# FOLIA VETERINARIA

The scientific journal of the
UNIVERSITY OF VETERINARY MEDICINE
AND PHARMACY IN KOŠICE — Slovakia

ISSN 0015-5748
eISSN 2453-7837


2

LXX • 2026


FOLIA VETERINARIA is a scientific journal issued by the University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia.
The journal is published quarterly in English (numbers 1–4) and distributed worldwide.



Contact: e-mail: folia.veterinaria@uvlf.sk

Electronic Publisher: De Gruyter Poland, Bogumila Zuga 32A
01-811 Warsaw, Poland

ISSN 2453-7837 on-line
ISSN 0015-5748 print
EV 3485/09

Publisher's Identification number: IČO 00397474

June 2026

# FOLIA VETERINARIA

PUBLISHED BY
THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE
SLOVAKIA


Folia Veterinaria
Vol. 70, 2, 2026

VYDÁVA
UNIVERZITA VETERINÁRSKEHO LEKÁRSTVA A FARMÁCIE V KOŠICIACH
2026

# FOLIA VETERINARIA, 70, 2, 2026

## CONTENTS

DOI: 10.2478/fv-2026-0011

FOLIA VETERINARIA, 70, 2: 1—9, 2026

ORIGINAL ARTICLE

# A SURVEY OF LOCAL CHEESE CONTAMINATION WITH *STREPTOCOCCI* IN KABUL CITY MARKETS

**Sayed Arif Ahmadi[1]*†, Mohammad Farzad Afshar[2†], Mahyar Mokhatab[3]**

[1]Department of Food Technology and Hygiene, Faculty of Veterinary Sciences, Kabul University, Jamal Mina, Kabul, Afghanistan; [2]Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; [3]Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

OPEN ACCESS

***Correspondence:** sayedarif.ahmadi@yahoo.com

†These authors contributed equally to this work.

**Citation:** Ahmadi, S. A., Afshar, M. F., Mokhatab, M., 2026: A survey of local cheese contamination with *Streptococci* in Kabul city markets. Folia Veterinaria, 70, 2, 1–9.

**Received:** August 16, 2025

**Accepted:** December 30, 2025

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**This study investigated the occurrence and distribution of potentially pathogenic streptococcal species in traditional cheese (semi-hard ripened cheese) sold in Kabul, a topic of significant relevance to veterinary science due to its implications for food safety, zoonotic transmission, and dairy hygiene management. To this end, cheese samples from nine regions of Kabul were analyzed for microbiological quality using Gram staining, catalase testing, hemolysis patterns, and disk sensitivity to optochin and bacitracin, supplemented by limited Analytical Profile Index (API) confirmation. Among 90 samples, 31 (34.4%) exhibited α-hemolysis with optochin sensitivity, suggesting *Streptococcus pneumoniae*, while 25 (27.8%) showed β-hemolysis with bacitracin sensitivity, consistent with Group A *Streptococcus* (GAS). Non-hemolytic isolates (37.8%) predominated overall; API identified *Streptococcus infantarius* in a subset. Results from the chi-square test of independence illustrated that regional differences were significant ($p < 0.001$): Company and Pole Sorkh had the highest optochin sensitivity, Kote Sangai had the highest bacitracin sensitivity, and three regions showed no target organisms. Standard plate counts were low ($10^2$–$10^3$ colony forming units (CFU·g$^{-1}$)) and well below international satisfactory limits (≤ $10^5$ CFU·g$^{-1}$), indicating generally good microbial quality. Despite low total counts, the detection of presumptive *S. pneumoniae* and GAS in specific areas highlights localized hygiene gaps.**

**Keywords: Afghanistan; cheese microbiology; Group A *Streptococci*; standard plate count; *Streptococcus pneumoniae***

## INTRODUCTION

Cheese has been produced by humans for centuries to preserve and concentrate milk, making it one of the earliest forms of processed food [1]. Cheese provides a range of essential nutrients, including fat-soluble vitamins (A, D, E, and K) and water-soluble vitamin B12, which contribute to immune function, energy metabolism, and blood cell formation [2]. It also contains diverse fatty acids—saturated, monounsaturated, and polyunsaturated—with some, like conjugated linoleic acid (CLA), showing potential anti-inflammatory and anticancer effects despite concerns linked to saturated fat intake [2, 3].

Raw milk cheeses are especially valued for their intense and robust flavor, which is stronger than that of cheeses made from pasteurized milk [1]. However, making cheese out of raw milk poses a threat to public health. In Afghanistan, such types of cheese have been made for a long time and have been reported to be contaminated with dangerous bacteria such as *Staphylococcus aureus* [4] and have been significantly associated with *Brucella* and *Coxiella burnetii* infections [5]. Indeed, worldwide outbreaks of foodborne illness have been linked to the consumption of raw milk cheese [1], such as outbreaks in Italy [6], England [7], Iran [8], and Pakistan [9].

*Streptococcus* species, a group of lactic acid bacteria, are facultative anaerobes that are catalase-negative, non-motile, non-spore-forming, and homofermentative cocci, generally consisting of Gram-positive, spherical or oval cells arranged in pairs or chains [10]. Numerous species within this genus are significant pathogens, with *S. bovis, S. pyogenes,* and *S. equi* subsp. *zooepidermicus* being linked to several serious medical conditions, including bacteremia, strep throat, scarlet fever, chills, hypotension, meningitis, endocarditis, sepsis, and colon cancer [11, 12]. These bacteria can enter dairy products through direct contamination during processing or via infected raw milk, particularly when hygienic practices are poor [13, 14].

Since raw milk cheese production is commonly practiced among Afghan families, this study aimed to investigate the potential presence of pathogenic *Streptococcus* species in ripened, semi-hard cheese sold across various markets in Kabul City and to analyze their microbiological quality using standard plate count (SPC). In addition, we hypothesize that the region may have a significant association with the streptococcal sensitivity pattern. To achieve these goals, a range of cheese samples was collected and subjected to biochemical testing to determine whether any zoonotic *Streptococci* were present.

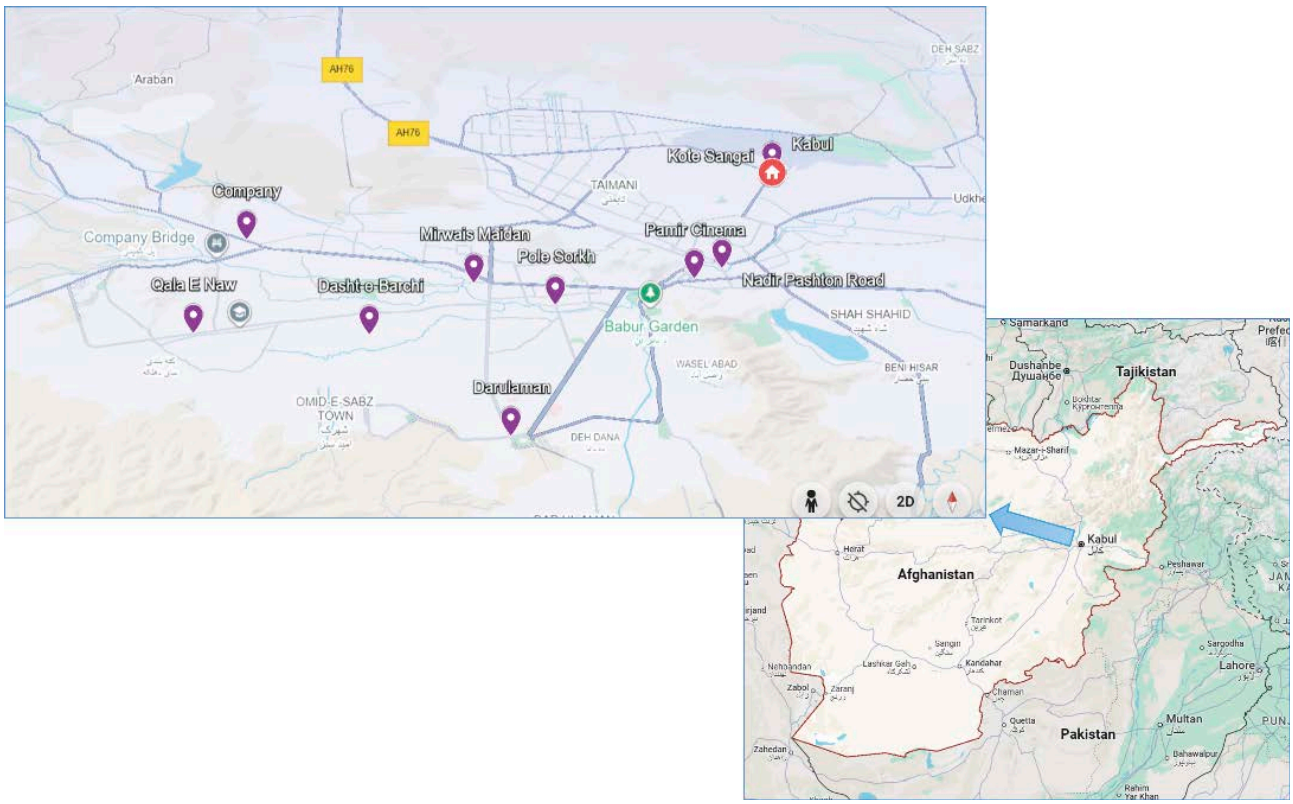


**Fig. 1. Illustration of different regions in Kabul City from which the samples were gathered**

## MATERIALS AND METHODS

### Sample collection

In this survey, a total of 90 ripened, semi-hard cheese samples, 400 g each, were collected from various dairy product shops across Kabul City (Figure 1). To prevent contamination, all samples were aseptically collected using sterilized tubes and bags [4]. The samples were transported in iceboxes to minimize bacterial growth and were promptly delivered to the Central Diagnostic Laboratories for Animal Disease, Ministry of Agriculture and Livestock, for further microbiological analysis [15].

### Sample preparation, culture, and microscopic examination

Five grams of each cheese sample were aseptically weighed and homogenized in 9 mL of sterile sodium citrate (Sigma Aldrich, Germany) using a mortar and pestle to create a uniform suspension [16]. For the standard plate count, a ten-fold serial dilution was performed by transferring 1 mL of the suspension sequentially into 10 sterile test tubes, each containing 9 mL of sodium citrate, to achieve $10^{-10}$ dilutions according to standard microbiological procedures. A sterile bent glass rod was used to spread 0.1 mL of diluted samples, and they were incubated at 37°C for 24 hours. After incubation, all colonies, including those of pin-point size, in standard plate count agar (SPCA) medium (HiMedia®, India) were counted [16, 17].

Blood agar plates (HiMedia®, India) were inoculated with 1 mL from each dilution and incubated at 37°C for 18–24 hours. After incubation, colonies were examined for hemolytic activity [18, 19]. Colonies showing hemolytic activity were subjected to Gram staining and examined microscopically. Gram-positive cocci appearing in chains were presumptively identified as the genus *Streptococci* [19].

### Biochemical identification within *Streptococcus* spp.

To differentiate between *Streptococcus* species, hemolytic colonies were subcultured on blood agar and incubated at 37°C for 24 hours. Optochin (5 µg) sensitivity testing was used (HiMedia®, India) to presumptively identify *Streptococcus pneumoniae* among α-hemolytic isolates, with a visible zone of inhibition indicating sensitivity (≥14 mm with a 6 mm disk containing 5 µg optochin) [20]. Bacitracin sensitivity testing using 0.04-unit disks (HiMedia®, India) was employed to presumptively identify *Streptococcus pyogenes* among β-hemolytic isolates, with the presence of a zone of inhibition interpreted as a positive result [18, 21]. These methods were strictly used for comparison within the *Streptococcus* genus.

Finally, to further confirm isolates to the species level, the API 20 STREP system (bioMérieux, France) were used. Colonies were suspended in sterile saline and inoculated into the API test strips following the manufacturer's instructions. The inoculated strips were incubated at 37°C for 24 hours. After incubation, color changes in the wells were recorded, and a numerical code was generated. This code was interpreted using the API database to identify the bacterial species with high precision [22].

### Statistical analysis

Statistical analysis was performed using SPSS 27 (IBM Inc., Chicago). Descriptive statistics were used to describe the sensitivity rate of antimicrobials in each region. Furthermore, A chi-square test of independence was performed to examine the association between region and *Streptococcus* sensitivity patterns (optochin sensitive or bacitracin sensitive separately) used for the presumptive identification of *Streptococci*. Standardized residuals were computed for post hoc analysis to identify cells contributing most to the overall association. Residuals with absolute values ≥ 1.96 were considered statistically significant at $p < 0.05$, indicating observed frequencies significantly different from expected. Residuals between ±1.5 and ±1.95 were interpreted as approaching significance.

## RESULTS

Gram staining, catalase testing, and hemolysis patterns combined with disk sensitivity testing (optochin and bacitracin) were used for the presumptive identification of streptococcal isolates from nine regions. Out of 90 total samples, 31 (34.4%) showed α-hemolysis and were sensitive to optochin, indicating a moderate presence of presumptive *Streptococcus pneumoniae*. Meanwhile, 25 samples (27.8%) exhibited β-hemolysis and bacitracin sensitivity, suggesting a putative low presence of Group A *Streptococci* (GAS). A notable 34 samples (37.8%) displayed no hemolysis, implying a higher proportion of

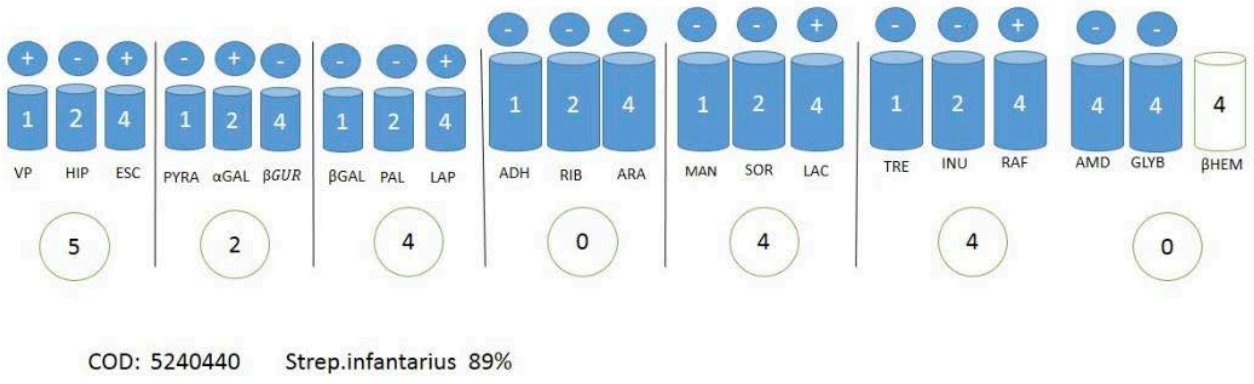


**Fig. 2. Identification of non-hemolytic-looking isolates on blood agar to the species level using the API system**

non-hemolytic bacteria. Due to limited access to the API system, it was only possible to test 4 samples (detected from Kote Sangai and Mirwais Maidan). The species of these non-hemolytic-looking samples was found to be *S. infantarius* (Figures 2 and 3).

Finally, it was found that there was a significant association between the region and the distribution of isolates showing optochin or bacitracin sensitivity patterns used for presumptive species differentiation ($p < 0.001$). Post-hoc analysis of standardized residuals indicated that cheese samples from Kote Sangai had a significantly higher frequency of bacitracin-sensitive isolates (presumptive presence of *S. pyogenes*) than expected (standardized residual = +2.3). For optochin sensitivity (presumptive presence of *S. pneumoniae*), samples from Company showed a higher-than-expected frequency, with a residual approaching significance (+1.9). Conversely, Dasht-e-Barchi, Qala-e-Naw, and Darulaman each showed lower-than-expected frequencies of optochin sensitivity, with residuals of –1.9, suggesting a marginal trend toward underrepresentation in these regions.

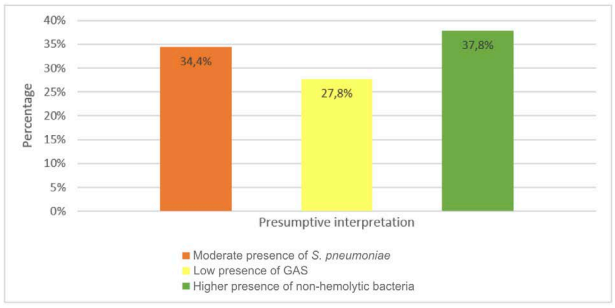


**Fig. 3. Presumptive identification of streptococcal isolates based on hemolysis patterns and disk sensitivity to bacitracin and optochin**

At the regional level, Company and Pole Sorkh showed the highest α-hemolytic activity with 7/10 (70%) optochin-sensitive isolates each, suggesting a strong likelihood of *S. pneumoniae* colonization. Pamir Cinema and Nadir Pashton Road exhibited moderate optochin sensitivity (5/10, 50%) and moderate bacitracin sensitivity (5/10, 50%), indicating potential mixed populations of S. pneumoniae and GAS. Kote Sangai demonstrated a high β-hemolytic bacitracin sensitivity (7/10, 75%), strongly pointing toward a predominant presence of GAS. In contrast, α-hemolysis and optochin sensitivity were lower in this region (2/10, 20%), suggesting a smaller proportion of tentative *S. pneumoniae*. Regions such as Darulaman, Dasht-e-Barchi, and Qala-e-Naw showed no hemolysis and no sensitivity to either antibiotic disk (0/10), indicating the absence of the target organisms. In Mirwais Maidan, moderate optochin sensitivity (5/10, 50%) and low bacitracin sensitivity (2/10, 20%) were observed, alongside a considerable proportion (3/10, 30%) of non-hemolytic samples. Overall, these findings highlight significant regional variations in streptococcal colonization patterns, with some areas dominated by presumptive *S. pneumoniae*, others by presumptive GAS, and several regions showing a predominance of non-hemolytic bacterial species. The overall means of standard plate count revealed that the samples gathered from Company had the highest CFU·g$^{-1}$: $6.0104 \times 10^2 \pm 0.44$, followed by Kote Sangai ($5.2104 \times 10^2 \pm 0.41$) and Mirwais Maidan ($5.0104 \times 10^2 \pm 0.52$), while no colony was detected from Darulaman, Dasht-e-Barchi, and Qala-e-Naw (Table 1). Thus, the standard plate counts across regions were uniformly low. These counts (on the order of $10^2$–$10^3$ CFU·g$^{-1}$) are well below common international "satisfactory" thresholds for total aerobic mesophilic counts in ready-to-eat dairy products (e.g., $\leq 1 \times 10^5$

CFU·g$^{-1}$ as per established microbiological criteria frameworks), indicating generally good microbial quality and low overall contamination levels in the positive samples [23].

## DISCUSSION

This study investigated the prevalence and distribution of streptococcal species in dairy products collected from nine regions, using classical microbiological methods complemented by limited confirmatory identification with the API system. The findings demonstrate notable heterogeneity in hemolysis patterns and overall bacterial load across regions, highlighting the variable colonization and potential public health risks associated with streptococcal contamination in locally sourced products.

The predominance of optochin sensitivity observed in multiple regions (Company, Pole-Sorkh, and Mirwais Maidan) contrasted with a relatively lower frequency of bacitracin sensitivity. The predominance of α-hemolytic, optochin-sensitive isolates in the mentioned regions strongly suggests the presumptive presence of *S. pneumoniae* in these regions. This finding is significant given the pathogenic potential of *S. pneumoniae* as a leading cause of pneumonia, meningitis, and septicemia worldwide, especially in populations with limited access to healthcare and vaccination [24].

On the other hand, Kote Sangai showed a predominance of β-hemolytic, bacitracin-sensitive isolates, consistent with the potential presence of Group A *Streptococci* (GAS). The presence of *S. pyogenes*, a member of GAS, in dairy products has remained a concern due to its ability to cause pharyngitis, skin infections, and invasive diseases such as necrotizing fasciitis [25]. The relatively high frequency of GAS in this region may reflect differences in hygienic practices, handling procedures, or local reservoirs of infection. Furthermore, detection of bacitracin sensitivity may reflect regional variations in antibiotic application or bacterial strain heterogeneity, paralleling findings by Perez-Trallero [26] and Katla et al. [27]. Besides, other Gram-positive bacteria (*Staphylococcus aureus*) have been detected in cheese in Kabul city (4.28%), which depicts the signs of lack of hygienic standards as stated in the study as well [4]. This mirrors patterns reported in both regional and global settings, pointing toward a concerning trend in dairy microbiology that carries significant public health implications.

The statistically significant association between region and the distribution of isolates showing optochin or bac-

**Table 1. Hemolysis pattern, disk test results, and interpretation of streptococcal isolates from different regions**

| Region | Presumed Hemolysis | Disk Test Applied | Result (No. & % of sensitive samples) | Overall mean of CFU·g$^{-1}$ |
|---|---|---|---|---|
| **Company** | α-hemolysis | Optochin | 7/10 (70) | $6.0104 \times 10^2 \pm 0.44$ |
| | β-hemolysis | Bacitracin | 3/10 (30) | |
| **Darulaman** | No hemolysis / none | N/A[a] | 0/10 (0) | Nil[b] |
| **Dasht-e-Barchi** | No hemolysis / none | N/A | 0/10 (0) | Nil |
| **Qala-e-Naw** | No hemolysis / none | N/A | 0/10 (0) | Nil |
| **Pamir Cinema** | α-hemolysis | Optochin | 5/10 (50) | $4.7104 \times 10^2 \pm 0.21$ |
| | β-hemolysis | Bacitracin | 5/10 (50) | |
| **Pole Sorkh** | α-hemolysis | Optochin | 7/10 (70) | $4.0104 \times 10^2 \pm 0.17$ |
| | β-hemolysis | Bacitracin | 3/10 (30) | |
| **Nadir Pashton Road** | α-hemolysis | Optochin | 5/10 (50) | $4.8104 \times 10^2 \pm 0.23$ |
| | β-hemolysis | Bacitracin | 5/10 (50) | |
| **Mirwais Maidan** | α-hemolysis | Optochin | 5/10 (50) | $5.0104 \times 10^2 \pm 0.52$ |
| | β-hemolysis | Bacitracin | 2/10 (20) | |
| | No hemolysis | N/A | 3/10 (30) | |
| **Kote Sangai** | β-hemolysis | Bacitracin | 7/10 (75) | $5.2104 \times 10^2 \pm 0.41$ |
| | α-hemolysis | Optochin | 2/10 (20) | |
| | No hemolysis | N/A | 1/10 (5) | |

[a]: Not applicable.
[b]: No bacterial growth or no colonies detected in the samples from the respective areas.

itracin sensitivity patterns used for presumptive species differentiation categories ($p < 0.001$) indicates that local environmental and production factors influence contamination and presence of various streptococcal species. The higher-than-expected bacitracin sensitivity in Kote-Sangai and increased optochin sensitivity in Company and Pole Sorkh may reflect localized differences in the presence of a specific type of *Streptococci* and cheese handling practices, which warrant further investigation. Meanwhile, the marginal underrepresentation of optochin and bacitracin sensitivity in regions such as Dasht-e-Barchi, Darulaman, and Qala-e-Naw suggests differential impacts of local hygiene practices, processing methods, or pasteurization, aligning with international evidence underscoring how proper sanitary measures mitigate microbial contamination [28]. Similarly, one study in Herat province of Afghanistan reported the presence of antibiotic residues (14.1%) in milk collected from shops and open markets of Herat city with the highest residue proportion related to beta-lactam antibiotics (12.8%) [15]. Thus, the presence of antibiotic residues previously reported in raw milk from Herat suggests an underlying selective pressure that may influence the occurrence of streptococcal species such as *S. pyogenes*. In particular, sustained antimicrobial exposure may enrich resistance mechanisms, including Bce-type transporters that detect bacitracin and promote efflux or inactivation, thereby reducing its inhibitory effect on cell wall synthesis [29]. Comparative studies further reinforce the relevance of these findings; for instance, one study in Iraq reported the presence of *S. pneumoniae* from cheese [30].

Interestingly, non-hemolytic isolates comprised the largest proportion overall (37.8%). API testing of a subset of four samples identified *S. infantarius*, a member of the *Streptococcus bovis*/*Streptococcus equinus* complex, which has been associated with fermented dairy products in Africa and Asia [31]. While often considered commensal or involved in traditional fermentation, *S. infantarius* has also been implicated in endocarditis and other opportunistic infections [32]. Its detection suggests that traditional dairy practices in Kabul may foster colonization by non-pathogenic or opportunistic *Streptococci*, a finding consistent with studies documenting *S. infantarius* dominance in raw and fermented dairy products in resource-limited settings [33]. Regions such as Darulaman, Dasht-e-Barchi, and Qala-e-Naw demonstrated no bacterial growth, which may indicate either effective hygienic practices during milk handling or environmental conditions less conducive to streptococcal survival. One limitation of the current results, however, is that only 4 isolates could be identified as *S. infantarius* using the API kit and therefore, these results are indicative and not definitive.

The standard plate count results provide reassuring evidence of generally acceptable microbiological quality across the positive samples. The CFU measurements ranging from approximately $4.01 \times 10^2$ to $6.01 \times 10^2$ per gram in bacitracin-resistant samples fall well below established international thresholds for ready-to-eat dairy products ($\leq 1 \times 10^5$ CFU·g$^{-1}$) [34, 35] and indicate the good quality and generally hygienic production of cheese, which is in contrast of previous findings in Herat and Kabul reporting low-hygiene dairy products [4, 17, 15]. However, the combined low standard plate counts together with the regional differentiation in hemolysis and disk sensitivity profiles underscore localized patterns of streptococcal occurrence rather than widespread heavy contamination; this supports a risk-based focus on areas (e.g., Company and Kote Sangai) where presumptive pathogens co-occur with detectable bacterial burden.

Future work should expand confirmatory identification beyond biochemical testing, incorporating molecular approaches such as 16S rRNA sequencing or MALDI-TOF for more accurate species-level classification since these results are considered presumptive. In addition, safety assessment, exploration of virulence genes, and antimicrobial susceptibility testing against a set of antibiotics would help clarify potential resistance patterns in these isolates. Such studies are crucial for developing region-specific risk mitigation strategies, especially in areas like Kote Sangai and Company, where contamination appears most pronounced.

## CONCLUSION

This study revealed notable regional variation in streptococcal contamination of ripened, semi-hard cheese in Kabul, with Company and Pole Sorkh showing a predominance of presumptive *S. pneumoniae* and Kote Sangai dominated by Group A *Streptococci*. Although overall bacterial loads were low and within international microbiological quality standards, the presence of potential pathogens in specific areas highlights localized hygiene and

handling deficiencies. The detection of *S. infantarius* further underscores the influence of traditional dairy practices on microbial profiles. Targeted interventions, improved hygienic measures, and molecular confirmation of isolates are warranted to better assess public health risks and guide region-specific control strategies.

### Data Availability Statement

The raw data of this article will be made available by the authors upon request.

### Ethical Statement

No ethical approval was necessary for this study.

### Conflict of Interest

There was no conflict of interest among the authors.


### Funding

This study did not receive any funding.


### Generative AI Statement

No generative AI or AI-assisted technologies were used in the writing process of this study.

### Authors' Contributions

All authors contributed to the study conception and design. The conceptualization was performed by Sayed Arif Ahmadi (SAA) and Mohammad Farzad Afshar (MFA). The methodology was developed by SAA and MFA. Data collection and analysis were performed by SAA, MFA, and Mahyar Mokhatab (MM). Supervision of the study was carried out by SAA. The original draft of the manuscript was written by SAA, MFA, and MM. The manuscript was reviewed and edited by MFA and MM. All authors read and approved the final manuscript.

## REFERENCES


1. **Yoon, Y., Lee, S., Choi, K.-H., 2016:** Microbial benefits and risks of raw milk cheese. *Food Control*, 63, 201–215. DOI: 10.1016/j.foodcont.2015.11.013
2. **Gülhan, A., 2024:** The Health Benefits of the Cheese. In *Global Perspectives on Cheese Tourism*, IGI Global Scientific Publishing,149–160. DOI: 10.4018/979-8-3693-3490-4.ch009
3. **Summer, A., Formaggioni, P., Franceschi, P., Di Frangia, F., Righi, F., Malacarne, M., 2017:** Cheese as Functional Food: The Example of Parmigiano Reggiano and Grana Padano. *Food. Technol. Biotechnol.*, 55, 3, 277–289. DOI: 10.17113/FTB.55.03.17.5233
4. **Ahmadi, S. A., Sakha, M. Z., Ebadi, S., Panda, A. K., 2021:** Study of milk and dairy products *Staphylococcus* contamination and antimicrobial susceptibility sold in local markets around Kabul University. *Int. J. Innov. Res. Sci. Stud.*, 4, 1, 20–24. DOI: 10.53894/ijirss.v4i1.51
5. **Saeed, K. M. I., Ahadi, J., Sahak, M. N., Ghiasi, A. F., Ashgar, R. J., 2014:** Concurrent brucellosis and Q fever infection: A case control study in Bamyan Province, Afghanistan. *Cent. Asian J. Glob. Health*, 2, 2, 58. DOI: 10.5195/cajgh.2013.58
6. **Cardamone, C., Castello, A., Oliveri, G., Costa, A., Sciortino, S., Nia, Y., et al., 2024:** Staphylococcal food poisoning outbreaks occurred in Sicily (Italy) from 2009 to 2016. *Ital. J. Food Saf.*, 13, 2,11667. DOI: 10.4081/ijfs.2024.11667
7. **Heinsbroek, E., Blakey, E., Simpson, A., Verlander, N. Q., Greig, D. R., Jørgensen, F., et al., 2024:** An outbreak of Shiga toxin-producing *Escherichia coli* serotype O103:H2 associated with unpasteurized soft cheese, England and Wales, 2022. *Epidemiol. Infect.*, 152, e172. DOI: 10.1017/s0950268824001523
8. **Montazeri, R., Rahimi, E., Shakerian, A., 2024:** The prevalence of *Staphylococcus aureus* and its enterotoxin genes in raw milk and dairy products of Isfahan Province. *J. Nutr. Fast. Health*, 12, 4, 305–313.
9. **Zahra, N., Sarwar, A., Waseem, M., Rashid, M. S., Ahmad, A., Qammar, A., et al. 2020:** Microbial analysis and health risk of *Listeria spps* and *S. aureus* isolated from cheese and raw milk marketed in Lahore, Pakistan. *Life. Sci. J.*, 17, 7, 55–59. DOI: 10.7537/marslsj170720.09
10. **Özkan, E. R., Öztürk, H. İ., Demirci, T., Akın, N., 2021:** Detection of biofilm formation, virulence factor genes, antibiotic-resistance, adherence properties, and some beneficial properties of cheese origin *S. infantarius*, *S. gallolyticus*, and

*S. lutetiensis* strains belonging to the *S. bovis*/*S. equinus* complex. *L.W.T.*, 150, 112077. DOI: 10.1016/j.lwt.2021.112077

11. **Pelkonen, S., Lindahl, S. B., Suomala, P., Karhukorpi, J., Vuorinen, S., Koivula, I., et al., 2013:** Transmission of *Streptococcus equi* subspecies *zooepidemicus* infection from horses to humans. *Emerg. infect. Dis*., 19, 7, 1041–1048. DOI: 10.3201/eid1907.121365
12. **Krzyściak, W., Pluskwa, K. K., Jurczak, A., Kościelniak, D., 2013:** The pathogenicity of the *Streptococcus* genus. *Eur. J. Clin. Microbiol. Infect. Dis.*, 32, 1361–1376. DOI: 10.1007/s10096-013-1914-9
13. **Raemy, A., Meylan, M., Casati, S., Gaia, V., Berchtold, B., Boss, R., Wyder, A. Graber, H. U., 2013:** Phenotypic and genotypic identification of *Streptococci* and related bacteria isolated from bovine intramammary infections. *Acta Vet. Scand*., 55, 53, 1–9. DOI: 10.1186/1751-0147-55-53
14. **Götz, F., 2016:** Antibiotic resistance in Streptococci: Implications for dairy microbiology. *J. Dairy. Sci.*, 99, 2, 512–520.
15. **Ebner, P. D., Ghoryar, M. A., McNamara, K., Amini, R., Faqiri, E., Azizi, A. E., et al., 2016:** Quality and safety assessment of raw bovine milk in Herat province, Afghanistan. *J. Food Saf. Hyg.*, 2, 1/2, 15–20.
16. **Shakhes, S. A., Wasim, W. A., Nasiry, Z., Tookhy, N. A., 2023:** Coliform contamination of raw beef at the slaughterhouse and butchery levels in Herat City, Afghanistan. *N.U.I. J.B.*, 2, 4, 137–144. DOI: 10.70436/nuijb.v2i04.128
17. **Sarwary, N. A., Farahmand, M. Y., Afshar, F., Sakha, M. Z., 2025:** Effect of storage temperature on the microbiological, physicochemical, and sensory properties of semi-hard cheese. *J.N.S.R.*, 3, 1, 70–81. DOI: 10.62810/jnsr.v3i1.174
18. **ISO, I., 2017:** 6887-1 Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 1: General rules for the preparation of the initial suspension and decimal dilutions**.** International Organization for Standardization. Geneva, Switzerland – 8–25.
19. **Taye, Y., Degu, T., Fesseha, H., Mathewos, M., 2021:** Isolation and identification of lactic acid bacteria from cow milk and milk products. *Sci. World J*., 2021, 1, 4697445. DOI: 10.1155/2021/4697445
20. **Ercibengoa, M., 2021:** Assessment of the optochin susceptibility test to differentiate *Streptococcus pneumoniae* from other viridans group *Streptococci*. *Clin. Lab.*, 67, 3, 857–861. DOI: 10.7754/clin.lab.2020.200438
21. **Mondkar, A. D., Kelkar, S. S., 1981:** The bacitracin sensitivity test for identifying beta-haemolytic *Streptococci* of Lancefield group A. *J. Postgrad. Med.*, 27, 2, 86–89.
22. **Yousufi, H., Hamid, H., Habibi Khishki, K., Haidari, N., Mohammadi, S., 2024:** Evaluation of contamination and antibiotic susceptibility pattern of bacteria isolated from bottled water in Kabul, Afghanistan. *W. E. S.*, 4, 1, 39–44. DOI: 10.52293/WES.4.1.3944
23. **Institute of Medicine (US) and National Research Council (US) Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food**, **Appendix E, International Microbiological Criteria., 2003:** *Scientific Criteria to Ensure Safe Food*, National Academies Press, Washington DC, 317–358. DOI: 10.17226/10690
24. **Ruan, J., Fu, Z., Ying, L., 2024:** Clinical differences between *Mycoplasma pneumoniae* pneumonia and *Streptococcus pneumoniae* pneumonia: a case control study. *Front. Pediatr.*, 12, 1409687. DOI: 10.3389/fped.2024.1409687
25. **Avire N. J., Whiley H., Ross, K., 2021:** A review of *Streptococcus pyogenes*: public health risk factors, prevention and control. *Pathogens*, 10, 2, 248. DOI: 10.3390/pathogens10020248
26. **Perez-Trallero, E., Garcia, C., Orden, B., Marimon, J. M., Montes, M., 2004:** Dissemination of emm28 erythromycin-, clindamycin- and bacitracin-resistant *Streptococcus pyogenes* in Spain. *Eur. J. Clin. Microbiol. Infect. Dis.*, 23, 123–126. DOI: 10.1007/s10096-003-1069-1
27. **Katla, A. K., Kruse, H., Johnsen, G., Herikstad, H., 2001:** Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. *Int. J. Food. Microbiol.*, 67, 1–2, 147–152. DOI: 10.1016/s0168-1605(00)00522-5
28. **Murray, P. R., Rosenthal, K. S., Pfaller, M. A., 2015:** *Medical Microbiology E-Book*. Elsevier Health Sciences, Philadelphia., 277 pp.
29. **Sachi, S., Ferdous J., Sikder, M. H., Azizul Karim Hussani, S. M., 2019:** Antibiotic residues in milk: past, present, and future. *J. Adv. Vet. Anim. Res.,* 6, 3, 315–332. DOI: 10.5455/javar.2019.f350
30. **Mounam, M. A. W. A., Razook, B. R. F., Madi, E. H., 2025:** Antimicrobial susceptibility patterns of infective *Streptococci* recovered from local and imported mozzarella cheese from different markets in Baghdad. *Open. Vet. J.*, 15, 3, 1178–1186. DOI: 10.5455/ovj.2025.v15.i3.10
31. **Jans, C., Meile, L., Kaindi, D. W. M., Kogi-Makau, W., Lamuka, P., Renault, P., et al**., **2017:** African fermented dairy products–overview of predominant technologically im-

portant microorganisms focusing on African *Streptococcus infantarius* variants and potential future applications for enhanced food safety and security. *Int. J. Food Microbiol*., 250, 27–36. 10.1016/j.ijfoodmicro.2017.03.012

32. **Lakshmi, S. S. J., Leela, K. V., 2022:** A review on updated species list of viridans *Streptococci* causing infective endocarditis. *J. Pure Appl. Microbiol.*, 16, 3, 1590–1594. DOI: 10.22207/JPAM.16.3.26
33. **Maitha, I. M., Kaindi, D. W. M., Cheruiyot, C., 2021:** The functionality of african *Streptococcus thermophillus* and *Streptococcus infantarius* Sub Sp. *infantarius* in milk fermentation. *Curr. Res. Nutr. Food Sci*., 9, 1, 111–121. DOI: 10.12944/CRNFSJ.9.1.11
34. **European Commission, 2005:** Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. O. J. E. U., L 338, 1–26.
35. **International Commission on Microbiological Specifications for Foods (ICMSF), 2011:** *Microorganisms in foods 8: Use of data for assessing process control and product acceptance*, Springer Science & Business Media, New York, 389 pp. DOI: 10.1007/978-1-4419-9374-8

DOI: 10.2478/fv-2026-0012

FOLIA VETERINARIA, 70, 2: 10—22, 2026

ORIGINAL ARTICLE

# *IN VITRO* INVESTIGATION OF THE POTENTIAL OF *FICUS EXASPERATA* EXTRACTS AGAINST BACTERIAL PATHOGENS ISOLATED FROM CHICKENS

**Olasunkanmi Olawuwo[1]*, Oyinlola Olaokun[3], Abimbola Aro[2], Gift Omokhua-Uyi[2], Ibukun Famuyide[2], Prudence Kayoka-Kabongo[1], Lyndy McGaw[2]**

[1]Department of Agriculture and Animal Health, University of South Africa, South Africa; [2]Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, South Africa; [3]Department of Biology, School of Science and Technology, Sefako Makgatho Health Sciences University, South Africa

OPEN ACCESS

***Correspondence:** lasunstephen1@gmail.com

**Citation:** Olawuwo, O., Olaokun, O., Aro, A., Omokhua-Uyi, G., Famuyide, I., Kayoka-Kabongo, P., McGaw, L., 2026: In vitro investigation of the potential of *Ficus exasperata* extracts against bacterial pathogens isolated from chickens. Folia Veterinaria, 70, 2, 10–22.

