	1	0	1	1	1	0
	1	1	1	1	0	0	1	0
	0	1	1	1	1	1	1	0
Great	1	1	1	1	1	1	1	0
Soppo	1	1	1	1	1	1	1	0
	1	1	0	1	1	0	1	0
	1	1	1	1	1	1	1	0
	1	1	0	0	1	1	1	0
	1	1	1	1	1	1	1	0
Untarred	1	1	1	1	1	1	1	0
Malingo	1	1	1	1	1	1	1	0
	1	1	0	0	1	0	1	0
	1	0	0	0	1	0	1	0
	1	1	0	1	0	0	1	0
	1	1	0	1	0	1	1	0
	1	1	1	1	0	1	1	0
	1	1	1	1	1	1	1	0
	0	1	1	1	1	1	1	0
	1	1	0	1	0	1	1	0
	1	1	0	1	1	1	1	0
	0	1	0	1	1	1	1	0
Lower	1	0	1	1	0	1	1	0
Muea	0	1	0	1	1	1	1	0
	0	0	1	0	0	1	1	0
	1	1	1	1	0	1	1	0
	1	0	1	1	0	1	1	0
	0	1	1	1	1	0	1	0
	1	1	1	1	1	0	1	0
	1	1	1	1	1	1	1	0
	1	0	0	1	0	1	1	0
	0	1	0	0	0	1	1	0
	0	1	1	1	1	1	1	0
	0	1	0	1	1	1	1	0
	1	1	1	1	0	0	1	0
	0	1	1	1	0	1	1	0

4b: Farms

Location	*STX1*	*STX2*	*Campylobacter*	*Listeria*	*Salmonella*	*Yersinia*	*16S*	*Pseudomonas*
Mile 16	0	1	1	0	1	0	1	0
	1	1	1	1	1	1	1	0
	1	1	0	1	0	1	1	0
	0	1	0	1	1	1	1	0
	0	1	1	1	1	1	1	0
	0	0	0	1	0	1	1	0
Bokwango	1	1	1	0	1	1	1	0
	0	1	1	0	0	0	1	0
	1	1	0	0	0	0	1	0
	1	1	0	0	1	0	1	0
	0	1	1	1	1	0	1	0
	1	1	1	1	1	1	1	0
	1	1	0	1	1	0	1	0
	1	0	0	0	0	1	1	0
	0	0	0	0	1	0	1	0
	1	1	1	0	0	1	1	0
	1	1	1	1	1	1	1	0
Mile 18	1	1	1	1	1	0	1	0
	1	1	1	0	1	1	1	0
	1	0	0	0	1	1	1	0
	0	1	0	0	1	1	1	0

4c: Local markets

Location	*STX1*	*STX2*	*Campylobacter*	*Listeria*	*Salmonella*	*Yersinia*	*16S*	*Pseudomonas*
Great	1	1	0	1	0	1	1	0
Soppo	0	1	1	1	0	1	1	0
	1	1	0	1	0	1	1	0
	0	1	0	1	0	1	1	0
	0	1	1	1	0	1	1	0
	0	1	1	1	0	1	1	0
Muea	0	1	1	1	0	1	1	0
	0	1	0	1	0	1	1	0
	0	1	1	1	1	1	1	0
	0	1	1	1	0	1	1	0
	1	1	0	1	1	1	1	0
	1	1	1	1	0	1	1	0
	0	0	1	1	1	1	1	0
	0	1	0	0	1	1	1	0
	0	1	1	0	1	1	1	0
Molyko	0	0	1	1	1	1	1	0
	0	0	1	1	1	1	1	0
	0	1	0	1	1	0	1	0
	1	0	1	1	1	1	1	0
	1	1	0	1	1	1	1	0
	1	0	0	1	1	1	1	0
	1	1	0	1	1	1	1	0
	1	0	1	0	1	0	1	0
	0	1	0	1	1	0	1	0
Mile	0	0	1	1	1	1	1	0
16/17	0	1	0	1	1	1	1	0
	0	1	1	1	1	0	1	0
	1	1	0	1	1	1	1	0
	1	0	0	1	1	0	1	0
	0	0	1	0	1	0	1	0
	1	1	1	1	1	0	1	0
	0	1	0	1	1	1	1	0

