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References

1. Armstrong C, Lillie RD. Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St. Louis encephalitis epidemic. Public Health Reports (1896–1970). 1934;49:1019–27.
2. Lewis JM, Utz JP. Orchitis, parotitis and meningoencephalitis due to lymphocytic-choriomeningitis virus. N Engl J Med. 1961;265:776–80. https://doi.org/10.1056/NEJM196110192651604
3. Palacios G, Druce J, Du L, Tran T, Birch C, Briese T, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. N Engl J Med. 2008;358:991–8. https://doi.org/10.1056/NEJMoa073785
4. Bonthius DJ, Wright R, Tseng B, Barton L, Marco E, Karacay B, et al. Congenital lymphocytic choriomeningitis virus infection: spectrum of disease. Ann Neurol. 2007;62:347–55. https://doi.org/10.1002/ana.21161
5. Gregg MB. Recent outbreaks of lymphocytic choriomeningitis in the United States of America. Bull World Health Organ. 1975;52:549–53.
6. Holdsworth RL, Downie E, Georgiades MJ, Bradbury R, Druce J, Collett J. Lymphocytic choriomeningitis virus in western New South Wales. Med J Aust. 2022;216:71–2. https://doi.org/10.5694/mja2.51383
7. Kafetzopoulou LE, Efthymiadis K, Lewandowski K, Crook A, Carter D, Osborne J, et al. Assessment of metagenomic Nanopore and Illumina sequencing for recovering whole genome sequences of chikungunya and dengue viruses directly from clinical samples. Euro Surveill. 2018;23:23. https://doi.org/10.2807/1560-7917.ES.2018.23.50.1800228
8. Gabriel SI, Stevens MI, Mathias Mda L, Searle JB. Of mice and 'convicts': origin of the Australian house mouse, Mus musculus. PLoS One. 2011;6:e28622. doi:10.1371/journal.pone.0028622
9. Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 2018;4:vey016. https://doi.org/10.1093/ve/vey016
10. Albariño CG, Palacios G, Khristova ML, Erickson BR, Carroll SA, Comer JA, et al. High diversity and ancient common ancestry of lymphocytic choriomeningitis virus. Emerg Infect Dis. 2010;16:1093–100. https://doi.org/10.3201/eid1607.091902
11. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37:1530–4. https://doi.org/10.1093/molbev/msaa015

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

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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

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

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

*STEC, Shiga toxin–producing *Escherichia coli*.

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 air freighting 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

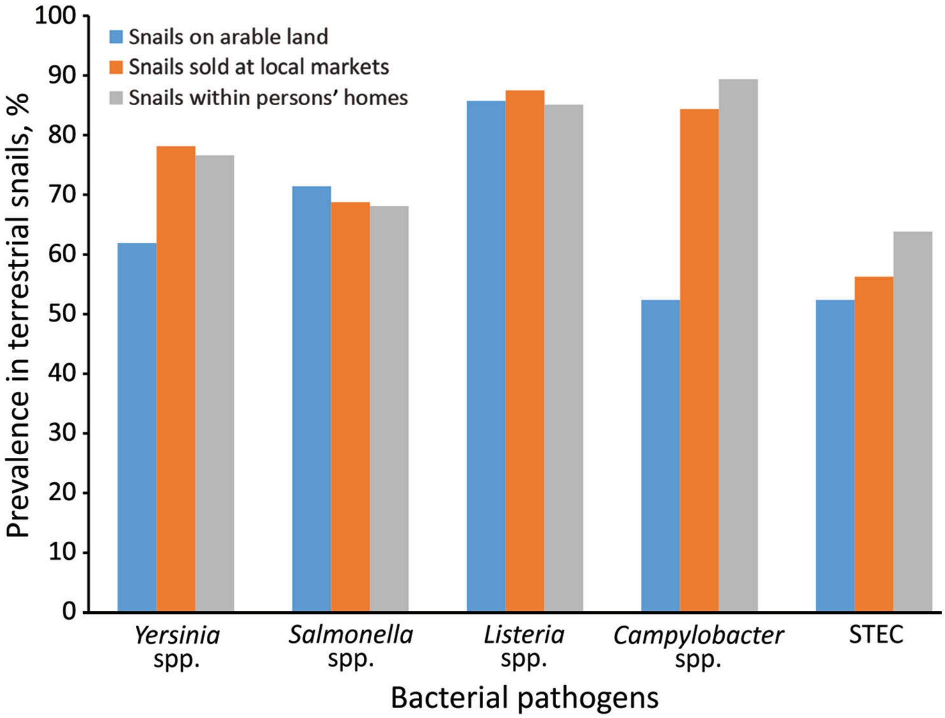

Figure. Prevalence of foodborne pathogens in land snails sampled in 3 selected locations, Buea, Cameroon. June–October 2019. STEC, Shiga toxin–producing *Escherichia coli*.

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*).

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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.