**Received:** August 28, 2025

**Accepted:** December 30, 2025

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

***Campylobacter* species and *Escherichia coli* are leading causes of bacterial diarrhea in humans and livestock globally, often transmitted through food and water with poultry, pigs, and cattle, as reservoirs, are increasingly resistant to antibiotics, necessitating alternative therapeutic strategies. Building on previous reports of the antibacterial, cytotoxic, phytochemical, and antioxidant properties of *Ficus exasperata* leaf extracts as phytogenic feed additives for chickens, this study expands the scope by evaluating additional clinical *E. coli* isolates with their selectivity index, genotoxicity assays and profiling the methanol extract using standard bioassays and GC-MS. The methanol extract demonstrated broad and best efficacy across all tested bacteria, though the aqueous had the highest total antibacterial activity (2 308 mL/g). The reference strains (ATCC) were more resistant than clinical isolates. The methanol extract showed the lowest cytotoxicity ($LC_{50}$: 0.99 mg/mL on Vero cells and 2.69 mg/mL on Caco-2 cells) and the highest selectivity indices (Vero: 6.33; Caco-2: 14.17). It was non-mutagenic to *Salmonella typhimurium* TA98 compared to acetone extract. The GC-MS analysis of methanol extract revealed 9-Octadecen-1-ol (24.52%) as the most abundant compound. The methanol extract showed promising potential for further *in vivo* testing as phytogenic feed additives and alternatives for managing campylobacteriosis and colibacillosis in chickens.**

**Keywords: antibacterial; *E. coli* isolates; *Ficus exasperata*; GC-MS; methanol extract; selectivity index**

## INTRODUCTION

Poultry comprises the greatest reservoir of *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, which are the most common causes of bacterial diarrhoea in humans worldwide [1]. Similarly, the disease is zoonotic, and domestic animals such as poultry, pigs, and cattle may act as reservoirs. One of the critical public health problems in recent years is antimicrobial resistance in food-borne pathogens [2, 3]. Reports have shown the resistance of poultry and human *Campylobacter* isolates to the antibiotic ciprofloxacin due to veterinary application of enrofloxacin and other quinolones [3, 4]. According to the 2017 report of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the most common causes of food-borne zoonotic diseases were *Campylobacter* and *Salmonella* bacteria [5]. In Canada, the annual incidence of food poisonings ranges between 3.1 and 5 million. In Australia, the incidence is 5.4 million [6, 7].

Avian pathogenic *Escherichia coli* (APEC) infection is zoonotic and also pathogenic to chickens. It has been responsible for great economic losses to the poultry industry worldwide [8]. The pathological presentations vary from localized, such as salpingitis or omphalitis, to systemic infections. Antimicrobial drugs can be used to reduce mortality in birds diagnosed with a localized or generalized bacterial infection. However, selection of antimicrobial drugs must be based on the susceptibility of isolated bacteria, as *E. coli* may be resistant to commonly used antibacterial drugs such as tetracycline, sulfonamides, ampicillin, and streptomycin [9, 10]. In humans, the enteropathogenic strains of *E. coli* (EPEC) cause diarrhoeal illness, especially in young infants, presenting symptoms such as watery diarrhoea with low-grade fever and vomiting and more severe cases resulting in death [11].

The ban on the use of antibiotics as growth promoters in livestock by the European Union occurred as a result of the emergence of antibiotic resistance in bacteria colonizing humans [12, 13], due to the development of resistant strains against conventional antibiotics. Therefore, feed companies and researchers are in search of alternative products and strategies that can help to maintain animal gut health in order to prevent or reduce the prevalence of pathogens in the food chain [14].

*Ficus exasperata* has been reported as a potential source of antimicrobial compounds. Major ethnomedicinal usage has been reported throughout Africa, with Nigeria, Cameroon, Ivory Coast, and Sierra Leone being the folkloric hubs. Crude extracts have been reported to exhibit a wide spectrum of *in vitro* and *in vivo* pharmacological activities like antidiabetic, anticonvulsant, anti-inflammatory, antimicrobial, hypolipidemic, antioxidant, antiulcer, anxiolytic, and hypotensive [15, 16].

The consumption of anticarcinogens and antimutagens in the diet may be the most effective way of preventing human cancer [17]. Therefore, incorporating non-genotoxic, antimutagenic plants like *Ficus* leaf-based feed additives may contribute not only to poultry health and productivity but also to improved food safety, public health, and protection against diet-related carcinogenesis in humans.

Previous studies by Olawuwo et al. [18, 19] demonstrated the antibacterial and cytotoxic effects of *F. exasperata* leaf extracts against reference strains of *E. coli* and *Campylobacter* species and reported phytochemical and antioxidant activities of the acetone and aqueous extracts. The present study extends this work by including additional clinical isolates of *E. coli* with their selectivity index, genotoxicity assays and chemical profiling of the methanol extract investigating the antibacterial potential and safety of *F. exasperata* in the treatment of colibacillosis and campylobacteriosis in chickens.

## MATERIALS AND METHODS

### Plant collection

Fresh leaves of *F. exasperata* were collected from Ibadan Metropolis in the Lagelu Local Government Area of Oyo State, the southwestern part of Nigeria. The plant was identified by Mr Esimekhinai, D. P. O with a voucher specimen (UIH - 22626) and was deposited after identification in the herbarium of the Department of Botany, University of Ibadan.

### Plant preparation and extraction

The fresh leaves were rinsed, air dried, and ground into powder. Three grams of the powdered material were weighed into centrifuge tubes, and 30 ml of acetone, methanol, and distilled water were added to separate aliquots and allowed to macerate for 24 h. The mixtures were centrifuged at 300×g for 10 min, and filtration was carried out using Whatman No. 1 filter paper. The resultant

extracts were transferred into pre-weighed, labelled glass vials. Resultant extracts were placed under a stream of air to dry completely and stored in a dark room at 4 °C while preparing for the experiment. The resultant extracts were reconstituted in their respective solvents to give the desired concentrations used in the study.

**Microbial strains**

Of the 11 pathogens used in this study, three were reference strains, American Type Culture Collection (ATCC), while eight were clinical isolates. The reference strains used were *Escherichia coli* (ATCC 25922), *Campylobacter coli* (ATCC 43478), and *Campylobacter jejuni* (ATCC 33560). Clinical isolates were (A) *Escherichia coli* (B 3427/16), (B) *Escherichia coli* (B 3584/16), (C) *Escherichia coli* (B 3412/16), (D) *Escherichia coli* (B 364/16), (E) *Escherichia coli* (B 3584/16), (F) *Escherichia coli* (B 3375/16), (G) *Escherichia coli* (B 3282/16), and (H) *Escherichia coli* (B 3397/16) from chickens. All were obtained from the Department of Veterinary Tropical Diseases (DVTD), Faculty of Veterinary Science, University of Pretoria.

**Antibacterial assay**

The antibacterial activity of acetone, methanol, and aqueous leaf extracts of Ficus exasperata against reference Campylobacter strains and clinical *E. coli* isolates from poultry was evaluated in vitro using a serial microdilution assay [20]. Clinical isolates were cultured in Mueller Hinton (MH) broth for *E. coli* and Brain Heart Infusion (BHI) broth for *Campylobacter*, incubated at 37 °C for 18–24 h, and adjusted to $3 \times 10^8$ cfu/ml (McFarland No. 1) by absorbance measurement at 560 nm. Plant extracts (10 mg/ml) were prepared and serially diluted in 96-well plates and inoculated with standardized bacterial suspensions; gentamicin (2 mg/ml) and acetone served as positive and negative controls, respectively. Plates containing *E. coli* were incubated at 37 °C for 24 h in a regular incubator, while those with *Campylobacter* were incubated anaerobically. Following incubation, 40 µl of p-iodonitrotetrazolium (INT, 0.2 mg/ml) was added to detect bacterial growth, and the minimum inhibitory concentration (MIC) was recorded as the lowest concentration with clear inhibition. The assay was conducted in triplicate and repeated twice for reliability.

**Toxicological assays**

***In vitro* cytotoxicity assay**

The cytotoxicity of the plant extracts was evaluated against Vero monkey kidney and human intestinal (Caco-2) cell lines using the MTT assay as described by Mossman [21] and modified by McGaw et al. [22]. Cells grown in Minimal Essential Medium (MEM) supplemented with gentamicin and foetal calf serum were harvested, adjusted to $5 \times 10^4$ cells/ml, and seeded into 96-well plates, with columns 1 and 12 containing only MEM to minimize edge effects. After 24 h incubation at 37°C with 5% $CO_2$, cells were treated in quadruplicate with varying concentrations of plant extracts (prepared from 100 mg/ml stock) for 48 h, while doxorubicin served as a positive control and untreated cells as a negative control. Following treatment, cells were washed with PBS, supplied with fresh MEM, and incubated with MTT solution (5 mg/ml) for 4 h to allow formazan crystal formation, which was then solubilized in dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader, with blank wells (no cells) for correction. Cytotoxicity was expressed as the lethal concentration ($LC_{50}$), defined as the extract concentration causing a 50% reduction in absorbance relative to untreated controls.

***In vitro* genotoxicity assay**

The plate incorporation assay, also known as the Ames test [23], was used to determine the genotoxicity of the selected plant extracts against *Salmonella typhimurium* strains TA98 and TA100. Acetone and methanol extracts were prepared at concentrations of 5, 0.5, and 0.05 mg/ml in DMSO under sterile conditions. The samples were tested against *S. typhimurium* strains TA98 and TA100 without an exogenous metabolic activation system. Briefly, 100 µl of each extract was added to the test tube, followed by 500 µl of phosphate buffer and 100 µl of a fresh overnight culture (prepared by inoculating 100 µl stock bacteria in 10 ml of Oxoid nutrient broth); then 2 ml of top agar with biotin/histidine was added. The mixture was centrifuged and poured into minimal agar plates. A positive control, 4-nitroquinoline-N-oxide (4-NQO) at a concentration of 2 µg/ml, negative controls of 10% DMSO and sterile distilled water were also prepared. The plates were incubated for 48 h at 37°C and a colony counter was used to count the colonies.

**Gas chromatography-mass spectrometry (GC-MS) of methanol plant extract**

Analysis of chemical constituents of methanol plant extract of *F. exasperata* with the best biological activities and industrial relevance was carried out in a LECO Pegasus 4D GC-TOFMS (LECO Africa (Pty) Ltd., Kempton Park, South Africa on a gas chromatography (GC) capillary column of dimension Rxi-1MS 30 m x 0.25 mm ID x 0.25 µm film thickness (Restek, Bellefonte, PA, USA). Gas chromatography (GC) UHP Helium (Afrox, South Africa) was used as a carrier gas at a flow rate of 1 ml/min. A 1 µL methanol solution of the sample was injected in a split mode at 10:1 with injector temperature at 250°C. The GC oven temperature was programmed at 40°C (held for 3 min), at 8°C/min to 300°C (hold for 5 min). The constant flow mode mass acquisition from 40-450 Da was maintained. The MS transfer line temperature was 280°C. The ion source temperature was maintained at 230°C. Compound spectra were detected by electron ionization system (70 eV), MS solvent delay 5 min and electron energy 70 eV in the electron ionisation mode (EI+). The relative quantity of the compounds present in the extract was expressed as a percentage based on the peak area produced in the chromatogram. The tentative identification was done by comparison of retention times with standard samples and by matching their mass spectra at a rate of 10 spectra/s and a detector voltage of 1750 V against mass spectra of the National Institute of Standards and Technology (NIST) 14 Library.

**Statistical analysis**

Some of the data obtained from experiments were expressed as mean ± standard deviation (SD) and were subjected to descriptive statistics using the Student's t- test to compare two sample means. A one-way ANOVA for the test of significance between groups that were more than two and Tukey's post-hoc test were used to compare means of all samples using GraphPad Prism, version 5.01, April 2016, statistical software Chicago, IL, USA, where applicable.

## RESULTS

**Antibacterial activity (MIC) and total antibacterial activity (TAA)**

The antibacterial activity of *F. exasperata* is presented in Table 1. The acetone and methanol extracts of *Ficus exasperata* were moderately active, with the lowest average MIC values for all tested organisms of 0.24 and 0.56 mg/

**Table 1. Minimum inhibitory concentration (MIC) of acetone, methanol, and aqueous extracts of *F. exasperata* against *Campylobacter* strains and eight selected *Escherichia coli* isolates**

| | MIC (mg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Extracts** | ***E. coli* R** | ***E. coli* (A)** | ***E. coli* (B)** | ***E. coli* (C)** | ***E. coli* (D)** | ***E. coli* (E)** | ***E. coli* *(F)*** | ***E. coli* (G)** | ***E. coli* (H)** | ***C. c.*** | ***C. j.*** | **Average MIC** |
| **Acetone** | 0.78 | **0.07** | **0.07** | 0.15* | 0.11* | 0.22* | 0.18 | 0.22* | 0.46* | **0.05** | 0.30* | 0.24* |
| **Methanol** | 0.78 | 0.15* | **0.07** | **0.07** | **0.07** | 0.62* | 0.30* | 0.15* | 0.15* | 2.50 | 1.25 | 0.56* |
| **Aqueous** | 1.87 | 0.15* | **0.03** | 0.34* | **0.03** | 0.30* | 0.62* | **0.03** | **0.03** | 2.50 | 1.87 | 0.71 |
| **Gentamicin** | **>0.01** | **0.001** | **0.0006** | **0.001** | **0.0006** | **0.0003** | **0.0002** | **0.0002** | **0.0006** | 0.62 | **0.001** | **0.06** |

MIC < 0.1 mg/ml – Significantly active (**Bold**), 0.1 ≤ MIC≤ 0.625 mg/ml – Moderately active*, MIC > 0.625 mg/ml, Gentamicin (2 mg/ml) – positive control, A–H – isolates, R – ATCC. *C. c. – C. coli*, *C. j. – C. jejuni.*

**Table 2. The total antibacterial activity (TAA) in ml/g of acetone, methanol, and aqueous extracts of *Ficus exasperata* against typed *Campylobacter* strains and 8 selected *Escherichia coli* isolates**

| | | Total Activity | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Extracts** | **Yield (%)** | ***E. coli* (R)** | ***E. coli* (A)** | ***E. coli* (B)** | ***E. coli* (C)** | ***E. coli* (D)** | ***E.coli* (E)** | ***E. coli* (F)** | ***E. coli* (G)** | ***E. coli* (H)** | ***C. c.*** | ***C. j.*** | **Average** |
| **Acetone** | 3.33 | 42 | 476 | 476 | 222 | 303 | 151 | 185 | 151 | 72 | 666 | 111 | 259 |
| **Methanol** | 9.33 | 119 | 622 | 1 333 | 1 333 | 1 333 | 150 | 311 | 622 | 622 | 37 | 74 | 596 |
| **Aqueous** | 17.00 | 90 | 1 133 | 5 666 | 500 | 5 666 | 566 | 274 | 5 666 | 5 666 | 68 | 90 | 2 308 |

A–H – isolates, R – ATCC. *C. c. – C. coli*, *C. j. – C. jejuni.*

ml, respectively. The findings indicated that the methanol extract had the best antibacterial activity against all the tested bacteria.

The total antibacterial activity (TAA) is a function of the extraction yield in mg per 1 gram of plant material and the minimal inhibitory concentration (MIC), expressed in milliliter per gram (ml/g). The results for the TAA values of the extracts against *E. coli* isolates and *Campylobacter* species are presented in Table 2. The aqueous extract showed average TAA values of 2,308 ml/g, followed by the methanol extract with average TAA values of 596 ml/g, while acetone extracts had the lowest % yield with the lowest average TAA values of 259 ml/g.

**Cytotoxicity and selectivity index (SI)**

The $LC_{50}$ values of the acetone, methanol, and aqueous extracts against Vero kidney cells were 0.09 ± 0.05, 0.99 ± 0.23, and 0.27 ± 0.21 mg/ml, respectively, while the $LC_{50}$ values of the acetone, methanol, and aqueous extracts against Caco-2 cells were 1.82 ± 0.56, 2.69 ± 0.44, and 0.05 ± 0.00 mg/ml, respectively (Figure 1). The methanol extract had the highest $LC_{50}$ (lowest toxicity) value against both Vero and Caco-2 cells (Figure 1).

The SI values for the acetone, methanol, and aqueous extracts against Vero and Caco-2 cells ranged from 0.10 to 14.14 and 0.02 to 38.42, respectively, with the methanol extract having the highest value. Selectivity index (SI) values for antimicrobial activity were calculated using the formula $SI = LC_{50}/MIC$, with units for $LC_{50}$ and MIC in mg/ml.

**Genotoxicity**

The methanol extract was non–mutagenic against TA98 at all concentrations but toxic to TA100 at 5 mg/ml, except for the acetone extract that showed clear mutagenicity with numbers of revertant colonies greater than twice that of the negative controls for the 5 mg/ml (the highest concentration tested) and also increased with increasing concentrations of extracts. The methanol extract was not toxic to *S. typhimurium* TA98 and TA100 though it showed a dose-dependent response against *S. typhimurium* TA100 (Table 4).

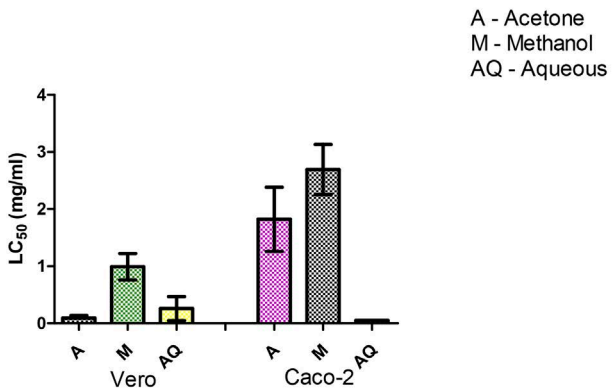


**Fig. 1. Cytotoxicity ($LC_{50}$) of extracts against Vero kidney cells and Caco-2 cells**

**Gas chromatography-mass spectrometry (GC-MS) of methanol plant extract**

The gas chromatographic-mass spectrometric analysis of the extract was done to elucidate the presence of any potential antibacterial and antioxidant compounds in the methanol extract of *F. exasperata* (Table 5, Figure 2). Out of ninety-eigth metabolites, only 58 were identifiable; the most abundant compound with the largest yields exhibiting the highest peak areas in the extract is 9-Octadecen-1-ol (24.52%).

## DISCUSSION

**Antibacterial activity**

Antibacterial activity was classified as active, moderately active, weak, or inactive following the benchmark established by Kuete [24] and Efferth and Kuete [25]. The findings indicated that the methanol extract had the best antibacterial activity against all chicken isolates but relatively weak activity against chicken *Campylobacter* species.

The total antibacterial activity (TAA) is a function of the extraction yield in mg per 1 gram of plant material and the minimal inhibitory concentration (MIC), expressed in ml per gram (ml/g) [26]. The TAA is a useful pharmacological criterion for the selection of plant species, and it also indicates which plant species could be the best source of extract for use by poor communities or for organic production [26].

The ethanol extract of the leaves of *F. exasperata* has been reported to have good inhibitory activity (300 mg/ml) against *E. coli* using a well diffusion assay [27]. The methanol extract of the bark of *Ficus religiosa* was found to be more active against all the enterotoxigenic *E. coli* using disc diffusion [28].

All the leaf extracts of *Ficus deltoidea* have been reported to show inhibitory activity against the fungus,

**Table 3. Selectivity index (SI) against Vero kidney cells and Caco-2 cells**

| | SI Vero cells | | | SI Caco-2 cells | | |
|---|---|---|---|---|---|---|
| | **Acetone** | **Methanol** | **Aqueous** | **Acetone** | **Methanol** | **Aqueous** |
| ***E. coli* (R)** | 0.12 | **1.27** | 0.14 | **2.33** | 3.45 | 0.03 |
| ***E. coli* (A)** | **1.29** | **6.60** | **1.73** | **26** | **17.93** | 0.33 |
| ***E. coli* (B)** | **1.29** | **14.14** | **8.67** | **26** | **38.42** | **1.66** |
| ***E. coli* (C)** | 0.60 | **14.14** | 0.76 | **12.1** | **38.42** | 0.14 |
| ***E. coli* (D)** | 0.82 | **14.14** | **8.67** | **16.5** | **38.42** | **1.66** |
| ***E. coli* (E)** | 0.41 | **1.60** | 0.87 | **8.20** | **4.33** | 0.16 |
| ***E. coli* (F)** | 0.50 | **3.30** | 0.42 | **10.1** | **8.96** | 0.08 |
| ***E. coli* (G)** | 0.41 | **6.60** | **8.67** | **8.27** | **17.93** | **1.66** |
| ***E. coli* (H)** | 0.20 | **6.60** | **8.67** | **3.95** | **17.93** | **1.66** |
| ***C. coli*** | **1.80** | 0.40 | 0.10 | **36.40** | **1.07** | 0.02 |
| ***C. jejuni*** | 0.30 | 0.80 | 0.14 | **6.06** | **2.15** | 0.02 |
| **Average** | 0.70 | **6.33** | **3.53** | **14.17** | **17.18** | 0.67 |

A selectivity index > 1 is regarded to be safe **(Bold)**, R – ATCC

**Table 4. Genotoxicity of selected extracts against *Salmonella typhimurium* strains (TA 98 and TA100) presented as mean ± standard deviations**

| | | ***Salmonella typhimurium* strains** | | | | | |
|---|---|---|---|---|---|---|---|
| **Plant Species** | **Extracts** | **TA98** | | | **TA100** | | |
| | | **Concentration mg/ml** | | | **Concentration mg/ml** | | |
| ***Ficus exasperata*** | | 5 mg/ml | 0.5 mg/ml | 0.05 mg/ml | 5 mg/ml | 0.5 mg/ml | 0.05 mg/ml |
| | | Number of revertant colonies per concentration | | | | | |
| | Acetone | 264.50±28.99 | 46.50±7.78 | 19.00±1.41 | 262.50±0.71 | 282.00±7.07 | 217.50±17.68 |
| | Methanol | 13.00±1.41 | 5.50±0.71 | 10.50±2.12 | 244.50±0.71 | 217.00±1.41 | 174.50±3.54 |
| **4NQO** | | 133 ± 1.21 | | | 491.50±4.95 | | |
| **10% DMSO** | 21.50±2.12 | | | | 260.00±2.83 | | |
| **Water** | 13.50±2.12 | | | | 244.50±6.36 | | |

DMSO – dimethyl sulphoxide, 4NQO – 4-nitroquinoline-N-oxide

gram-positive and gram-negative bacteria strains tested, except for the chloroform and aqueous extracts on *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa* using disc diffusion [29]. Koona and Rao [30] reported that the methanol extract of the leaf of *Ficus benghalensis* displayed high antibacterial activity against all tested bacteria, while Gayathri and Kannabiran [31] observed that the aqueous extract of the bark of the same plant also had active inhibition (0.04 mg/ml to 0.1 mg/ml) against the tested bacteria.

To the best of our knowledge the findings from this study comprise the first report on the antibacterial activity of *F. exasperata* against different chicken clinical isolates of *E. coli*. The different reported findings on the antibacterial activities of this plant and other related species could be attributed to geographical location, age of plant at harvest, season of harvest, and method of extraction, all of which affect the yield of active constituents of medicinal plants [32, 33]. This can also be due to differences in laboratory procedures and reagents used [34, 35].

## Cytotoxicity and selectivity index (SI)

Three extracts of *F. exasperata* leaves were tested against Vero kidney and Caco-2 cell lines for general and local toxicity, respectively. Makhafola et al. [36] stated that until cellular toxicity tests are conducted, no natural products or crude plant extracts can be considered safe for usage. It is expected that the sensitivity of the cell lines to the extracts will be different because of different metabolic activities and uptake capabilities [37]. The human intestinal cell line (Caco-2) has been reported for its known uptake capabilities [38]. Therefore, the choice of Caco-2 cells was attributable to its uptake capabilities and being

**Table 5. Major compounds detected in the methanol crude leaf extract of *F. exasperata***

| S/N | RT (s) | Compounds | MW | Molecular formula | % Peak Area | Similarity Index (%) |
|---|---|---|---|---|---|---|
| **1** | 739.6 | n-Hexane | 86 | $C_6H_{14}$ | 0.25251 | 95.5 |
| **2** | 801.3 | 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- | 139 | $C_7H_9NO_2$ | 0.037975 | 72.4 |
| **3** | 816 | Hexadecane | 226 | $C_{16}H_{34}$ | 0.075317 | 89.6 |
| **4** | 987.7 | Eicosane | 282 | $C_{20}H_{42}$ | 0.083463 | 85.2 |
| **5** | 1030.9 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- | 180 | $C_{11}H_{16}O_2$ | 0.063582 | 87.5 |
| **6** | 1059.8 | 1,3-Benzenediol, o-(2-methylbenzoyl)-o'-(3-cyclopentylpropionyl)- | 352 | $C_{22}H_{24}O_4$ | 0.048256 | 81.2 |
| **7** | 1069.4 | Diethyl Phthalate | 222 | $C_{12}H_{14}O_4$ | 0.13902 | 94.8 |
| **8** | 1116.7 | aR-Turmerone | 216 | $C_{15}H_{20}O$ | 1.0882 | 90.5 |
| **9** | 1117.1 | n-Tridecan-1-ol | 200 | $C_{13}H_{28}O$ | 1.0882 | 84.1 |
| **10** | 1175.7 | Tetradecanoic acid | 228 | $C_{14}H_{28}O_2$ | 0.20765 | 83.6 |
| **11** | 1190.3 | 3-Methylbenzoic acid, 2-formyl-4,6-dichlorophenyl ester | 308 | $C_{15}H_{10}Cl_2O_3$ | 0.050817 | 79.8 |
| **12** | 1191.2 | 2-Undecanethiol, 2-methyl- | 202 | $C_{12}H_{26}S$ | 0.050817 | 78.8 |
| **13** | 1196.4 | 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one | 196 | $C_{11}H_{16}O_3$ | 0.10085 | 81.5 |
| **14** | 1220.8 | Neophytadiene | 278 | $C_{20}H_{38}$ | 0.95137 | 91.0 |
| **15** | 1225.4 | 2-Pentadecanone, 6,10,14-trimethyl- | 268 | $C_{18}H_{36}O$ | 0,71471 | 85.1 |
| **16** | 1244.2 | Phthalic acid, isobutyl nonyl ester | 348 | $C_{21}H_{32}O_4$ | 0.004233 | 85.6 |
| **17** | 1247.3 | 1-Hexadecanol | 242 | $C_{16}H_{34}O$ | 12.262 | 94.0 |
| **18** | 1247.7 | Cyclohexene, 3-propyl- | 124 | $C_9H_{16}$ | 12.392 | 70.7 |
| **19** | 1272.9 | 2-Piperidinone, N-[4-bromo-n-butyl]- | 233 | $C_9H_{16}BrNO$ | 0.2513 | 79.7 |
| **20** | 1273.5 | Pentadecanoic acid, 14-methyl-, methyl ester | 270 | $C_{17}H_{34}O_2$ | 0.2513 | 80.6 |
| **21** | 1279.7 | Cyclotetradecane | 196 | $C_{14}H_{28}$ | 0.070195 | 89.4 |
| **22** | 1287.7 | Isophytol | 296 | $C_{20}H_{40}O$ | 0.086541 | 73.3 |
| **23** | 1288.7 | Benzenepropanoic acid, 3,5-bis(1,1 dimethylethyl)-4-hydroxy-, methyl ester | 292 | $C_{18}H_{28}O_3$ | 0.086541 | 74.4 |
| **24** | 1299.7 | n-Hexadecanoic acid | 256 | $C_{16}H_{32}O_2$ | 3.4626 | 92.0 |
| **25** | 1302 | Dibutyl phthalate | 278 | $C_{16}H_{22}O_4$ | 0.52773 | 93.8 |
| **26** | 1312.5 | Oxalic acid, allyl octadecyl ester | 382 | $C_{23}H_{42}O_4$ | 0.11053 | 79.9 |
| **27** | 1313.2 | Hexadecanoic acid, ethyl ester | 284 | $C_{18}H_{36}O_2$ | 0.11053 | 75.2 |
| **28** | 1349.9 | Cyclooctene, 3-ethenyl- | 136 | $C_{10}H_{16}$ | 0.12643 | 78.7 |
| **29** | 1354 | 9-Octadecen-1-ol, (E)- | 268 | $C_{18}H_{36}O$ | 24.521 | 95.4 |

**Table 5: (Continued)**

| S/N | RT (s) | Compounds | MW | Molecular formula | % Peak Area | Similarity Index (%) |
|---|---|---|---|---|---|---|
| **30** | 1365.8 | Hexadecen-1-ol, trans-9- | 240 | $C_{16}H_{32}O$ | 8.6044 | 94.1 |
| **31** | 1374.9 | 9-Octadecenoic acid (Z)-, methyl ester | 296 | $C_{19}H_{36}O_2$ | 0.089798 | 74.5 |
| **32** | 1376.1 | 3-Tetradecen-5-yne, (Z)- | 192 | $C_{14}H_{24}$ | 0.35967 | 74.3 |
| **33** | 1382.8 | Phytol | 296 | $C_{20}H_{40}O$ | 2.3539 | 90.7 |
| **34** | 1400.9 | 1,8,11,14-Heptadecatetraene, (Z,Z,Z)- | 232 | $C_{17}H_{28}$ | 0.87074 | 71.6 |
| **35** | 1409.9 | Octadecanoic acid | 284 | $C_{18}H_{36}O_2$ | 0.78268 | 87.6 |
| **36** | 1412.5 | Decanedioic acid, dibutyl ester | 314 | $C_{18}H_{34}O_4$ | 0.51516 | 89.5 |
| **37** | 1423.9 | Dodecanamide | 199 | $C_{12}H_{25}NO$ | 0.42652 | 80.7 |
| **38** | 1451.9 | cis-9,10-Epoxyoctadecan-1-ol | 284 | $C_{18}H_{36}O_2$ | 0.15911 | 85.7 |
| **39** | 1462 | 1-Octadecyne | 250 | $C_{18}H_{34}$ | 0.19828 | 79.5 |
| **40** | 1465.1 | cisZ-11,12-Epoxytetradecan-1-ol | 228 | $C_{14}H_{28}O_2$ | 0.2618 | 77.9 |
| **41** | 1473.5 | Cyclopentane, heneicosyl- | 364 | $C_{26}H_{52}$ | 0.12349 | 73.9 |
| **42** | 1473.7 | 1-Hexadecanol | 242 | $C_{16}H_{34}O$ | 0.12349 | 70.2 |
| **43** | 1493.9 | Oxirane, tetradecyl- | 240 | $C_{16}H_{32}O$ | 0.15779 | 79.4 |
| **44** | 1511.2 | 4,8,12,16-Tetramethylheptadecan-4-olide | 324 | $C_{21}H_{40}O_2$ | 0.21983 | 92.4 |
| **45** | 1515.8 | 9-Octadecenamide, (Z)- | 281 | $C_{18}H_{35}NO$ | 1.344 | 87.8 |
| **46** | 1524.9 | trans-Farnesol | 222 | $C_{15}H_{26}O$ | 0.14699 | 72.9 |
| **47** | 1527.7 | Dodecanamide | 199 | $C_{12}H_{25}NO$ | 0.43441 | 88.9 |
| **48** | 1602.9 | Bis(2-ethylhexyl) phthalate | 390 | $C_{24}H_{38}O_4$ | 0.12006 | 90.3 |
| **49** | 1690.6 | 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester | 390 | $C_{24}H_{38}O_4$ | 0.058469 | 77.0 |
| **50** | 1706.1 | 9-Octadecenamide, (Z)- | 281 | $C_{18}H_{35}NO$ | 0.53898 | 86.6 |
| **51** | 1724.1 | Squalene | 410 | $C_{30}H_{50}$ | 0.38526 | 92.2 |
| **52** | 1823.9 | γ-Tocopherol | 416 | $C_{28}H_{48}O_2$ | 0.002837 | 79.0 |
| **53** | 1855.2 | Cholesta-4,6-dien-3-ol, (3á)- | 384 | $C_{27}H_{44}O$ | 0.37283 | 75.3 |
| **54** | 1876.7 | DL-α-Tocopherol | 430 | $C_{29}H_{50}O_2$ | 0.13892 | 850 |
| **55** | 1879.7 | 26-Nor-5-cholesten-3á-ol-25-one | 386 | $C_{26}H_{42}O_2$ | 0.57127 | 74.8 |
| **56** | 1948.7 | 5-Cholestene-3-ol, 24-methyl- | 400 | $C_{28}H_{48}O$ | 0.37861 | 70.3 |
| **57** | 1970.4 | Stigmasterol | 412 | $C_{29}H_{48}O$ | 0.2277 | 81.6 |
| **58** | 2015.5 | á-Sitosterol | 414 | $C_{29}H_{50}O$ | 4.7134 | 89.3 |

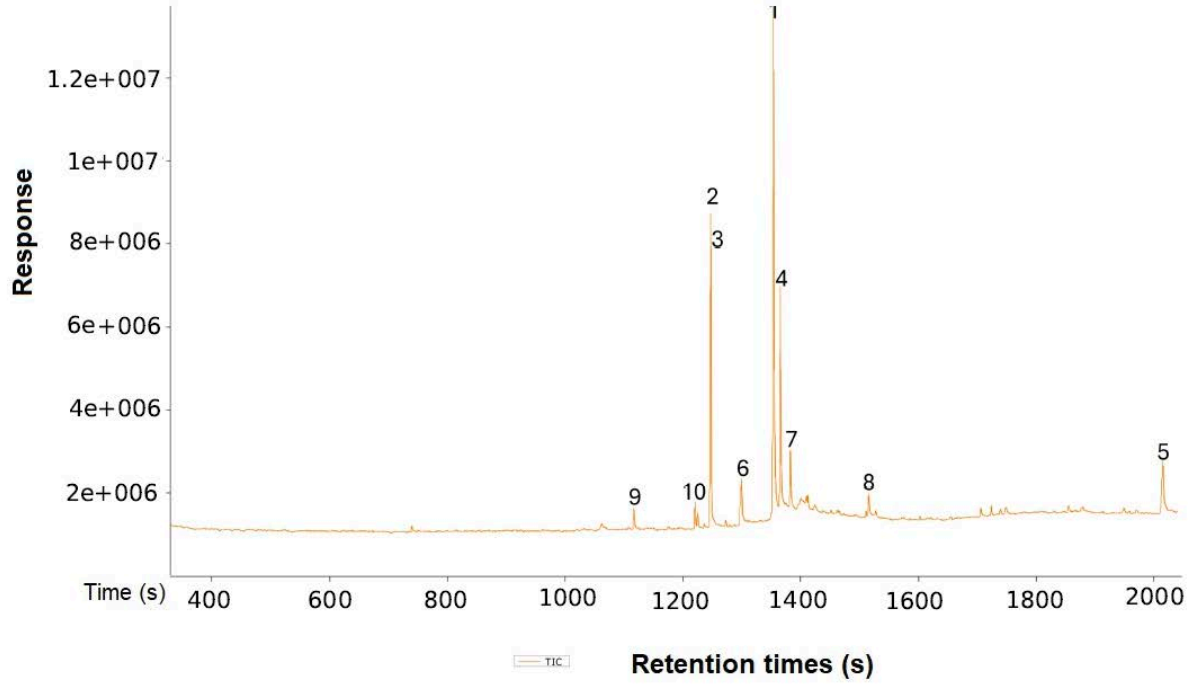


**Fig. 2. GC-MS chromatogram of methanolic extract of *F. exasperata***
(1) 9-Octadecen-1-ol (24.52%), (2) Cyclohexene,3-propyl- (12.39%), (3) 1-Hexadecanol (12.26%), (4) Hexadecen-1-ol,trans-9- (8.60%), (5) á-Sitosterol (4.71%), (6) n-Hexadecanoic acid (3.46%, (7) Phytol (2.35%), (8) 9-Octadecenamide,(Z)- (1.34%), (9) aR-Turmerone (1.08%) and (10) n-Tridecan-1-ol (1.08%).

an absorptive surface for the bioactive ingredients in the feed additives.