C.2 PCR cycling conditions

Table A.6. Single specific PCR primers and optimized conditions used in PCR

Pathogen	**Gene**	**Primer name**	**Primer sequence 5'--> 3'**	**Product size**	**Cycle conditions**	**References**
STEC	*Stx1*	Stx1-ET-F	CATTACAGACTATTTCATCAGGAG GT	68	95^0C/4 min, 95^0C/10 sec, 60^0C/5 min, 72^0C/2 sec, 72^0C/2 min, 10^0C/1 min, 35 cycles, cycling time, 37 min	Kawase et al. (2016)
		stx1-ET-R	CAAATTATCCCCTGAGCCACTA			
	Stx2	stx2-ET-F	CATGACAACGGACAGCAGTTAT	114	95^0C/4 min, 95^0C/10 sec, 60^0C/5 min, 72^0C/2 sec, 72^0C/2 min, 10^0C/1 min, 35 cycles, cycling time, 37 min	Kawase et al. (2016)
		stx2-ET-R	AACTCCATTAACGCCAGATATGA			
C. jejuni/coli	16S rRNA	CCCJ609F	AAT CTA ATG GCT TAA CCA TTA	854	94^0C/5min, 94^0C/1min, 55^0C/1min, 72^0C/1min, 72^0C/7min, 10^0C/1min, 25cycles, cycling time, 1h 44min	Linton et al. (1997)
		CCCJ1442R	GTA ACT AGT TTA GTA TTC CGG			

Positive control	16S rRNA	16SF	CCAgACTCCTACGGGAGGCAG	203	95^0C/4 min, 95^0C/10 sec, 60^0C/5 min, 72^0C/2 sec, 72^0C/2 min, 10^0C/1 min, 35 cycles, cycling time, 37 min	Chakravorty et al. (2007)
		16SR	CGTATTACCGCGGCTGCTG			
Listeria spp	Hly	Lm-hly-F	GGGAAATCTGTCTCAGGTGATGT	72	95^0C/4 min, 95^0C/10 sec, 60^0C/5 min, 72^0C/2 sec, 72^0C/2 min, 10^0C/1 min, 35 cycles, cycling time, 37 min	Kawase et al. (2016)
		Lm-hly-R-kai	GTAAATTACGGCTTTGAAGGAAG A			
Salmonella spp	Nested	Sal1-F	GTA GAA ATT CCC AGC GGG TAC TG	438	95^0C/3 min, 95^0C/30 sec, 60^0C/1 min, 72^0C/1.5 min, 72^0C/10 min, 10^0C/1 min, 20 cycles, cycling time, 2 h 40 min	Waage et al. (1999)
		Sal2-R	GTA TCC ATC TAG CCA ACC ATT GC			
		Sal3-F	TTT GCG ACT ATC AGG TTA CCG TGG	312	95^0C/3min, 95^0C/30sec, 67^0C/1min, 72^0C/2sec, 72^0C/1.5min,	

		Sal4-R	AGC CAA CCA TTG CTA AAT TGG CGC		10^0C/1min, 40cycles, cycling time, 1h 44min	
Yersinia spp	16S rRNA	LandzY1	GGAATTTAGCAGAGATGCTTTA	300	94^0C/5 min, 94^0C/30 sec, 58^0C/30 min, 72^0C/40 min, 72^0C/7 min, 10^0C/1 min, 30 cycles, cycling time, 1 h 21 min	Lantz et al. (1998)
		LandzY2	GGACTACGACAGACTTTATCT			

Appendix D. Laboratory model system

D.1 Trial experiments

Several trials with laboratory-grade disinfectants were conducted to determine a suitable medium for the surface decontamination of snails after inoculation. Here, I used Ultraviolet light (intensity = 2.1 mJ/cm^2) and three reagents: 5% sodium hypochlorite (bleach), 10% w/v povidone-iodine Betadine (Munipharma, Netherlands), 70% w/v ethanol.

