Occurrence of selected pathogenic microorganisms in raw and processed eggs of snails of the *Cornu* genus

Monika Maćkowiak-Dryka[✉], Michał Gondek, Krzysztof Szkucik

Department of Food Hygiene of Animal Origin, Faculty of Veterinary Medicine,
University of Life Sciences in Lublin, 20-950 Lublin, Poland
monika.mackowiak@up.lublin.pl

Received: July 12, 2021   Accepted: December 6, 2021

Abstract

Introduction: This study investigated the eggs of Polish-bred edible snails of the *Cornu* genus as a food and aimed to determine the presence of microorganisms in them of the *Salmonella* and *Listeria* genera and ascertain the number of coagulase-positive staphylococci. **Material and Methods:** Raw material, semi-finished products, and the final product were collected during the production cycle. Testing for the presence of *Salmonella* spp. and *Listeria* spp. and measuring of the pathogenic staphylococci contamination level were carried out in accordance with ISO standards. Commercial biochemical tests were used for species identification of bacteria of the *Enterobacteriaceae* family and *Staphylococcus* genus. An API kit and a PCR protocol were utilised for species confirmation of the microorganisms of the *Listeria* genus. **Results:** Neither *Salmonella* nor coagulase-positive staphylococci were found in any of the studied material. Bacteria of the *Listeria* genus were found in samples taken at every stage of production; however *L. monocytogenes* was confirmed in samples of the final product. **Conclusion:** The absence of *Salmonella* spp. and *Staphylococcus aureus* in samples of the final product indicates that the required hygiene standard was maintained in the production process of edible snail eggs. Nevertheless, the presence of *L. monocytogenes* in eggs of common garden snails may pose a potential risk to consumer health.

Keywords: snail eggs, *Cornu aspersum*, *Salmonella* spp., *Listeria* spp., coagulase-positive staphylococci.

Introduction

Land snails of the *Cornu* genus (common garden snails) are popular edible molluscs. They yield edible material as meat and eggs. Due to constant consumer demand and economic advantages, the dominant resource is meat (12). The eggs of common garden snails, on the other hand, when not intended for hatching are exploited for the production of a caviar substitute known as “white caviar”. These salted eggs constitute an alternative to caviar and due to their limited production, sensory and nutritional value, and relatively high price, they are regarded as an exclusive delicacy (4, 5, 6).

Diverse bacterial diseases of snails of the *Cornu* genus can be the cause of biological contamination of the raw material, at the same time affecting its food suitability and the safety of the final products for their consumers’ health.

One of the main microbiological criteria used in assessing the safety of individual types of food is the detection of no *Salmonella* in 25 g of the final product. The natural occurrence of bacilli of *Salmonella* in the

environment of common garden snail farms and in the production area necessitates studies on the occurrence of these bacteria in the entire production chain of edible eggs of this species. According to Regulation (EC) 2073/2005 (2), the only food safety criterion applying to consumable eggs of common garden snails (ready-to-eat foods) concerns *Listeria monocytogenes*, for which two limits have been established: that *L. monocytogenes* is undetectable in 25 g or its content is lower than 100 CFU/g. Another microorganism frequently isolated from both healthy and diseased common garden snails is *Staphylococcus aureus*. Coagulase-positive staphylococci contamination assessment of samples of the material taken at every stage of the caviar substitute production process will allow the safety of this type of food and the hygiene conditions of its production to be evaluated. The available literature lacks data regarding the microbiological quality of caviar substitute produced from eggs of common garden snails.

The aims of this study were to determine the occurrence of microorganisms of the *Salmonella* and *Listeria* genera and assess the number of coagulase-positive

staphylococci in raw eggs (harvested from two edible subspecies of common garden snails of the *Cornu* genus bred in Poland), semi-finished products, and the final product (substitute caviar) obtained from these eggs.

Material and Methods

Material for the study consisted of 50 samples taken at three stages of the snail caviar production cycle, namely raw material (20 samples), semi-finished product (20 samples), and final product (10 samples). The raw material included eggs of two subspecies of edible snails: the small common garden snail (*Cornu aspersum aspersum*), and the large common garden snail (*Cornu aspersum maxima*). The eggs were harvested directly after being laid by the snails into breeding cups filled with soil. The semi-finished product consisted of eggs of both subspecies that underwent initial processing in the form of manual segregation and repeated rinsing with cold water. The final product, *i.e.* caviar substitute marketed under the name of “Snail Eggs”, was a small glass jar containing 50 g of processed eggs of both snail subspecies of the *Cornu* genus. Immediately after collection, the samples were transported under cold storage conditions (at a temperature of 0°C to +4°C) to a laboratory, where tests for determining the presence of *Salmonella*, *Listeria monocytogenes*, and the number of coagulase-positive staphylococci were performed.

