About the Author
Dr. Caly is a senior medical scientist at the Peter Doherty Institute of Infection and Immunity in Melbourne, Australia. He is currently working toward validating whole-genome sequencing methodologies targeting viral pathogens for implementation into a public health diagnostic service.

References

Address for correspondence: Leon Caly, Victorian Infectious Diseases Reference Laboratory, Peter Doherty Institute for Infection and Immunity, 792 Elizabeth St, Melbourne, VIC 3000, Australia; email: leon.caly@mh.org.au

Public Health Risk of Foodborne Pathogens in Edible African Land Snails, Cameroon

Mary Nkongho Tanyitiku, Graeme Nicholas, Igor C. Njombissie Petcheu, Jon J. Sullivan, Stephen L.W. On


DOI: https://doi.org/10.3201/eid2808.220722

In tropical countries, land snails are an important food source; however, foodborne disease risks are poorly quantified. We detected Campylobacter spp., Yersinia spp., Listeria spp., Salmonella spp., or Shiga-toxigenic Escherichia coli in 57%–86% of snails in Cameroon. Snail meat is a likely vector for enteric diseases in sub-Saharan Africa countries.

African land snails (Achatina achatina, Achatina fulica, Archachatina marginata) are a source of food for many persons in sub-Saharan Africa (1-5). Snail meat contains 37%–51% protein, which is higher than the protein content in poultry (18.3%), fish (18.0%), cattle (17.5%), sheep (16.4%), and swine (14.5%) (1,2,5).

In rural settings, commercial snail farming is uncommon. Rural dwellers may spend up to 20 hours a week in search of edible snails in environments that include marshes, decaying vegetation, domestic wastes, roadsides, footpaths, and bushes (2,4-6). Those local practices of collecting, handling, and consuming snails could expose handlers and consumers to foodborne pathogens.

Although several studies (2,3,6) have highlighted the close association of edible snails with pathogenic microorganisms, their potential contribution to the burden of foodborne diseases in Africa has been overlooked. In Cameroon, no data on foodborne pathogens in snail meat are available, and their role in causing enteric diseases in the local population is unknown. Our study assessed the prevalence of potential foodborne pathogens in African land snails consumed in Buea, Cameroon.

We collected live snails from 3 locations (in persons’ homes, on arable land, and in local markets) during June–October 2019. We sampled within persons’ homes from 9 PM to 5 AM on rainy nights and on arable land during the day. In Buea, live snails are
found actively moving around at night, and during the day, they usually are present underneath decaying vegetation in farmlands (7). We purchased samples from local markets weekly from snail vendors. Our choice of these sampling locations emerged from participants’ responses 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?”; and “If you are to teach your daughter on how to get snails, what will you teach her?” (7)

We collected live snails weekly from the 3 locations and stored them at room temperature in a laboratory in 2-L sterile Sistema containers (Sistema Plastics, https://www.sistemaplastics.com). We aseptically collected the feces of 6–12 edible snails/sample within 12–18 hours, pooled them, and placed them in 15-mL sterile tubes manufactured by Eppendorf (https://corporate.eppendorf.com). We then stored the samples at –80°C before DNA extraction. We then stored DNA extracts at 4°C before airfreighting them to Lincoln University (Christchurch, New Zealand), for PCR analysis. We examined for the presence of Shiga toxin–producing Escherichia coli, Campylobacter spp., Salmonella spp., Listeria spp., and Yersinia spp. by using a high-fidelity DNA polymerase (repliQa HiFi toughmix; Quantabio, https://www.quantabio.com) (Appendix, https://wwwnc.cdc.gov/EID/article/28/8/22-0722-App1.pdf). We validated PCR methods in-house by using authenticated reference strains as positive and negative controls and then detecting them by electrophoresis. We recorded the presence of an amplicon of the appropriate size for each PCR in each sample as a positive result. For Shiga toxin–producing Escherichia coli, a positive result required the detection of both stx1 and stx2 genes. These criteria determined the occurrences of each pathogen in the samples (Table; Figure).