For each reagent, a post-inoculated snail was placed in several dilutions of 1:1, 1:10 and 1:50 for 5secs. The snail was removed and rinsed with sterile distilled water. The rinsed distilled water was then plated on Nutrient agar at 37^0C for 18h. as shown in Figure A.4, experiments were conducted in triplicates till a satisfactory result of no growth (with the snail being alive) was obtained for a 1:50 dilution of 5% sodium hypochlorite (bleach).

Experiments using Ultraviolet light were conducted based on Abedi-Moghaddam et al. (2004). Here, several post-inoculated snails were placed in closed petri dishes and placed in UV. Two snails were removed at intervals of 5mins, 10mins, 15mins, 20imins, 30mins, 60mins, 90mins, 120mins, and 180mins. The surfaces of the snails were rinsed with sterile distilled water. The rinsed distilled water was then plated on Nutrient agar at 37^0C for 18h. The use of UV light was unsatisfactory as a surface decontaminant for the experimental snails as continuous growth of microorganisms (*E. coli* inclusive) was observed up to 180mins.

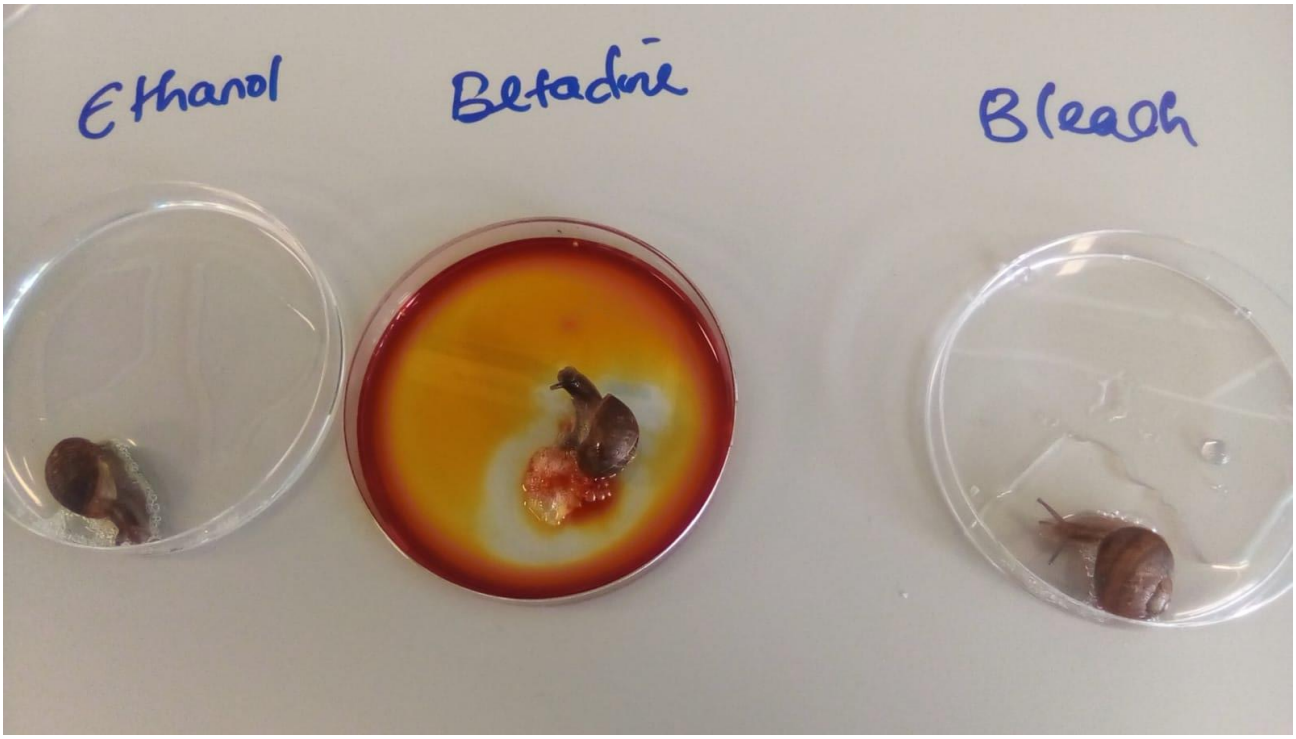


Figure A.4. Trial experiments showing survival of snail in bleach

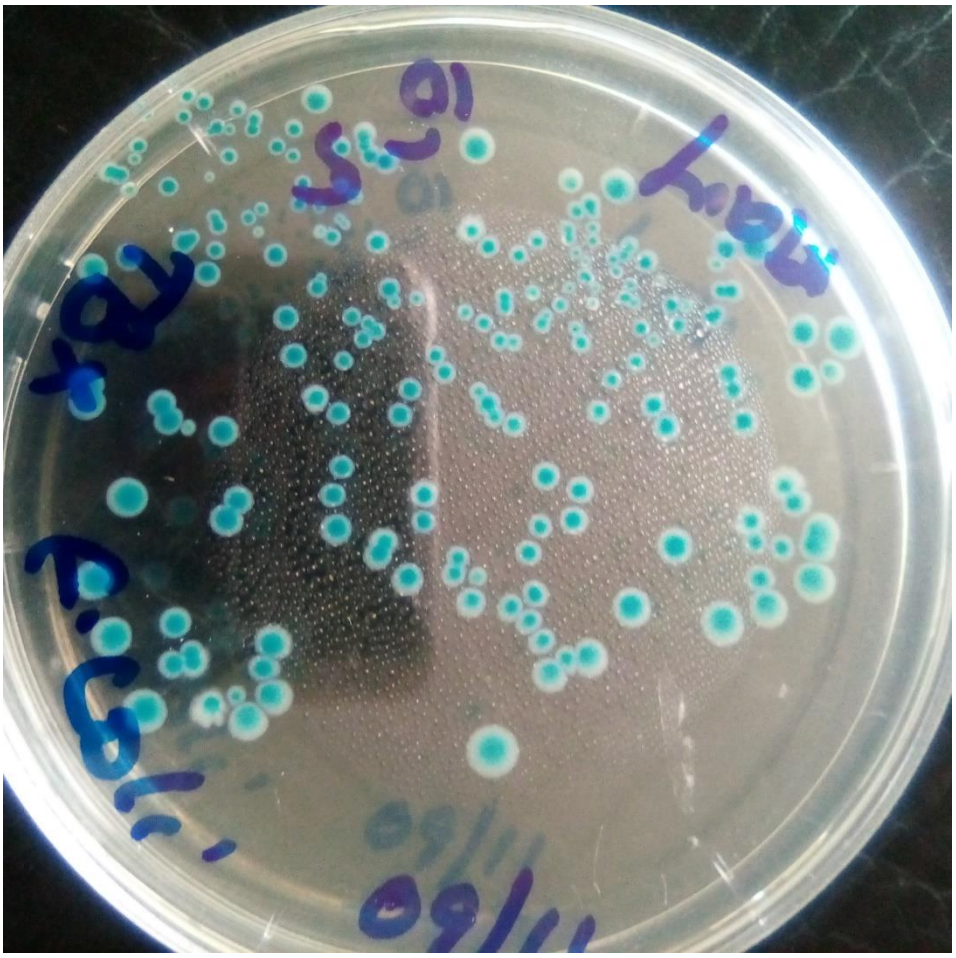


Figure A.5. *E. coli* colonies of interest on TBX agar

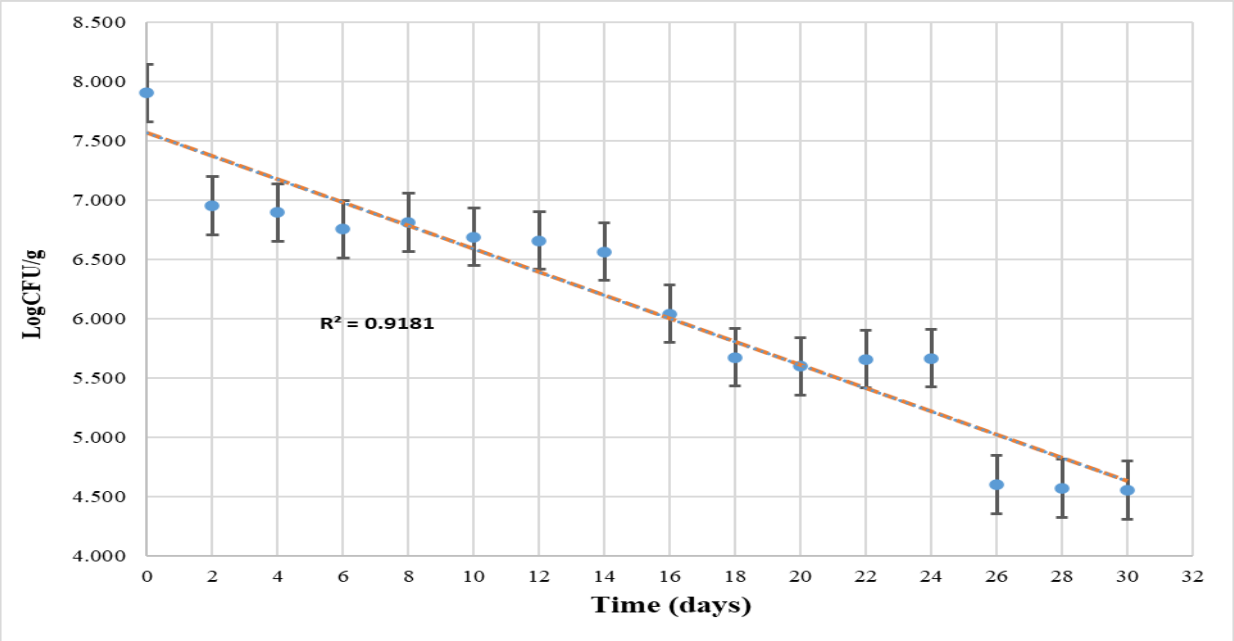
D.2 Results of plate counting experiments

Table A.7. Calculated Colony Forming Units (CFU) from plate counted colonies

Days	CFU/g (*E. coli* CSH62)			CFU/g (*E. coli* ERL 06-2503)		
	1st	2nd	3rd	1st	2nd	3rd
0	$77x10^6$	$77x10^6$	$86x10^6$	$72x10^6$	$66x10^6$	$68x10^6$
2	$98x10^5$	$102x10^5$	$72x10^5$	$108x10^5$	$104x10^5$	$99x10^5$