Microbiological analysis. The procedure for the detection of *Salmonella* was based on the protocol described in the PN-EN ISO 6579-1:2017 standard (14). Non-selective enrichment was carried out in buffered peptone water (catalogue no. PS52, BioCorp, Warsaw, Poland), and selective enrichment by using Rappaport–Vassiliadis soy broth (catalogue no. PS65, BioCorp), and Mueller–Kauffman tetrathionate medium with novobiocin and brilliant green broth base (catalogue no. PS147, Biocorp). The following step used selective agar growth media with xylose and lysine (X.L.D. LAB-AGAR, catalogue no. PS45, Biocorp) and with brilliant green and phenol red (B.G.A. LAB-AGAR, catalogue no. PS98, Biocorp). The biochemical profile of the isolated strains was based on their ability or inability to ferment glucose, lactose, and saccharose, produce H₂S (triple sugar iron LAB-AGAR, catalogue no. PS44, Biocorp) and urease (urea LAB-AGAR, catalogue no. PS211, Biocorp), and decarboxylate lysine (catalogue no. P-0129; BTL, Łódź, Poland). Optional tests were also performed to determine the ability to produce β-galactosidase using an O-nitrophenyl-beta-D-galactopyranoside (ONPG) test with an ONPG disc (catalogue no. DD 0013, Oxoid, Basingstoke, UK), and the ability to produce indole, assayed with peptone water with tryptophan (catalogue no. P-0078, BTL) and Kovacs reagent (catalogue no. C-018, BTL). In addition, an ENTEROtest 24N commercial identification kit (catalogue no. 10020290, Erba Lachema, Brno, Czech Republic) was used to identify bacteria from the

Enterobacteriaceae family in a Multiskan EX automatic microplate reader (catalogue no. 1507300, Thermo Fisher Scientific, Vantaa, Finland) and with TNW Pro Auto 6.5 software (catalogue no. 50002056, Erba Lachema).

The detection procedure for *Listeria monocytogenes* was carried out in accordance with the PN-EN ISO 11290-1:2017 standard (13). Primary enrichment was conducted using half Fraser broth (catalogue no. PS100, Biocorp), and secondary enrichment using Fraser broth (catalogue no. PS101, Biocorp). Agar *Listeria* according to Ottaviani & Agosti growth medium (chromogenic *Listeria* LAB-AGAR, catalogue no. PS165, Biocorp) and Oxford medium (Oxford LAB-AGAR, catalogue no. PS104, Biocorp) were used as selective media. The isolated microorganisms were classified as belonging to the *Listeria* genus, based on their typical morphological features (they were Gram-positive bacilli), positive results of catalase tests (H₂O₂, catalogue no. 118851934, Chempur, Piekary Śląskie, Poland), and motility (medium for testing movement ability of bacteria, catalogue no. P-0238, BTL). The next step involved determining the haemolytic properties of the strains (Columbia LAB-AGAR base, catalogue no. PS06, Biocorp) and their ability to ferment xylose and ramnose (xylose and ramnose broth, catalogue nos. 3000 and 3001, Biocorp). For the purpose of biochemical identification of bacteria from the *Listeria* genus an API *Listeria* kit (catalogue no. 10300, bioMérieux, Marcy-l'Étoile, France) was used in accordance with the manufacturer's instructions. Additionally, to confirm the presence of *L. monocytogenes*, a PCR technique was introduced. The procedure entailed the use of a DNA-Genomic Mini AX bacteria isolation kit (catalogue no. 060-60, A&A Biotechnology, Gdynia, Poland), a *Listeria monocytogenes* amplification PCR kit (catalogue no. K 069, Genekam Biotechnology, Duisburg, Germany), and Serva agarose gel (catalogue no. 11404, Serva Electrophoresis, Heidelberg, Germany). The electrophoretic separation was carried out in a 15-well Mini-Sub Cell GT System horizontal electrophoresis device (Bio-Rad Laboratories, Hercules, CA, USA) using a DNA Marker 1 size marker (from 100 to 1000 bp) (catalogue no. 3000-500, A&A Biotechnology, Gdynia, Poland).