We detected ≥1 pathogen in every sample examined; most samples contained multiple pathogens. We also calculated the prevalence of each pathogen within the 3 sampling locations (Figure). The overall pathogen prevalence among the samples examined was high, ranging from 57% to 86%.

Although detailed information regarding the consumption of snail meat is not available in Cameroon, live snails are sold in almost every local market in the country (8). As for other sub-Saharan countries, an increase in the demand for snail meat has

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**Table.** Frequency of pathogens detected by PCR in African land snails, Buea, Cameroon, June–October 2019*

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<tbody>
<tr>
<td>Frequency, %</td>
<td>57</td>
<td>75</td>
<td>69</td>
<td>86</td>
<td>71</td>
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</table>

*STEC, Shiga toxin–producing Escherichia coli.*
prompted the random collection of edible snails from locations that could be considered unhygienic (2,3,6). Our results identify the public health risks in the handling and consumption of raw or undercooked edible snails collected from natural habitats in Cameroon. Similar pathogenic microorganisms have been isolated in edible snails consumed in Nigeria (2) and Ghana (3,6).

Moreover, the pathogens isolated in this study are associated with many foodborne outbreaks in developed countries such as the United States (9). Higher prevalences 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 (3,6). Although previous studies highlighted that the local residents believed their practices of snail washing with aluminum sulfate or salt and lime in addition to boiling and then stewing could kill all microorganisms (3,7), Akpomie et al. (2) described substantial bacterial loads in snail meat after boiling, frying, smoking, and oven drying in Nigeria. Thus, our results strongly suggest that foodborne outbreaks from edible snail consumption may be occurring, but are unidentified, in Cameroon, and probably other sub-Saharan Africa countries. The situation 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 practices (7,10).

The New Zealand Aid Programme provided financial support during sample collection and analysis. M.N.T is the grateful recipient of a New Zealand Aid Scholarship.

### References


### About the Author

Ms. Tanyitiku is currently finishing her doctoral studies at Lincoln University, New Zealand. In combination with her experiences in food process engineering, her research interests are in the food safety of locally produced foods.

Address for correspondence: Stephen L.W. On, Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, PO Box 85084, Lincoln University, RFH Bldg, Rm 081, Lincoln 7647, New Zealand; email: stephen.on@lincoln.ac.nz
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Appendix

Detailed Methods

DNA Extraction

The manufacturers guidelines of the Presto stool gDNA extraction kit were followed. 200mg of snail feces was centrifuged at 8000 g 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°C for 5 min. The mixture was centrifuged at 16000 g for 3 minutes and a clear supernatant of 500μl of 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 sec. 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 16000 g for 2mins to obtain the eluted DNA.

PCR Amplification

PCR reactions and cycling conditions (Table 1) were performed on a 96-well GenePro thermocycler (BIOER technology, England). Each reaction mixture was prepared in a volume of 20 μl, consisting of 2μl 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 MgCl₂, dNTP’s and proprietarily formulated HiFi polymerase, hot start antibodies and ToughMix chemistry (repliQa Hifi toughmix: Quantabio, MA, USA).

Table 1 presents the PCR primers and optimal conditions. 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. The 16S rRNA gene (see Table 1) 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 Appendix Figure.

**Gel Electrophoresis**

Each electrophoretic setup is composed of 0.8% agarose gel stained with 2µl SYBR Safe. 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. 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).