4	$86x10^5$	$100x10^5$	$57x10^5$	$89x10^5$	$94x10^5$	$87x10^5$
6	$70x10^5$	$30x10^5$	$87x10^5$	$40x10^5$	$56x10^5$	$69x10^5$
8	$88x10^5$	$69x10^5$	$65x10^5$	$32x10^5$	$44x10^5$	$50x10^5$
10	$45x10^5$	$42x10^5$	$31x10^5$	$60x10^5$	$54x10^5$	$64x10^5$
12	$38x10^5$	$71x10^5$	$35x10^5$	$42x10^5$	$48x10^5$	$52x10^5$
14	$31x10^5$	$50x10^5$	$32x10^5$	$50x10^5$	$49x10^5$	$56x10^5$
16	$59x10^4$	$67x10^4$	$34x10^5$	$30x10^5$	$41x10^5$	$50x10^5$
18	$67x10^4$	$40x10^4$	$50x10^4$	$48x10^5$	$44x10^5$	$61x10^5$
20	$49x10^4$	$46x10^4$	$30x10^4$	$60x10^4$	$54x10^5$	$58x10^5$
22	$51x10^4$	$39x10^4$	$48x10^4$	$49x10^4$	$64x10^4$	$40x10^5$
24	$39x10^4$	$40x10^4$	$65x10^3$	$32x10^5$	$56x10^4$	$60x10^5$
26	$41x10^4$	$33x10^3$	$48x10^3$	$39x10^4$	$44x10^4$	$42x10^5$
28	$39x10^4$	$36x10^3$	$37x10^3$	$35x10^4$	$39x10^3$	$32x10^4$
30	$43x10^4$	$34x10^3$	$32x10^3$	$41x10^3$	$33x10^3$	$47x10^3$