In another study by Pinto [39], it was demonstrated that these cells, upon differentiation, expressed several morphological and biochemical characteristics of small intestinal enterocytes. In order to reduce the use of experimental animals for toxicity testing, the Caco-2 cell model has been considered for the development of alternative *in vitro* toxicity tests. Moreover, the gastrointestinal tract can be a direct target for several toxicants. The extracts with $LC_{50}$ > 0.1 mg/ml are considered not cytotoxic [40]. Two cell lines were evaluated in this study since it may be misleading to determine the safety and utility of a plant extract using just one.

Similar to our findings, Sowemimo et al. [41] reported that the ethanol extract of *F. exasperata* was not toxic to brine shrimps and rat lymphocyte chromosomes with no inhibition in the telomerase activity. Likewise, Bafor and Igbinuwen [42] observed no toxicity on the haematological parameters and changes in physiological activity after oral and intraperitoneal administration of the aqueous extract in mice during the acute toxicity test.

Kudumela et al. [43] and McGaw et al. [44] reported that SI values greater than or equal to 10 are preferred; however SI above 1 is an indication that the biological activity of the plant extracts is higher than their cellular toxicity. The higher the SI values, the safer the extracts. Therefore, the methanol extract is the safest of all the tested extracts.

Similarly, it has been reported that cell line sensitivity in Caco-2 cells decreased progressively from more distal regions of the gastrointestinal tract: gastric > duodenal > ileal > colonic, although further study would be required to determine whether this is a phenomenon that might be relevant to intestinal uptake rates [37].

**Genotoxicity**

For an extract to be genotoxic, the number of revertant colonies should be equal to or greater than twice the average of the negative control and a clear dose-dependent response should be observed for the various concentrations tested [23]. The methanol extract was not toxic to *S. typhimurium* TA98 and TA100, though it showed a dose-dependent response against *S. typhimurium* TA100. Our findings in this study are similar to those of Satish et al. [45], they reported that *Ficus benghalensis* stem bark exhibited non-toxicity in *S. typhimurium* strains against induced mutagenicity of sodium azide ($NaN_3$).

Flavonoids have been reported to possess significant antimutagenic activity, such as glaberene, quercetin, myricetin, kaempferol, and hesperidin [46]. Natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for human cancer prevention [47].

**Gas Chromatography-Mass Spectrometry (GC-MS)**

The solvent used for the extraction [48] and the seasonal variation and soil type [49] are among the determining factors for the chemical composition of a plant. It has been reported that phenolic compounds are more soluble in highly polar solvents, such as methanol [50]. Methanol extract from this current study had better antibacterial activity across all the tested pathogens and was industrially relevant and the safest among the tested extracts with good total antibacterial activity (TAA). In addition, it has the highest selectivity index (SI) against Vero and Caco-2 mammalian cells.

Therefore, the methanol extract of *F. exasperata* was chosen for analysis using Gas Chromatography-Mass Spectrometry (GC-MS); it reveals the presence of several bioactive compounds with notable antimicrobial and antioxidant activities and, in some cases, anticancer activities. Among the most abundant compounds identified are 9-Octadecen-1-ol which has demonstrated antimicrobial activity as reported by Mugayi and Mukanganyame [51], suggesting its potential role in the inhibitory effect of the extract against microbial pathogens. Cyclohexene, 3-propyl- was previously identified in the essential oil of *Cyperus articulates*; this compound exhibited significant antimicrobial (antibacterial and antifungal) and antioxidant activities against a wide range of pathogens, including *E. coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Aspergillus flavus*, and *Trichoderma harzianum* [52], indicating its broad-spectrum bioactivity.

1-Hexadecanol was known for its antimicrobial and antioxidant properties; its compound supports the potential of the methanol extract of *F. exasperata* in combating oxidative stress and microbial infections [53]. Hexadecen-1-ol, trans-9- was identified in *Indigofera longeracemosa*; this compound showed specific antimicrobial activity with agar disc diffusion, particularly against fish pathogens such as *Aeromonas hydrophila* (3 ± 0.2 mm) and *Aeromonas sobria* (9 ± 0.1 mm), although it was inactive against *Vibrio harveyi* [54]. Its presence in *F. exasperata* suggests selective antimicrobial capabilities. The α-Sitosterol was detected in the methanol extract of *Heliotropium bacciferum* Forssk, this compound has been widely reported for its antibacterial and antifungal effects [55].

Generally, these compounds highlight the multifaceted therapeutic potential of the methanol extract of *F. exasperate,* especially its antimicrobial and antioxidant properties. Their individual and synergistic actions could explain the biological efficacy of the extract and support its possible use in pharmaceutical or feed additive formulations targeting microbial infections and oxidative stress.

## CONCLUSION

This study demonstrated the antibacterial potential of *F. exasperata* leaf extracts against antibiotic-resistant *Campylobacter* species and *E. coli*. The methanol extract showed superior efficacy, low cytotoxicity, high selectivity indices, and no mutagenic effects, indicating good safety. Alhough the aqueous extract had higher total antibacterial activity, it was less biologically robust overall. Clinical isolates were more susceptible than reference strains, highlighting practical relevance. GC-MS analysis identified 9-Octadecen-1-ol as the dominant compound likely contributing activity. Despite *in vitro* limitations, the methanol extract showed promise as a phytogenic feed additive, warranting further in vivo and formulation studies.

**Data Availability Statement**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

**Generative AI Statement**

I acknowledge using Grammarly (https://grammarly.com/) to improve the grammar and punctuation of this research paper.

**Authorship**

Conceptualization, supervision and reviewing: Olasunkanmi Olawuwo, Ibukun Famuyide and Lyndy McGaw; Data analysis: Olasunkanmi Olawuwo, Abimbo-

la Aro and Oyinlola Olaokun; Manuscript writing, reviewing and editing: Olasunkanmi Olawuwo, Ibukun Famuyide, Abimbola Aro, Gift Omokhua-Uyi, Prudence Kayoka-Kabongo and Lyndy McGaw.

## Conflict of Interest

All authors of this study declare no conflict of interest.

## Ethical Consideration

The presented work did not avail human participation and animal use. However, the study, protocol number REC 232-19, was approved by the faculty ethics committee, Faculty of Veterinary Science, University of Pretoria.

## Acknowledgements

*The efforts of Balungile Madikizela in assisting with genotoxicity assays, Dr. Yvette Naude of the Department of Chemistry, University of Pretoria, for GC-MS analysis and Mr Esimekhinai, D. P.O in the herbarium of the Department of Botany, University of Ibadan, for identification of the plant are well acknowledged. The National Research Foundation (NRF), South Africa (Grant no. 105993) is thanked for providing research funding through Lyndy McGaw.*

## REFERENCES

1. **Olson, C. K., Ethelberg, S., van Pelt, W., Tauxe, R. V., 2008:** Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. *Campylobacter*, 121–38. DOI: 10.1128/9781555815554.ch9
2. **Gupta, A., Nelson, J. M., Barrett, T. J., Tauxe, R.V., Rossiter, S. P., Friedman, C. R., et al., 2004:** Antimicrobial resistance among *Campylobacter* strains, United States, 1997–2001. *Emerg. Infect. Dis.*, 10, 6, 1102–1109. DOI: 10.3201/eid1006.030635
3. **EFSA, 2014:** The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012. *EFSA Journal*, 12, 3, 3590. DOI: 10.2903/j.efsa.2014.3590
4. **Engberg, J., Neimann, J., Nielsen, E. M., Aarestrup, F. M., Fussing, V., 2004:** Quinolone-resistant Campylobacter Infections: Risk Factors and Clinical Consequences. *Emerg. Infect. Dis.*, 10, 6, 1056–1063. DOI: 10.3201/eid1006.030669
5. **Guillier, L., Gourmelon, M., Lozach, S., Cadel-Six, S., Vignaud, M. L., Munck, N., et al., 2019:** Tracing the source of bacterial strains based on accessory genes. Application to *Salmonella* Typhimurium environmental strains. *bioRxiv*, 814459. DOI: 10.1101/814459
6. **Keerthirathne, T. P., Ross, K., Fallowfield, H., Whiley, H., 2016:** A review of temperature, pH, and other factors that influence the survival of *Salmonella* in mayonnaise and other raw egg products. *Pathogens*, 5, 4, 63. DOI: 10.3390/pathogens5040063
7. **Rohde, A., Hammerl, J. A., Al Dahouk, S., 2016:** Detection of foodborne bacterial zoonoses by fluorescence *in situ* hybridization. *Food Control*, 69, 297–305. DOI: 10.1016/j.foodcont.2016.05.008
8. **Hussein, A. H. M., Ghanem, I. A. I., Eid, A. A. M., Ali, M. A., Sherwood, J. S., Li, G., et al., 2013:** Molecular and phenotypic characterization of *Escherichia coli* isolated from broiler chicken flocks in Egypt. *Avian Dis*., 57, 3, 602–611. DOI: 10.1637/10503-012513-Reg.1
9. **Miles, T. D., McLaughlin, W., Brown, P. D., 2006:** Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. *BMC Vet. Res.*, 2, 7, 1–9. DOI: 10.1186/1746-6148-2-7
10. **Vandemaele, F., Assadzadeh, A., Derijcke, J., Vereecken, M., Goddeeris, B. M., 2002:** Avian pathogenic *Escherichia coli* (APEC). *Tijdschr Diergeneeskd.*, 127, 19, 582–588.
11. **Nataro, J. P., Kaper, J. B., 1998:** Diarrheagenic *Escherichia coli*. *Clin. Micro. Rev.*, 11, 1, 142–201. DOI: 10.1128/cmr.11.1.142
12. **Schwarz, S., Kehrenberg, C., Walsh, T. R., 2001:** Use of antimicrobial agents in veterinary medicine and food animal production. *Int. J. Antimicrob. Agents*, 17, 6, 431–437. DOI: 10.1016/S0924-8579(01)00297-7
13. **Ezerskis, Z., Robouch, P., von Holst, C., 2012:** EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003.
14. **Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia D., et al., 2010:** Characterization of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*. *Int. J. Food Microbiol.*, 141, S98–S108. DOI: 10.1016/j.ijfoodmicro.2010.03.039
15. **Ahmed, F., Ahmed, M. K. K., Abedin, M. Z., Karim, A. A., 2012:** Traditional uses and pharmacological potential of *Ficus exasperata* Vahl. *Syst. Rev. Pharm.*, 3, 1, 15–23.

16. **Singh, D., Singh, B., Goel, R. K., 2011:** Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: A review. *J. Ethnopharmacol.*, 134, 3, 565–583. DOI: 10.1016/j.jep.2011.01.046
17. **Farombi, E. O., 2004:** Diet-related cancer and prevention using anticarcinogens. *Afr. J. Biotechnol.*, 3, 12, 651–661. Available from: https://www.ajol.info/index.php/ajb/article/view/15034
18. **Olawuwo, O. S., Abdalla, M. A., Mühling, K. H, McGaw, L. J., 2021:** Proximate analysis of nutrients and in vitro radical scavenging efficacy in selected medicinal plant powders with potential for use as poultry feed additives. *S. Afr. J. Bot.*, 146, 103–110. DOI: 10.1016/j.sajb.2021.09.038
19. **Olawuwo, O. S., Famuyide, I. M., McGaw, L. J., 2022:** Antibacterial and antibiofilm activity of selected medicinal plant leaf extracts against pathogens implicated in poultry diseases. *Front. Vet. Sci.*, 9. DOI: 10.3389/fvets.2022.820304
20. **Eloff, J. N., 2007:** A Sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64, 8, 711–713. DOI: 10.1055/s-2006-957563
21. **Mosmann, T., 1983:** Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 1–2, 55–63. DOI: 10.1016/0022-1759(83)90303-4
22. **McGaw, L. J., Elgorashi, E. E., Eloff, J. N., 2007:** Cytotoxicity of african medicinal plants against normal animal and human cells. In: Kuete, V., editor. *Toxicological Survey of African Medicinal Plants*, 181–233. DOI: 10.1016/B978-0-12-800018-2.00008-X
23. **Maron, D. M., Ames, B. N., 1983:** Revised methods for the Salmonella mutagenicity test. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 113, 3–4, 173–215. DOI: 10.1016/0165-1161(83)90010-9
24. **Kuete, V., 2010:** Potential of cameroonian plants and derived products against microbial infections: A Review. *Planta Medica*, 76, 14, 1479–1491. DOI: 10.1055/s-0030-1250027
25. **Efferth, T., Kuete, V., 2010:** Cameroonian medicinal plants: pharmacology and derived natural products. *Front Pharmacol.*, 1, 123, 1–19. DOI: 10.3389/fphar.2010.00123
26. **Eloff, J. N., McGaw, L. J., 2006:** Plant extracts used to manage bacterial, fungal, and parasitic infections in Southern Africa. In: Ahmad, I, Aqil, F., Owais, M., editors. *Modern Phytomedicine*, John Wiley & Sons, Ltd., pp. 97–121. DOI: 10.1002/9783527609987.ch5
27. **Odunbaku, O. A., Ilusanya, O. A., Akasoro, K. S., 2008:** Antibacterial activity of ethanolic leaf extract of *Ficus exasperata* on *Escherichia coli* and *Staphylococcus albus*. *Sci. Res. Essay*, 3, 11, 562–564.
28. **Uma, B., Prabhakar, K., Rajendran, S., 2009:** In vitro Antimicrobial activity and phytochemical analysis of *Ficus religiosa* L. and *Ficus bengalensis* L. against diarrhoeal enterotoxigenic *E. coli*. *Ethnobotanical Leaflets*, 2009, 4.
29. **Abdsamah, O. A., Zaidi, N. T. A., Sule, A. B., 2012:** Antimicrobial activity of *Ficus deltoidea* Jack (mas cotek). *Pak. J. Pharm. Sci.*, 25, 3, 675–678.
30. **Koona, S. J., Rao, B. S., 2012:** In vitro evaluation of antibacterial activity of crude extracts of *Ficus benghalensis* Linn., the banyan tree leaves. *Indian J. Nat. Prod. Resour.*, 3, 2, 281–284.
31. **Gayathri, M., Kannabiran, K., 2009:** Antimicrobial activity of *Hemidesmus indicus*, *Ficus bengalensis* and *Pterocarpus marsupium* roxb. *Indian J. Pharm. Sci.*, 71, 5, 578–81. DOI: 10.4103/0250-474X.58182
32. **Calixto, J. B., 2000:** Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz. J. Med. Biol. Res.*, 33, 2, 179–189. DOI: 10.1590/S0100-879X2000000200004
33. **Evans, W.C., 2009:** *Trease and Evans' Pharmacognosy*. Elsevier Health Sciences; 16th ed. 2009. pp. 3322.
34. **Bonini, P., Plebani, M., Ceriotti, F., Rubboli, F., 2002:** Errors in laboratory medicine. *Clin. Chem.*, 48, 5, 691–698. DOI: 10.1093/clinchem/48.5.691
35. **Wallach, J. B., 2007:** *Interpretation of Diagnostic Tests*. Lippincott Williams & Wilkins., pp. 1202.
36. **Makhafola, T. J., Samuel, B. B., Elgorashi, E. E., Eloff, J. N., 2012:** Ochnaflavone and Ochnaflavone 7-O-Methyl Ether two antibacterial biflavonoids from *Ochna pretoriensis* (Ochnaceae). *Nat. Prod. Comm.*, 7, 12. DOI: 10.1177/1934578X1200701216
37. **Froscio, S. M., Fanok, S., Humpage, A. R., 2009:** Cytotoxicity screening for the cyanobacterial toxin cylindrospermopsin. *J. Toxicol. Environ. Health*, 72, 5, 345–349. DOI: 10.1080/15287390802529906
38. **Meunier, V., Bourrié, M., Berger, Y., Fabre, G., 1995:** The human intestinal epithelial cell line Caco-2; pharmacological and pharmacokinetic applications. *Cell Biol. Toxicol.*, 11, 3, 187–194. DOI: 10.1007/BF00756522
39. **Pinto, M., Robine-Leon, S., Appay, M. D., Kedinger, M., Triadou, N., Dussaulx, E., et al., 1983:** Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol. Cell*, 47, 323–330.
40. **Sambuy, Y., De Angelis, I., Ranaldi, G., Scarino, M. L., Stammati, A., Zucco, F., 2005:** The Caco-2 cell line as

a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol. Toxicol*., 21, 1–26. DOI: 10.1007/s10565-005-0085-6

41. **Sowemimo, A. A., Fakoya, F. A., Awopetu, I., Omobuwajo, O. R., Adesanya, S. A., 2007:** Toxicity and mutagenic activity of some selected Nigerian plants. *J. Ethnopharm.*, 113, 3, 427–432. DOI: 10.1016/j.jep.2007.06.024
42. **Bafor, E. E., Igbinuwen, O., 2009:** Acute toxicity studies of the leaf extract of *Ficus exasperata* on haematological parameters, body weight and body temperature. *J. Ethnopharm*., 123, 2, 302–307. DOI: 10.1016/j.jep.2009.03.001
43. **Kudumela, R. G., Masoko, P., 2018:** In vitro assessment of selected medicinal plants used by the Bapedi community in South Africa for treatment of bacterial infections. *J. Evid. -Based Integr. Med.*, 23. DOI: 10.1177/2515690X18762736
44. **McGaw, L. J., Steenkamp, V., Eloff, J. N., 2007:** Evaluation of *Athrixia* bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. *J. Ethnopharm.*, 110, 1, 16–22. DOI: 10.1016/j.jep.2006.08.029
45. **Satish, A., Punith Kumar, R., Rakshith, D., Satish, S., Ahmed, F., 2013:** Antimutagenic and antioxidant activity of *Ficus benghalensis* stem bark and *Moringa oleifera* root extract. *Int. J. Chem. Anal. Sci.*, 4, 2, 45–48. DOI: 10.1016/j.ijcas.2013.03.008
46. **Miyazawa, M., Hisama, M., 2003:** Antimutagenic activity of flavonoids from *Chrysanthemum morifolium*. *Biosci. Biotechnol. Biochem*., 67, 10, 2091–2099. DOI: 10.1271/bbb.67.2091
47. **Malik, S., Kaur, K., Prasad, S., Jha, N. K., Kumar, V., 2022:** A perspective review on medicinal plant resources for their antimutagenic potentials. *Environ. Sci. Pollut. Res.*, 29, 62014–62029. DOI: 10.1007/s11356-021-16057-w
48. **Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., Lightfoot, D. A., 2017:** Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6, 4, 42. DOI: 10.3390/plants6040042
49. **Saravanakumar, A., Ganesh, M., Peng, M. M., Aziz, A. S., Jang, H. T., 2015:** Comparative antioxidant and antimycobacterial activities of *Opuntia ficus-indica* fruit extracts from summer and rainy seasons. *Frontiers in Life Sci.*, 8, 2, 182–191. DOI: 10.1080/21553769.2015.1028655
50. **Osorio-Tobón, J. F., 2020:** Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *J. Food Sci. Technol.*, 57, 12, 4299–4315. DOI: 10.1007/s13197-020-04433-2
51. **Mugayi, L. C., Mukanganyama, S., 2024:** Antimycobacterial and antifungal activities of leaf extracts from *Trichilia emetica. Scientifica*, 1, 8784390. DOI: 10.1155/sci5/8784390
52. **Azzaz, N. A. E., El-Khateeb, A. Y., Farag, A. A., 2014:** Chemical Composition and Biological Activity of the Essential Oil of Cyperus articulatus. *Int. J. Acad. Res.*, 6, 5, 265–269.
53. **Omotoso, D. R., Olubowale, V. O., Aina, F. M., Daramola, O. O. O., 2024:** Phytochemical Profiling of Basella alba Using Gas Chromatography-Mass Spectrometry. *Trop. J. Nat. Prod. Res*., 8, 6, 7561–7565.
54. **Subash, N., Raju, G., 2014:** GC-MS analysis and antibacterial activity of stem of *Indigofera longeracemosa* Boiv. Ex Baill. *Nat. Pharm. Technol*., 4, 2, 1–6.
55. **Abdelkhalek, A., Elshaer, M., Al-Askar, A., Kowalczewski, P., Behiry, S., 2024:** Antimicrobial activities and metabolites profiling of Heliotropium bacciferum Forssk. methanolic extract. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 52, 3, 13602. DOI: 10.15835/nbha52313602

DOI: 10.2478/fv-2026-0013

FOLIA VETERINARIA, 70, 2: 23—33, 2026

ORIGINAL ARTICLE

# FIRST MOLECULAR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN APPARENTLY HEALTHY DROMEDARY CAMELS FROM KEBBI STATE, NIGERIA

**Mohammed Sani Gaddafi[1,2]*, Kelvin Olutimilehin Jolayemi[3]*, Habiba Lawal[1], Shamsuddeen Abubakar Alhaji[1,2], Muhammad Zaharaddeen Muhammad[4], Abdulrahman Hassan Jibril[2], Mohammed Salihu Jibrin[2], Muhammed Danlami Salihu[2], Abdulgaffar Usman El-Yakub[5], Ismail Ayoade Odetokun[6]**

[1]Department of Public Health, Ministry of Animal Health, Husbandry and Fisheries, Kebbi, Nigeria; [2]Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria; [3]Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria; [4]Department of Veterinary Services, Ministry of Livestock and Fisheries Development, Sokoto, Nigeria; [5]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Kaduna State University, Kaduna, Nigeria; [6]Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria

OPEN ACCESS

*Correspondence: jolayemikelvin@yahoo.com; gaddafimohammedsani@yahoo.com

**Citation:** Gaddafi, M. S., Jolayemi, K. O., Lawal, H., Alhaji, S. A., Muhammad, M. Z., Jibril, A. H., Jibrin, M. S., Salihu, M. D., El-Yakub, A. U., Odetokun, I. A., 2026: First molecular detection of methicillin-resistant *Staphylococcus aureus* in apparently healthy dromedary camels from Kebbi State, Nigeria. Folia Veterinaria, 70, 2, 23–33.

**Received:** October 24, 2025

**Accepted:** January 21, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a critical global health concern due to its extensive resistance to β-lactam antibiotics and zoonotic transmission potential. However, data on MRSA carriage among camels in Nigeria are scarce. This study investigated nasal colonization of *Staphylococcus aureus* and MRSA among apparently healthy dromedary camels in Kebbi State, Nigeria. A cross-sectional study was conducted in two pastoral communities, where 120 nasal swabs were collected. Conventional culture and biochemical tests were used for preliminary identification, while PCR detection targeted the *nuc* and *mecA* genes. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Overall, 66.7% (80/120) of samples were positive for *S. aureus*, while 38.3% (46/120) were confirmed as MRSA. Prevalence was significantly higher ($p < 0.05$) in young (80%) and female (70%) camels compared to adults (53.3%) and males (63.3%). All MRSA isolates carried the *mecA* gene and showed multidrug resistance, with high resistance to penicillin, tetracycline, erythromycin, chloramphenicol, and gentamicin, whereas all isolates remained susceptible to vancomycin. This study provides the first molecular evidence of MRSA colonization in dromedary camels in Nigeria, highlighting the need for routine surveillance and prudent antimicrobial use within a One Health framework.**

**Keywords: dromedary camels; Kebbi; methicillin-resistant *Staphylococcus aureus*; Nigeria; *Staphylococcus aureus***

## INTRODUCTION

Camels play a vital role in the socio-economic fabric of pastoralist communities across arid and semi-arid regions of Nigeria, serving as crucial sources of food, transport, and income [1]. Dromedary camels, often referred to as the "ships of the desert," are uniquely adapted to harsh climatic conditions where other livestock species struggle to thrive [2]. Their increasing use for milk, meat, and even traditional medicine such as camel milk and urine for their purported therapeutic value has amplified their significance in food security and livelihoods [3]. Recognizing these contributions, the Food and Agriculture Organization (FAO) declared 2024 as the International Year of the Camel, underscoring the species' global relevance in climate resilience and sustainable food production [4].

Despite their importance, camels can serve as asymptomatic reservoirs for zoonotic pathogens, including *Staphylococcus aureus* and its methicillin-resistant variant (MRSA) [5]. MRSA poses a major global health threat due to its resistance to β-lactam antibiotics and its capacity for cross-species transmission, complicating both human and veterinary treatments [6, 7]. In livestock systems, particularly among pastoralists, limited biosecurity measures and frequent animal-human interactions increase the risk of MRSA circulation within and between species [8]. In Nigeria, the rapid expansion of camel husbandry and the growing demand for camel-derived products necessitate stronger surveillance of zoonotic and antimicrobial-resistant pathogens [9]. However, MRSA has been documented in Nigerian livestock such as poultry, cattle and pigs [10]; data on camels are limited. Although a study of camels slaughtered at Kano abattoir reported MRSA occurrence [11], most Nigerian studies of camels have used phenotypic approaches and molecular data remain scarce; molecular detection of MRSA in camels has, however, been demonstrated in neighbouring countries like Algeria [12], highlighting an important knowledge gap for camels in Nigeria given the increasing cross-border movement and trade of camels in north-western Nigeria.

This study therefore aimed to detect and characterize *Staphylococcus aureus* isolates, including methicillin-resistant strains, among apparently healthy dromedary camels in Kebbi State, Northwestern Nigeria, using both phenotypic and molecular methods. To the best of our knowledge, it provides the first molecular evidence of MRSA carriage in Nigerian camels and underscores the public health significance of antimicrobial resistance at the livestock-human interface. The findings are expected to inform One Health-based strategies for antimicrobial resistance surveillance, biosecurity enhancement, and prudent antimicrobial use in pastoral production systems.

## MATERIALS AND METHODS

### Study Area

The study was conducted in Argungu Local Government Area (LGA) of Kebbi State, Northwestern Nigeria (Fig. 1), located at latitude 11°26′6.79″ N and longitude 5°14′5.78″ E. Argungu serves as the administrative headquarters of the Argungu Emirate and shares an international border with the Republic of Niger. The area is predominantly agrarian, with extensive agricultural lands, fertile soils, and abundant rivers that support mixed farming and livestock rearing. According to the 2006 National Population Census, Argungu has a population of 24,338, mainly from the Arawa and Hausa ethnic groups, with Islam as the major religion. This LGA hosts a major camel market that attracts traders and pastoralists from both within and outside Nigeria. Its proximity to an international animal control post and seasonal transhumance activities makes it a suitable site for the epidemiological surveillance of transboundary zoonotic pathogens, including *Staphylococcus aureus* and MRSA.

### Study Design and Sample Size Determination

A cross-sectional study was conducted between March and May 2024 to estimate the prevalence of *S. aureus* and MRSA in dromedary camels. The sample size was determined using the formula of Thrusfield [13]:

$$n = \frac{t^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

where $t$ = 1.96 (for 95% confidence), $P_{exp}$ = 5% (estimated prevalence from Yusuf [11]), and $d$ = 0.05 (desired precision). The calculated minimum sample size was 73, but this was increased to 120 to enhance statistical precision and account for potential non-responses.

### Sample Collection

Nasal swabs were collected from 120 apparently healthy camels randomly selected from two pastoral com-

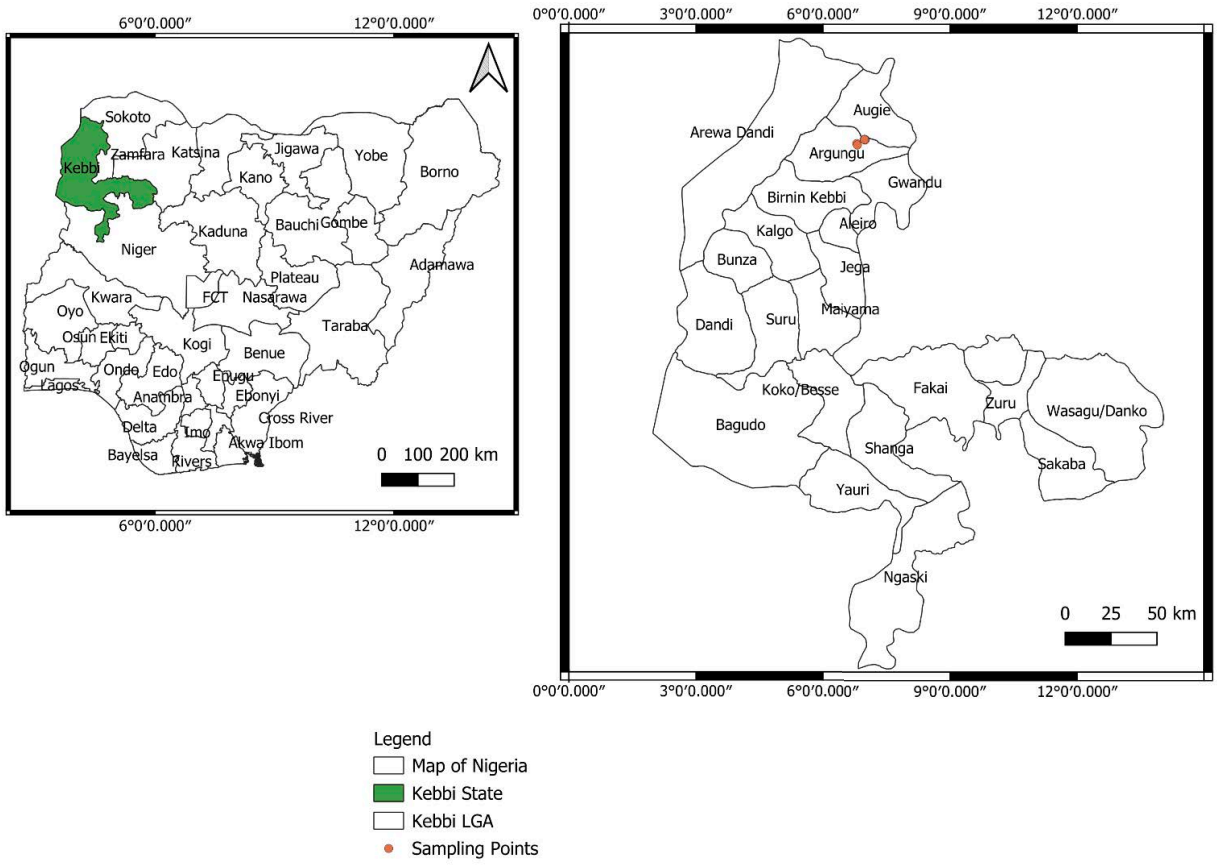


**Fig. 1. A map of Nigeria showing the Kebbi states and Argungu LGA, where samples were collected (designed using QGIS, version 3.44.0)**

munities (Community A and Community B). Each community contributed 60 samples, stratified equally by sex (30 males, 30 females) and age group (15 young, 15 adults per sex). Camels were humanely restrained, and sterile commercial cotton swabs were gently inserted 5–10 cm into the anterior nares and rotated along the mucosal surface for 5–10 seconds. Each swab was placed in 5 mL of tryptic soy broth supplemented with 6.5% NaCl, transported in an ice box, and processed within 6 hours at the Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. However, camels in the study area are managed under extensive pastoral systems with frequent informal and undocumented antimicrobial use, so reliable individual treatment histories were unavailable. Consequently, apparently healthy camels were sampled irrespective of prior antibiotic exposure.

**Bacteriological Isolation and Identification**

Samples were cultured on 5% horse blood agar and incubated aerobically at 37 °C for 24 hours. Colonies presumptively identified as *Staphylococcus* spp. were characterized based on morphology and Gram staining. Typical colonies were sub-cultured on Mannitol Salt Agar (Oxoid, Basingstoke, UK) and re-incubated at 37 °C for 24 hours. Isolates showing smooth, golden-yellow colonies were further tested biochemically using catalase and coagulase tests. Confirmatory identification of *S. aureus* was achieved through PCR amplification of the thermostable nuclease (*nuc*) gene.

**Phenotypic Detection of MRSA**

Presumptive *S. aureus* isolates were inoculated on Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid) to determine methicillin resistance phenotypically. Colonies that appeared intense blue on a colorless background were considered presumptive MRSA.

**Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, Manchester, UK), following Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. A bacterial suspension equivalent to the 0.5 McFarland standard was spread uniformly on agar plates,

and antibiotic discs were placed aseptically on the surface. Plates were incubated at 37 °C for 18 hours, and inhibition zones were measured in millimeters. The antibiotic panel included: Aztreonam (30 µg), Cefotaxime (30 µg), Amoxicillin-clavulanic acid (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Trimethoprim-sulfamethoxazole (25 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Gentamicin (10 µg), Kanamycin (30 µg), Ampicillin (10 µg), Ofloxacin (5 µg), Imipenem (10 µg), Erythromycin (15 µg), and Vancomycin (30 µg). Quality control was ensured using *Staphylococcus aureus* ATCC 25923 as a reference strain obtained from the American type culture collection (ATCC) through the Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. The measured inhibition zone diameters were interpreted and categorized as susceptible, intermediate, or resistant in accordance with the Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial susceptibility testing [14].

**Multiple Antibiotic Resistance Index (MARI)**

The MARI of each isolate was determined as described by Furtula [15]:

$$\text{MARI} = \frac{\text{Number of antibiotics resisted}}{\text{Total antibiotics tested}}$$

Values above 0.2 indicate isolates originating from environments with high antimicrobial use pressure.

**Genomic DNA Extraction**

Genomic DNA was extracted using the boiling method [16]. Briefly, a loopful of fresh overnight culture was suspended in 100 µL of sterile distilled water, heated at 96 °C for 10 min, and centrifuged at 12,000 × g for 5 min. The supernatant containing DNA was transferred to a sterile tube and stored at 4 °C until PCR analysis.

**PCR Detection of the *nuc* and *mecA* Genes**

PCR amplification was performed in 25 µL reaction mixtures containing 12.5 µL of 2× Master Mix (Qiagen), 1 µL (0.2 µM) each of forward and reverse primers (Table 1), 5 µL of DNA template, and 5.5 µL of nuclease-free water. The amplification program consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing (*nuc*: 55 °C; *mecA*: 57 °C) for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. PCR products were electrophoresed in 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination. Positive controls (*S. aureus* ATCC 43300 for *mecA* and *S. aureus* ATCC 25923 for *nuc*, obtained from the American Type Culture Collection (ATCC) through the Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto) and a negative control (PCR-grade water) were included in each run.

**Table 1. Primers used for the study**

| Gene name | Primer Sequence | Band Size (bp) | Reference |
|---|---|---|---|
| Nuc- F | 5′- GCGATT GAT GGT GAT ACG GTT-3′ | 270 | [24] |
| Nuc- R | 5′-AGCCAAGCCTTGACGAACTAAAGC-3′ | | |
| Mec- F | 5′- AAAATCGATGGTAAAGTTGGC-3′ | 533 | [24] |
| Mec- R | 5′-AGTTCTGCAGTACCGGATTTGC-3′ | | |

**Data Analysis**

Data were entered and analyzed using Microsoft Excel (version 2016) and InVivoStat (version 4.1). Descriptive statistics were used to compute prevalence and proportions. Associations between MRSA carriage and host factors (age, sex, and community) were tested using the Chi-square test at $p < 0.05$. Measures of association were expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

## RESULTS

**Nasal Carriage of *Staphylococcus aureus* and MRSA in Dromedary Camels**

Out of 120 nasal swabs examined, 66.7% (80/120) yielded *Staphylococcus aureus* based on culture, biochemical, and PCR confirmation of the *nuc* gene (Table 2). The overall prevalence of MRSA, confirmed by phenotypic growth on ORSAB and detection of the *mecA* gene, was 38.3% (46/120). The prevalence of *S. aureus* and MRSA varied across the two pastoral communities sampled. Community A recorded *S. aureus* in 60% (36/60) of camels and MRSA in 33.3% (20/60), whereas Community B recorded *S. aureus* in 73.3% (44/60) and MRSA in 43.3% (26/60) (Table 2). The difference in *S. aureus* carriage between communities was statistically significant ($\chi^2 = 6.79$, $p = 0.01$), while MRSA occurrence did not differ significantly ($\chi^2 = 3.15$, $p = 0.08$). Age- and sex-related differences were observed (Table 2). Younger camels showed

significantly higher colonization rates for both *S. aureus* (80%) and MRSA (53.3%) compared to adults (53.3% and 23.3%, respectively; $p < 0.001$). Similarly, females had higher prevalence rates for *S. aureus* (70%) and MRSA (46.7%) than males (63.3% and 30%; $p < 0.05$) (Table 2). These findings indicate that young and female camels were more likely to be colonized by *S. aureus* and MRSA.

### Molecular Confirmation of *nuc* and *mecA* Genes

PCR amplification confirmed all phenotypically identified *S. aureus* isolates as *nuc*-positive, generating the expected 270 bp amplicon (Fig. 2). Similarly, all 46 MRSA isolates carried the *mecA* gene (533 bp), validating their methicillin resistance (Fig. 3). No amplification was observed in negative controls.