The level of pathogenic staphylococci contamination was determined according to the method described in the PN-EN ISO 6888-1:2001 standard (15). The procedure involved the use of Baird-Parker LAB-AGAR growth medium (catalogue no. PS33, Biocorp), brain heart infusion broth LAB-AGAR, (catalogue no. PS04, Biocorp) and rabbit coagulase plasma (catalogue no. PL.850-3, Pro-Lab Diagnostics, Bromborough, UK) in order to assess the possibility of coagulase production by the isolated strains. Finally, a STAPHYtest 24 commercial biochemical kit (catalogue no. 10010233, Erba Lachema) was used to identify the *Staphylococcus* species.

Statistical analysis. The obtained results were analysed statistically with SAS Enterprise Guide 5.1

(SAS Institute, Cary, NC, USA) and expressed as arithmetic means and standard deviation. The normal distribution in each group was examined with the Shapiro–Wilk test. Levene’s test determined the homogeneity of their variance. The influence of each variability factor on the determined parameters was established using the one-way analysis of variance (ANOVA) for groups for which the assumption of homogeneous variances was fulfilled, and Tukey’s test was used as post-hoc analysis. Statistical significance was assumed at $P \leq 0.05$.

Results

Bacilli of the *Salmonella* genus were not found in any of the 25 g samples of raw material, semi-finished products, or samples of the final “Snail Eggs” product of either snail species.

Bacteria of the *Listeria* genus were found in 25 g samples taken at every stage of caviar substitute production. The origin species and number of common garden snail egg samples that contained *Listeria* spp. are presented in Table 1.

Table 1. The origin species and number of common garden snail egg samples in which *Listeria* spp. were detected

	Raw material	Semi-finished product	Final product “Snail Eggs” (n=10)
CAA eggs (n=10)	4	8	8
CAM eggs (n=10)	0	9	

CAA – *Cornu aspersum aspersum* (small common garden snail); CAM – *Cornu aspersum maxima* (large common garden snail)

Table 2. Level of contamination log₁₀ (CFU/g) with bacteria of the *Staphylococcus* genus in common garden snail eggs, depending on production stage and origin species

	Raw material	Semi-finished product	Final product “Snail Eggs” (n=10)
CAA eggs (n=10)	4.27 ± 0.01	4.17 ± 0.03	Not detected
CAM eggs (n=10)	4.29 ± 0.004	4.14 ± 0.03	

CAA – *Cornu aspersum aspersum* (small common garden snail); CAM – *Cornu aspersum maxima* (large common garden snail)

No significant differences emerged between the eggs from the two snail subspecies nor between the product categories, $P < 0.05$

In the raw eggs of the common garden snail, *Listeria* bacteria were found in 4 samples from the small common garden snail (CAA), whereas in 10 samples of raw large common garden snail eggs (CAM) no occurrence of this microorganism was confirmed. In the CAA semi-finished product, *Listeria* spp. were confirmed in 8 samples, and in the CAM semi product in 9 samples. Samples of the final product revealed *Listeria* spp. in 8 out of 10.

Based on the biochemical test using the API *Listeria* kit, *Listeria monocytogenes* was confirmed only

in six samples of the final product in which microorganisms of the *Listeria* genus had initially been detected. Strains isolated from one of these six samples of the final product were subjected to PCR analysis, which confirmed that they belonged to the *L. monocytogenes* species. In the remaining samples of the final product, the API *Listeria* test confirmed the presence of *L. innocua*. There was no *L. monocytogenes* found in raw product or semi-finished product samples; however, *L. innocua* was demonstrated in these samples.

The number of *Staphylococcus* bacteria in raw product, semi product, and final product samples is presented in Table 2. The average number of these bacteria in samples of raw product from both origin species was 1.98×10^4 CFU/g (4.28 log CFU/g), and in the semi-finished product this number varied between 1.4×10^4 and 1.5×10^4 CFU/g (4.14–4.17 log CFU/g). There were no statistically significant differences in the level of *Staphylococcus* contamination between the raw material and semi-product either from CAA or from CAM snails. Moreover, no statistically significant differences were found in terms of the number of staphylococci between the CAA raw material and the CAM raw material. Similarly, the species of snail did not influence the level of semi-product contamination with these microorganisms. In addition, *Staphylococcus* spp. were not detected in the samples of the final product. Only coagulase-negative strains were isolated from the samples of raw and preliminarily processed common garden snail eggs. The commercial STAPHYtest 24 biochemical kit most often confirmed the presence of *S. epidermidis* and *S. lentus* in raw product samples, whereas in semi-product samples *S. warneri* and *S. lentus* were the most prevalent species.