D.3 Linear regressions

Figure A.6. Reduction in *E. coli* CSH-62 population in the faeces of live snails.



Note. Each point represents the mean of three replicates. Error bars indicate standard deviations at each sampling time

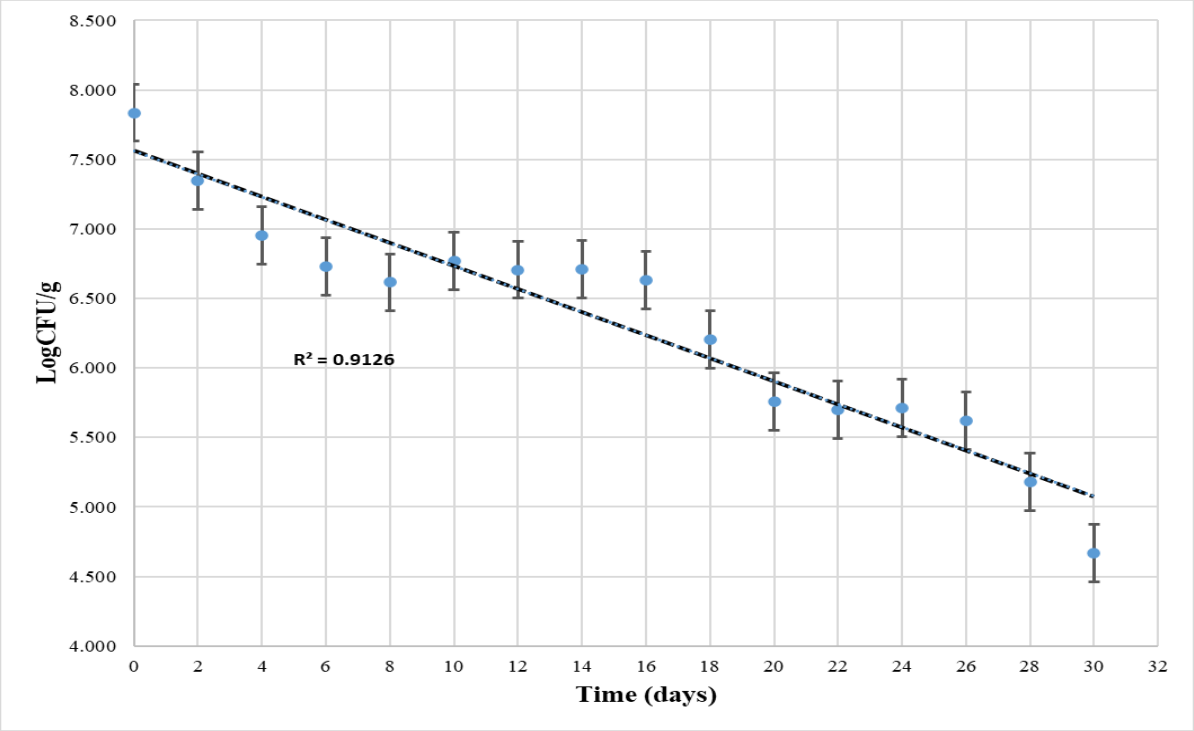


Figure A.7. Reduction in *E. coli* ERL 06-2503 population in the faeces of live snails Each point represents the mean of three replicates.

Note. Error bars indicate standard deviations at each sampling time