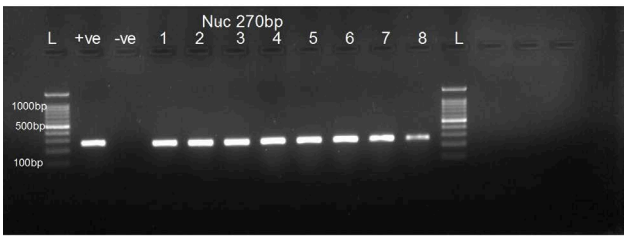


**Fig. 2. Agarose gel electrophoresis showing amplification of the *nuc* gene (270 bp) from *S. aureus* isolates using a 100 bp DNA ladder. (Lane legends: L = 100 bp DNA marker; +VE = positive control; -VE = negative control)**

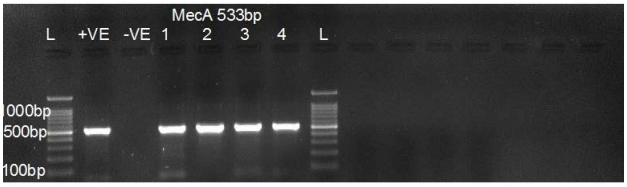


**Fig. 3. Agarose gel electrophoresis showing amplification of the *mecA* gene (533 bp) from MRSA isolates using a 100 bp DNA ladder. (Lane legends: L = 100 bp DNA marker; +VE = positive control; -VE = negative control)**

### Antimicrobial Resistance Profiles of MRSA

All MRSA isolates exhibited resistance to at least three classes of antibiotics, confirming their multidrug-resistant (MDR) status. The highest resistance frequencies were observed against penicillin (100%), ampicillin-sulbactam (100%), tetracycline (100%), chloramphenicol (100%), gentamicin (100%), erythromycin (100%), and kanamycin (100%). Moderate resistance was observed to cefotaxime (78.3%), ceftriaxone (65.2%), and ofloxacin (56.5%), while all isolates remained susceptible to vancomycin (Fig. 4).

### Multiple Antibiotic Resistance Index (MARI)

Table 3 summarizes the antibiotic resistance patterns and multiple antibiotic resistance index (MARI) of MRSA isolates recovered from dromedary camels. All MRSA isolates (46/46; 100%) were multidrug resistant, exhibiting resistance to three or more classes of antibiotics. Resistance consistently involved β-lactams (penicillin, ampicillin-sulbactam, amoxicillin–clavulanic acid, ceftriaxone), tetracyclines (tetracycline), aminoglycosides (gentamicin, kanamycin, amikacin, neomycin), macrolides (erythromycin), phenicols (chloramphenicol), and fluoroquinolones (ofloxacin), with some isolates also resistant to carbapenems (imipenem).

Although all isolates were classified as multidrug resistant, their resistance profiles were heterogeneous. Five distinct MDR patterns were identified, differing in both the

**Table 2. Prevalence of *Staphylococcus aureus* and MRSA among dromedary camels in Argungu, Kebbi State**

| Variable | No. sampled | *S. aureus* positive (%) | MRSA positive (%) | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|
| **Community** | | | | | |
| A | 60 | 36 (60.0) | 20 (33.3) | 6.79 | 0.01* |
| B | 60 | 44 (73.3) | 26 (43.3) | 3.15 | 0.08 |
| **Age group** | | | | | |
| Young | 60 | 48 (80.0) | 32 (53.3) | 12.22 | <0.001*** |
| Adult | 60 | 32 (53.3) | 14 (23.3) | | |
| **Sex** | | | | | |
| Female | 60 | 42 (70.0) | 28 (46.7) | 8.11 | 0.005** |
| Male | 60 | 38 (63.3) | 18 (30.0) | | |

*Significance levels:* $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $\chi^2$: Chi-square.

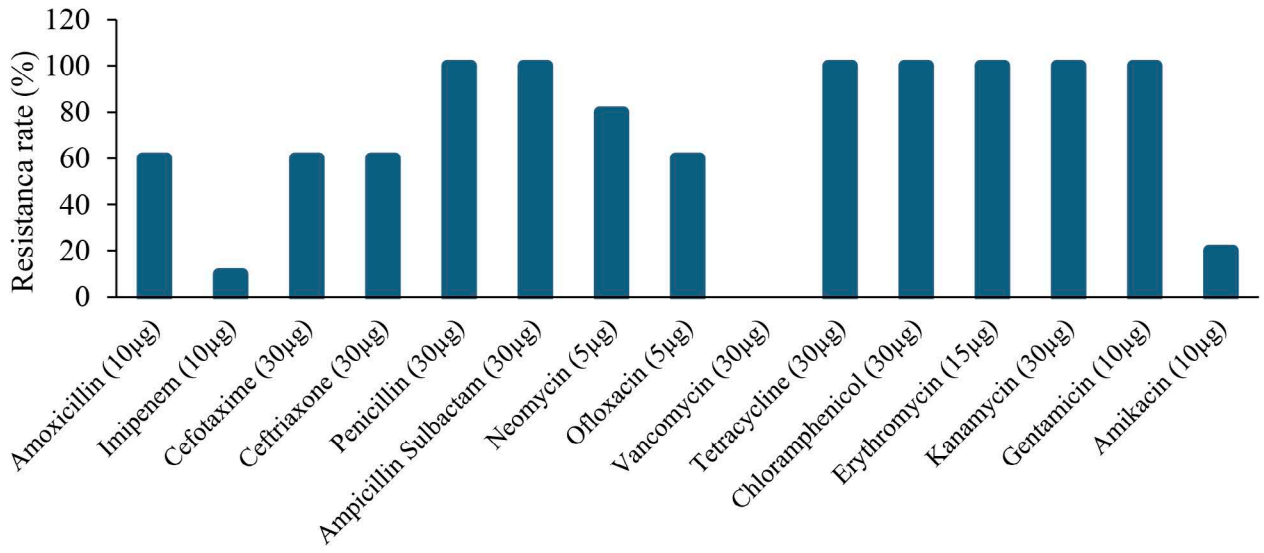


**Fig. 4. Antimicrobial resistance profile of MRSA isolates from camel nasal swabs, showing percentage resistance to tested antibiotics (n = 15)**

number and combination of antibiotic classes involved. The most frequent pattern, observed in 20 isolates, included resistance to β-lactams, tetracyclines, aminoglycosides, macrolides, phenicols, and fluoroquinolones, yielding a MARI value of 0.67. Other isolates exhibited narrower but still extensive resistance spectra, with MARI values ranging from 0.60 to 0.73. All resistance patterns were derived from antibiotics tested in this study, indicating that multidrug resistance was not limited to a single antimicrobial class or fixed resistance phenotype but reflected diverse exposure histories and selective pressures within the study population. The most common resistance patterns included PEN-AMS-TET-CHL-ERY-KAN-GEN-AMC-NEO-OFL (n = 20) and PEN-AMS-TET-CHL-ERY-KAN-GEN-NEO-AMK (n = 14).

**Table 3. Antibiotic Resistance Patterns and MARI of MRSA Isolates**

| Resistance pattern | No. of isolates | MARI |
|---|---|---|
| PEN-AMS-TET-CHL-ERY-KAN-GEN-AMC-NEO-OFL | 20 | 0.67 |
| PEN-AMS-TET-CHL-ERY-KAN-GEN-NEO-AMK | 14 | 0.60 |
| PEN-AMS-TET-CHL-ERY-KAN-GEN-AMC-IMP-CRO-OFL | 5 | 0.73 |
| PEN-AMS-TET-CHL-ERY-KAN-GEN-IMP-CRO | 4 | 0.60 |
| PEN-AMS-TET-CHL-ERY-KAN-GEN-IMP-OFL-AMK | 3 | 0.67 |

PEN = Penicillin; AMS = Ampicillin-sulbactam; TET = Tetracycline; CHL = Chloramphenicol; ERY = Erythromycin; KAN = Kanamycin; GEN = Gentamicin; AMC = Amoxicillin-clavulanic acid; NEO = Neomycin; OFL = Ofloxacin; AMK = Amikacin; IMP = Imipenem; CRO = Ceftriaxone.

## DISCUSSION

Antimicrobial resistance (AMR) continues to threaten global health, food security, and animal production systems, particularly in developing countries where antibiotic use is often unregulated [17]. The nasal cavity of camels harbors a diverse and dynamic microbial community that plays a critical role in respiratory health and microbial colonization. Previous studies in camels and other livestock species have demonstrated that the nasal microflora is predominantly composed of commensal Gram-positive bacteria, including coagulase-negative staphylococci, *Micrococcus* spp., *Corynebacterium* spp., and *Bacillus* spp., with *Staphylococcus aureus* commonly occurring as both a commensal and opportunistic pathogen [5, 18]. In addition, Gram-negative bacteria such as *Pasteurella* and other respiratory-associated organisms have been reported in camels, particularly under conditions of environmental stress, crowding, and exposure to dust typical of arid and semi-arid pastoral systems [18, 21].

The composition of this nasal microbial ecosystem influences the ability of *S. aureus*, including methicillin-resistant strains, to establish and persist within the host. Commensal bacteria may competitively inhibit pathogenic colonization through competition for adhesion sites and nutrients; however, disruption of this balance by antimicrobial exposure or management-related stressors can facilitate MRSA carriage [5, 6]. In pastoral camel production systems, factors such as close animal-to-animal contact,

shared watering points, frequent human handling, and unregulated antimicrobial use are likely to alter the nasal microflora and create selective pressure favoring multidrug-resistant organisms [8, 22].

The detection of MRSA in apparently healthy camels in the present study therefore reflects not only antimicrobial selection pressure but also ecological shifts within the nasal microflora that permit resistant strains to persist asymptomatically. Such silent carriage is epidemiologically important, as colonized camels may serve as reservoirs for onward transmission to humans, other livestock species, and the environment, consistent with previous One Health observations of livestock-associated MRSA [6, 23].

Beyond direct animal-to-animal and human-to-animal contact, the environment plays a critical role in the transmission and maintenance of *Staphylococcus aureus*, including methicillin-resistant strains, within pastoral production systems. *S. aureus* is well recognized for its ability to survive for prolonged periods on abiotic surfaces such as soil, dust, bedding materials, watering troughs, feeding equipment, and animal housing structures, thereby facilitating indirect transmission through environmental contamination [5, 6]. In arid and semi-arid regions such as Northwestern Nigeria, frequent exposure of camels to dust-laden environments, communal resting grounds, and shared watering points creates ideal conditions for the persistence and dissemination of environmentally derived staphylococci.

Environmental contamination is further amplified by the continuous shedding of *S. aureus* from colonized but clinically healthy animals through nasal secretions, skin contact, and faecal material. These organisms can accumulate in the surrounding environment and subsequently be reintroduced into susceptible hosts via inhalation of contaminated dust particles, direct contact with contaminated surfaces, or indirect transfer through handlers and husbandry tools [6, 8]. Such transmission pathways are particularly relevant in extensive pastoral systems, where biosecurity measures are minimal and animals from multiple herds frequently congregate at grazing fields, markets, and watering sites. The environment also acts as an important ecological interface for the selection and spread of antimicrobial-resistant *S. aureus*. Antibiotic residues introduced into soil and water through improper disposal of veterinary drugs, excreta from treated animals, and runoff from livestock holding areas can exert selective pressure on environmental bacterial populations, promoting the survival of resistant strains and resistance genes [7, 9]. These resistant organisms may subsequently colonize camels, contributing to the high prevalence of multidrug-resistant S. aureus observed in the present study. This mechanism is consistent with previous One Health observations that link environmental antimicrobial contamination with increased resistance burdens in livestock and humans [6, 7].

Environmental transmission blurs the distinction between community-associated, livestock-associated, and environmentally maintained *S. aureus* strains. Camels may therefore function not only as reservoirs but also as amplifiers of environmentally acquired *S. aureus*, sustaining a cycle of contamination between animals, humans, and shared ecosystems [6, 23]. The detection of MRSA in apparently healthy camels underscores the epidemiological significance of this pathway, as asymptomatic carriers can perpetuate environmental contamination without triggering disease-based control measures.

The present study provides the first molecular evidence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in apparently healthy dromedary camels in Nigeria, revealing a high prevalence and multidrug-resistant (MDR) phenotype. These findings underscore the growing risk of AMR dissemination at the human–livestock interface in pastoral settings. The 66.7% prevalence of *S. aureus* observed in this study is higher than previously reported among dromedary camels in Kano, Nigeria (14%; [11]) and other African and Middle Eastern studies, including Tunisia (6.6%; [18]), Egypt (17.9%; [19]), Saudi Arabia (10.7%; [20]), and Ethiopia (38.5%; [21]). Such variation may be attributed to differences in sample size, husbandry systems, hygienic practices, and antibiotic use intensity. The high prevalence recorded here likely reflects limited biosecurity and close human–animal contact typical of nomadic and transhumant camel-herding systems in Northwestern Nigeria. Similarly, the MRSA prevalence of 38.3% is markedly higher than earlier reports from Nigeria (5%; [11]), Egypt (6.8%; [19]), and Saudi Arabia (1.6%; [20]). This discrepancy could arise from differences in detection sensitivity (molecular versus phenotypic methods), the extent of antimicrobial exposure, or ecological and management factors influencing pathogen transmission. Frequent self-medication of camels with broad-spectrum antibiotics, unregulated use of veterinary drugs, and environmental contamination may contribute to the high

MRSA burden in this study area [22]. The significantly higher colonization rates among younger and female camels may reflect immunological and physiological factors. Young animals are immunologically naïve and more susceptible to bacterial colonization, while females, especially lactating ones, are more frequently handled and medicated, potentially increasing exposure to resistant strains [18, 23]. Furthermore, close animal grouping during milking or watering could facilitate nasal MRSA transmission through aerosols or fomites.

The high resistance rates of MRSA isolates to commonly used antibiotics such as tetracycline, erythromycin, chloramphenicol, gentamicin, and penicillin mirror findings in livestock from the same region [23, 24]. These antimicrobials are extensively used in both veterinary and human medicine in Northwestern Nigeria, often without prescription, promoting selection pressure and horizontal gene transfer of resistance determinants. The detection of complete susceptibility to vancomycin is reassuring, as it remains a critical "last-resort" drug for MRSA infections [7]. However, its exclusive reliance could precipitate resistance if misuse extends to veterinary applications. The Multiple Antibiotic Resistance Index (MARI) > 0.3 recorded in this study indicates substantial and sustained exposure of these bacterial populations to antimicrobial agents, consistent with reports from intensive livestock production systems [15]. The presence of multidrug resistance in *S. aureus* can be partly attributed to the acquisition of mobile genetic elements such as plasmids, transposons, and staphylococcal cassette chromosome mec (SCCmec) that carry resistance genes [25]. All MRSA isolates in this study harbored the *mecA* gene, confirming the molecular basis for their phenotypic resistance to β-lactam antibiotics and supporting earlier observations that phenotypic resistance strongly correlates with *mecA* carriage [26].

The detection of MRSA in apparently healthy camels highlights the potential for zoonotic transmission to pastoralists, herders, traders, and abattoir workers through direct contact or contaminated milk and meat. This finding is of particular concern in regions like Kebbi State, where veterinary infrastructure is limited and humans frequently share close environments with livestock. MRSA strains of livestock origin (*LA-MRSA*) have previously been reported to colonize humans, blurring the distinction between hospital, community, and animal-associated strains [6]. From a One Health perspective, this study reinforces the interconnectedness of animal health, human health, and environmental contamination in sustaining the AMR cycle. Antibiotic residues in the environment, combined with poor hygiene and unrestricted access to antimicrobials, create selective conditions for resistant bacteria to thrive. Implementing integrated surveillance and antimicrobial stewardship programs, particularly within transboundary livestock corridors, is essential to prevent further dissemination of MRSA and other resistant pathogens.

## CONCLUSION

This study provides the first molecular evidence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization among apparently healthy dromedary camels in Nigeria, revealing a high prevalence and extensive multidrug resistance pattern. The universal detection of the *mecA* gene among isolates confirms the molecular basis for methicillin resistance and suggests that camels may serve as important reservoirs and potential disseminators of resistant *S. aureus* strains within pastoral ecosystems. The high multiple antibiotic resistance index observed reflects substantial and sustained exposure of camel-associated bacterial populations to antimicrobial agents, likely resulting from unregulated antibiotic use and weak veterinary oversight. These findings have significant One Health implications, emphasizing the potential for cross-species transmission of MRSA between camels and humans, especially in pastoral communities where close animal contact and limited biosecurity are common.

The results of this study highlight the urgent need for continuous surveillance of MRSA in livestock populations, stricter antimicrobial stewardship, and strengthened collaboration between human and animal health sectors. Integrating molecular epidemiological approaches into national antimicrobial resistance monitoring frameworks will be vital for tracking transmission dynamics and informing control strategies. By establishing a molecular baseline for MRSA in camels, this study contributes valuable insights to the growing body of knowledge on zoonotic antimicrobial resistance in sub-Saharan Africa and highlights the necessity of adopting a unified One-Health approach to protect both animal and public health.

## Study Limitations

The sample was restricted to two pastoral communities within a single LGA, which may not fully represent the broader camel population in Nigeria. Additionally, the absence of molecular typing methods such as *spa*-typing, SCCmec typing, or multi-locus sequence typing (MLST) limited the ability to infer genetic relatedness or potential human-animal transmission. Future studies incorporating genomic epidemiology and comparative host sampling (camels, handlers, and the environment) are warranted to elucidate MRSA transmission dynamics.

## Data Availability Statement

The data sets used and/or analyzed during the current study are available within the manuscript and supplementary file.

## Ethical Statement

All procedures involving animals were approved by the Animal Research Ethics Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (Approval No.: UDUS/FAREC/03/2019). Animal handling followed institutional and international ethical standards for research involving animals.

## Conflict of Interest

None to be declared.


## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.


## Generative AI Statement

No generative AI was used in writing the manuscript, however Grammarly was used to ensure grammatical consistency of the manuscript.

## Authors' Contributions

Conceptualization: MSG, AHJ, MSJ, MDS. Investigation and data analysis: MSG, KOJ, MZM, SAA, AUE. Original draft preparation: MSG, KOJ. Review and editing: MSG, KOJ, MSJ, MDS, AHJ, MZM, SAA. All authors have read and agreed to the final version of the manuscript.


## Acknowledgement

*We appreciated the communities and the owners of the camels that allowed us to collect these samples.*


## REFERENCES


1. **Asiimwe, R., Ainembabazi, J. H., Egeru, A., Isoto, R., Aleper, D. K., Namaalwa, J., Diiro, G. M., 2020:** The role of camel production on household resilience to droughts in pastoral and agro-pastoral households in Uganda. *Pastoralism*, 10, 5, 1–12. DOI: 10.1186/s13570-020-0160-x
2. **Ait-El-Alia, O., Zine-Eddine, Y., Chaji, S., Boukrouh, S., Boutoial, K., Faye, B., 2025:** Global camel milk industry: A comprehensive overview of production, consumption trends, market evolution, and value chain efficiency. *Small Rumin. Res.,* 243, 107441. DOI: 10.1016/j.smallrumres.2025.107441
3. **Tharwat, M., Almundarij, T. I., Sadan, M., Khorshid, F., Swelum, A., 2023:** Is camel's urine friend or enemy? Review of its role in human health or diseases. *Open Vet. J.,* 13, 10, 1228–1238. DOI: 10.5455/OVJ.2023.v13.i10.1
4. **Amsidder, L., Alary, V., Duteurtre, G., Mnaouer, I., 2024:** The economic contribution of camel-based livestock systems in North-African drylands: The case of East and South Moroccan provinces. *Pastoralism: Res. Policy Pract*., 14, 13600. DOI: 10.3389/past.2024.13600
5. **Pal, M., Kerorsa, G. B., Marami, L. M., Kandi, V., 2020:** Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of *Staphylococcus aureus*: A comprehensive review. *Am. J. Public Health Res.,* 8, 1, 14–21.
6. **Abdullahi, I. N., Fernández-Fernández, R., Juárez-Fernández, G., Martínez-Álvarez, S., Eguizábal, P., Zarazaga, M., et al., 2021:** Wild animals are reservoirs and sentinels of *Staphylococcus aureus* and MRSA clones: A problem with "One Health" concern. *Antibiotics,* 10, 12, 1556. DOI: 10.3390/antibiotics10121556

7. **Silva, V., Correia, S., Pereira, J.E., Igrejas, G., Poeta, P. 2020:** Surveillance and environmental risk assessment of antibiotics and AMR/ARGs related with MRSA: One Health perspective. In: Hashmi, M. (eds) *Antibiotics and Antimicrobial Resistance Genes*. *Emerging Contaminants and Associated Treatment Technologies*. Springer, Cham. Pp. 271–295. DOI: 10.1007/978-3-030-40422-2_13
8. **Hussein, H. A., Abdi, S. M., Ahad, A. A., 2023:** Factors and challenges contributing to antimicrobial resistance in East African pastoral settings and importance of One Health approach. *CABI One Health*, ohcs202300025. DOI: 10.1079/cabionehealth.2023.0025
9. **Ifedinezi, O. V., Nnaji, N. D., Anumudu, C. K., Ekwueme, C. T., Uhegwu, C. C., Ihenetu, F. C., et al., 2024:** Environmental antimicrobial resistance: Implications for food safety and public health. *Antibiotics,* 13, 11, 1087. DOI: 10.3390/antibiotics13111087
10. **Gaddafi, M. S., Yakubu, Y., Junaidu, A. U., Bello, M. B., Garba, B., Bitrus, A. A., Lawal, H., 2021:** Nasal colonization of pigs and farm attendants by *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in Kebbi, Northwestern Nigeria. *Thai J. Vet. Med.,* 51, 1, 119–124. DOI: 10.56808/2985-1130.3100
11. **Yusuf, S. T., Kwaga, J. K. P., Okolocha, E. C., Bello, M., 2017:** Phenotypic occurrence of methicillin-resistant *Staphylococcus aureus* in camels slaughtered at Kano abattoir, Kano, Nigeria. *Sokoto J. Vet. Sci.,* 15, 2, 29–35. DOI: 10.4314/sokjvs.v15i2.4
12. **Belhout, C., Boyen, F., Vereecke, N., Theuns, S., Taibi, N., Stegger, M., et al., 2023:** Prevalence and molecular characterization of methicillin-resistant staphylococci (MRS) and mammaliicocci (MRM) in dromedary camels from Algeria: First detection of SCC*mec-mecc* hybrid in methicillin-resistant *Mammaliicoccus lentus*. *Antibiotics.,* 12, 4, 674. DOI: 10.3390/antibiotics12040674
13. **Thrusfield, M., 2018:** *Veterinary Epidemiology.* 4th ed. Oxford: Blackwell Scientific Publications, 434–440.
14. **Clinical and Laboratory Standards Institute (CLSI), 2023:** *Performance Standards for Antimicrobial Susceptibility Testing.* 33rd ed. CLSI supplement M100. Wayne, PA: CLSI.
15. **Furtula, V., Jackson, C. R., Farrell, E. G., Barrett, J. B., Hiott, L. M., Chambers, P. A., 2013:** Antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. *Int. J. Environ. Res. Public Health,* 10, 3, 1020–1036. DOI: 10.3390/ijerph10031020
16. **Chen, H., Rangasamy, M., Tan, S. Y., Wang, H., Siegfried, B. D., 2010:** Evaluation of five methods for total DNA extraction from western corn rootworm beetles. *PLoS One.,* 5, 8, e11963. DOI: 10.1371/journal.pone.0011963
17. **Coque, T. M., Cantón, R., Pérez-Cobas, A. E., Fernández-de-Bobadilla, M. D., Baquero, F., 2023:** Antimicrobial resistance in the global health network: Known unknowns and challenges for efficient responses in the 21st century. *Microorganisms.,* 11, 4, 1050. DOI: 10.3390/microorganisms11041050
18. **Chehida, B. F., Gharsa, H., Tombari, W., Selmi, R., Khaldi, S., Daaloul, M., et al., 2021:** First report of antimicrobial susceptibility and virulence gene characterization associated with *Staphylococcus aureus* carriage in healthy camels from Tunisia. *Animals.,* 11, 9, 2754. DOI: 10.3390/ani11092754
19. **El-Ghareeb, W. R., Almathen, F. S., Fayez, M. M., Alsultan, R. A., 2019:** Methicillin-resistant *Staphylococcus aureus* (MRSA) in camel meat: Prevalence and antibiotic susceptibility. *Slov. Vet. Res.,* 56, Suppl. 22, 249–256. DOI: 10.26873/SVR-764-2019
20. **Mahran, S., Elhelw, R., El-Jakee, J., 2020:** Characterization of *Staphylococcus aureus* isolated from camel and human samples from Aswan Governorate in Egypt. *Int. J. Vet. Sci.,* 9, 3, 397–402.
21. **Tasse, I., Mengistu, D. A., Belina, D., Girma, S., 2022:** Detection and determination of *Staphylococcus aureus* in camel milk and associated factors in Fedis, eastern Hararghe, Ethiopia. *Microbiol. Insights,* 15, 11786361221099876. DOI: 10.1177/117863612210998
22. **Mohmed, S. A., Barre, A., Mohamud, A. H., Gaciye, M. M., Hirsi, F. I., 2024:** Knowledge, attitude, and practice related to antibiotic use and resistance towards dairy camel farmers in the Banadir Region, Somalia. *J. Anim. Vet. Sci.,* 9, 1, 20–27. DOI: 10.20431/2455-2518.0901004
23. **Gaddafi, M. S., Yakubu, Y., Junaidu, A. U., Bello, M. B., Bitrus, A. A., Musawa, A. I., et al., 2023:** Occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) from dairy cows in Kebbi, Nigeria. *Iran. J. Vet. Med.,* 17, 1, 19–26. DOI: 10.22059/ijvm.17.1.1005256
24. **Gaddafi, M. S., Yakubu, Y., Bello, M. B., Bitrus, A. A., Musawa, A. I., Garba, B., et al., 2022:** Occurrence and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* (*MRSA*) in layer chickens in Kebbi, Nigeria. *Folia Vet.,* 66, 2, 37–45. DOI: 10.2478/fv-2022-0016
25. **Bitrus, A. A., Zunita, Z., Khairani-Bejo, S., Othman, S., Nadzir, N. A. A., 2018:** Staphylococcal cassette chromo-

some *mec* (SCC*mec*) and characterization of the attachment site (*att*B) of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates. *Microb. Pathog.,* 123, 323–329. DOI: 10.1016/j.micpath.2018.07.033

**26. Plata, K. B., Riosa, S., Singh, C. R., Rosato, R. R., Rosato, A. E., 2013:** Targeting of PBP1 by β-lactams determines *recA*/SOS response activation in heterogeneous MRSA clinical strains. *PLoS One.,* 8, 4, e61083. DOI: 10.1371/journal.pone.0061083

DOI: 10.2478/fv-2026-0014

FOLIA VETERINARIA, 70, 2: 34—46, 2026

REVIEW ARTICLE

# A SYSTEMATIC REVIEW AND META-ANALYSIS OF THE BACTERIAL PREVALENCES AND RESISTANCE PROFILES IN SELECTED FOOD-PRODUCING ANIMALS AND ANIMAL-DERIVED PRODUCTS IN NIGERIA (2000–2025)

**Mark Musa Hamman[1], Franklyn Ayomide Oluwadare[2], Terese Gabriel Orum[3], Blessing Seun Agbajelola[4], Adeola Mariam Lateef[1], Anthony Jide Ogunbadewa[5], Olubodun Michael Lateef[6], Victor Ibukun Agbajelola[1]***

[1]Department of Pathobiology and Integrative Biomedical Sciences, University of Missouri-Columbia, Missouri, USA; [2]Vaccine Production and Quality Control Program, Pan African University Life and Earth Sciences Institute (Including Health and Agriculture) - PAULESI, University of Ibadan, Oyo State, Nigeria; [3]Nigeria Centre for Disease Control and Prevention, Abuja, Nigeria; [4]Department of Health Promotion and Education, Faculty of Public Health, University of Ibadan, Ibadan, Oyo State, Nigeria; [5]Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia, Missouri, USA; [6]Department of Medical Pharmacology and Physiology, University of Missouri-Columbia, Missouri, USA

OPEN ACCESS

***Correspondence:** vianh2@missouri.edu

**Citation:** Hamman, M. M., Oluwadare, F. A., Orum, T. G., Agbajelola, B. S., Lateef, A. M., Ogunbadewa, A. J., Lateef, O. M., Agbajelola, V. I., 2026: A systematic review and meta-analysis of the bacterial prevalences and resistance profiles in selected food-producing animals and animal-derived products in Nigeria (2000–2025). Folia Veterinaria, 70, 2, 34–46.

**Received:** November 21, 2025

**Accepted:** April 14, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**Bacterial pathogens such as *Escherichia coli* and *Salmonella* spp. are widely reported in food-producing animals in Nigeria and contribute to animal disease, food contamination, and public health risk. However, evidence on their prevalence and antimicrobial resistance (AMR) patterns remains fragmented. This study systematically reviewed and meta-analyzed studies published between January 2000 and March 2025 on bacterial prevalence and resistance profiles in food-producing animals and related products in Nigeria. Data on pathogen type, host species, sample source, and antimicrobial susceptibility were extracted. Pooled prevalence estimates were calculated using random-effects meta-analysis (REML), and heterogeneity was assessed using Cochran's Q and $I^2$ statistics. Thirty-two studies met the inclusion criteria. *E. coli* and *Salmonella* spp. were the most frequently reported pathogens and exhibited substantial multidrug resistance (resistance to ≥3 antimicrobial classes), particularly to tetracyclines, fluoroquinolones, sulphonamides, and *β*-lactams. The pooled prevalence of bacterial detection was 25% (95% CI: 14%–41%), with high heterogeneity ($I^2$ = 96.9%). Higher prevalence was observed in poultry systems, and meta-regression indicated that sample size significantly influenced prevalence estimates. These findings highlight a substantial burden of resistant bacteria in food-animal systems and underscore the need for strengthened antimicrobial stewardship, improved surveillance, and coordinated One Health interventions in Nigeria.**

**Key words: antimicrobial resistance; *Escherichia coli*; food animals; meta-analysis; One Health; *Salmonella*; surveillance**

## INTRODUCTION

Bacterial pathogens of major importance to food-animal production in Nigeria—particularly *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp. and several others—have been widely reported in poultry, cattle, swine, and small ruminants across diverse regions of the country [1–7]. These organisms contribute not only to poor animal health, reductions in productivity, and increased economic losses for farmers but also to substantial challenges in veterinary clinical management [1, 3]. Rising antibiotic resistance has made the treatment of these infections increasingly difficult, with multiple studies documenting resistance to commonly used antibiotics, such as tetracyclines, fluoroquinolones, $\beta$-lactams, and aminoglycosides [2, 3, 5, 8]. Reports from several parts of Nigeria have consistently described multidrug-resistant *E. coli* and *Salmonella* serovars, including extended-spectrum $\beta$-lactamase (ESBL)-producing isolates, recovered from farm environments, slaughter facilities, and retail animal products [2, 6, 8, 9]. These findings underscore the growing clinical complexity of treating bacterial infections in livestock and the broader concerns for animal health systems in Nigeria.

A variety of structural and systemic factors contribute to the emergence and persistence of antimicrobial-resistant (AMR) bacteria in Nigerian food animals—antibiotics are widely accessible, frequently purchased without prescription, and commonly used for routine prophylaxis, metaphylaxis, or empirical mass treatment, particularly within commercial livestock operations [8–9]. These practices are often accompanied by inconsistent observance of withdrawal periods, insufficient veterinary oversight, and limited laboratory infrastructure for routine antibiotic susceptibility testing [2, 9]. Such conditions not only facilitate the development of antibiotic resistance but also hinder the generation of reliable data to guide antibiotic stewardship. As injudicious antibiotic use increases and production systems continue to expand, the challenges of understanding the national AMR landscape have become more pressing.

Despite the growing body of primary research, the existing evidence remains highly fragmented, as many studies focus on specific states, isolated production systems, or single pathogens, and their findings vary widely in methodology, sample size, and reporting standards [7]. Several narrative reviews have addressed aspects of AMR in Nigeria, but these are generally limited in analytical depth and have not provided formal meta-analytic estimates. Crucially, there is no comprehensive synthesis covering the entire 21st-century evidence base that integrates prevalence and resistance data across bacterial pathogens, regions, animal species, time periods, and antibiotic classes. In a country with a rapidly expanding livestock sector and significant public health concerns around antibiotic effectiveness, this gap has restricted the ability of policymakers, veterinary authorities, and public health practitioners to identify consistent national trends or to understand the magnitude and distribution of antibiotic-resistant bacteria [7, 8].

This systematic review and meta-analysis were undertaken to address these limitations by collating and rigorously synthesizing published evidence from 2000 to 2025. By bringing together data from multiple regions, animal hosts and animal-derived food products and applying standardized analytical methods, this study provides a comprehensive national assessment of bacterial pathogen prevalence and resistance patterns in Nigerian food-producing animals and animal-derived products. Beyond producing pooled estimates, the review evaluates the extent of heterogeneity across studies and explores the antibiotic resistance patterns of the bacteria identified. In doing so, it offers critical insights into the epidemiology of AMR in Nigeria's livestock sector, identifies gaps in surveillance and reporting, and highlights areas where targeted investment and policy interventions may have the greatest impact. The review represents a more detailed and methodologically robust synthesis of its kind for Nigeria and contributes essential evidence to guide antibiotic stewardship. Also, it improves surveillance frameworks and supports One Health–aligned strategies that safeguard the efficacy of antibiotics across both human and animal health.

## METHODS

This systematic review and meta-analysis were conducted and reported in accordance with the PRISMA 2020 statement and followed methodological guidance for prevalence reviews and meta-analyses. The review protocol was developed using the PRISMA-P checklist [10]. The methods described below summarize the stepwise approach used for literature identification, study selection, data extraction and validation, risk-of-bias assessment, quantitative and qualitative synthesis, and sensitivity anal-

yses to ensure a rigorous and transparent approach for prevalence reviews and meta-analyses.

**Eligibility criteria**

Studies were eligible if they reported primary data on bacterial pathogens isolated from food-producing animals in Nigeria (including but not limited to chickens, cattle, pigs, ducks, and quails) or from animal-derived products from these animals (including eggs, milk, and meat) and presented prevalence estimates or sufficient numerator/denominator data to calculate prevalence. Eligible studies also included those that reported detailed antibiotic susceptibility results for isolates, using phenotypic or molecular methods, because a principal aim was to synthesize resistance patterns. We included observational studies (cross-sectional surveys, surveillance reports, and cohort studies) and peer-reviewed articles as well as grey literature if primary data were available. We limited the search to publications in English between 1 January 2000 and 30 March 2025 and excluded reviews, editorials, commentaries and conference abstracts (without primary data), experimental infection (challenge) studies, studies of companion or wild animals that did not include food animals, studies not conducted in Nigeria, and reports that duplicated data published elsewhere. Studies reporting only antibiotic residue concentrations without isolates or organism-level susceptibility data were also excluded.

**Search strategy and information sources**

A comprehensive search was performed on 30 March 2025 in PubMed, Web of Science, ScienceDirect, and African Journals Online (AJOL) and supplemented by screening the reference lists of included studies and relevant reviews. The PubMed search combined Medical Subject Headings and free-text terms and used Boolean operators; a representative query was: ("food animals" OR livestock OR poultry OR cattle OR swine OR goats OR sheep) AND (Nigeria) AND ("*Salmonella*" OR "*Campylobacter*" OR "*Escherichia coli*" OR "*Listeria*" OR "*Staphylococcus*") AND ("antimicrobial resistance" OR "antibiotic resistance" OR "AMR") AND (prevalence OR occurrence OR epidemiology). The same conceptual search was adapted to other databases to match their syntax and filters; for example, MeSH terms were omitted in AJOL, and search fields were adjusted in Web of Science to search within Title/Abstract/Keywords. Where databases supported it, searches were limited by publication date (2000–2025) and language (English). Search logs were maintained, documenting the exact query used in each database and the number of records retrieved.

**Study selection and data management**

All retrieved records were exported to Microsoft Excel for Office 2019 (Microsoft Corp.) and then imported into a reference manager for de-duplication. After removal of duplicates, three reviewers (MMH, FAO, TGO) independently screened titles and abstracts against the eligibility criteria and subsequently reviewed full texts for inclusion. The reviewers used a pilot-tested screening form to ensure consistency, and discrepancies at either stage were resolved by discussion and, where necessary, adjudication by four other reviewers (AML, OML, BSA, and VIA). This multi-reviewer approach ensured consistency and minimized selection bias during study inclusion. The process of identification, screening, eligibility assessment, and inclusion was documented using a PRISMA flow diagram.

**Data extraction and validation**

Data were extracted using a standardized, pilot-tested abstraction form. Extracted variables included study characteristics (author, year, location), animal host, sample type, bacterial organism(s) identified, and prevalence data. Animal-derived samples (e.g., milk, eggs, and meat) were retained where studies explicitly linked them to food-producing animals, as these represent important points along the production-to-consumption continuum. Where reported, antimicrobial susceptibility testing (AST) methods and interpretive standards were recorded; however, due to inconsistent reporting, these variables were not included in quantitative synthesis. Antimicrobial resistance (AMR) was defined as resistance to any antimicrobial agent, including antibiotics and non-antibiotic agents such as sulphonamides. Information on resistance assessment approaches (phenotypic or genotypic) was noted where reported in the included studies. Phenotypic resistance was generally determined using antimicrobial susceptibility testing (AST) methods, including disk diffusion and minimum inhibitory concentration (MIC)-based approaches, although reporting of these methods and associated interpretive standards (e.g., CLSI or EUCAST) was inconsistent. Given this variability and incomplete reporting across

studies, AST methodologies were not analyzed as part of the synthesis, so the review focused on the reported antimicrobial resistance patterns.

### Risk-of-bias assessment

The methodological quality of included studies was assessed using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Prevalence Studies [11]. This tool evaluates sampling methods, population representativeness, measurement reliability, and completeness of reporting. Studies were classified as having low, moderate, or high risk of bias, and disagreements were resolved through consensus.