**References**


### Appendix Table. Single specific PCR primers and optimized conditions used in the laboratory analyses

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Gene</th>
<th>Primer name</th>
<th>Primer sequence 5′→ 3′</th>
<th>Product size</th>
<th>Cycle conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEC</td>
<td>Stx1</td>
<td>Stx1-ET-F</td>
<td>CATTACAGACTATTTCATCAGGAGGT</td>
<td>68</td>
<td>95°C / 4 min, 95°C / 10 s, 60°C / 5 min, 72°C / 2 s, 72°C / 2 min, 100°C / 1 min, 35 cycles, cycling time: 37 min</td>
<td>Kawase et al. (1)</td>
</tr>
<tr>
<td></td>
<td>Stx1</td>
<td>Stx1-ET-R</td>
<td>CAAATTATCCCCTGAGCCACTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stx2</td>
<td>stx2-ET-F</td>
<td>CATGACAACGGACAGCATATT</td>
<td>114</td>
<td>95°C / 4 min, 95°C / 10 s, 60°C / 5 min, 72°C / 2 s, 72°C / 2 min, 100°C / 1 min, 35 cycles, cycling time: 37 min</td>
<td>Kawase et al. (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stx2-ET-R</td>
<td>AACTCCATTAACGAGATATG</td>
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<tr>
<td>C. jejuni/coll</td>
<td>16S rRNA*</td>
<td>CCCJ609F</td>
<td>AAT CTA ATG GCT TAA CCA TTA</td>
<td>854</td>
<td>94°C/5mins, 94°C/1min, 55°C/1min, 72°C/1min, 72°C/7mins, 10°C/1min, 25cycles, cycling time: 1h44mins</td>
<td>Linton et al. (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCCJ1442R</td>
<td>GTA ACT AGT TTA GTA TTA CCC</td>
<td></td>
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<tr>
<td>Positive control</td>
<td>16S rRNA</td>
<td>16SF</td>
<td>CCAGACTCTACGGAGCGCAG</td>
<td>203</td>
<td>95°C / 4 min, 95°C / 10 s, 60°C / 5 min, 72°C / 2 s, 72°C / 2 min, 100°C / 1 min, 35 cycles, cycling time: 37 min</td>
<td>Chakravorty et al. (3)</td>
</tr>
<tr>
<td>Listeria spp</td>
<td>hly</td>
<td>Lm-hly-F</td>
<td>GGGAAATCTGTCAGGTGATGT</td>
<td>72</td>
<td>95°C / 4 min, 95°C / 10 s, 60°C / 5 min, 72°C / 2 s, 72°C / 2 min, 100°C / 1 min, 35 cycles, cycling time: 37 min</td>
<td>Kawase et al. (1)</td>
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<td></td>
<td></td>
<td>Lm-hly-R-kai</td>
<td>GTAAATTACGGGCTTGAAGGAAGA</td>
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<tr>
<td>Salmonella spp</td>
<td>Nested</td>
<td>Sal1-F</td>
<td>GTA GAA ATT CCC AGC GGG TAG TG</td>
<td>438</td>
<td>95°C / 3 min, 95°C / 30 s, 60°C / 1 min, 72°C / 1.5 min, 72°C / 10 min, 100°C / 1 min, 20 cycles, cycling time: 2 h 40 min</td>
<td>Waage et al. (4)</td>
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<tr>
<td></td>
<td></td>
<td>Sal2-R</td>
<td>GTA TCC ATC TAG CCA ACC ATT GC</td>
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<td></td>
<td>Sal3-F</td>
<td>TTT GGC ACT ATG TTA CCG TGG</td>
<td>312</td>
<td>95°C/3mins, 95°C/30secs, 67°C/1min, 72°C/2secs, 72°C/1.5mins, 10°C/1min, 40cycles, cycling time: 1h44mins</td>
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<tr>
<td></td>
<td>Sal4-R</td>
<td>AGC CAA CCA TTG CTA AAT TGG GC</td>
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<tr>
<td>Yersinia spp</td>
<td>16S rRNA**</td>
<td>LandzY1</td>
<td>GGAATTAGCAGAGATTATAAGTTTA</td>
<td>300</td>
<td>94°C / 5 min, 94°C / 30 s, 580°C / 30 min, 72°C / 40 min, 72°C / 7 min, 100°C / 1 min, 30 cycles, cycling time: 1 h 21 min</td>
<td>Landz et al. (5)</td>
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<tr>
<td></td>
<td></td>
<td>LandzY2</td>
<td>GGACTACGCGACAGCTTTATCT</td>
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</table>
**Appendix Figure.** Specific bands at optimized PCR conditions. Lane L: 1kb plus DNA ladder, Lane 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*. 