References

1. Adeyeye SAO, Bolaji OT, Abegunde TA, Adesina TO. Processing and utilization of snail meat in alleviating protein malnutrition in Africa: a review. Nutr Food Sci. 2020;50:1085–97. https://doi.org/10.1108/NFS-08-2019-0261
2. Akpomie OO, Akponah E, Onoharigho I, Isiakpere OP, Adewuyi IS. Microbiological analysis and nutritional constituents of *Achatina achatina* subjected to various cooking methods. Nigerian Journal of Microbiology. 2019;33:4415–22.
3. Barimah MNYS. Microbiological quality of edible land snails from selected markets in Ghana: MPHIL Thesis; Department of Microbiology, University of Ghana Medical School, Ghana; 2013 [cited 2020 Jun 17]. http://ugspace.ug.edu.gh/handle/123456789/23446
4. Mohammed S, Ahmed A, Adjei D. Opportunities for increasing peasant farmers income through snail production in Ghana Sch J Agric. Vet Sci. 2014;1:195–200.
5. Ndah NR, Celestine FCL, Chia EL, Enow EA, Yengo T, Ngwa AD. Assessment of snail farming from selected villages in the Mount Cameroon Range, South West Region of Cameroon. Asian Research Journal of Agriculture. 2017;6:1–11. https://doi.org/10.9734/ARJA/2017/35113
6. Nyoagbe LA, Appiah V, Josephine N, Daniel L, Isaac A. Evaluation of African giant snails (*Achatina* and *Archachatina*) obtained from markets (wild) and breeding farms. Afr J Food Sci. 2016;10:94–104. https://doi.org/10.5897/AJFS2015.1320
7. Tanyitiku MN, Nicholas G, Sullivan JJ, Njombissie Petcheu IC, On SLW. Snail meat consumption in Buea, Cameroon: the methodological challenges in exploring its public health risks. Int J Qual Methods. 2022;21:1–12. https://doi. org/10.1177/16094069221078132
8. Meffowoet CP, Kouam KM, Kana JR, Tchakounte FM. Infestation rate of African giant snails (*Achatina fulica* and *Archachatina marginata*) by parasites during the rainy season in three localities of Cameroon. Journal of Veterinary Medicine and Research. 2020;7:1–8.
9. Centers for Disease Control and Prevention. Reports of selected *E. coli* outbreak investigations, 2021 [cited 2022 Mar 12]. https://www.cdc.gov/ecoli/outbreaks.html
10. Kaldjob MC, Enangue NA, Siri BN, Etchu K. Socio-economic perception of snail meat consumption in Fako Division, South-West Region Cameroon. Int J Livest Prod. 2019;10:143–50. https://doi.org/10.5897/IJLP2018.0543

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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 $MgCl_2$, 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

1. Kawase J, Etoh Y, Ikeda T, Yamaguchi K, Watahiki M, Shima T, et al. Yamaguch i, Watahiki M, Shima T. An improved multiplex real-time SYBR Green PCR assay for the analysis of 24 target genes from 16 bacterial species in fecal DNA samples from patients with foodborne illnesses. Jpn J Infect Dis. 2016;69:191–201. PubMed https://doi.org/10.7883/yoken.JJID.2015.027

2. Linton D, Lawson AJ, Owen RJ, Stanley J. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J Clin Microbiol. 1997;35:2568–72. PubMed https://doi.org/10.1128/jcm.35.10.2568-2572.1997

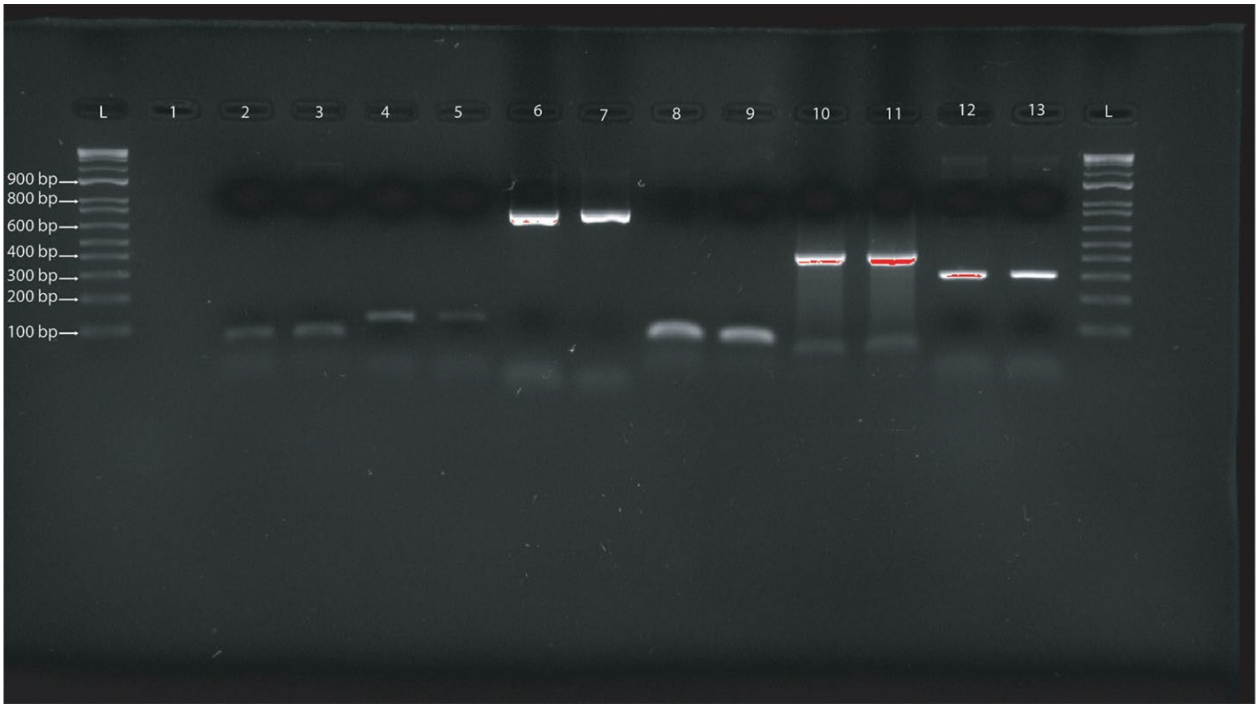
3. Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. J Microbiol Methods. 2007;69:330–9. PubMed https://doi.org/10.1016/j.mimet.2007.02.005