### Statistical analysis

Quantitative analyses were conducted in R (version 4.3.2) using the *meta* and *metafor* packages [12]. Study-level prevalence was calculated as the proportion of samples yielding the bacterial organism(s) of interest. The primary outcome was the bacterial pooled prevalence and the resistance profiles of the bacterial isolates. Proportions were logit-transformed prior to pooling, and random-effects meta-analysis was performed using restricted maximum likelihood (REML) to estimate between-study variance ($\tau^2$). Heterogeneity was assessed using Cochran's Q and quantified with the $I^2$ statistic. Subgroup analyses were conducted by host species, reclassified sample types, and geographic regions. Meta-regression was used to assess the influence of study-level variables, including sample size, study year, and host category, on prevalence estimates. Given the expected methodological and epidemiological variability across studies, pooled estimates were interpreted cautiously in the presence of substantial heterogeneity.

Publication bias was evaluated using funnel plots and Egger's regression test. Sensitivity analyses were performed to assess robustness by excluding high-risk studies and examining the influence of individual studies. In addition to quantitative synthesis, a qualitative analysis of AMR patterns was conducted. Resistance profiles across bacterial species and antimicrobial classes were summarized and visualized using a heatmap generated in *ggplot2*, with antimicrobial classes and organism names standardized to ensure comparability.

## RESULTS

### Literature search outcome

The database search identified 281 records across PUBMED, AJOL, and Web of Science, with 10 additional studies located through grey literature and manual searches. After removing 85 duplicates, 206 records were screened by title and abstract, and 169 were excluded for lacking extractable data or presenting duplicated datasets. Of the 37 studies assessed at full text, five were excluded due to unclear or undefined sampling procedures, resulting in thirty-two studies meeting all eligibility criteria for inclusion in the review and meta-analysis (Fig. 1; Table 1).

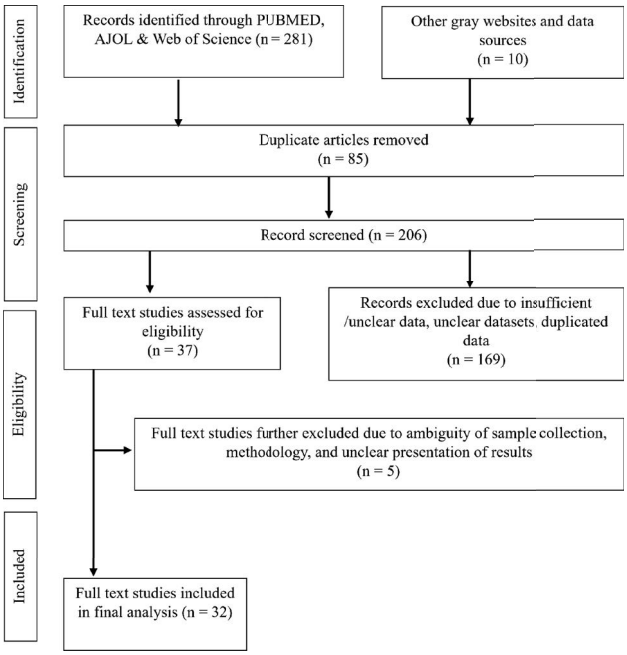


**Fig. 1. PRISMA flowchart of the selection of eligible studies**

### Descriptive characteristics of included studies

The 32 included studies covered food-producing animals and animal-derived samples across all geopolitical zones of Nigeria. In total, 9,811 samples were analyzed, of which 1,865 tested positive for the bacterial organism(s) reported in the studies (19.01%; 95% CI: 18.24%–19.8%). Chickens were the most frequently investigated host species, followed by cattle, pigs, and mixed avian populations. The South-west (27.1%) and South-south (20%) zones contributed the largest proportion of samples.

Across bacterial groups, studies focusing on single organisms reported moderate prevalence estimates. *Escherichia coli* was the most frequently isolated organism, with

**Table 1. Characteristics of eligible studies reporting the prevalence of resistant bacteria in food-producing animals and animal-derived products in Nigeria**

| Year | State | Geographic region | Animal/sample source | Positive samples | Reported bacterial prevalence (%) | No of isolates | Bacteria found | References |
|---|---|---|---|---|---|---|---|---|
| **2010** | Ogun | South-west | Dead chickens | 5 | 100.0 | 5 | *Salmonella* spp. | [32] |
| **2010** | Plateau | North-central | Dead-in-shell embryos, dead chicks, sick chicks and eggshells | 45 | 9.0 | 45 | *Salmonella* spp. | [34] |
| **2010** | Oyo | South-west | Live chickens | 70 | 10.9 | 70 | *Salmonella* spp. | [19] |
| **2011** | Osun | South-west | Live chickens | 2 | 1.3 | 2 | *Arcobacter butzleri*, *Arcobacter cryaerophilus* | [40] |
| **2014** | Oyo and Borno | South-west and North-east | Live chickens | 45 | 7.0 | 45 | *Salmonella* spp. | [26] |
| **2014** | Borno | North-east | Dead fishes | 23 | 11.5 | 23 | *Salmonella* spp. | [41] |
| **2017** | Ogun | South-west | Live quails | 14 | 3.5 | 14 | *Salmonella* spp. | [33] |
| **2018** | Ebonyi | South-east | Live cattle | 48 | 40.0 | 48 | *Escherichia coli* | [29] |
| **2018** | Oyo, Ondo, Ogun, Ekiti and Osun | South-west | Live chickens and pigs | 240 | 100.0 | 350 | *Klebsiella pneumoniae*, *Morganella morganii*, *Leclercia adecarboxylata* and *Citrobacter freundii* | [38] |
| **2018** | Enugu | South-east | Live chickens and pigs | 20 | 3.9 | 18 | *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter asburiae* and *Providencia* spp. | [39] |
| **2019** | Kwara | North-central | Live chickens | 58 | 6.4 | 58 | *Salmonella* spp. | [31] |
| **2020** | Kwara | North-central | Live and dead chickens | 42 | 11.8 | 58 | *Salmonella* spp. | [35] |
| **2021** | Enugu and Ebonyi | South-east | Live and dead chickens and cattle | 55 | 18.3 | 55 | *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas* spp. | [17] |
| **2021** | Kwara | North-central | Live pigs | 16 | 2.7 | 16 | *Salmonella* spp. | [18] |
| **2021** | Nasarawa | North-central | Cattle meat (suya samples) | 40 | 13.3 | 40 | *Escherichia coli* | [27] |
| **2021** | Oyo | South-west | Live cattle | 53 | 21.2 | 56 | *Escherichia coli* | [21] |
| **2021** | Ogun | South-west | Egg samples | 14 | 14.0 | 14 | *Salmonella* spp. | [3] |
| **2021** | Kwara | North-central | Dead chickens and ready-to-eat gizzards | 43 | 8.6 | 43 | *Salmonella* spp. | [37] |
| **2021** | Oyo | South-west | Milk samples from live cattle | 40 | 23.8 | 40 | *Escherichia* coli, *Enterobacter amnigenus* and *Pseudomonas aeruginosa* | [42] |
| **2022** | Ogun | South-west | Live chickens | 32 | 80.0 | 32 | *Escherichia coli* | [13] |
| **2022** | Plateau | North-central | Live and dead chickens and cattle | 132 | 28.0 | 132 | *Campylobacter* spp. | [14] |
| **2022** | Abuja and Lagos | North-central and South-west | Dead cattle | 44 | 16.2 | 43 | *Escherichia coli* | [15] |
| **2022** | Nasarawa | North-central | Live chickens | 120 | 100.0 | 120 | *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp. and *Pseudomonas* spp. | [16] |
| **2022** | Kwara | North-central | Dead chickens | 73 | 40.3 | 73 | *Escherichia coli* | [23] |
| **2022** | Oyo | South-west | Live chickens | 77 | 21.4 | 77 | *Salmonella* spp. | [1] |
| **2022** | Oyo | South-west | Live chickens and ducks | 156 | 83.9 | 156 | *Escherichia coli* and *Salmonella* spp. | [30] |
| **2023** | Edo and Delta | South-south | Live chickens | 10 | 20.0 | 10 | *Salmonella* spp. | [21] |
| **2023** | Sokoto, Kebbi and Zamfara | North-west | Day-old chickens | 32 | 10.7 | 32 | *Salmonella* spp. | [23] |
| **2023** | Kwara | North-central | Dead chickens | 37 | 10.5 | 37 | *Escherichia coli* | [24] |
| **2024** | Plateau | North-central | Live chickens | 178 | 99.4 | 178 | *Escherichia coli* | [20] |
| **2025** | Ebonyi | South-east | Dead pigs | 24 | 32.0 | 24 | *Escherichia coli* | [28] |
| **2025** | Oyo | South-west | Live ducks and pigeons | 78 | 22.9 | 30 | *Escherichia coli* | [36] |

a prevalence of 28.7% (606/2,111), while *Salmonella* spp. showed a lower prevalence of 8.9% (494/5,554). A subset of studies reported multiple bacterial organisms, commonly combinations of *E. coli*, *Salmonella* spp., *Klebsiella* spp., and *Pseudomonas* spp. with higher reported prevalence values, typically based on smaller sample sizes (Supplementary Table 2).

To enhance comparability, sample types were reclassified into four categories: live, dead, products (dead-derived), and others (live-derived). Live samples accounted for the largest proportion (5,895 samples; 60.1%), followed by dead samples (3,216 samples; 32.8%). Products derived from dead animals comprised 300 samples (3.0%), while other live-derived samples accounted for 400 samples (4.1%).

**Meta-analysis**

Random-effects meta-analysis of the 32 studies yielded a pooled prevalence of bacterial detection of 25% (95% CI: 14%–41%), with substantial heterogeneity observed across studies (Q = 999.21, p < 0.0001; $I^2$ = 96.9%; $\tau^2$ = 4.29) (Fig. 2).

Subgroup analysis showed variation in bacterial pooled prevalence across host species; among chickens, the pooled prevalence was 35% (95% CI: 13%–66%) based on 18 studies (n = 5,618). In cattle, the pooled prevalence was 22% (95% CI: 15%–31%) from five studies (n = 1,110). Other host categories showed a wide range of estimates, including pigs (10%; 95% CI: 1%–65%), Japanese quails (4%; 95% CI: 2%–6%), chickens and ducks (84%; 95% CI: 78%–89%), ducks and pigeons (23%; 95% CI: 19%–28%), and chickens and pigs (4%; 95% CI: 2%–96%) (Fig. 3A and B).

Meta-regression analysis indicated that total sample size significantly influenced reported prevalence estimates ($\beta$ = −0.0051, p = 0.0012), with larger studies reporting lower prevalence values. Residual heterogeneity remained high after adjustment ($\tau^2$ = 3.1; $I^2$ = 98.9%). The model explained 27.9% of between-study variability.

**Publication bias and sensitivity analysis**

Funnel plot inspection showed a broadly symmetrical distribution of studies around the pooled estimate (Fig. 4). Egger's regression test did not indicate significant small-study effects (p = 0.3109). Sensitivity analyses, including exclusion of studies with high risk of bias or extreme estimates, yielded pooled estimates comparable to the primary analysis.

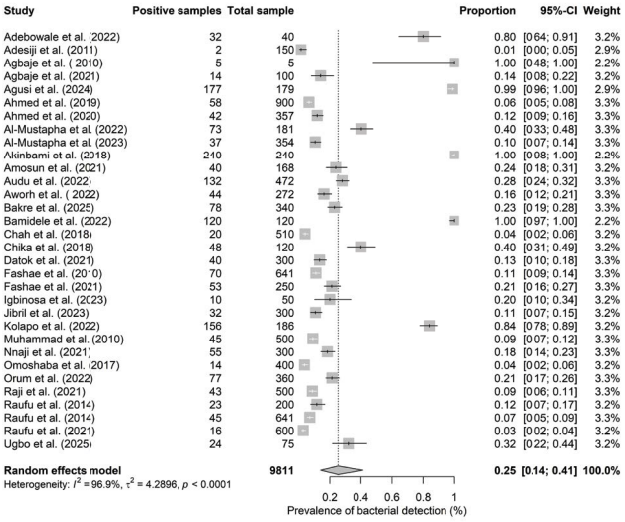


**Fig. 2. Forest plot showing the estimated pooled prevalence of bacterial detection/isolation among sampled food animals and related products**

**Resistance profiles of bacteria detected in studies included in the systematic review**

Across the included studies, AMR was frequently reported among *Escherichia coli*, *Salmonella* spp., and other enteric bacteria isolated from food animals and related samples. *E. coli* isolates demonstrated particularly broad antibiotic resistance profiles, with high resistance to tetracyclines, sulphonamides, and *β*-lactams (Fig. 5), and many studies reported multidrug resistance (MDR) rates above 70% [13, 20, 28]. Extended-spectrum *β*-lactamase (ESBL)-producing and cefotaxime-resistant *E. coli* were also widely documented [15, 21, 23].

Similarly, *Salmonella* spp. exhibited notable resistance to multiple antimicrobial classes, including *β*-lactams, fluoroquinolones, and sulphonamides, with several studies reporting multidrug-resistant phenotypes [18, 24, 30]. Other bacterial species, including *Klebsiella* spp., *Pseudomonas* spp., and *Enterobacter* spp., also demonstrated resistance to commonly used antimicrobials, particularly *β*-lactams and tetracyclines, although the magnitude of resistance varied across studies [17, 26, 31]. Molecular analyses further identified key resistance determinants, including *tetA*, *tetB*, *sul1*, *ampC*, and *qnr* genes, as well as mutations in *gyrA* and *parC*, supporting the observed phenotypic resistance patterns and indicating the circulation

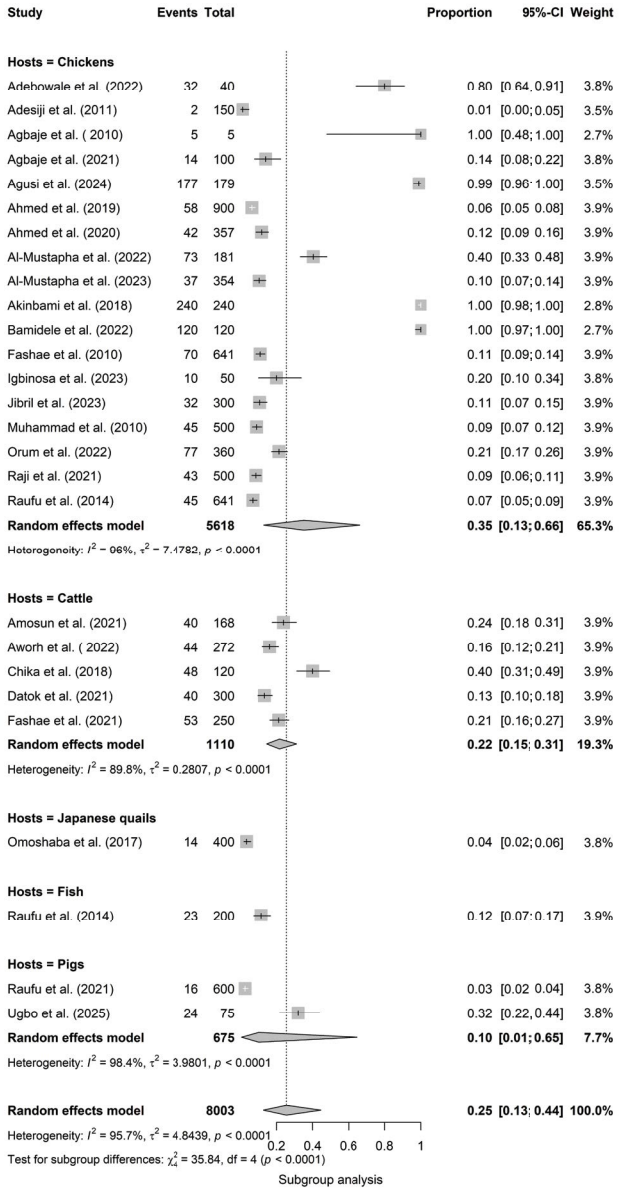


**Fig. 3A. Forest plot showing pooled bacterial prevalence in studies of isolates recovered from single-host categories (chicken, cattle, pigs, Japanese quails, and fishes)**

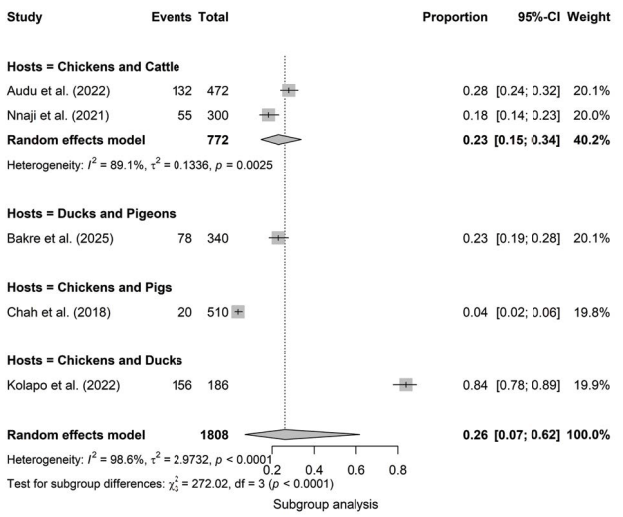


**Fig. 3B. Forest plot showing pooled bacterial prevalence in studies involving multiple-host categories (including ducks and pigeons, chickens and pigs, and chickens and ducks)**

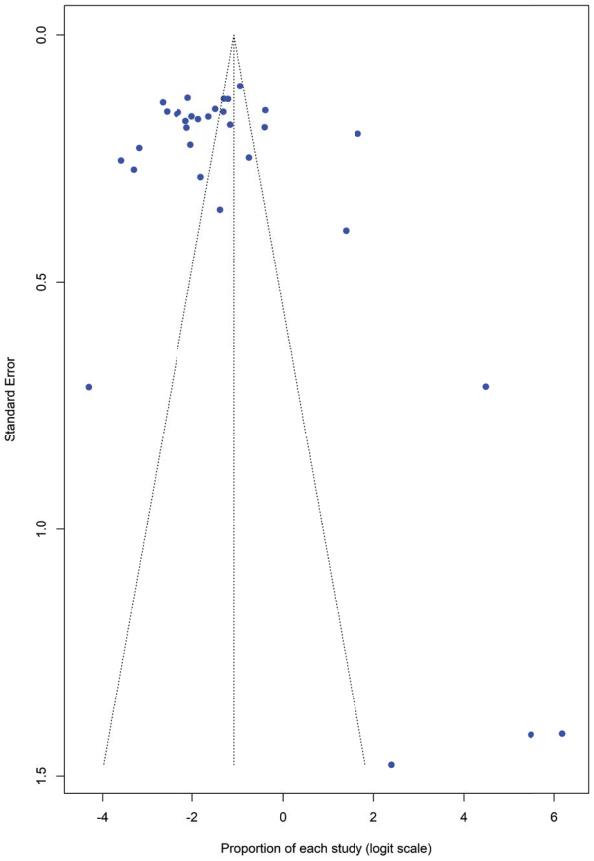


**Fig. 4. Funnel plot displaying the proportion of each study against the standard error of each study**

of resistant bacterial lineages within food-animal production systems [15, 21, 23, 25, 42].

## DISCUSSION

The studies included in this review provide a contemporary synthesis of bacterial prevalence and antimicrobial resistance (AMR) in food-producing animals and related samples in Nigeria. The pooled prevalence of the bacteria identified and resistance patterns observed are consistent with earlier reports from Nigeria [43–44] and other African countries, including Ghana, Kenya, and Ethiopia [45–47], confirming that antimicrobial-resistant bacteria in livestock remain a significant and persistent public health concern across the continent.

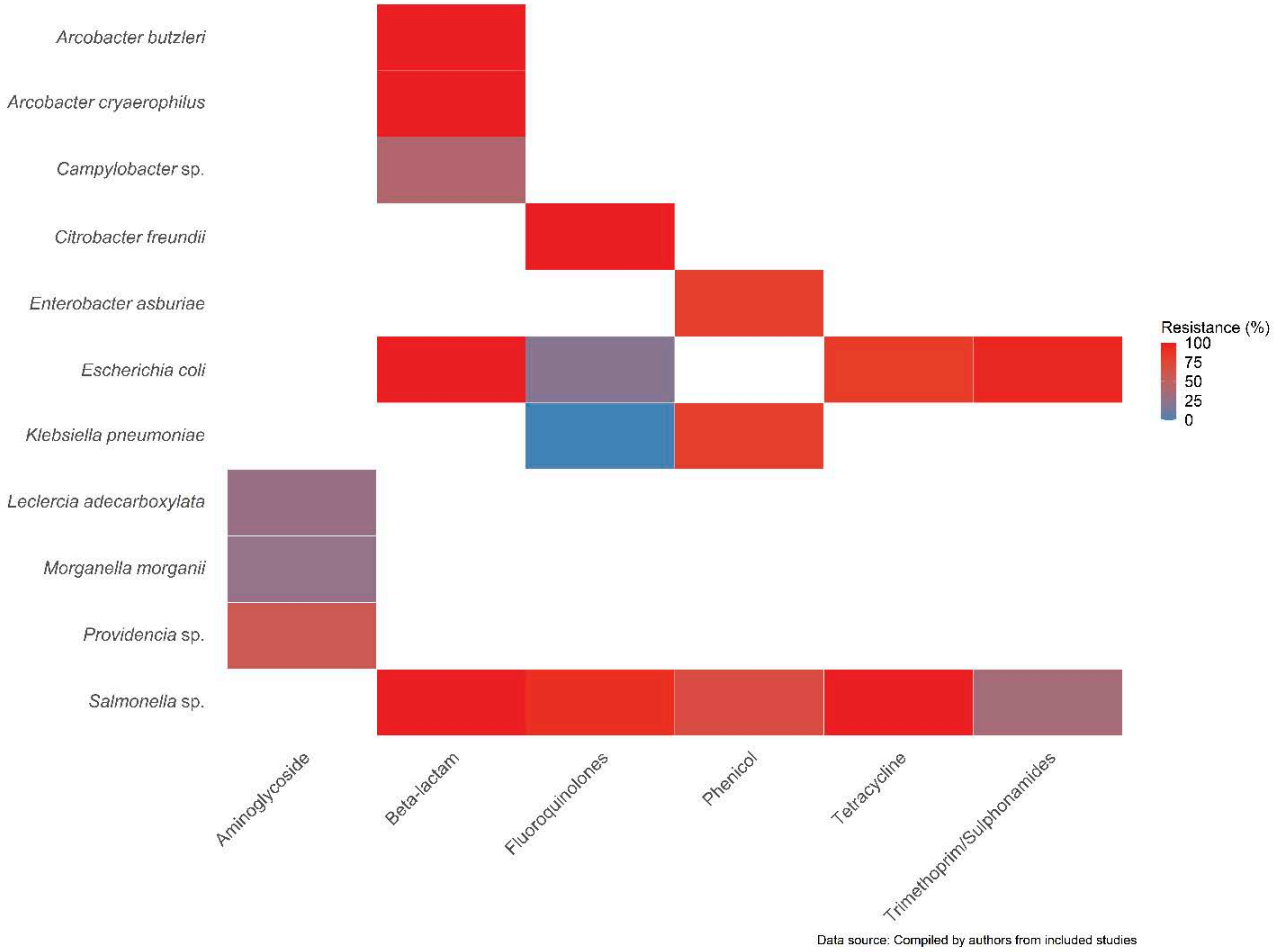


**Fig. 5. Resistance patterns of microorganisms isolated from food-producing animals and animal-derived products in Nigeria (2000–2025)**

Comparable trends are also reported globally. In Europe and other high-surveillance settings, AMR in food-producing animals continues to be driven by the emergence of multidrug-resistant (MDR) bacteria, defined as resistance to three or more antimicrobial classes, and the widespread occurrence of extended-spectrum $\beta$-lactamase (ESBL)-producing *Escherichia coli*. The detection of such organisms in this review highlights the continued relevance of food animals as reservoirs of resistance and underscores the potential movement of resistant bacteria and resistance determinants along the farm-to-fork continuum.

Geographical variation in bacterial prevalence was evident, with higher levels observed in the South-west and North-central regions. These areas are characterized by intensive livestock production, dense market networks, and informal slaughter systems, which may increase opportunities for bacterial transmission and environmental contamination. Similar spatial clustering has been reported in East African production systems with comparable intensification patterns [48–49].

Across host species, poultry, particularly chickens and ducks, demonstrated the highest burden of bacterial detection and AMR. This finding aligns with global evidence indicating that poultry systems, often associated with high stocking densities and frequent antimicrobial exposure, act as major reservoirs of resistant bacteria [50–51]. The predominance of *E. coli* and *Salmonella* spp. across studies is consistent with their recognized role as indicator organisms in AMR surveillance and their capacity to acquire and disseminate resistance determinants [52]. The detection of resistant bacteria in meat, milk, and eggs further reflects contamination risks along the production chain, as reported in other African settings [53–54].

Host-specific differences were also observed, with higher prevalence in poultry compared with cattle. This likely reflects differences in production systems and antimicrobial-use practices, a pattern consistently reported across African livestock systems [49, 55]. The high prevalence observed in mixed chicken–duck systems may be associated with increased opportunities for cross-species

transmission, whereas lower prevalence in quail populations may reflect differences in management practices and stocking density [56].

Substantial between-study heterogeneity was observed, indicating marked variability in prevalence estimates across studies. This variability is likely influenced by differences in production systems, sampling strategies, antimicrobial use, and laboratory methodologies [57]. The inverse relationship between sample size and reported prevalence observed in meta-regression suggests a small-study effect, whereby smaller studies, often conducted in localized or higher-risk settings, report higher prevalence estimates, while larger studies provide more conservative and representative estimates, and this pattern is well documented in epidemiological and AMR meta-analyses [58].

Despite the absence of significant publication bias based on funnel plot symmetry and Egger's test, these findings should be interpreted with caution in the context of high heterogeneity. Furthermore, the limited explanatory power of included moderators suggests that key drivers of AMR, such as antimicrobial usage patterns, farm-level biosecurity, and environmental conditions, remain insufficiently reported in primary studies [59].

The resistance profiles observed across bacterial species in this review are consistent with regional and global trends. High resistance to tetracyclines, fluoroquinolones, sulphonamides, and *β*-lactams was frequently reported, alongside the detection of ESBL-producing *E. coli* and resistance to third-generation cephalosporins. The presence of resistance determinants such as *tetA*, *sul1*, *qnr*, and mutations in *gyrA* and *parC* reflects the circulation of resistant bacterial lineages within animal production systems and aligns with patterns described in global AMR surveillance studies [60–62].

**Study limitations**

This review has several limitations that should be considered when interpreting the findings. First, the included studies varied widely in design, sampling strategies, laboratory methods, antimicrobial susceptibility testing approaches, and antibiotic testing panels, which contributed to substantial heterogeneity and limited the comparability of resistance estimates across settings. Differences in AST methodology, including the use of disk diffusion versus MIC-based methods, as well as variation in breakpoint interpretation and reporting standards such as CLSI or EUCAST, may have influenced the classification of susceptibility results across studies. In addition, some studies did not clearly report quality-control procedures, breakpoints, or interpretive criteria, raising the possibility of misclassification or inconsistency in resistance reporting. Furthermore, most included studies relied on phenotypic susceptibility testing, while only a limited number reported genotypic resistance determinants, which constrained direct comparison between phenotypic and molecular resistance findings across studies.

Additionally, several studies reported incomplete metadata, such as host species, sample type, production system, or antibiotic exposure history, which constrained the depth of subgroup and meta-regression analyses. Publication bias is also likely, as studies with high antibiotic resistance levels may be more readily published than those reporting low or null findings, although statistical tests for asymmetry were inconclusive due to underlying heterogeneity. Finally, the reliance on phenotypic data in most included studies limited our ability to link AMR patterns to specific genetic determinants comprehensively, and the scarcity of molecular analyses restricted inferences about transmission pathways and the emergence of extensively resistant bacterial pathogens. Despite these limitations, the synthesis provides a robust and regionally relevant overview of bacterial prevalence and antibiotic resistance patterns across the animal, food, and environmental interface.

## CONCLUSION AND RECOMMENDATIONS

This review demonstrates that antibiotic resistance is widespread across animal, food, and environmental reservoirs, with particularly high burdens of MDR *Escherichia coli*, *Salmonella* spp., and other enteric pathogens among food animals in Nigeria. The consistency of these patterns across diverse hosts, sample types, and geographic settings underscores the extent to which AMR has become entrenched within livestock production systems and along the broader One Health interface. The high prevalence of resistance to critically important antibiotic classes—including *β*-lactams, fluoroquinolones, and tetracyclines—signals a growing threat to both animal health and public health, especially in regions where surveillance capacity and regulatory enforcement remain limited. The substantial heterogeneity observed across the 32 studies in this work further

highlights the fragmented nature of AMR monitoring in Nigeria and the urgent need for more standardized, coordinated, and longitudinal data to capture trends over time.

Considering these findings, strengthening integrated AMR surveillance should be a priority, with emphasis on harmonized laboratory protocols, routine reporting of both phenotypic and genotypic data, and improved geographic representativeness. Policies aimed at reducing inappropriate antibiotic use in livestock, including restrictions on non-therapeutic use, improved access to veterinary oversight, and promotion of vaccination, biosecurity, and hygiene—are essential to prevent the emergence and spread of antibiotic-resistant bacteria. Investment in laboratory capacity, data management systems, and intersectoral collaboration will be critical to operationalizing effective One Health AMR control strategies. Finally, future research should focus on filling existing data gaps, characterizing antibiotic resistance genes and transmission pathways, and evaluating intervention effectiveness to inform evidence-based policy and practice.

## Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

## Ethical Statement

No ethical approval was necessary for this study.

## Conflict of Interest

Authors declare that no conflicts of interest are directly or indirectly related to the work submitted for publication.


## Funding

No funding was received for this study.


## Authors' Contributions

Agbajelola VI conceived and designed the study. Hamman MM, Oluwadare FA, and Orum TG worked on data screening and selection. Agbajelola VI, Lateef AM, Ogunbadewa AJ, Agbajelola BS, and Lateef OM validated the method and reviewed the manuscript. Agbajelola VI carried out the analysis and drafted the initial version of the manuscript. All the authors read and approved the final manuscript.


## Acknowledgments

*The authors would like to acknowledge all anonymous reviewers for their constructive feedback.*


## REFERENCES


1. **Orum, T. G., Ishola, O. O., Adebowale, O. O., 2022:** Occurrence and antimicrobial susceptibility patterns of *Salmonella* species from poultry farms in Ibadan, Nigeria. *Afr. J. Lab. Med.,* 11, 1, a1606. DOI: 10.4102/ajlm.v11i1.1606
2. **Aworh, M. K., Kwaga, J., Okolocha, E., Harden, L., Hull, D., Hendriksen, R. S., Thakur, S., 2020:** Extended-spectrum *β*-lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. *One Health Outlook*, 2, 8. DOI: 10.1186/s42522-020-00014-7
3. **Agbaje, M., Ayo-Ajayi, P., Kehinde, O., Omoshaba, E., Dipeolu, M., Fasina, F. O., 2021:** *Salmonella* characterization in poultry eggs sold in farms and markets in relation to handling and biosecurity practices in Ogun State, Nigeria. *Antibiotics*, 10, 7, 773. DOI: 10.3390/antibiotics10070773
4. **Ogbor, O., Ajayi, A., Zautner, A. E., Smith, S. I., 2019:** Antibiotic susceptibility profiles of *Campylobacter coli* isolated from poultry farms in Lagos, Nigeria – a pilot study. *Eur. J. Microbiol. Immunol.*, 9, 2, 32–34. DOI: 10.1556/1886.2019.00007
5. **Sati, N. M., Card, R. M., Barco, L., Muhammad, M., Luka, P. D., Chisnall, T., et al., 2024:** Antimicrobial resistance and phylogenetic relatedness of *Salmonella* serovars in indigenous poultry and their drinking water sources in North Central Nigeria. *Microorganisms,* 12, 8, 1529. DOI: 10.3390/microorganisms12081529
6. **Adeyanju, G. T., Ishola, O., 2014:** *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *SpringerPlus*, 3, 139. DOI: 10.1186/2193-1801-3-139
7. **Njoga, E. O., Nnaemeka, V. C., Jaja, I. F., Oguttu, J. W., Nwanta, J. A., Chah, K. F., 2025:** Systematic review and meta-analysis of *Campylobacter* species infections in humans and food-producing animals in Nigeria, 2002–2023: The imperative of a One Health control approach. *One Health*, 20, 101029. DOI: 10.1016/j.onehlt.2025.101029

8. **Jibril, A. H., Okeke, I. N., Dalsgaard, A., Olsen, J. E., 2021:** Association between antimicrobial usage and resistance in *Salmonella* from poultry farms in Nigeria. *BMC Vet. Res.,* 17, 234, 1–10. DOI: 10.1186/s12917-021-02938-2
9. **Hambolu, D. A., Olatoye, O. I., Besong, M. A., Call, D. R., 2024:** Low-cost biosecurity measures are associated with reduced detection of non-typhoidal *Salmonella* in Nigerian poultry while inappropriate antibiotic use is widespread. *Sci. Rep*., 14, 20974. DOI: 10.1038/s41598-024-72317-y
10. **Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., The PRISMA Group, 2009:** Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med*., 6, 7, e1000097. DOI: 10.1371/journal.pmed.1000097
11. **Munn, Z., Moola, S., Lisy, K., Riitano, D., Tufanaru, C., 2015:** Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. *Int. J. Evid. Based Healthc.*, 13, 3, 147–153. DOI: 10.1097/XEB.0000000000000054
12. **R Core Team, 2020:** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/
13. **Adebowale, O., Makanjuola, M., Bankole, N., Olasoju, M., Alamu, A., Kperegbeyi, E., et al., 2022:** Multi-drug resistant *Escherichia coli*, biosecurity and antimicrobial use in live bird markets, Abeokuta, Nigeria. *Antibiotics*, 11, 2, 253. DOI: 10.3390/antibiotics11020253
14. **Audu, B. J., Norval, S., Bruno, L., Meenakshi, R., Marion, M., Forbes, K. J., 2022:** Genomic diversity and antimicrobial resistance of *Campylobacter* spp. from humans and livestock in Nigeria. *J. Biomed. Sci*., 29, 7, 1–15. DOI: 10.1186/s12929-022-00786-2
15. **Aworh, M. K., Ekeng, E., Nilsson, P., Egyir, B., Owusu-Nyantakyi, C., Hendriksen, R. S., 2022:** Extended-spectrum *β*-lactamase-producing *Escherichia coli* among humans, beef cattle, and abattoir environments in Nigeria. *Front. Cell. Infect. Microbiol*., 12, 869314. DOI: 10.3389/fcimb.2022.869314
16. **Bamidele, O., Yakubu, A., Joseph, E. B., Amole, T. A., 2022:** Antibiotic resistance of bacterial isolates from smallholder poultry droppings in the Guinea Savanna Zone of Nigeria. *Antibiotics*, 11, 7, 973. DOI: 10.3390/antibiotics11070973
17. **Nnaji, J. O., Moses, I. B., Ejikeugwu, P. C., Nwakaeze, E. A., Ude-Ude, I., Iroha, I. R., 2021:** Antibiogram and molecular characterization of AmpC and ESBL-producing Gram-negative bacteria from poultry and abattoir samples. *Pak. J. Biol. Sci*., 24, 2, 193–198. DOI: 10.3923/pjbs.2021.193.198
18. **Raufu, I. A., Ahmed, O. A., Aremu, A., Ameh, J. A., Timme, R. E., Hendriksen, R. S., Ambali, A. G., 2021:** Occurrence, antimicrobial resistance and whole genome sequence analysis of *Salmonella* serovars from pig farms in Ilorin, North-central Nigeria. *Int. J. Food Microbiol*., 350, 109245. DOI: 10.1016/j.ijfoodmicro.2021.109245
19. **Fashae, K., Ogunsola, F., Aarestrup, F. M., Hendriksen, R. S., 2010:** Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. *J. Infect. Dev. Ctries*., 4, 8, 484–494. DOI: 10.3855/jidc.909
20. **Agusi, E. R., Kabantiyok, D., Mkpuma, N., Atai, R. B., Okongwu-Ejike, C., Bakare, E. L., et al., 2024:** Prevalence of multidrug-resistant *Escherichia coli* isolates and virulence gene expression in poultry farms in Jos, Nigeria. *Front. Microbiol*., 15, 1298582. DOI: 10.3389/fmicb.2024.1298582
21. **Fashae, K., Engelmann, I., Monecke, S., Braun, S. D., Ehricht, R., 2021:** Molecular characterisation of extended-spectrum *β*-lactamase-producing *Escherichia coli* in wild birds and cattle, Ibadan, Nigeria. *BMC Vet. Res*., 17, 33, 1–12. DOI: 10.1186/s12917-020-02734-4
22. **Igbinosa, I. H., Amolo, C. N., Beshiru, A., Akinnibosun, O., Ogofure, A. G., El-Ashker, M., et al., 2023:** Identification and characterization of MDR virulent *Salmonella* spp. isolated from smallholder poultry production environments in Edo and Delta States, Nigeria. *PLoS ONE*, 18, 2, e0281329. DOI: 10.1371/journal.pone.0281329
23. **Al-Mustapha, A. I., Alada, S. A., Raufu, I. A., Lawal, A. N., Eskola, K., Brouwer, M. S. M., et al., 2022:** Co-occurrence of antibiotic and disinfectant resistance genes in extensively drug-resistant *Escherichia coli* isolated from broilers in Ilorin, North Central Nigeria. *J. Glob. Antimicrob. Resist*., 31, 337–344. DOI: 10.1016/j.jgar.2022.11.002
24. **Jibril, A. H., Okeke, I. N., Dalsgaard, A., Olsen, J. E., 2023:** Prevalence and whole genome phylogenetic analysis reveal genetic relatedness between antibiotic resistance *Salmonella* in hatchlings and older chickens from farms in Nigeria. *Poult. Sci*., 102, 3, 102427. DOI: 10.1016/j.psj.2022.102427
25. **Al-Mustapha, A. I., Raufu, I. A., Ogundijo, O. A., Odetokun, I. A., Tiwari, A., Brouwer, M. S. M., et al., 2023:** Antibiotic resistance genes, mobile elements, virulence genes, and phages in cultivated ESBL-producing *Escherichia coli* of poultry origin in Kwara State, North Central Nigeria. *Int. J. Food Microbiol*., 389, 110086. DOI: 10.1016/j.ijfoodmicro.2023.110086
26. **Raufu, I. A., Fashae, K., Ameh, J.A., Ambali, A., Ogunsola, F. T., Coker, A. O., Hendriksen, R. S., 2014:** Persistence of

fluoroquinolone-resistant *Salmonella enterica* serovar Kentucky from poultry and poultry sources in Nigeria. *J. Infect. Dev. Ctries*., 8, 3, 384–388. DOI: 10.3855/jidc.3495