Discussion

Bacilli of *Salmonella* are considered a natural component of the microbiota of the environment of farms and production areas of common garden snails and are therefore a health hazard to people (18). These bacteria were found in 84 of 270 examined samples of raw meat of farmed snails of the *Cornu* genus that came from Morocco, and among the isolated serotypes the dominant ones were: *S. Gatuni*, *S. Montevideo*, *S. Newport*, and *S. Bredeney* (1). On the other hand, other authors are of the opinion that controlled breeding of common garden snails decreases the possibility of occurrence of these pathogens in raw snail meat (9, 10). It was confirmed by the studies performed by Parlapani *et al.* (9), who isolated *Salmonella* only from the raw meat and bowels of common garden snails living in the wild.

In the authors’ own research, the presence of *Salmonella* was not confirmed in the eggs of common garden snails of the *Cornu* genus at any studied stage of the production cycle. Nunes Almeida (8) arrived at similar results, having not isolated these microorganisms

from an analogous final product by the name of “Caviar Pérola”. Importantly, studies performed in the 1990s assessing the health status of common garden snails detected *Salmonella* in raw eggs of snails belonging to *Cornu* species (16). These microorganisms were also isolated from diseased snails during their breeding period, and together with the *Streptococcus* spp. and *Arcanobacterium* spp. were considered to be opportunistic bacteria of common garden snails (7).

The present research results suggest that the procedures of the caviar substitute production process do not guarantee inactivation of *L. monocytogenes* in the final product ready for human consumption. Nonetheless, it should be noted that the results of studies performed by other authors did not confirm the presence of *L. monocytogenes* in the final product (8). The absence of this microorganism in samples of raw common garden snail egg does not exclude the possibility of its occurrence in the soil used to fill the breeding cups, which may constitute the primary source of contamination of the environment of the processing plant and, indirectly, of the final product. Literature data confirm that *Listeria* spp. is a regular component of the microflora naturally occurring in the farming environment of snails of the *Cornu* genus and, therefore it should be considered a potential hazard to consumer health (10).

Previous studies recognised *Staphylococcus aureus* as the most common bacterium causing disease in common garden snails (7). This microorganism was also isolated from breeder snails which did not show any visible symptoms of the disease (7). The sources of pathogenic bacteria, including staphylococci which pose a threat during not only the breeding period but also the entire farming cycle, are sites of deficient hygiene in the snails' environment, and rodents, flies, and other insects (3). Therefore, it is vital to implement proper prophylaxis against bacterial diseases principally in measures to disinfect the rooms, tools, tables, and crates used for breeding snails. It is also pivotal to maintain cleanliness during the production process of common garden snail eggs and, most importantly, to purchase healthy breeder snails with appropriate documentation confirming their health status. No pathogenic staphylococci were confirmed in the raw meat of the snails of the *Cornu* genus (18), and among the strains which were isolated from the meat, the dominant ones were coagulase-negative, such as *S. epidermidis*, *S. warneri* and *S. haemolyticus* (11). Similarly, the authors' own research regarding the contamination of common garden snail eggs did not confirm the presence of coagulase-positive staphylococci, and the most frequently isolated strains in this instance also included *S. epidermidis* and *S. warneri*, a third being *S. lentus*. The absence of *S. aureus* in the consumption-ready product was also confirmed in studies performed by other authors (8). As is evident from data in the literature, an increase in staphylococci contamination is noted after every production stage in which processing of snail meat

requires manual operations (17). Such a situation may also pertain during the manual production of consumable snail eggs. However, our research did not confirm this thesis, which may indicate that the appropriate hygiene standard was carried through the production process.

In conclusion, *Salmonella* and *Listeria* bacteria are present in the habitat of edible common garden snails of the *Cornu* genus and therefore may constitute a potential health hazard to the consumer. The absence of bacilli from the *Salmonella* genus in the studied material indicates a high hygiene standard both at the stage of farming of common garden snails and during the harvesting and processing of their eggs. According to Regulation (EC) 2073/2005 (2), the only safety criterion for the studied caviar substitute has regard to *Listeria monocytogenes*, for which the established standard is the detection of none of the microorganism in 25 g of the product or its concentration below 100 CFU/g. However, since the presence of this bacterium was demonstrated in a sample of the final product, it seems justified to use the criterion of the number of bacteria instead of their presence. An appropriately hygienic process for the production of this caviar substitute was confirmed by the absence of coagulase-positive staphylococci (*Staphylococcus aureus*) in the studied material.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The studies and preparation of the manuscript was supported by Grant for Young Scientists no. WKH/MN/1 of the University of Life Sciences in Lublin, Poland.