4. Waage AS, Vardund T, Lund V, Kapperud G. Detection of low numbers of *Salmonella* in environmental water, sewage and food samples by a nested polymerase chain reaction assay. J Appl Microbiol. 1999;87:418–28. PubMed https://doi.org/10.1046/j.1365-2672.1999.00835.x

5. Lantz P-G, Knutsson R, Blixt Y, Al Soud WA, Borch E, Rådström P. Detection of pathogenic *Yersinia enterocolitica* in enrichment media and pork by a multiplex PCR: a study of sample preparation and PCR-inhibitory components. Int J Food Microbiol. 1998;45:93–105. PubMed https://doi.org/10.1016/S0168-1605(98)00152-4

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

Pathogen	Gene	Primer name	Primer sequence 5′→ 3′	Product size	Cycle conditions	References
STEC	*Stx1*	Stx1-ET-F stx1-ET-R	CATTACAGACTATTTCATCAGGAGGT CAAATTATCCCCTGAGCCACTA	68	950C / 4 min, 950C / 10 s, 600C / 5 min, 720C / 2 s, 720C / 2 min, 100C / 1 min, 35 cycles, cycling time: 37 min	Kawase et al. (*1*)
	Stx2	stx2-ET-F stx2-ET-R	CATGACAACGGACAGCAGTTAT AACTCCATTAACGCCAGATATGA	114	950C / 4 min, 950C / 10 s, 600C / 5 min, 720C / 2 s, 720C / 2 min, 100C / 1 min, 35 cycles, cycling time: 37 min	Kawase et al. (*1*)
C. jejuni/coli	16S rRNA*	CCCJ609F CCCJ1442R	AAT CTA ATG GCT TAA CCA TTA GTA ACT AGT TTA GTA TTC CGG	854	94°C/5mins, 94°C/1min, 55°C/1min, 72°C/1min, 72°C/7mins, 10°C/1min, 25cycles, cycling time: 1h44mins	Linton et al. (*2*)
Positive control	16S rRNA	16SF 16SR	CCAgACTCCTACGGGAGGCAG CGTATTACCGCGGCTGCTG	203	950C / 4 min, 950C / 10 s, 600C / 5 min, 720C / 2 s, 720C / 2 min, 100C / 1 min, 35 cycles, cycling time: 37 min	Chakravorty et al. (*3*)
Listeria spp	hly	Lm-hly-F Lm-hly-R-kai	GGGAAATCTGTCTCAGGTGATGT GTAAATTACGGCTTTGAAGGAAGA	72	950C / 4 min, 950C / 10 s, 600C / 5 min, 720C / 2 s, 720C / 2 min, 100C / 1 min, 35 cycles, cycling time: 37 min	Kawase et al. (*1*)
Salmonella spp	Nested	Sal1-F Sal2-R	GTA GAA ATT CCC AGC GGG TAC TG GTA TCC ATC TAG CCA ACC ATT GC	438	950C / 3 min, 950C / 30 s, 600C / 1 min, 720C / 1.5 min, 720C / 10 min, 100C / 1 min, 20 cycles, cycling time: 2 h 40 min	Waage et al. (*4*)
		Sal3-F Sal4-R	TTT GCG ACT ATC AGG TTA CCG TGG AGC CAA CCA TTG CTA AAT TGG CGC	312	95°C/3mins, 95°C/30secs, 67°C/1min, 72°C/2secs, 72°C/1.5mins, 10°C/1min, 40cycles, cycling time: 1h44mins	
Yersinia spp	16S rRNA**	LandzY1 LandzY2	GGAATTTAGCAGAGATGCTTTA GGACTACGACAGACTTTATCT	300	940C / 5 min, 940C / 30 s, 580C / 30 min, 720C / 40 min, 720C / 7 min, 100C / 1 min, 30 cycles, cycling time: 1 h 21 min	Landz et al. (*5*)



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.*