27. **Datok, D. W., Ishaleku, D., Tsaku, P. A., Agya, E. O., Adoga, M. P., 2021:** Multidrug resistance to commonly prescribed antibiotics in *Escherichia coli* isolated from barbecued beef (Suya) sold in a Nigerian city. *Pan Afr. Med. J*., 39, 50, 1–8. DOI: 10.11604/pamj.2021.39.50.25502
28. **Ugbo, E. N., Effendi, M. H., Ugbo, A. I., Tyasningsih, W., Agumah, B. N., Raharjo, H. M., et al., 2025:** Molecular identification of tetracycline resistance genes in *Escherichia coli* isolates from internal organs of swine sold in Abakaliki, Nigeria. *Open Vet. J.*, 15, 1, 171–178. DOI: 10.5455/OVJ.2025.v15.i1.16
29. **Chika, E., Charles, E., Ifeanyichukwu, I., Michael, A., 2018:** First detection of FOX-1 AmpC *β*-lactamase gene expression among *Escherichia coli* isolated from abattoir samples in Abakaliki, Nigeria. *Oman Med. J*., 33, 3, 243–249. DOI: 10.5001/omj.2018.44
30. **Kolapo, A., Amosun, E., Olatoye, O., Adeoye, F., Oladele, O., 2022:** *Escherichia coli* O157:H7 and Salmonella species isolated from ducks and indigenous chickens in live-bird markets in Ibadan, Oyo State, Nigeria. *Veterinaria Italiana*, 58, 4. DOI: 10.12834/VetIt.2553.16733.2
31. **Ahmed, A. O., Raji, M. A., Mamman, P. H., Kwanashie, C. N., Raufu, I. A., Aremu, A., Akorede, G. J., 2019:** Salmonellosis: serotypes, prevalence and multidrug-resistant profiles of *Salmonella enterica* in selected poultry farms, Kwara State, North Central Nigeria. *Onderstepoort J. Vet. Res*., 86, 1, 1–8. DOI: 10.4102/ojvr.v86i1.1667
32. **Agbaje, M., Davies, R., Oyekunle, M. A., Ojo, O. E., Fasina, F. O., Akinduti, P. A., 2010:** Observation on the occurrence and transmission pattern of *Salmonella gallinarum* in commercial poultry farms in Ogun State, South Western Nigeria. *Afr. J. Microbiol. Res.,* 4, 9, 796–800.
33. **Omoshaba, E. O., Olufemi, F. O., Ojo, O. E., Sonibare, A. O., Agbaje, M., 2017:** Multidrug-resistant *Salmonellae* isolated in Japanese quails reared in Abeokuta, Nigeria. *Trop. Anim. Health Prod*., 49, 7, 1455–1460. DOI: 10.1007/s11250-017-1347-z
34. **Muhammad, M., Muhammad, L. U., Ambali, A., Mani, A. U., Azard, S., Barco, L., 2010:** Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents. *Vet. Microbiol*., 140, 1–2, 131–135. DOI: 10.1016/j.vetmic.2009.07.009
35. **Ahmed, A. O., Raji, M. A., Mamman, P. H., Kwanashie, C. N., Raufu, I. A., Akorede, G. J., et al., 2020:** *Salmonella enterica* isolated from selected poultry farms in Kwara State, Nigeria between 2015 and 2016 showed resistance to critical antimicrobials. *J. Cent. Eur. Agric*., 21, 1, 14–24. DOI: 10.5513/JCEA01/21.1.2298
36. **Bakre, A. A., Adekanmbi, A. O., Ajani, I., Festus, P., 2025:** Genetic diversity and antibiogram of ESBL-producing *Escherichia coli* isolated from apparently healthy birds sold at two selected live bird markets in Nigeria. *Mol. Biol. Rep*., 52, 357. DOI: 10.1007/s11033-025-10445-y
37. **Raji, M. A., Kazeem, H. M., Magyigbe, K. A., Ahmed, A. O., Lawal, D. N., Raufu, I. A., 2021:** *Salmonella* serovars, antibiotic resistance, and virulence factors isolated from intestinal content of slaughtered chickens and ready-to-eat chicken gizzards in the Ilorin Metropolis, Kwara State, Nigeria. *Int. J. Food Sci*., 2021, 8872137, 1–11. DOI: 10.1155/2021/8872137
38. **Akinbami, O. R., Olofinsae, S., Ayeni, F. A., 2018:** Prevalence of extended spectrum beta-lactamase and plasmid-mediated quinolone-resistant genes in strains of *Klebsiella pneumoniae*, *Morganella morganii*, *Leclercia adecarboxylata* and *Citrobacter freundii* isolated from poultry in South Western Nigeria. *PeerJ*, 6, e5053. DOI: 10.7717/peerj.5053
39. **Chah, K. F., Ugwu, I. C., Okpala, A., Adamu, K. Y., Alonso, C. A., Ceballos, S., et al., 2018:** Detection and molecular characterisation of extended-spectrum *β*-lactamase-producing enteric bacteria from pigs and chickens in Nsukka, Nigeria. *J. Glob. Antimicrob. Resist*., 15, 36–40. DOI: 10.1016/j.jgar.2018.06.002
40. **Adesiji, Y. O., Coker, A. O., Oloke, J. K., 2011:** Detection of *Arcobacter* in feces of healthy chickens in Osogbo, Nigeria. *J. Food Prot*., 74, 1, 119–121. DOI: 10.4315/0362-028X.JFP-10-231
41. **Raufu, I. A., Lawan, F. A., Bello, H. S., Musa, A. S., Ameh, J. A., Ambali, A. G., 2014:** Occurrence and antimicrobial susceptibility profiles of *Salmonella* serovars from fish in Maiduguri, sub-Saharan Nigeria. *Egypt. J. Aquat. Res*., 40, 1, 59–63. DOI: 10.1016/j.ejar.2014.01.003
42. **Amosun, E. A., Ogunbadewa, A. J., Ojo, O. E., Akinade, S. A., 2021:** Investigation of extended spectrum beta-lactamase producing *Escherichia coli* and other cefotaxime-resistant bacteria in cow milk in Nigeria. *Isr. J. Vet. Med.*, 76, 3, 116–125.
43. **Adesokan, H. K., Akanbi, I. O., Akanbi, I. M., Obaweda, R. A., 2015:** Pattern of antimicrobial usage in livestock animals in South-Western Nigeria: The need for alternative plans. *Onderstepoort J. Vet. Res*., 82, 1, 816. DOI: 10.4102/ojvr.v82i1.816

44. **Fagbamila, I. O., Barco, L., Mancin, M., Kwaga, J., Ngulukun, S. S., Zavagnin, P., et al., 2017:** *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms. *PLoS ONE*, 12, 3, e0173097. DOI: 10.1371/journal.pone.0173097
45. **Donkor, E. S., Odoom, A., Osman, A. H., Darkwah, S., Kotey, F. C. N., 2024:** A systematic review on antimicrobial resistance in Ghana from a One Health perspective. *Antibiotics*, 13, 7, 662. DOI: 10.3390/antibiotics13070662
46. **Mitema, E. S., Kikuvi, G. M., Wegener, H. C., Stohr, K., 2001:** An assessment of antimicrobial consumption in food producing animals in Kenya. *J. Vet. Pharmacol. Ther*., 24, 6, 385–390. DOI: 10.1046/j.1365-2885.2001.00360.x
47. **Gemeda, B. A., Wieland, B., Alemayehu, G., Knight-Jones, T. J. D., Wodajo, H. D., Tefera, M., et al., 2023:** Antimicrobial resistance of *Escherichia coli* isolates from livestock and the environment in extensive smallholder livestock production systems in Ethiopia. *Antibiotics*, 12, 5, 941. DOI: 10.3390/antibiotics12050941
48. **Katakweba, A. A. S., Muhairwa, A. P., Lupindu, A. M., Damborg, P., Rosenkrantz, J. T., Minga, U. M., et al., 2018:** First report on a randomized investigation of antimicrobial resistance in fecal indicator bacteria from livestock, poultry, and humans in Tanzania. *Microb. Drug Resist*., 24, 3, 260–268. DOI: 10.1089/mdr.2016.0297
49. **Ssemakadde, T., Pauline Petra, N., Busingye, J. C., Bazira, J., Taseera, K., 2025:** Prevalence and antimicrobial resistance of *Salmonella* and pathogenic *E. coli* in broiler farms, Wakiso district, Uganda. *PLoS One*, 20, 7, e0309599. DOI: 10.1371/journal.pone.0309599
50. **Oloso, N. O., Fagbo, S., Garbati, M., Olonitola, S. O., Awosanya, E. J., Aworh, M. K., et al., 2018:** Antimicrobial resistance in food animals and the environment in Nigeria: A review. *Int. J. Environ. Res. Public Health*, 15, 6, 1284. DOI: 10.3390/ijerph15061284
51. **World Bank, 2017:** Drug-resistant infections: A threat to our economic future. World Bank Report, Washington DC. Retrieved from https://documents1.worldbank.org/curated/en/455311493396671601/pdf/executive-summary.pdf on Nov. 15. 2025
52. **WHO, 2025:** Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2015–2022. World Health Organization, Geneva. Retrieved from https://www.flemingfund.org/wp-content/uploads/af70da7dd8db123c9f-32392fa550975f.pdf on Nov. 15. 2025
53. **Eagar, H., Swan, G., van Vuuren, M., 2012:** A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. *J. S. Afr. Vet. Assoc*., 83, 1, 16. DOI: 10.4102/jsava.v83i1.16
54. **Ayukekbong, J. A., Ntemgwa, M., Atabe, A. N., 2017:** The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrob. Resist. Infect. Control*, 6, 47, 1–8. DOI: 10.1186/s13756-017-0208-x
55. **Abo-Shama, U. H., Abo-Amer, A. E. S., Ahmed, E. A. E., Alsaadawy, R. M., Sayed, H. H., 2025:** Prevalence of MDR and ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among meat products sold at Sohag Governorate, Egypt. *BMC Microbiol*., 25, 636, 1–12. DOI: 10.1186/s12866-025-04392-8
56. **Bata, S. I., Karshima, N. S., Yohanna, J., Dashe, M., Pam, V. A., Ogbu, K. I., 2016:** Isolation and antibiotic sensitivity patterns of *Salmonella* species from raw beef and quail eggs from farms and retail outlets in Jos, Plateau State, Nigeria. *J. Vet. Med. Anim. Health*, 8, 4, 29–34. DOI: 10.5897/JVMAH2014-0315
57. **Agbajelola, V. I., Agbajelola, B. S., 2025:** A systematic review and meta-analysis on the prevalence of bovine fascioliasis in Nigeria. *Acta Parasit*., 70, 57, 1–13. DOI: 10.1007/s11686-025-00991-7
58. **Andrel, J. A., Keith, S. W., Leiby, B. E., 2009:** Meta-analysis: A brief introduction. *Clin. Transl. Sci.*, 2, 5, 374–378. DOI: 10.1111/j.1752-8062.2009.00152.x
59. **Awoyomi, O. J., Olasoju, M. I., Kehinde, O. O., Adebowale, O. O., Awoyomi, F. S. O., Agbajelola, V. I., Ogugua, A. J., 2022:** Impact of COVID-19 on livestock production and marketing in Nigeria. *Nig. J. Anim. Prod*., 49, 3, 215–232.
60. **Onduru, O. G., Mkakosya, R. S., Aboud, S., Rumisha, S. F., 2021:** Genetic determinants of resistance among ESBL-producing *Enterobacteriaceae* in East, Central, and Southern Africa: A systematic review and meta-analysis of prevalence. *Can. J. Infect. Dis. Med. Microbiol*., 2021, 515323, 1–9. DOI: 10.1155/2021/5153237
61. **Widodo, A., Khairullah, A. R., Effendi, M. H., Moses, I. B., Agustin, A. L. D., 2024:** Extended-spectrum $\beta$-lactamase-producing *Escherichia coli* from poultry: A review. *Vet. World*, 17, 9, 2017–2027. DOI: 1 0.14202/vetworld.2024.2017-2027
62. **van den Bogaard, A. E., Stobberingh, E. E., 2000:** Epidemiology of resistance to antibiotics: Links between animals and humans. *Int. J. Antimicrob. Agents*, 14, 4, 327–335. DOI: 10.1016/s0924-8579(00)00145-x

DOI: 10.2478/fv-2026-0015

FOLIA VETERINARIA, 70, 2: 47—50, 2026

CASE REPORT

# MICROSPORIDIAL GRANULOMATOUS CONJUNCTIVITIS IN A BEARDED DRAGON (*POGONA VITTICEPS*): A CASE REPORT

**Ladislav Novotný[1,2]*, Petr Soukup[3,4], Martin Květoň[5,6], Jan Misík[1]**

[1]School of Veterinary Medicine, St. George's University, True Blue, Grenada, West Indies; [2]Department of Veterinary Disciplines, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic, [3]EyeVET Veterinary Ophthalmology, Jablonec nad Nisou, Czech Republic; [4]Animal Eye Practice, Berlin, Federal Republic of Germany; [5]Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic; [6]Third Faculty of Medicine, Charles University, Prague, Czech Republic

OPEN ACCESS

***Correspondence:** ladislavnovotnyvet@gmail.com

**Citation:** Novotný, L., Soukup, P., Květoň, M., Misík, J., 2026: Microsporidial granulomatous conjunctivitis in a bearded dragon (*Pogona vitticeps*): A case report. Folia Veterinaria, 70, 2, 47–50.

**Received:** December 14, 2025

**Accepted:** January 29, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**An eight-month-old female bearded dragon (*Pogona vitticeps*) was presented with chronic nodular conjunctivitis of the left lower eyelid that failed to respond to previous topical antibiotic and anti-inflammatory therapy. Histopathological examination of an excisional biopsy revealed marked granulomatous inflammation in the lamina propria and submucosa. Special stains (Gram, PAS) demonstrated numerous microsporidia within sporophorous vacuoles, while Ziehl–Neelsen staining for acid-fast bacteria was negative. Based on the histopathological findings and results of special stains, a diagnosis of microsporidial granulomatous conjunctivitis was established. Postoperatively, the patient was treated with tobramycin–dexamethasone eye drops in a tapering regimen for four weeks, resulting in local resolution of the lesion. However, the animal died two months after the initial ophthalmological examination. As post-mortem examination was not performed, systemic microsporidial infection, previously reported in bearded dragons, cannot be excluded. This case highlights microsporidia as a potential cause of chronic conjunctival lesions in bearded dragons and emphasizes histopathology with special stains as a key diagnostic tool.**

**Key words: *Encephalitozoon pogonae*; histopathology; ocular infections; reptiles**

## INTRODUCTION

Ocular disorders are relatively common in bearded dragons (*Pogona vitticeps*); however, infections caused by microsporidia are reported only sporadically. Microsporidia are obligate intracellular eukaryotic parasites that, based on molecular phylogenetic studies, are currently classified within the kingdom Fungi [1]. They can infect a broad range of vertebrate hosts, including reptiles [2]. In reptiles, microsporidial infections are

typically associated with chronic, multisystemic granulomatous inflammation and high mortality, most often due to granulomatous arteritis and subsequent organ failure [3, 4]. Ophthalmic manifestations of microsporidial infection include keratitis, conjunctivitis [5], and blepharoconjunctivitis [6]. The present report describes a rare case of microsporidial granulomatous conjunctivitis in a juvenile bearded dragon and discusses its diagnostic and clinical relevance.

## CASE PRESENTATION

An eight-month-old female bearded dragon (*Pogona vitticeps*) was examined for a several-week history of conjunctival thickening of the left eye. The animal was housed individually in a terrarium with appropriate temperature gradients and ultraviolet (UV) lighting and was fed crickets supplemented with calcium and vitamin $D_3$.

Ophthalmological examination revealed marked conjunctival hyperaemia and chemosis, predominantly affecting the lower eyelid, with no other ocular abnormalities detected (Fig. 1). Visual function appeared to be preserved. A clinical diagnosis of nodular conjunctivitis was made. Previous treatment with topical antibiotics (tobramycin eye drops) and anti-inflammatory agents failed to induce regression of the lesion.

An excisional biopsy of the thickened conjunctiva (6 × 3 × 3 mm) was performed, and the tissue was submitted for histopathological examination. No additional laboratory investigations were undertaken.

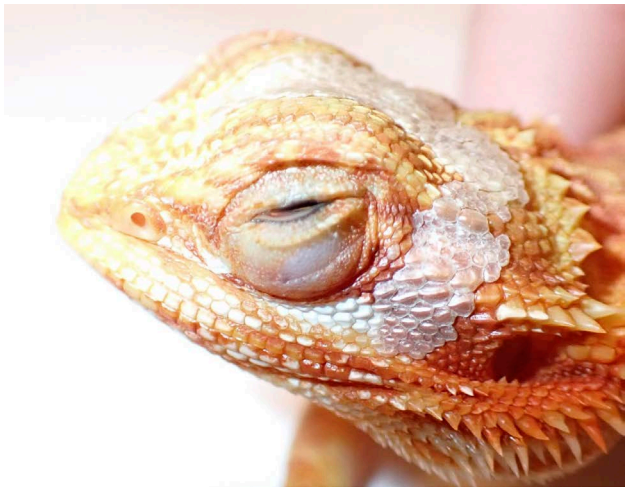

**Fig. 1. Bearded dragon (*Pogona vitticeps*) with marked swelling of the conjunctiva of the left lower eyelid.**

## RESULTS

### Histopathology

In the haematoxylin-eosin-stained sections, the conjunctival epithelium was focally attenuated. The lamina propria and submucosa were expanded by multifocal to coalescing, dense inflammatory infiltrates composed predominantly of macrophages, with fewer small lymphocytes and heterophils. The inflammatory infiltrate multifocally formed granulomas with central necrosis (Fig. 2). The cytoplasm of a subset of macrophages was conspicuously pale and abundant, suspicious for the presence of intracellular microorganisms (Fig. 3). The inflammatory process extended to the surgical margins. No neoplastic tissue was identified.

Special stains: Gram stain revealed numerous microsporidia within sporophorous vacuoles in the cytoplasm of macrophages (Fig. 4). Ziehl–Neelsen staining for acid-fast bacteria was negative. Microsporidial spores were lightly basophilic with a darker polar cap and measured approximately 3–5 µm. A histopathological diagnosis of microsporidial granulomatous conjunctivitis was established.

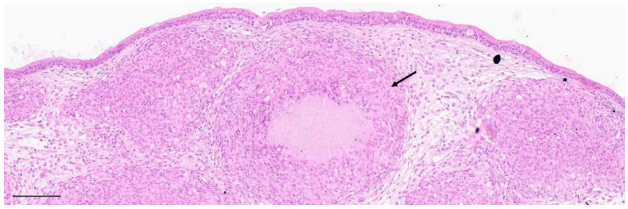

**Fig. 2. Histological section of conjunctiva with multiple granulomas; larger granulomas show central necrosis (arrow). Haematoxylin and eosin. Scale bar = 100 µm.**

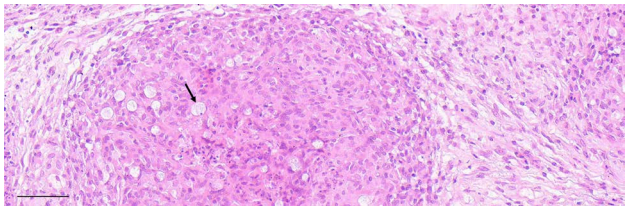

**Fig. 3. Higher magnification showing weakly staining organisms within the cytoplasm of macrophages in a granuloma (arrow). Haematoxylin and eosin. Scale bar = 50 µm.**

## MANAGEMENT AND OUTCOMES

Following excisional biopsy, topical therapy with tobramycin–dexamethasone eye drops (TOBRADEX 3 mg/ml +

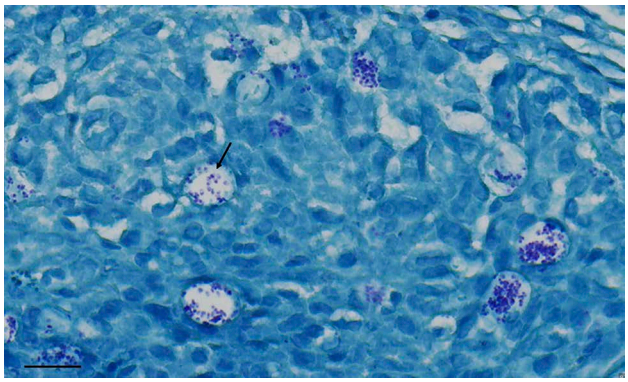

**Fig. 4. Gram stain revealing numerous microsporidial spores within macrophage cytoplasm (arrow). Scale bar = 20 µm.**

1 mg/ml, Novartis Pharma GmbH) was instituted in a tapering regimen over four weeks, resulting in complete local healing of the conjunctival lesion. Nevertheless, the animal died approximately two months after the initial ophthalmological examination. A necropsy was not permitted.

## DISCUSSION

The findings in the present case are consistent with chronic granulomatous conjunctivitis caused by microsporidia, a condition that is rare but documented in lizards [6, 7]. In bearded dragons, microsporidial infections have been reported not only as localized ocular disease but also as systemic infections affecting multiple organs [5], frequently with a fatal outcome [3, 4]. In the present case, death occurred two months after the initial ophthalmological examination, despite complete local resolution of the conjunctival lesion following surgical excision and topical therapy.

Because post-mortem examination was not performed, systemic microsporidial infection could not be confirmed, and the exact cause of death remains undetermined. In previously published cases, microsporidial keratoconjunctivitis required combined surgical and antifungal therapy, including enucleation and systemic antifungal treatment (itraconazole, fenbendazole, voriconazole) [7, 8]. In another report, the diagnosis of ocular microsporidiosis was supported by histopathology and PCR with genomic sequencing, leading to definitive identification of *Encephalitozoon pogonae* [6].

In the present case, the diagnosis was established by direct histological demonstration of microsporidia within sporophorous vacuoles. The morphology and intracellular localization of the spores are consistent with *Encephalitozoon pogonae*; however, definitive species identification would require PCR-based molecular typing. Differential diagnoses for granulomatous conjunctivitis in reptiles include atypical bacterial and fungal infections, mycobacteriosis, protozoal infections, and non-infectious causes such as foreign bodies or keratin accumulation.

## CONCLUSION

Chronic inflammatory lesions of the eyelids, conjunctiva, and cornea in bearded dragons may be caused by microsporidial infection and should therefore be considered in the differential diagnostic work-up of chronic ocular disease in reptiles. Conjunctival biopsy, followed by histopathological examination with appropriate special stains, is a crucial step in establishing an aetiological diagnosis.

### Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

### Ethical Statement

This case report is based on the examination of a privately owned animal presented for clinical care. No experimental procedures were performed; therefore, specific ethical approval was not required.

### Conflict of Interest

The authors declare no conflict of interest.


### Funding

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.


### Generative AI Statement

Generative AI was used exclusively for language editing and stylistic refinement of the manuscript. The authors

take full responsibility for the scientific content, interpretation, and conclusions presented in this article.

### Authors' Contributions

L.N. and J.M. performed the histopathological evaluation and interpretation. P.S. conducted the ophthalmological examination and clinical management. M.K. performed the special stains, contributed to the interpretation of findings and literature review. All authors contributed to manuscript preparation and approved the final version.

### Acknowledgement

*The authors thank the animal caretakers for their cooperation and the technical staff for assistance with histological processing.*

## REFERENCES

1. **Keeling, P., 2009:** Five questions about microsporidia. *PLoS Pathog.*, 5, 9, e1000489. DOI: 10.1371/journal.ppat.1000489
2. **Vergneau-Grosset, C., Larrat, S., 2016:** Microsporidiosis in vertebrate companion exotic animals. *J. Fungi*, 2, 1, 3. DOI: 10.3390/jof2010003
3. **Wünschmann, A., Armién, A. G., Childress, A. L., Wellehan, J. F. X., Giannitti, F., 2019:** Intrapericardial *Encephalitozoon pogonae*–associated arteritis with fatal hemopericardium in two juvenile central bearded dragons. *J. Vet. Diagn. Invest.*, 31, 3, 467–470. https://journals.sagepub.com/doi/pdf/10.1177/1040638719834330
4. **Kaiser, N. C., Greenwood, S. J., Gouchie, G. M., Martinson, S. A., 2021:** *Encephalitozoon pogonae*–associated systemic vasculitis in a central bearded dragon (*Pogona vitticeps*). *J. Herpetol. Med. Surg.*, 31, 4, 168–172.
5. **Jacobson, E. R., Green, D. E., Undeen, A. H., Mauldin, M. R., Farmer, M., Reavill, D. R., 1998**: Systemic microsporidiosis in inland bearded dragons (*Pogona vitticeps*). *J. Zoo Wildl. Med.*, 29, 3, 315–323.
6. **Raphtis, V., Roeder, M., Jimenez-Romero, A., Meritet, D., Chan, K., Ozawa, S., de Linde Henriksen, M., 2026:** An ocular manifestation of a systemic disease with *Encephalitozoon pogonae* in a juvenile central bearded dragon (*Pogona vitticeps*). *Vet. Ophthalmol.*, 29, 2, e70081. https://onlinelibrary.wiley.com/doi/pdf/10.1111/vop.70081
7. **Martel-Arquette, A., Chen, S., Hempstead, J., Pacheco, R., Antinoff, N., Teixeira, L., 2017:** Microsporidial keratoconjunctivitis in a pet bearded dragon (*Pogona vitticeps*). *J. Exotic Pet Med.*, 26, 4, 257–262. DOI: 10.1053/j.jepm.2017.09.002
8. **Ramatchandirane, B., Kumar, M. A., Marimuthu, Y., Nicodemus, D. S., Yarra, M. C., 2023:** Successful treatment of microsporidial keratoconjunctivitis with a combination of topical voriconazole 1% and gatifloxacin 0.5%: A large case series of 29 patients. *Cureus*, 15, 11, e49247. DOI: 10.7759/cureus.49247

DOI: 10.2478/fv-2026-0016

FOLIA VETERINARIA, 70, 2: 51—59, 2026

ORIGINAL ARTICLE

# PREVALENCE AND SPECIES COMPOSITION OF ENDOPARASITES IN HOBBY AND MEAT RABBITS IN EASTERN SLOVAKIA WITH SPECIAL FOCUS ON *EIMERIA* SPP.

**Andrea Schreiberová, Nikola Janošková, Gabriela Štrkolcová***

Department of Epizootiology, Parasitology and Protection of One Health, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

OPEN ACCESS

***Correspondence:** gabriela.strkolcova@uvlf.sk

**Citation:** Schreiberová, A., Janošková, N., Štrkolcová, G., 2026: Prevalence and species composition of endoparasites in hobby and meat rabbits in Eastern Slovakia with special focus on *Eimeria* spp. Folia Veterinaria, 70, 2, 51–59.

**Received:** December 15, 2025

**Accepted:** April 9, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**Endoparasitosis in rabbits often represents a hidden problem and causes serious financial loss to breeders as it occurs without any evident clinical symptoms. The subject of the present investigation was to identify the rate of prevalence of endoparasites, with special attention being paid to coccidiosis. The investigation was carried out at selected rabbit farms in five villages in the Gelnica District in Eastern Slovakia in the period from September 2019 to August 2020. A total of 87 faecal samples were examined using the flotation method and the McMaster technique. Of these, 83 samples tested positive for intestinal parasites, representing a positivity rate of 95.4%. Coccidian oocysts of *Eimeria* spp. were present in all positive samples, and *Passalurus ambiguus* eggs were found in six cases (7.2%). The overall oocyst counts for *Eimeria* spp. ranged from 50 to 27,000 oocysts per gram (OPG), while the egg counts of *P. ambiguus* ranged from 50 to 250 eggs per gram (EPG). To determine the species composition of the genus *Eimeria*, molecular analysis of the first internal transcribed spacer (ITS-1) region of ribosomal DNA was performed on selected samples with the highest concentrations of oocysts. The analysis revealed the presence of five coccidian species: *Eimeria magna, Eimeria flavescens, Eimeria piriformis*, *Eimeria irresidua,* and one unidentified *Eimeria* sp.**

**Keywords: coccidiosis; coprological methods; *Eimeria* spp.; PCR; rabbit**

## INTRODUCTION

Coccidiosis is a gastrointestinal parasitic infection that affects rabbits and hares, and it has a notably negative economic impact, especially in farmed rabbit populations (*Oryctolagus cuniculus*) worldwide [1, 2]. Coccidiosis in rabbits is caused by one or more species of unicellular parasites belonging to the genus *Eimeria*, subphylum Apicomplexa. Globally, a large number of *Eimeria* species have been described; however, most commonly, 15 species of the genus *Eimeria* are believed to be responsible for rabbit coccidiosis, including: *E. stiedae, E. magna, E. matsubayashi,*

*E. neoleporis, E. nagpurensis, E. irresidua, E. flavescens, E. piriformis, E. intestinalis, E.exigua, E. roobroucki, E. perforans, E. vejdovskyi, E. coecicola,* and *E. media.* The only exception is *E. stiedae*, which parasitises the bile ducts of the liver and is considered one of the most pathogenic species, while *E. magna*, *E. irresidua*, *E. perforans*, and *E. media* belong to the most frequently diagnosed species in domestic rabbits [3, 4, 5, 6, 7, 8, 9, 10, 11, 12].

Young rabbits, especially after weaning and between the ages of 6 weeks and 6 months, represent the most vulnerable group [13]. Another group of susceptible individuals includes older and immunocompromised ones, while adult rabbits are often asymptomatic carriers and rarely present with clinical symptoms of coccidiosis. No gender- and breed-dependent predisposition to that disease has been observed [13, 14].

The life cycle of that parasite is direct and comprises the exogenous phase (sporogony) and the endogenous phases (schizogony and gametogony). They are transmitted through the faecal-oral route, and the severity of infection depends on the number of ingested oocysts [15]. The intake of sporulated oocysts, containing 8 sporozoites, is followed by the schizogony phase [16]. The sporozoites are released from sporocysts and invade the epithelial cells of the small intestine and appendix of rabbits. The exception is the *Eimeria stiedae* species, which colonises the cells of the liver and bile ducts. During the gametogony phase (sexual reproduction), a new generation of oocysts is produced at the parasite's predilection sites. Upon completion of the cycle, these oocysts are excreted in the faeces into the external environment. The exogenous phase (sporogony) takes place in the external environment and leads to the creation of a new generation of infectious oocysts [17, 18]. Clinical symptoms of intestinal and hepatic coccidiosis are very similar. Generally, the clinical condition of affected rabbits is characterised by apathy, anorexia, and weight loss, primarily caused by a reduced intake of food during the first four weeks. In untreated cases, the condition may progress to a chronic stage [19].

*Post-mortem* observations in cases of infection caused by *E. stiedae* reveal hepatomegaly accompanied by typical multifocal necrotic lesions on the hepatic parenchyma. The lesions are 1 – 2 mm large, of white or cream colour, filled with an exudate dispersed across the entire parenchyma [14, 17, 20].

Diagnosis of coccidial infection is primarily based on the detection of oocysts in examined faecal samples. Species identification within the genus *Eimeria* is traditionally performed through morphological and morphometric evaluation of oocysts, sporocysts, and sporozoites [21]. However, microscopic identification requires considerable expertise and is often time-consuming, particularly when distinguishing closely related species. For this reason, molecular techniques such as polymerase chain reaction (PCR) have increasingly been employed to improve the accuracy and reliability of species identification [18].

In addition to protozoa, rabbits also present with helminths. *Passalurus ambiguus* (an oxyurid worm) is the most frequently detected intestinal nematode in rabbits and hares and the second most common intestinal parasite after *Eimeria* spp. [22]. *P. ambiguus* parasitises the appendix and large intestine of infected individuals, completing a direct life cycle. Infection occurs when rabbits ingest these eggs from the environment, commonly through contaminated feed, water, or materials, or via caecotrophy, resulting in autoinfection. Adult females deposit sticky, embryonated eggs – typically oval, asymmetrical, light brown in colour, and featuring a polar plug – around the perineal region [14, 17, 23, 24, 25]. In most cases, rabbits infected with *P. ambiguus* are asymptomatic, even when the intensity of infection is high. However, in young and weakened rabbits, the risk of clinical symptoms is higher. Those may include intensive itching around the rectum, digestive disorders, diarrhoea, weight loss, and even rectal prolapse [17, 26, 27].

The aim of the present study was to determine the prevalence and species composition of endoparasites in hobby and meat rabbits in selected localities of Eastern Slovakia, with particular emphasis on coccidial infections caused by *Eimeria* spp., using coprological and molecular diagnostic methods, in order to contribute to a better understanding of the epidemiological situation of these infections in rabbit breeding. In addition, this study provides the first PCR-based molecular confirmation of *Eimeria* species infecting rabbits in Slovakia.

## MATERIAL AND METHODS

### Sample collection

The investigation was carried out from September 2019 to August 2020. A total of 87 faecal samples were analysed, including 76 samples collected from individu-

al rabbits and 11 pooled samples obtained from groups of breeding females with young. The study focused on selected farms in the town of Gelnica ($n$ = 43), followed by the villages of Mníšek nad Hnilcom ($n$ = 19), Prakovce ($n$ = 15), Jaklovce ($n$ = 5), and Kojšov ($n$ = 5), all located within a 20-kilometer radius in the Gelnica District of Eastern Slovakia. The majority of the samples originated from hobby rabbits, particularly those involved in rabbit hopping, a popular regional activity, and from rabbits raised for meat production. A detailed overview of the distribution of rabbits by location and category is presented in Table 1. The examined faecal samples were mostly collected from the Czech Spotted Rabbit ($n$ = 18/87), New Zealand White Rabbit ($n$ = 13/87), and Rhön Rabbit ($n$ = 18/87) breeds. All hobby and meat rabbits included in the study were in the range of 6 months to 3 years.

**Coprological examination**

After collection, the faecal samples were stored at refrigerator temperature (4 °C) and subsequently transported to the laboratory for coprological examination. The samples were examined using a qualitative flotation method to confirm the presence of protozoan oocysts and helminth eggs. To assess the intensity of infection, the quantitative McMaster technique was applied according to the method described by Taylor (2007) [14].

**Molecular identification of *Eimeria* spp.**

The examined faecal samples with the highest concentrations of *Eimeria* spp. oocysts (ranging from 10,000 to 27,000 OPG) were subjected to molecular analysis in order to identify the *Eimeria* species infecting rabbits in the studied region. The total genomic DNA extraction was carried out using a ZR Faecal DNA MiniPrep™ commercial kit (ZymoResearch, USA) following the manufacturer's instructions and subsequently stored in a freezer at -20 °C and later used for the PCR analysis. DNA samples were used for amplification of the internal transcribed spacer 1 (ITS-1) region of ribosomal DNA according to the procedure published by Oliveira (2011) [18] using universal primers for the *Eimeria* species: forward ITS1-F (GGGAAGTTGCGTAAATAGA) and reverse ITS1-R (CTGCGTCCTTCATCGAT) with resulting length fragments of about 400 bp. The total PCR reaction mixture, with a volume of 50 µl, consisted of 25 µL of OneTaq® 2X Master Mix with Standard Buffer (New England Biolabs), 21 µL of nuclease-free water, 1 µL of each of the two primers (Sigma, GB), and 2 µL of DNA template. The amplification program consisted of the initial denaturation step at 94 °C lasting for 2 min, with 30 cycles repeated at 94 °C for 1 min, at 58 °C for 1 min, at 72 °C for 1 min, and a final extension phase at 72 °C for 7 min [18] in a programmable Mastercycler Nexus X2 (Eppendorf, Germany).

The amplified PCR products were visualized by electrophoresis on a 1% agarose gel and observed under a UV transilluminator. All positive products were sent to the commercial laboratory Microsynth Seqlab (Vienna, Austria) for purification and bidirectional sequencing using the same primers as in the PCR. Sequencing was performed using the Sanger method. The obtained sequences were edited using MEGA X software [28] and assembled using GeneTool Lite 1.0 (BioTools Inc., USA). The resulting sequences were compared with reference sequences in the GenBank database (NCBI) using the BLAST algorithm.

## RESULTS

**Coprological analysis**

Flotation analysis revealed the overall presence of *Eimeria* spp. oocysts in 83 out of 87 (95.4%) (Table 2).