Animal Rights Statement: None required.

References

1. Andrews W.H., Wilson C.R., Romero A., Poelma P.L.: The Moroccan food snail *Helix aspersa* as a source of Salmonella. Appl Microbiol 1975, 29, 328–330, doi: 10.1128/am.29.3.328-330.1975.
2. Commission of the European Communities: Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 2005, 338, 48, 22/12/2005, 1–26.
3. Ligaszewski M., Pol P.: Selected aspects of the heliciculture (in Polish), Wydawnictwo Instytutu Zootechniki PIB, Kraków, 2019.
4. Maćkowiak-Dryka M., Paszkiewicz W., Szkucik K.: Characteristics and nutritional value of selected caviar products. Med Weter 2020, 76, 690–693, doi: 10.21521/mw.6486.
5. Maćkowiak-Dryka M., Pyz-Łukasik R., Ziomek M., Szkucik K.: Nutritional value of a new type of substitute caviar. Med Weter 2020, 76, 285–288, doi: 10.21521/mw.6404.
6. Maćkowiak-Dryka M., Szkucik K., Pyz-Łukasik R.: Snail eggs as a raw material for the production of a caviar substitute. J Vet Res 2020, 64, 543–547, doi: 10.2478/jvetres-2020-0062.
7. Moncada Reyes F.A., Veloza Martínez P.A., Rodríguez Martínez G., Reyes Herrera L.: Sanitary diagnosis in different snail breeding

- places in Colombia: main pathogen agents affecting the snail *Helix aspersa* (O.F. Muller, 1774) in every phase of its biological process (in Spanish). *Rev Med Vet* 2007, 14, 17–35.
8. Nunes Almeida G.: Caviar Pearl, study of physical-chemical and microbiological stability. Master's Dissertation in Food Security. Faculty of Veterinary Medicine, Technical University of Lisbon, Lisbon, 2014.
  9. Parlapani F.F., Neofitou Ch., Boziaris I.S.: Microbiological quality of raw and processed wild and cultured edible snails. *J Sci Food Agric* 2014, 94, 768–772, doi: 10.1002/jsfa.6438.
  10. Paszkiewicz W., Szkucik K., Ziomek M., Gondek M., Pyz-Lukasik R.: Occurrence of *Salmonella* spp. and *Listeria* spp. in snail meat. *Med Weter* 2018, 74, 110–113, doi: 10.21521/mw.6074.
  11. Paszkiewicz W., Szkucik K., Ziomek M., Pyz-Lukasik R., Drozd Ł., Belkot Z.: Variability of microbial contamination of edible snail meat depending on species and place of their collection. *Med Weter* 2018, 74, 591–598, doi: 10.21521/mw.5970.
  12. Paszkiewicz W., Ziomek M., Szkucik K., Maćkowiak-Dryka M.: Production and quality of snail meat. *Med Weter* 2014, 70, 673–679.
  13. Polish Committee for Standardization: PN-EN ISO 11290-1:2017 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 1: Detection method. Polski Komitet Normalizacyjny, Warsaw, 2017.
  14. Polish Committee for Standardization: PN-EN ISO 6579-1:2017 Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. Polski Komitet Normalizacyjny, Warsaw, 2017.
  15. Polish Committee for Standardization: PN-EN ISO 6888-1:2001 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium. Polski Komitet Normalizacyjny, Warsaw, 2001.
  16. Sadzikowski A., Tomczuk K.: Snails of *Helix* spp. - patients in veterinary practice. *Med Weter* 1997, 53, 630–637.
  17. Temelli S., Dokuzulu C., Cem Sen M.K.: Determination of microbiological contamination sources during frozen snail meat processing stages. *Food Control* 2006, 17, 22–29, doi: 10.1016/j.foodcont.2004.08.004.
  18. Walczak Z., Czerwieńska E.: Microbiological quality of snail meat (*Helix aspersa* MULLER) after initial processing and its changes during freezing storage (in Polish). *Zesz Probl Postęp Nauk Rol* 2011, 569, 285–293.