**Table 1. Distribution of rabbits by category and location**

| Location | Hobby rabbits | Meat rabbits | Breeding females with young | Total |
|---|---|---|---|---|
| **Gelnica** | 24 | 16 | 3 | **43** |
| **Mníšek nad Hnilcom** | 5 | 9 | 5 | **19** |
| **Prakovce** | 15 | - | - | **15** |
| **Jaklovce** | - | 3 | 2 | **5** |
| **Kojšov** | - | 4 | 1 | **5** |
| **Total** | **44** | **32** | **11** | **87** |

Note: Dash (–) indicates that no animals of the given category were reported in the respective location

**Table 2. Positivity for *Eimeria* spp. in the Gelnica District**

| Location | Positive Hobby Rabbits / Total Examined | Positive Meat Rabbits / Total Examined | Positive Breeding Females with Young / Total Examined | Overall positivity (%) |
|---|---|---|---|---|
| **Gelnica** | 23/24 | 14/16 | 3/3 | **93.0** |
| **Mníšek nad Hnilcom** | 5/5 | 8/9 | 5/5 | **94.7** |
| **Prakovce** | 15/15 | - | - | **100** |
| **Jaklovce** | - | 3/3 | 2/2 | **100** |
| **Kojšov** | - | 4/4 | 1/1 | **100** |

Eggs of *P. ambiguus* were confirmed in 6 samples (7.2%) originating from both hobby and meat rabbits of three breeds (Czech Spotted Rabbit, New Zealand White Rabbit, and Rhön Rabbit) kept in Gelnica, Mníšek nad Hnilcom, and Prakovce. In contrast, no *P. ambiguus* eggs were detected in any of the individual or pooled samples collected from breeding females with young in Jaklovce and Kojšov.

The McMaster quantitative technique was employed to determine the intensity of infection caused by *Eimeria* spp. oocysts (OPG – oocysts per gram of faeces) and to detect the presence of *P. ambiguus* eggs (EPG – eggs per gram). Across all examined samples, the intensity of coccidian infection ranged from 50 to 27,000 OPG (Table 3).

### Molecular analysis of *Eimeria* spp.

Molecular analysis of the ITS-1 ribosomal DNA region was performed on 15 samples with a high concentration of oocysts in rabbit faeces in order to detect the presence of *Eimeria* spp. and determine their species composition. Sequencing and subsequent sequence editing yielded eight high-quality sequences, which showed 96.45-100% identity to *Eimeria* species in the GenBank database. The sequences obtained in this study were deposited in GenBank under accession numbers PZ160625–PZ160632. In these eight isolates, five *Eimeria* species were identified: *E. piriformis*, *E. magna, E. irresidua*, *E. flavescens*, and one unidentified *Eimeria* sp. (Table 4). The first sample (E1) was collected from breeding females with young of the Czech Spotted Rabbit breed from Gelnica, which exhibited a high intensity of infection caused by *Eimeria* spp. oocysts (16,400 OPG). Two isolates from this sample were identified: E1a as *E. piriformis* and E1b as *E. magna*. The second positive sample (E2) originated from a female of the diminutive Lionhead breed from Gelnica. Sample 3 (E3) was collected from a diminutive male Hermelin rabbit from Gelnica. In both cases (E2 and E3), *Eimeria* sp. were detected; however, species-level identification was not achieved due to low sequence similarity (96.45% and 96.63%) with reference sequences available in the GenBank database. The fourth sample (E4) originated from a male Rhön Rabbit from Prakovce, which was used for breeding and kept under extensive rearing conditions. The fifth sample (E5) was collected from a female Czech Spotted Rabbit from Mníšek nad Hnilcom, housed together with her kits, and showing the highest intensity of coccidian infection (27,000 OPG). The E6 sample was obtained from a male Hermelin rabbit from Gelnica and the E7 sample from a pregnant female Czech Spotted rabbit from the Mníšek nad Hnilcom area (Table 4).

## DISCUSSION

Coccidiosis in rabbits is a major parasitic disease that significantly increases breeding costs. In the present study, 87 faecal samples from rabbits were examined microscopically using the flotation method. The samples showed a high positivity rate of 95.4% for *Eimeria* spp. oocysts. This is notably higher compared to a 2015 study that in-

**Table 3. Intensity of infection with *Eimeria* spp. and *Passalurus ambiguus* in examined rabbit breeds**

| Rabbit breed | Locality | *Eimeria* spp. (OPG range) | *Passalurus ambiguus* (EPG range) |
|---|---|---|---|
| **Czech Spotted Rabbit** | Gelnica, Mníšek nad Hnilcom | 250 – 16,400 (max. 27,000 in pooled sample) | 50 – 150 |
| **New Zealand Rabbit** | Jaklovce, Kojšov | 50 – 4,500 | not detected |
| **Rhön Rabbit** | Prakovce | 50 – 4,400 | 200 – 250 |

**Table 4. Identification of *Eimeria* spp. based on ITS-1 rDNA and GenBank accession numbers**

| Sample code | Eimeria spp. | GenBank accession no. | Highest identity with GenBank | |
|---|---|---|---|---|
| | | | Percent identity | Accession number |
| **E1a** | *E. piriformis* | PZ160625 | 100% | HM768889.1 host: Rabbit Czech Republic [18] |
| **E1b** | *E. magna* | PZ160626 | 98.60% | MK590197.1 host: Rabbit China |
| **E2** | *Eimeria* sp. | PZ160627 | *Eimeria perforans* 96.45% | HM768888.1 host: Rabbit France [18] |
| **E3** | *Eimeria* sp. | PZ160628 | *Eimeria perforans* 96,63% | HM768888.1 host: Rabbit France [18] |
| **E4** | *E. irresidua* | PZ160629 | 100% | HM768885.1 host: Rabbit Czech Republic [18] |
| **E5** | *E. irresidua* | PZ160630 | 100% | HM768885.1 host: Rabbit Czech Republic [18] |
| **E6** | *E. magna* | PZ160631 | 98.60% | MK590197.1 host: Rabbit China |
| **E7** | *E. flavescens* | PZ160632 | 99.18% | MN535227.1 host: Rabbit Mexico |

vestigated endoparasite prevalence in rabbits from 23 breeder farms in Eastern Slovakia, which reported a 72.5% infection rate with *Eimeria* spp. [29].

In Romania, 236 rabbit faecal samples were examined, revealing a 77.56% infection rate with *Eimeria* spp. [12]. In wild European brown hares from Austria and the Czech Republic, Chroust et al. (2012) reported very high prevalence in young animals (97.9% in Austria and 95.5% in the Czech Republic), while adults showed lower rates (60.0% and 64.6%, respectively) [30]. In Poland, a survey of 14 small rabbit farms identified ten *Eimeria* species, with prevalence ranging from 8.0% to 98.5% depending on the species, and the highest mean oocyst count reached 21,100 OPG in mid-May [31].

Research conducted in Romania showed that domestic rabbits exhibited a prevalence of *Eimeria* spp. infection of 52.3% [32]. In three Algerian regions, Maziz-Bettahar et al. (2018) reported a post-weaning coccidian prevalence of 90%. Across the examined farms, two to six *Eimeria* species were detected *per* location, with eight species identified in total. The most prevalent were *E. magna* (42.5%), *E. media* (17.6%), and *E. irresidua* (14.9%). High oocyst excretion was significantly associated with limited anticoccidial use (25.0% of farms) and poor hygienic conditions (65.0% of farms) [33]. In the Sichuan Province of southwestern China, an investigation based on 110 samples collected from 11 farms reported an overall *Eimeria* prevalence of 56.4%, with the highest infection rate occurring in weaned rabbits (74.0%). These results indicate that kits and recently weaned rabbits constitute the most vulnerable age category, representing the period of greatest susceptibility to eimeriosis [34].

Using a specifically designed pair of primers for ITS-1 region amplification and applying the PCR analysis proposed by Oliveira, eight samples from rabbits reared in the Czech Republic and France were examined, revealing the highest presence of oocysts of *E. magna*, *E. flavescens*, *E. piriformis*, *E. perforans*, and *E. irresidua*. The scientific study conducted by Oliveira (2011) [18] was used as the basis for subsequent published studies. In China, Brazil and Mexico, the species that were most frequently detected included *E. flavescens* (61.82%), *E. magna* (42.5%), *E. intestinalis* (11.3%), and *E. perforans* (35.2%) [1, 35, 36].

In Germany, *E. media*, *E. magna*, and *E. perforans* were the most commonly detected species, whereas *E. exigua* was observed relatively infrequently [37]. These findings demonstrate the widespread occurrence of *Eimeria*

infections in European rabbit and hare populations and underscore the importance of ongoing monitoring and control measures. Although numerous studies have identified *Eimeria* spp. in domestic rabbits solely on the basis of oocyst morphology [8, 21, 38], accurate species differentiation remains challenging due to the considerable morphological overlap among the many *Eimeria* species capable of infecting rabbits [39].

Infections caused by *P. ambiguus* may affect the production performance, especially in young and weaned kits. The intensity of infection may also vary depending on the season and the age of the rabbits [22]. Among all examined samples collected in the Gelnica District, the prevalence of *Passalurus* eggs was 7.2%, with egg counts ranging from 50 to 200 EPG. This represents a 3.0% lower prevalence compared to a 2015 study conducted in Eastern Slovakia, which reported a prevalence of 10.2% [29]. In a study from Poland, Szkucik et al. (2014) detected *P. ambiguus* eggs with a prevalence of 5.83% [40]. In Southern Italy, infection intensity was high, with 82.3% of examined rabbits testing positive using the FLOTAC technique [41]. The most recent study from Ukraine reported morbidity rates of passalurosis ranging from 37.70% to 41.67% in domestic rabbits, with the highest infection intensity (82.76%) observed in animals aged 1–2 years [42]. A study conducted in south-western Romania reported a 4.4% prevalence of *P. ambiguus* eggs in domestic rabbits, based on coproscopic examination of 111 individuals [32]. In contrast, research performed in Hungary on rabbit farms revealed a different pattern, showing a significantly lower infection rate in the growing stage (5–11 weeks old); the highest infection rate was detected in samples collected from young rabbits during the lactation period [43].

It is important to highlight that the post-weaning period represents the highest risk phase for digestive diseases in rabbits, where endoparasitism and bacterial infections are among the most common health issues [44].

## CONCLUSION

The present study aimed to investigate the occurrence of intestinal parasites in rabbits from selected rabbit farms in the Gelnica District, focusing on hobby and meat rabbits raised on these farms. A total of 87 faecal samples were collected from individual rabbits as well as from groups of breeding females with their young. Coprological examination revealed a high overall prevalence of oocysts of *Eimeria* spp. (95.4%), despite the absence of overt clinical signs in most animals. The intensity of infection varied across breeds and locations, with the highest oocyst counts observed in breeding females and their kits. Eggs of the *P. ambiguus* were also detected, although at a lower prevalence (7.2%), mainly in hobby and meat rabbits from specific breeds and sites. Molecular analysis using PCR allowed species-level identification of *Eimeria* spp., providing new insights into the genetic diversity of these parasites in Slovakian rabbit populations. This represents the first molecular characterization of *Eimeria* spp. in rabbits in Slovakia. Given the high prevalence and potential impact on rabbit health and breeder economy, routine parasitological monitoring and early intervention strategies are strongly recommended. Regular screening for intestinal parasites, particularly *Eimeria* spp. and *P. ambiguus* can help mitigate financial losses and improve animal welfare in both hobby and commercial rabbit production systems. Effective prevention of coccidiosis in commercial rabbit production requires strict hygiene management, including regular removal of faeces, sanitation of cages and equipment, and environmental conditions. In addition, regular parasitological monitoring and the appropriate use of anticoccidial drugs can help reduce the spread of infection, minimize economic losses, and improve animal welfare.

### Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

### Ethical Statement

No ethical approval was necessary for this study.

### Conflict of Interest

The authors declare no conflicts of interest.

### Funding

This study was supported by the VEGA 1/0709/23.

## Generative AI Statement

The authors declare that no generative AI was used in the creation of this manuscript.

## Authors' Contributions

Conceptualization: A. S., G. Š.; methodology: A. S., G. Š., N. J.; sample collection: N. J.; data curation: A. S., G. Š.; manuscript writing: A. S., G. Š., N. J.; molecular identification: A. S.; funding acquisition: G. Š. All authors have read and agreed to the published version of the manuscript.

## REFERENCES


1. **Jing, F., Yin, G., Liu, X., Suo, X., Qin, Y., 2012:** Large-scale survey of the prevalence of *Eimeria* infections in domestic rabbits in China. *Parasitol. Res.*, 110, 1495–1500. DOI: 10.1007/s00436-011-2653-4
2. **Petrova, J. P., Iliev, P. T., Ivanov, A. I., Petrov, V. S., Kalkanov, I. I., Kostadinov, N. N., et al., 2022:** Biochemical and pathomorphological investigations on rabbits with experimentally induced hepatic eimeriosis. *Maced. Vet. Rev.*, 45, 1, 19–28. DOI: 10.2478/macvetrev-2022-0013
3. **Gill, B. S., Ray, H. N., 1960:** The coccidia of domestic rabbit and the common field hare of India. *Proc. Zool. Soc. Calcutta*, 13, 129–143.
4. **Kvičerová, J., Pakandl, M., Hypša, V., 2008:** Phylogenetic relationships among *Eimeria* spp. (Apicomplexa, Eimeriidae) infecting rabbits: evolutionary significance of biological and morphological features. *Parasitology*, 135, 4, 443–452. DOI: 10.1017/S0031182007004106
5. **Ming-Hsien, L., Hai-I, H., Hong-Kean, O., 2010:** Prevalence, infectivity and oocyst sporulation time of rabbit coccidia in Taiwan. *Trop. Biomed.*, 27, 3, 424–429.
6. **Duszynski, D. W., Couch, L., 2013:** Coccidia (Eimeriidae) of the family Leporidae: Genus *Oryctolagus.* In: Duszynski, D. W., Couch, L. (Eds.), *The Biology and Identification of the Coccidia (Apicomplexa) of Rabbits of the World.* Elsevier, Oxford, pp. 121–187. DOI: 10.1016/B978-0-12-397899-8.00006-8
7. **Hassan, K. M., Arafa, W. M., Mousa, W. M., Shokier, K. A., Shany, S. A., Aboelhadid, S. M., 2016:** Molecular diagnosis of *Eimeria stiedae* in hepatic tissue of experimentally infected rabbits. *Exp. Parasitol.*, 169, 1–5. DOI: 10.1016/j.exppara.2016.07.001
8. **Pakandl, M., Liu, X., 2019:** Coccidiosis in rabbits *(Oryctolagus cuniculus).* In: Dubey, J. P. (Ed.), *Coccidiosis in Livestock, Poultry, Companion Animals, and Humans.* 1st ed. CRC Press, Boca Raton, pp. 159–168. DOI: 10.1201/9780429294105-14
9. **Xie, Y., Xiao, J., Zhou, X., Gu, X., He, R., Xu, J., Jing, B., Peng, X., Yang, G., 2021:** Global transcriptome landscape of the rabbit protozoan parasite *Eimeria stiedae. Parasit. Vectors*, 14, 308, 1–17. DOI: 10.1186/s13071-021-04811-5
10. **Qin, Z., Zhang, J., Zhang, K., Lang, J., Wang, N., Li, J., Zhang, L., 2023:** Morphological and molecular characteristics of a single oocyst for the identification of *Eimeria* species in domestic rabbits (*Oryctolagus cuniculus f. domesticus*). *Vet. Parasitol.*, 321, 109986. DOI: 10.1016/j.vetpar.2023.109986
11. **Shi, Y., Fang, S., Gu, X., Hao, C., Du, F., Cui, P., Tang, X., 2024:** The complete mitochondrial genome sequence of *Eimeria kongi* (Apicomplexa: Coccidia). *Life*, 14, 6, 699. DOI: 10.3390/life14060699
12. **Jitea, B. A.-M., Morariu, S., Imre, M., Florea, T., Sîrbu, C. B., Luca, I., et al., 2025:** Microscopic and molecular identification of *Eimeria* species in domestic rabbits (*Oryctolagus cuniculus*) in Romania. *Animals*, 15, 8, 1109. DOI: 10.3390/ani15081109
13. **Pilarczyk, B., Tomza-Marciniak, A., Pilarczyk, R., Januś, E., Stanek, P., Seremak, B., Sablik, P., 2020:** The effect of the sex, age, and breed of farmed rabbits and the choice of management system on the extensity and intensity of *Eimeria* infection. *Vet. World*, 13, 8, 1654–1660. DOI: 10.14202/vetworld.2020.1654-1660
14. **Taylor, M. A., Coop, R. L., Wall, R. L., 2007:** *Veterinary Parasitology.* 3rd ed. Blackwell Publishing, Oxford, 872. ISBN: 978-1-4051-1964-1
15. **Pakandl, M., Hlásková, L., Poplštein, M., Chromá, V., Vodička, T., Salát, J., Mucksová, J., 2008:** Dependence of the immune response to coccidiosis on the age of rabbit suckling. *Parasitol. Res.*, 103, 1265–1271. DOI: 10.1007/s00436-008-1123-0
16. **Bochyńska, D., Lloyd, S., Restif, O., Hughes, K., 2022:** *Eimeria stiedae* causes most of the white-spotted liver lesions in wild European rabbits in Cambridgeshire, United Kingdom. *J. Vet. Diagn. Invest.*, 34, 2, 199–205. DOI: 10.1177/10406387211066923
17. **Deplazes, P., Eckert, J., Mathis, A., Samson-Himmelstjerna, G., Zahner, H., 2016:** *Parasitology in Veterinary Med-*

*icine.* Wageningen Academic Publishers, Wageningen, 650. ISBN: 978-90-8686-274-0

18. **Oliveira, U. C., Fraga, J. S., Licois, D., Pakandl, M., Gruber, A., 2011:** Development of molecular assays for the identification of the 11 *Eimeria* species of the domestic rabbit (*Oryctolagus cuniculus*). *Vet. Parasitol.,* 176, 2–3, 275–280. DOI: 10.1016/j.vetpar.2010.10.054
19. **Varga, M., 2013:** *Textbook of Rabbit Medicine*. 2nd. edition. Elsevier, 494. ISBN 978-0-7020-4979-8
20. **Hrženjak, N. M., Zadravec, M., Švara, T., Račnik, J., 2021:** Hepatic coccidiosis in two pet rabbits. *J. Exot. Pet Med.*, 36, 53–56. DOI: 10.1053/j.jepm.2020.12.009
21. **El-Shahawi, G. A., El-Fayomi, H. M., Abdel-Haleem, H. M., 2012:** Coccidiosis of domestic rabbit (*Oryctolagus cuniculus*) in Egypt: light microscopic study. *Parasitol. Res.*, 110, 251–258. DOI: 10.1007/s00436-011-2479-0
22. **Sioutas, G., Evangelou, K., Vlachavas, A., Papadopoulos, E., 2021**: Deaths due to mixed infections with *Passalurus ambiguus*, *Eimeria* spp. and *Cyniclomyces guttulatus* in an industrial rabbit farm in Greece. *Pathogens*, 10, 6, 756. DOI: 10.3390/pathogens10060756
23. **Boag, B., Lello, J., Fenton, A., Tompkins, D. M., Hudson, P. J., 2001**: Patterns of parasite aggregation in the wild European rabbit (*Oryctolagus cuniculus*). *Int. J. Parasitol.*, 31, 13, 1421–1428. DOI: 10.1016/S0020-7519(01)00270-3
24. **Abdel-Gaber, R., Ataya, F., Fouad, D., Daoud, M., Alzuhairy, S., 2019:** Prevalence, morphological and molecular phylogenetic analyses of the rabbit pinworm, *Passalurus ambiguus* Rudolphi 1819, in the domestic rabbits *Oryctolagus cuniculus*. *Acta Parasitol.*, 64, 316–330. DOI: 10.2478/s11686-019-00047-7
25. **Khorolskyi, A., Yevstafieva, V., Kravchenko, S., Pishchalenko, M., Vakulenko, Y., Gutyj, B., 2021:** Specifics of the morphological identification of the pathogen of passaluariasis of rabbits. *Regul. Mech. Biosyst.*, 12, 4, 702–709. DOI: 10.15421/022197
26. **Taylor, M. A., Coop, R. L., Wall, R. L., 2015:** *Veterinary Parasitology.* John Wiley & Sons, Hoboken, 1032.
27. **Schoeb, T. R., Baker, R. A., 2007:** Parasites of rabbits. In: Baker, D. G. (Ed.), *Flynn's Parasites of Laboratory Animals*, 2nd edition. Blackwell Publishing, Hoboken, pp. 451–499. ISBN 978-0-8138-1202-1
28. **Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018:** MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.*, 35, 6, 1547–1549. DOI: 10.1093/molbev/msy096
29. **Bukovszki, V., Kočišová, A., 2015:** Surveillance of rabbit parasitoses on selected farms in eastern Slovakia. *Folia Vet.*, 59, 2, 74–76. ISSN 0015-5748
30. **Chroust, K., Vodnansky, M., Pikula, J., 2012:** Parasite load of European brown hares in Austria and the Czech Republic. *Vet. Med. (Praha)*, 57, 10, 551–558. DOI: 10.17221/6367-VETMED
31. **Balicka-Ramisz, A., Laurans, Ł., Pohorecki, K., Batko, M., Ramisz, A., 2020:** Short communication: Prevalence of *Eimeria* spp. infection in domestic rabbits of Polish farms. *World Rabbit Sci.*, 28, 4, 283–288. DOI: 10.4995/wrs.2020.10758
32. **Sîrbu, C.B., Florea, T., Sîrbu, B. A.-M., Plotuna A.-M., Hotea, I., 2022:** Research on endoparasites of domestic and wild rabbits from south-western Romania. *Int. Multidiscip. Sci. GeoConf.: SGEM*, 22, 6.2, 707–713. DOI: 10.5593/sgem2022V/6.2/s29.89
33. **Maziz-Bettahar, S., Aissi, M., Ainbaziz, H., Bachene, M. S., Zenia, S., Ghisani, F., 2018:** Prevalence of coccidian infection in rabbit farms in North Algeria. *Vet. World*, 11, 11, 1569–1573. DOI: 10.14202/vetworld.2018.1569-1573
34. **Yin, G., Goraya, M. U., Huang, J., Suo, X., Huang, Z., Liu, X., 2016:** Survey of coccidial infection of rabbits in Sichuan Province, Southwest China. *SpringerPlus*, 5, 870, 1–4. DOI: 10.1186/s40064-016-2586-6
35. **Heker, M. M., Nakamura, A. A., Santana, B. N., Meireles, M. V., 2017**: Etiological aspects of *Eimeria* spp. infection in Brazilian rabbit (*Oryctolagus cuniculus*) farms. *Vet. Parasitol.: Reg. Stud. Rep.*, 8, 78–81. DOI: 10.1016/j.vprsr.2017.02.004
36. **Ladron de Guevara, O. S., Perez-Rivero, J. J., Perez-Martinez, M., Flores-Perez, F. I., Romero-Callejas, E., 2019:** *Eimeria* spp. in broiler rabbit: seasonal prevalence in the backyard farms of the State of Mexico. *Vet. Ital.*, 55, 2, 183–187. DOI: 10.12834/VetIt.443.2154.3
37. **Lohkamp, F., Hankel, J., Beineke, A., Kamphues, J., Strube, C., 2024:** A field study evaluating the effects of diclazuril and oregano oil for the prevention of coccidiosis in fattening rabbits. *Parasitologia*, 4, 1, 47–60. DOI: 10.3390/parasitologia4010004
38. **Tokiwa, T., Chou, S., Kitazoe, H., Ito, K., Torimoto, R., Shoshi, Y., et al., 2022:** Three new species of *Eimeria* (Apicomplexa: Eimeriidae) from the Amami rabbit, *Pentalagus furnessi* (Mammalia: Leporidae). *Int. J. Parasitol.: Parasites Wildl.*, 18, 194–200. DOI: 10.1016/j.ijppaw.2022.05.006

39. **Zahedi, A., Liu, D., Yang, R., Austen, J. M., Potter, A., Ryan, U., 2023:** Next-generation sequencing amplicon analysis of the genetic diversity of *Eimeria* populations in livestock and wildlife samples from Australia. *Parasitol. Res.*, 122, 615–624. DOI: 10.1007/s00436-022-07764-5

40. **Szkucik, K., Pyz-Łukasik, R., Szczepaniak, K. O., Paszkiewicz, W., 2014:** Occurrence of gastrointestinal parasites in slaughter rabbits. *Parasitol. Res.,* 113, 59–64. DOI: 10.1007/s00436-013-3625-7

41. **Rinaldi, L., Russo, T., Schioppi, M., Pennacchio, S., Cringoli, G., 2007:** *Passalurus ambiguus*: new insights into copromicroscopic diagnosis and circadian rhythm of egg excretion. *Parasitol. Res.*, 101, 557–561. DOI: 10.1007/s00436-007-0513-z

42. **Prus, M., Duda, Y., Koreyba, L., Borisevich, B., Lisova, V., 2022:** Seasonal and age dynamics of passalurosis invasion of rabbits and pathological and histological changes in this nematodosis. *Sci. Horizons*, 25, 11, 9–19. DOI: 10.48077/scihor.25(11).2022.9-19

43. **Demeter, C., Német, Z. L., Gerencsér, Z., Demeter-Jeremiás, A., Sándor, F., Matics, Z., 2022:** Survey of *Passalorus ambiguus* infections at indrustial rabbit farms according to ages and seasons. *Acta Agraria Kaposváriensis*, 26, 2, 63–74, 19396. DOI: 10.31914/aak.3077

44. **El-Ashram, S., Aboelhadid, S. M., Abdel-Kafy, E. S. M., Hashem, S. A., Mahrous, L. N., Farghly, E. M., Kamel, A. A., 2020:** Investigation of pre-and post-weaning mortalities in rabbits bred in Egypt, with reference to parasitic and bacterial causes. *Animals*, 10, 3, 537. DOI: 10.3390/ani10030537

DOI: 10.2478/fv-2026-0017

FOLIA VETERINARIA, 70, 2: 60—66, 2026

ORIGINAL ARTICLE

# SEROLOGICAL AND EPIZOOTIC DYNAMICS OF SWINE INFLUENZA A IN INDUSTRIAL SWINE FARMS OF UKRAINE (2021–2024)

**Anatolii Kovalenko[1]*, Jana Mojžišová Vaščinec[1], Vasil Koziy[2], Nataliia Kozii[2], Boris Vojtek[1], Anna Ondrejková[1], Silvia Kapľavka[1], Ľuboš Korytár[1], Marián Prokeš[1], Monika Drážovská[1]**

[1]Department of Epizootiology, Parasitology and Protection of One Health, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; [2]Bila Tserkva National Agrarian University, School of Veterinary Medicine, Bila Tserkva, Kiev region, Ukraine

OPEN ACCESS

***Correspondence:** anatolii.kovalenko@uvlf.sk

**Citation:** Kovalenko, A., Mojžišová Vaščinec, J., Koziy, V., Kozii, N., Vojtek, B., Ondrejková, A., Kapľavka, S., Korytár, Ľ., Prokeš, M., Drážovská, M., 2026: Serological and epizootic dynamics of swine influenza A in industrial swine farms of Ukraine (2021–2024). Folia Veterinaria, 70, 2, 60–66.

**Received:** January 9, 2026

**Accepted:** February 25, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**Swine Influenza A virus (SIV-A) is a significant zoonotic pathogen with the potential to cause pandemics. In Ukraine, surveillance is crucial due to the risk of interspecies transmission between pigs and humans. SIV-A circulates within swine populations and undergoes frequent genetic changes, increasing its variability and pathogenicity. In this study, we monitored the presence of SIV-A on 92 pig farms in the Lviv and Kyiv regions during the period 2021–2024. Serological testing was performed using the IDEXX Influenza A Virus Ab Test Kit, revealing 24 seropositive farms, with a decreasing trend observed over the years. Additionally, 422 pigs from seropositive farms were tested for SIV-A using real-time RT-PCR, and the virus was detected in 59 individuals. These results suggest that while epizootic control measures were largely effective, the virus continues to circulate among susceptible animals. Continuous surveillance and timely intervention remain essential for detecting emerging subtypes and preventing potential outbreaks.**

**Keywords: influenza A virus; pigs; prevalence; Ukraine**

## INTRODUCTION

Swine flu, or porcine influenza, is a respiratory viral infection caused by the swine influenza A virus (SIV-A) that predominantly affects pigs. This infection is important both for humans and for animals.

SIV-A was first isolated in 1930; since then, it has become endemic in pigs worldwide. The major challenge of the disease is the big number of different subtypes and genotypes of the virus circulating among pigs worldwide [1]. The other scientists [2–5] emphasize the potential pandemic risk of circulating SIV-A. Mon et al. [6] found that the genetic diversity of SIV-A possesses zoonotic and reverse zoonotic potentials. Even though only a few hundred confirmed human cases caused by SIV-A have been

reported, Abdelwhab et al. [7] claim that a high vigilance is needed to prevent the next pandemic caused by animal influenza viruses.

The study done by Meng et al. [8] demonstrates that SIV-A may become pathogenic and transmissible by acquiring key mutations in acidic polymerase. The authors especially emphasize the possible pathogenicity development in different species of animals.

Surveillance of occupational exposure among persons working with pigs showed that up to 20.5% of humans may become seropositive, indicating the acquired human infectivity of SIV-A [9]. The authors further claim that such high infectivity enhances the possibility for viruses to acquire humans' pathogenicity and raises concerns for the possible pandemic effect.

The lack of attention to swine influenza makes it possible for the animals at exhibitions and fairs to be the spreading source of the virus. There were identified deficiencies of routine event biosecurity that may have important public and animal health consequences [10, 11]. Furthermore, the complexity of viral detection and control in exhibition swine indicates that the viruses are likely to continually reemerge, presenting threats to human and animal health [12].

The global monitoring of swine influenza SIV-A is important for human and animal health and represents a vital 'One Health' challenge for human and veterinary medicine [13, 14]. Yet, the collective knowledge regarding the occurrence of influenza among swine is still incomplete. It is mainly due to lack and inconsistency of surveillance data on SIV-A prevalence among the world's swine populations [15]. Monitoring the viral pathogens is important for disease control and is critical from a preventive standpoint [16].

With this, the main aim of our article was to estimate the viral RNA and seropositivity prevalence of SIV-A on industrial pig farms in Ukraine.

## MATERIALS AND METHODS

The study period encompasses four years (2021–2024). The pig's samples (nasal swabs and serum) came from 92 farms from the Lviv and Kyiv regions, with a total of 422 animals examined.

For the antibody detection, the IDEXX Influenza A Virus Ab Test Kit (IDEXX, USA), a blocking ELISA test that recognizes serum antibodies to any influenza A subtype of swine influenza virus, was used. The test was performed according to the manufacturer´s instructions.

For virus detection, total RNA was extracted from samples using the MagMAX Core Nucleic Acid Purification Kit (ThermoFisher Scientific, Applied Biosystems, USA) according to the manufacturer's instructions. The presence of the Influenza A virus was detected using the Kylt® Influenza A Real-Time RT-PCR Kit (Kylt®, AniCon Labor GmbH, Germany). The assay was performed following the protocol provided by the manufacturer. Reactions were run on a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific, USA). Each reaction included an internal control provided with the Kylt® kit to validate RNA extraction and PCR performance.

## RESULTS

In 2021, serological positivity for SIV-A was detected in 41.2% (7 seropositive farms from 17 industrial tested farms). In 2022 and 2023, the rate decreased from 26.1% (6/23) to 21.8% (7/32). In 2024, seropositivity reached 20.0% in 20 farms. Trend line of serological testing of farms among years is shown in Figure 1.

Results of virus detection are shown in Table 1. In 2021, 103 clinical samples from seven farms were analyzed, with 16.5% testing positive for SIV-A. In subsequent years, the prevalence of viral RNA detection in clinical samples remained high: 15.9% in 2022, 13.7% in 2023, and 10.2% in 2024. On average, 14% of tested samples from affected farms were positive for viral RNA, demonstrating ongoing circulation and persistence of the virus.

## DISCUSSION

Analysis of data from 92 industrial pig farms over the study period confirmed a decreasing trend in the occurrence of the influenza A virus, indicating an intensification of epizootic activity during the monitoring years. Nevertheless, given that the infection rate ranged from 20% to 41.2%, our findings indicate sustained transmission of the virus among susceptible individuals. The persistent detection of viral RNA (10.2–16%) among seropositive animals underscores the heightened epizootic risk and facilitates rapid reinfection cycles within affected farms.

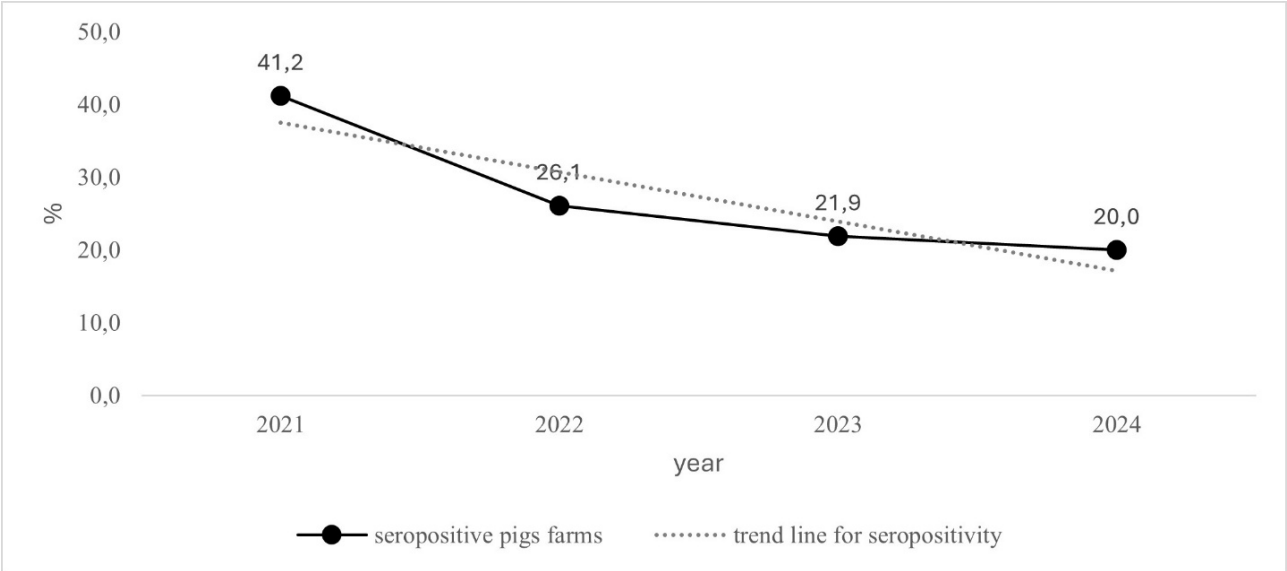


**Fig. 1. Dynamics of detection of antibodies to the influenza A virus on industrial pig farms of Ukraine**

Influenza viruses remain important pathogens affecting human and animal health. They have relatively low pathogenicity for swine, but in conjunction with other pathogens, the outcome can be unfavorable or even mortal [17]. It is also known that SIV-A circulates in swine populations worldwide, and has the ability for reverse zoonosis from humans to swine, and for interspecies transmission [8, 13, 14]. It was also clearly shown the rising historical drug resistance level of SIV-A around the world that is mediated by the evolution of adamantanes-resistant mutations [18]. The authors also emphasize the importance of properly monitoring the adamantanes' susceptibility of SIV-A and the necessity to draw attention to the evolution of drug-resistant H1N1 influenza variants.

Swine influenza is considered one of the most important pathogens of swine respiratory disease in Europe. This infection is primarily caused by H1N1, H1N2, and H3N2 influenza A viruses that have remained endemic in European pig populations for many years. Significant differences in the circulation of these strains occur in different regions across Europe [19].

The result of our study presents an analysis of the serological prevalence and epizootic trends of swine influenza A virus among industrial pig farms in Ukraine from 2021 to 2024. The data indicate a progressive decrease in seropositivity rates, highlighting the worsening epizootic situation and the risk of viral reassortment with potential zoonotic implications. Since the 2009 influenza pandemic caused by the A(H1N1) virus, swine populations have been recognized as important reservoirs for diverse influenza A virus lineages and reassortment events [20, 21]. In Ukraine, although systematic large-scale surveillance has been limited [22], available reports from national veterinary monitoring systems and WOAH notifications indicate sporadic detection of swine influenza A virus in the post-pandemic period prior to 2021. Regional studies and serological surveys conducted in different parts of the country also suggest the circulation of influenza A virus among pig populations (unpublished data), though data remain fragmented and geographically heterogeneous. These limitations underscore the importance of longitudinal studies, such

**Table 1. Detection of influenza A virus in animals from seropositive pig farms in Ukraine**

| Year | Number of samples tested | Number of positive samples | Prevalence of Influenza A virus (%) |
|---|---|---|---|
| **2021** | 103 | 17 | 16.5 |
| **2022** | 63 | 10 | 15.9 |
| **2023** | 168 | 23 | 13.7 |
| **2024** | 88 | 9 | 10.2 |
| **Total** | 422 | 59 | 14.0 |

as the present work, for assessing temporal trends in seroprevalence and epizootic dynamics.

In Germany, present enzootic swine influenza viruses include several co-circulating, antigenically different viruses of each of the H1N1 and H1N2 subtypes [23]. The data obtained by Trombetta et al. [24] suggest that swine influenza viruses might have circulated in Italy as early as 2004, at least in the swine population. According to the authors, this finding highlights the importance of continuing monitoring the influenza viruses' spread in animals and humans for more detailed surveillance. Van Reeth et al. [25] suggested that SIV-A are enzootic in swine-producing regions of Western European countries, while in Central Europe SIV-A activity is low.

Bakre et al. [26] analyzed the diversity of SIV-A in nasal washes and oral fluids from commercial swine farms in the USA using influenza M qRT-PCR and hemagglutinin and neuraminidase subtyping. It was found a predominance of H1 HAs and N2 NAs in the samples examined. There were also identified HAs of the H1 alpha cluster of human novel pandemic origin. The authors claim that SIV-A surveillance can aid the understanding of viral transmission dynamics and help clarify the diversity of its human-swine interaction.

SIV-A subtypes in Brazilian commercial pig herds were studied from the year 2012 to 2019. A higher occurrence of H1N1 was observed from the year 2012 to 2015, H3N2 in 2017, and H1hu in the years 2017 to 2019. Serological data suggested a cyclical character of infection occurrence between the H3N2 and H1N1 virus subtypes over time [27]. The authors conclude that monitoring SIV-A circulation provides the relevant information for field veterinarians to apply proper and timely control measures on the sites.

The results obtained by López-Robles et al. [28] demonstrated a high circulation of strains similar to North American linage among commercial farms in Mexico. The influenza virus H1 circulating in northwestern Mexico showed 97–100% identity among them, 89% identity with other North American strains, 88% – with strains from central Mexico, and 85% with the pandemic A/H1N1p2009 virus.

The results of another study [29] demonstrated circulation of SIV-A throughout Guatemala. The authors determined the virus's identity on commercial farms, animal health status, and age as potential risk factors associated with swine influenza virus infection and exposure. Detection of human-origin viruses in pigs leads to suggestions about a human role in the molecular epidemiology of virus influenza in swine.

Prevalence and distribution results obtained by Li et al. [30] helped to recognize the risk factors of swine influenza infection on commercial farms in China. The open access of wild birds to piggeries, the presence of wild or domestic poultry on a pig farm and no proper biosecurity measures applied to workers working on the farms were found to increase the probability of swine virus influenza in commercial pigs. The surveillance data also allowed Zhao et al. [31] to find out that the occurrences of coinfections from two or more subtypes foster their capacity to produce new viruses. The authors emphasized the need for continuous surveillance and analysis of influenza viruses.

A pilot study on influenza A viruses' prevalence in South Africa suggests that they are likely highly prevalent in the country's swine farms [32]. The authors concluded that South Africa would benefit from periodic surveillance for influenza viruses in swine farms. It was also noted the importance of education and seasonal influenza vaccine programs for the workers on swine farms.

Epidemiological features of swine influenza circulation in farm populations anticipate the continuous serological and systematic molecular surveillance to monitor the status of swine influenza virus circulating in swine populations worldwide [33–35].

The above-stated monitoring data on the prevalence of swine influenza A viruses are extremely useful for familiarizing ourselves with their spread, which makes it possible to better understand the epizootology of these infections and their ability to increase their pathogenicity and prevalence. This data makes it possible to approach the development of appropriate diagnostic and preventive measures.

## CONCLUSIONS

The long-term persistence of swine influenza virus A in industrial pig farms of Ukraine poses a serious risk of viral reassortment and increased virulence, with potential zoonotic consequences. This situation is exacerbated by the lack of systematic vaccination and the uncontrolled movement of animals and humans, particularly in conflict zones. Strengthened biosecurity measures, enhanced sur-

veillance, and targeted vaccination programs are urgently needed to mitigate the threat of widespread outbreaks and potential transmission to humans. The monitoring of the prevalence of swine influenza viruses is useful not only for familiarizing oneself with their geographical distribution but also for providing a deeper understanding of the particularity of their epidemiology, spreading ability, and pathogenicity. It can improve the development and implementation of relevant preventive and control measures for the persistence of SIV-A in industrial pig farms.

## Ethical Statement

This study did not require any Ethical approval.

## Conflict of Interest

The authors declare no conflicts of interest.


## Funding

Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V01-00151.


## Generative AI Statement

Authors declare that no generative AI was used in the creation of this manuscript.

## Authors' Contributions

Material preparation, data collection, results analysis and manuscript writing: A.K., V.K., and N.K.; critical review of manuscript: A.O., J.M.V., B.V., S.K., L.K., M.D., and M.P.; literature review: A.K., M.P., M.D. All authors contributed to the study revision. All authors read and approved the final manuscript.


## Acknowledgements

*We gratefully acknowledge the support provided by Irina Olexandrivna Sobko from the Diagnostic Center of Veterinary Medicine in Kyiv, Ukraine.*


## REFERENCES


1. **Ma, W., 2020:** Swine influenza virus: Current status and challenge. *Virus Res.*, 288, 198118. DOI: 10.1016/j.virusres.2020.198118
2. **Anderson, T. K., Chang, J., Arendsee, Z. W., Venkatesh, D., Souza, C. K., Kimble, J. B., et al., 2021:** Swine influenza A viruses and the tangled relationship with humans. *Cold Spring Harb. Perspect. Med.*, 11, a038737. DOI: 10.1101/cshperspect.a038737
3. **Ang, L. W., Cui, L., Mak, T. M., Ng, Y., Leo, Y. S., Lee, V. J. M., Lin, R. T.-P., 2019:** Differential age-specific distribution of influenza virus types and subtypes in tropical Singapore, 2011 to 2017. *J. Med. Virol.,* 91, 8, 1415–1422. DOI: 10.1002/jmv.25473
4. **Kanji, J. N., Pabbaraju, K., Croxen, M., Detmer, S., Bastien, N., Li, Y., et al., 2021:** Characterization of swine influenza A(H1N2) variant, Alberta, Canada, 2020. *Emerg. Infect. Dis.*, 27, 12, 3045–3051.
5. **Le Sage, V., Rockey, N. C., French, A. J., McBride, R., McCarthy, K. R., Rigatti, L. H., et al., 2024:** Potential pandemic risk of circulating swine H1N2 influenza viruses. *Nat. Commun.*, 15, 5025. DOI: 10.1038/s41467-024-49117-z
6. **Mon, P. P., Thurain, K., Janetanakit, T., Nasamran, C., Bunpapong, N., Aye, A. M., et al. 2020:** Swine influenza viruses and pandemic H1N1-2009 infection in pigs, Myanmar. *Transbound. Emerg. Dis.*, 67, 2653–2666. DOI: 10.1111/tbed.13616
7. **Abdelwhab, E. M., Mettenleiter, T. C., 2023:** Zoonotic animal influenza virus and potential mixing vessel hosts. *Viruses*, 15, 4, 980. DOI: 10.3390/v15040980
8. **Meng, F., Yang, H., Qu, Z., Chen, Y., Zhang, Y., Zhang, Y., et al., 2022:** A Eurasian avian-like H1N1 swine influenza reassortant virus became pathogenic and highly transmissible due to mutations in its PA gene. *Proc. Natl. Acad. Sci. USA*, 119, 34, e2203919119. DOI: 10.1073/pnas.2203919119
9. **Sun, H., Xiao, Y., Liu, J., Wang, D., Li, F., Wang, C., et al., 2020:** Prevalent Eurasian avian-like H1N1 swine influenza virus with 2009 pandemic viral genes facilitating human infection. *Proc. Natl. Acad. Sci. USA*, 117, 29, 17204–17210. DOI: 10.1073/pnas.1921186117
10. **Bliss, N., Nelson, S. W., Nolting, J. M., Bowman, A. S., 2016:** Prevalence of influenza A virus in exhibition swine during arrival at agricultural fairs. *Zoonoses Public Health,* 63, 6, 477–485. DOI: 10.1111/zph.12252
11. **Bliss, N., Stull, J. W., Moeller, S. J., Rajala-Schultz, P. J., Bowman, A. S., 2017:** Movement patterns of exhibition

swine and associations of influenza A virus infection with swine management practices. *J. Am. Vet. Med. Assoc.*, 251, 6, 706–713. DOI: 10.2460/javma.251.6.706

12. **Nelson, M. I., Wentworth, D. E., Das, S. R., Sreevatsan, S., Killian, M. L., Nolting, J. M., et al., 2016:** Evolutionary dynamics of influenza A viruses in US exhibition swine. *J. Infect. Dis.,* 213, 2, 173–182. DOI: 10.1093/infdis/jiv399
13. **Gopinath, S. C. B., Tang, T.-H., Chen, Y., Citartan, M., Tominaga, J., Lakshmipriya, T., 2014:** Sensing strategies for influenza surveillance. *Biosens. Bioelectron.*, 61, 357–369. DOI: 10.1016/j.bios.2014.05.024
14. **Mollett, B. C., Everett, H. E., van Diemen, P. M., Byrne, A. M. P., Ramsay, A., James, J., et al., 2023:** JMM profile: Swine influenza A virus: a neglected virus with pandemic potential. *J. Med. Microbiol.*, 72, 1, 1–5. DOI: 10.1099/jmm.0.001623
15. **Detmer, S., Gramer, M., Goyal, S., Torremorell, M., Torrison, J., 2012:** Diagnostics and surveillance for swine influenza. *Curr. Top. Microbiol. Immunol.,* 370, 85–112. DOI: 10.1007/82_2012_220
16. **Graaf-Rau, A., Hennig, C., Lillie-Jaschniski, K., Koechling, M., Stadler, J., Boehmer, J., et al., 2023:** Emergence of swine influenza A virus, porcine respirovirus 1 and swine orthopneumovirus in porcine respiratory disease in Germany. *Emerg. Microbes Infect.,* 12, 2, 2239938. DOI: 10.1080/22221751.2023.2239938
17. **Klivleyeva, N., Saktaganov, N., Glebova, T., Lukmanova, G., Ongarbayeva, N., Webby, R., 2024:** Influenza A viruses in the swine population: ecology and geographical distribution. *Viruses*, 16, 11, 1728. DOI: 10.3390/v16111728
18. **He, W., Zhang, W., Yan, H., Xu, H., Xie, Y., Wu, Q., et al., 2021:** Distribution and evolution of H1N1 influenza A viruses with adamantanes-resistant mutations worldwide from 1918 to 2019. *J. Med. Virol.*, 93, 6, 3473–3483. DOI: 10.1002/jmv.26670
19. **Brown, I. H., 2013:** History and epidemiology of swine influenza in Europe. *Curr. Top. Microbiol. Immunol.*, 370, 133–146. DOI: 10.1007/82_2011_194
20. **Simon, G., Larsen, L. E., Dürrwald, R., Foni, E., Harder, T., Reeth, K. V., et al., 2014:** European surveillance network for influenza in pigs: surveillance programs, diagnostic tools and swine influenza virus subtypes identified in 14 european countries from 2010 to 2013. *PLoS One*, 9, 12, e115815. DOI: 10.1371/journal.pone.0115815
21. **Lewis, N. S., Russell, C. A., Langat, P., Anderson, T. K., Berger, K., Bielejec, F., et al., 2016:** The global antigenic diversity of swine influenza A viruses. *eLife*, 5, e12217. DOI: 10.7554/eLife.12217
22. **Stegniy, B. T., Rula, O. M., Muzyka, N. M., Dunaiev, Yu. K., Busol, V. O., Kolchyk, O. V., Muzyka, D. V., 2023:** Risk of swine influenza for veterinary medicine and human health in Ukraine. *J. Vet. Med. Biotechnol. Biosafety*, 9, 3, 33–41. DOI: 10.36016/JVMBBS-2023-9-3-6
23. **Zell, R., Groth, M., Krumbholz, A., Lange, J., Philipps, A., Dürrwald, R., 2020:** Cocirculation of swine H1N1 influenza A virus lineages in Germany. *Viruses*, 12, 7, 762. DOI: 10.3390/v12070762
24. **Trombetta, C. M., Montomoli, E., Di Bartolo, I., Ostanello, F., Chiapponi, C., Marchi, S., 2022:** Detection of antibodies against influenza D virus in swine veterinarians in Italy in 2004. *J. Med. Virol.*, 94, 6, 2855–2859. DOI: 10.1002/jmv.27466
25. **Van Reeth, K., Brown, I. H., Dürrwald, R., Foni, E., Labarque, G., Lenihan, P., et al., 2008:** Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002–2003. *Influenza Other Respir. Viruses*, 2, 3, 99–105. DOI: 10.1111/j.1750-2659.2008.00043.x
26. **Bakre, A. A., Jones, L. P., Kyriakis, C. S., Hanson, J. M., Bobbitt, D. E., Bennett, H. K., et al., 2020:** Molecular epidemiology and glycomics of swine influenza viruses circulating in commercial swine farms in the southeastern and midwest United States. *Vet. Microbiol.*, 251, 108914. DOI: 10.1016/j.vetmic.2020.108914
27. **Fraiha, A. L. S., Matos, A. C. D., Cunha, J. L. R., Santos, B. S. Á., da S., Peixoto, M. V. C., Oliveira, A. G. G., et al., 2021:** Swine influenza A virus subtypes circulating in Brazilian commercial pig herds from 2012 to 2019. *Braz. J. Microbiol.*, 52, 2421–2430. DOI: 10.1007/s42770-021-00550-y
28. **López-Robles, G., Montalvo-Corral, M., Burgara-Estrella, A., Hernández, J., 2014:** Serological and molecular prevalence of swine influenza virus on farms in northwestern Mexico. *Vet. Microbiol.*, 172, 323–328. DOI: 10.1016/j.vetmic.2014.05.017
29. **Gonzalez-Reiche, A. S., Ramírez, A. L., Müller, M. L., Orellana, D., Sosa, S. M., Ola, P., et al., 2017:** Origin, distribution, and potential risk factors associated with influenza A virus in swine in two production systems in Guatemala. *Influenza Other Respir. Viruses,* 11, 2, 182–192. DOI: 10.1111/irv.12437
30. **Li, Y., Edwards, J., Wang, Y., Zhang, G., Cai, C., Zhao, M., et al., 2019:** Prevalence, distribution and risk factors of farmer reported swine influenza infection in Guangdong

Province, China. *Prev. Vet. Med.*, 167, 1–8. DOI: 10.1016/j.prevetmed.2019.03.011

31. **Zhao, Y., Han, L., Chen, T., Sang, H., Ding, G., Li, Y., et al., 2022:** Serological surveillance of the H1N1 and H3N2 swine influenza A virus in chinese swine between 2016 and 2021. *Biomed. Res. Int.*, 2022, 5833769, 1–8. DOI: 10.1155/2022/5833769
32. **El Zowalaty, M. E., Abdelgadir, A., Borkenhagen, L. K., Ducatez, M. F., Bailey, E. S., Gray, G. C., 2022:** Influenza A viruses are likely highly prevalent in South African swine farms. *Transbound. Emerg. Dis.*, 69, 2373–2383. DOI: 10.1111/tbed.14255
33. **Baudon, E., Peyre, M., Peiris, M., Cowling, B. J., 2017:** Epidemiological features of influenza circulation in swine populations: A systematic review and meta-analysis. *PLoS One*, 12, 6, e0179044. DOI: 10.1371/journal.pone.0179044
34. **Senthilkumar, D., Kulkarni, D. D., Venkatesh, G., Gupta, V., Patel, P., Dixit, M., et al., 2021:** Widespread prevalence of antibodies against swine influenza A (pdm H1N1 09) virus in pigs of eastern Uttar Pradesh, India. *Curr. Microbiol.,* 78, 2753–2761. DOI: 10.1007/s00284-021-02520-x
35. **Harima, H., Okuya, K., Kajihara, M., Ogawa, H., Simulundu, E., Bwalya, E., et al., 2022:** Serological and molecular epidemiological study on swine influenza in Zambia. *Transbound. Emerg. Dis.*, 69, e931–e943. DOI: 10.1111/tbed.14373

DOI: 10.2478/fv-2026-0018

FOLIA VETERINARIA, 70, 2: 67—74, 2026

REVIEW ARTICLE

# METABOLIC AND DIGESTIVE FUNCTIONS OF GLUCAGON-LIKE PEPTIDE-1 IN PIGS

**Ľubomír Čulka, Zuzana Krepelková, Katarína Bárdová, Jaroslav Novotný***
Clinic of Swine, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

OPEN ACCESS

***Correspondence:** jaroslav.novotny@uvlf.sk

**Citation:** Čulka, Ľ., Krepelková, Z., Bárdová, K., Novotný, J., 2026: Metabolic and digestive functions of glucagon-like peptide-1 in pigs. Folia Veterinaria, 70, 2, 67–74.

**Received:** January 12, 2026

**Accepted:** April 1, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**Glucagon-like peptide-1 (GLP-1) is a hormone with multiple biological effects that plays a key role in glucose metabolism, digestion, and energy homeostasis. In pigs, GLP-1 serves as an important metabolic regulator with relevance for both production efficiency and translational research. This review summarizes current knowledge on GLP-1 physiology in pigs, including synthesis, secretion, metabolism, and biological actions. Particular emphasis is placed on the role of GLP-1 in insulin secretion, digestive function, including gastric mucosal protection and ulcer prevention, appetite regulation, and lipid metabolism. Understanding GLP-1 mechanisms in pigs has practical implications for optimizing feeding strategies in swine production and provides insights relevant to human metabolic disease research.**

**Keywords: digestion; GLP-1; glucose homeostasis; incretin; insulin; metabolism; pigs; stomach ulcers**

## INTRODUCTION

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid incretin hormone originating in intestinal enteroendocrine L-cells where proglucagon undergoes tissue-specific enzymatic processing [1]. It is an incretin hormone that enhances glucose-dependent insulin secretion following nutrient intake. Since its discovery in the 1980s, GLP-1 has become the focus of intensive research due to its therapeutic potential for treating type 2 diabetes and obesity in humans together with other emerging therapeutic areas [2, 3].

Although most GLP-1 research has been conducted in humans and laboratory rodents, pigs also represent a valuable model for studying GLP-1 physiology. Due to their comparable anatomical and physiological features to humans, pigs provide relevant biomedical models for metabolic disease research [4]. Additionally, understanding GLP-1's role in pig metabolism has practical significance for improving feed efficiency and growth performance in commercial pig production [5]. Research on GLP-1 in various animal models, including pigs, has greatly contributed to the development of GLP-1-based therapeutics now widely used in clinical practice, especially for treating type 2 diabetes and obesity [2, 6].

In pigs, proglucagon is processed to GLP-1 and other bioactive peptides through prohormone convertase (PC) 1/3 in intestinal L-cells [1]. The bioactive forms include GLP-1(7-36) amide and GLP-1(7-37), with the amidated form pre-

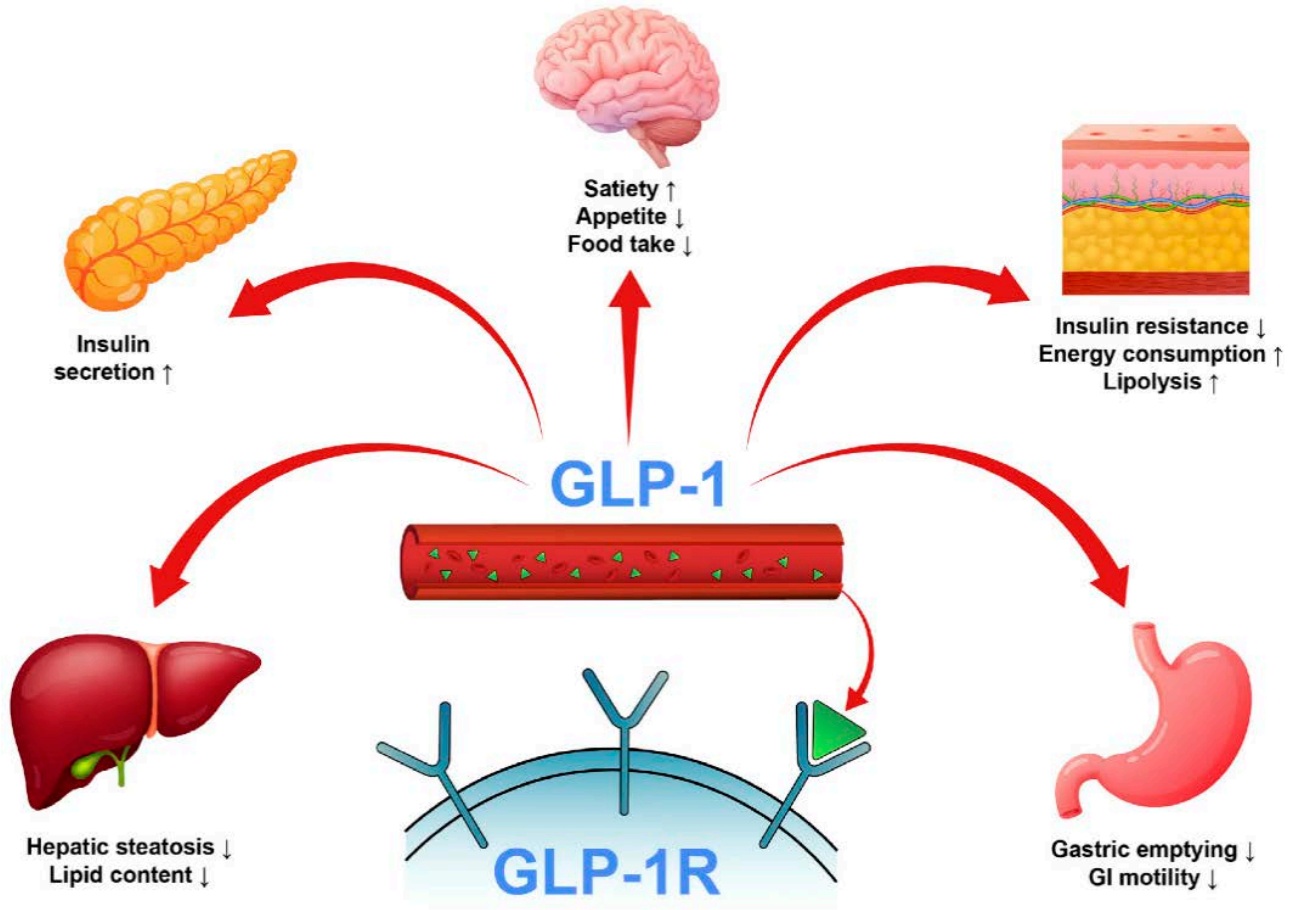


**Fig. 1. Schematic representation of GLP-1 biological multi-organ effects mediated through the GLP-1 receptor (GLP-1R). GLP-1 regulates multiple physiological processes, including insulin secretion from pancreatic β-cells, appetite control in the brain, gastric emptying and GI motility, hepatic lipid metabolism, and adipose tissue energy consumption. Adapted from Alturki et al. (2025) [46] (CC BY 4.0 license)**

dominating in pigs as in humans [7]. Eissele et al. [8] demonstrated a distribution of L-cells (GLP-1-releasing cells) along the gastrointestinal tract, with the highest accumulation in the distal part of the small intestine and colon (Figure 1). Like other mammals, pigs exhibit rapid GLP-1 degradation by dipeptidyl peptidase-4 (DPP-4), resulting in a plasma half-life of approximately 1–2 minutes [9, 10, 11].

## LITERATURE SEARCH STRATEGY

Relevant scientific literature was identified through systematic searches of the PubMed, Web of Science, Scopus, and Google Scholar databases. The search strategy employed combinations of the following keywords and their variants: *GLP-1*, *glucagon-like peptide-1*, *pigs*, *porcine*, *incretin*, *glucose metabolism*, *insulin secretion*, *digestive function*, *gastric emptying*, *gastric ulcers*, *gastroprotection*, *gut hormones*, and *intestinal microbiota*.

Publications published primarily between 1990 and 2025 were considered, with emphasis placed on peer-reviewed original research articles and review papers written in English. Additional relevant references were identified through manual screening of the reference lists of selected articles. Studies focusing on GLP-1 physiology, secretion, metabolism, and biological effects in pigs were prioritized, while key comparative studies in humans and other animal models were included where they provided important mechanistic or translational insights. Only publications directly relevant to metabolic, digestive, or gastrointestinal aspects of GLP-1 were included in the final synthesis.

## GLP-1 SECRETION AND REGULATION

### Nutrient and Neural Regulation

Nutrient intake, particularly carbohydrates and lipids, has been shown to stimulate GLP-1 secretion in pigs [12].

Perfused porcine ileum studies demonstrate dose-dependent GLP-1 release in response to glucose [13]. Long-chain fatty acids are especially potent secretagogues, acting through G-protein-coupled receptors (GPCRs) on L-cells [14]. Also, fermentable dietary fibers promote GLP-1 secretion through short-chain fatty acid production by gut microbiota [14, 15].

Glucagon-like peptide-1 secretion in pigs is also modulated by neural mechanisms. Sympathetic innervation inhibits secretion via α-adrenergic receptors, while β-adrenergic receptor activation and cholinergic stimulation enhance GLP-1 release [16]. However, vagal nerve stimulation shows minimal effect in anesthetized pigs, suggesting intrinsic cholinergic nerves play a more important role than extrinsic vagal pathways [16]. These findings indicate complex local and systemic regulation of GLP-1 secretion.

Glucagon-like peptide-1 secretion exhibits both nutrient-stimulated and basal autonomous patterns. While acute secretory responses to nutrients represent the primary regulatory mechanism, recent evidence indicates that in humans, GLP-1 also follows an intrinsic circadian rhythm. L-cells have been demonstrated to possess autonomous molecular clocks regulated by identified core circadian genes (BMAL1 and CLOCK) modulating time-dependent GLP-1 secretion patterns independently of immediate nutrient stimuli [17]. The basal autonomous pattern as described in humans may be disrupted by various factors, including high-fat diets, irregular sleep patterns, and gut microbiota dysbiosis, potentially contributing to metabolic dysfunction [17]. Characterizing these temporal secretion patterns should be considered when developing guide evidence-based feeding strategies in pig production and providing comparative insights into circadian metabolic regulation across mammals.

**Metabolism and Clearance**

Glucagon-like peptide-1 metabolism in pigs exhibits tissue-specific patterns. Studies in anesthetized pigs reveal substantial renal extraction of endogenous GLP-1 (approximately 33%), indicating the kidneys play a ma-

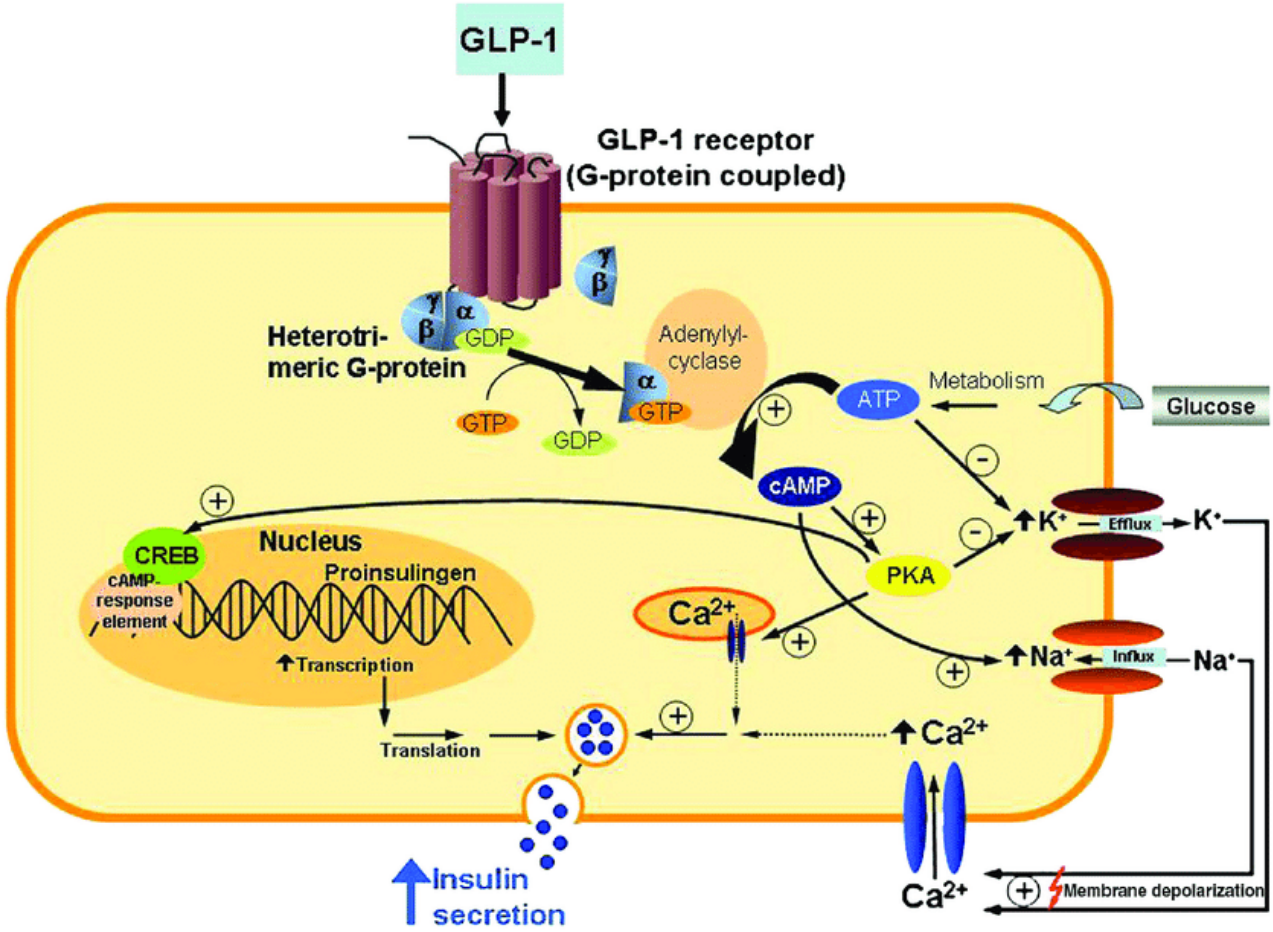


**Fig. 2. Molecular mechanism of GLP-1 receptor signalling in pancreatic β-cells. Adapted from Sheikh (2013) [34] (CC by 2.0 license).**

jor role in GLP-1 clearance, as confirmed by subsequent studies in the same model [9, 18]. The liver and lungs also contribute to elimination through N-terminal cleavage by DPP-4 [9, 10]. Peripheral tissues including skeletal muscle show significant GLP-1 extraction, suggesting widespread hormone metabolism [9]. This rapid degradation necessitates continuous secretion to maintain physiological effects [1].

## GLUCOSE METABOLISM AND INSULIN SECRETION

### The Incretin Effect

The incretin effect has attracted growing interest since the identification of GLP-1 as a physiological incretin in humans by Kreymann et al. [19]. In pigs, glucagon-like peptide-1 contributes significantly to the incretin effect, whereby oral glucose results in greater insulin secretion than intravenous glucose at equivalent blood glucose levels [20]. This effect is comparable in magnitude to the one observed in humans, supporting the pig's validity as a translational model [4, 21]. Both GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) cooperate to increase insulin secretion [20].

The glucagon-like peptide-1 receptor on pancreatic β-cells activates adenylyl cyclase via Gs proteins, increasing intracellular cAMP and activating protein kinase A (PKA) [2, 22]. This signalling pathway is shared across mammals (Figure 2). Importantly, the insulinotropic action of GLP-1 is glucose-dependent, stimulating insulin secretion only at elevated glucose levels and thereby minimizing the risk of hypoglycemia [20]. This safety feature differentiates GLP-1 from conventional insulin secretagogues [23].

### β-Cell Effects

Beyond acute insulin secretion, GLP-1 exerts long-term trophic effects on pancreatic β-cells. Studies using porcine islets demonstrate that GLP-1 promotes β-cell proliferation, inhibits apoptosis, and increases insulin gene expression [24]. These protective effects involve PKA and EPAC (exchange protein directly activated by cAMP) pathways. Such trophic actions may be particularly relevant during metabolic challenges and developmental transitions in pigs, such as weaning [12, 25]. The capacity to maintain β-cell mass and function suggests GLP-1 plays a role beyond immediate glucose regulation.

## DIGESTIVE FUNCTION

### Gastric Emptying and Motility

Glucagon-like peptide-1 significantly slows gastric emptying in pigs [26]. This effect contributes to postprandial glucose control by moderating nutrient delivery to the small intestine, thereby influencing the rate of glucose absorption. The mechanism involves both direct effects on gastric smooth muscle as well as indirect neural pathways [27, 28]. Delayed gastric emptying also enhances satiety and reduces food intake. Additionally, GLP-1 inhibits gastric acid secretion, complementing its effects on gastric emptying [26].

Glucagon-like peptide-1 also influences intestinal function beyond the stomach. It modulates intestinal motility and may affect pancreatic enzyme secretion, though these effects vary with physiological conditions [29]. Recent evidence suggests GLP-1 supports intestinal barrier integrity and reduces intestinal permeability [2, 4]. This is particularly relevant during weaning stress and dietary transitions in commercial pig production, where maintaining gut health is crucial for animal welfare and performance [30].

### Nutrient Absorption

Emerging evidence indicates GLP-1 may influence intestinal nutrient absorption, though the specific mechanisms in pigs remain incompletely understood. Results from postnatal preterm pig studies indicate that intestinal maturation is associated with changes in hexose absorptive capacity and brush-border enzyme activities, processes in which gut hormone signalling from intestinal L-cells plays an important role in postnatal intestinal development [12]. Furthermore, GLP-1's effects on intestinal blood flow may indirectly support nutrient uptake [25]. These actions have important implications for feed efficiency in swine production, though practical applications require further investigation.

### Gastric Ulcers

Gastric ulcers are a frequent finding in pigs, especially in those raised in large-scale farms, significantly affecting productivity. Development of gastric ulcers in pigs has been extensively studied. Key contributing factors include

infectious agents (*Helicobacter suis*), feeding strategies, environmental factors inducing stress, and non-steroidal anti-inflammatory drugs (NSAIDs) administration [31].

Despite commonly reported gastrointestinal adverse effects associated with GLP-1 receptor agonist therapy in patients with type 2 diabetes, several beneficial actions beyond glycaemic control have been documented [32, 33, 34].

Glucagon-like peptide-1 may exert gastroprotective properties via several mechanisms, including delayed gastric emptying [35], inhibition of gastric acid secretion [36, 37], enhanced mucosal barrier function [38, 39], and anti-inflammatory effects [32, 33], as demonstrated in multiple species, including humans and pigs. Understanding the relationship between GLP-1 and gastric ulcers may be of particular relevance in swine production, where gastric ulceration is highly prevalent [31].

## APPETITE REGULATION AND FOOD INTAKE

Glucagon-like peptide-1 functions as a satiety signal in pigs, reducing voluntary feed intake when administered exogenously [40]. This effect involves peripheral mechanisms like delayed gastric emptying and central mechanisms mediated through GLP-1 receptors in the hypothalamus and brainstem [25, 28]. GLP-1 receptors are present in brain regions that control food intake and energy homeostasis, including the *nucleus tractus solitarius* and *area postrema* [25, 28].

From a production perspective, modulating the GLP-1 system could theoretically optimize feed efficiency by improving the growth-to-feed ratio. Dietary strategies promoting endogenous GLP-1 secretion, such as fermentable fiber supplementation, may influence food intake and metabolic efficiency [14, 15]. The gut microbiota plays a key role, producing short-chain fatty acids that stimulate GLP-1 release [14, 15].

## LIPID METABOLISM

Glucagon-like peptide-1 influences lipid metabolism through multiple pathways. In porcine models of metabolic syndrome [4], GLP-1 receptor activation associates with reduced hepatic lipid accumulation and improved hepatic lipid metabolism (including increased fatty acid oxidation and decreased lipogenesis), consistent with effects observed across diverse mammalian models [41]. These effects may result from direct hepatocyte actions or indirect mechanisms involving insulin secretion and food intake changes [41].

Glucagon-like peptide-1 may also affect adipose tissue metabolism by modulating lipolysis and lipogenesis [42]. However, results across studies show some inconsistency, possibly reflecting differences in experimental protocols, pig breeds, or metabolic states. Understanding GLP-1's role in lipid metabolism is important for optimizing meat quality and metabolic health in pig production, as well as for developing pig models of lipid disorders relevant to human health [4].

## DEVELOPMENTAL AND GENETIC ASPECTS

Glucagon-like peptide-1 physiology changes throughout pig development. Studies examining blood plasma and intestinal GLP-1 concentrations during suckling and post-weaning reveal significant developmental changes [43]. Neonatal and weaned piglets exhibit different GLP-1 secretory patterns and receptor sensitivity compared to adult pigs [43]. These changes reflect enteroendocrine system maturation and adaptation to dietary transitions that occur across key production stages in pigs.

Genetic variability in the GLP-1 system exists among pig breeds. Evidence from human and animal studies suggests that differences in body composition and metabolic status may influence incretin responses and GLP-1 sensitivity [27, 44]. Understanding this genetic variation could inform breeding strategies aimed at improving metabolic efficiency and production traits. The interaction between genotype and nutrition in modulating the GLP-1 system represents an important area for future investigation, potentially enabling precision feeding strategies tailored to genetic background.

## TRANSLATIONAL SIGNIFICANCE AND FUTURE PERSPECTIVES

Pigs serve as excellent translational models for GLP-1 research due to anatomical, physiological, and metabolic similarities to humans [4]. Porcine GLP-1 research has

contributed to understanding incretin physiology and developing GLP-1-based therapeutics for type 2 diabetes and obesity [2]. In past years, GLP-1-based therapy and DPP-4 inhibitors have become a standard clinical treatment, and porcine models have played important roles in their development [45, 3]. Conversely, insights from human clinical studies can inform strategies to optimize metabolic health and production efficiency in swine [5, 10].

Future research directions include investigating interactions between GLP-1 and other gut hormones, exploring the gut microbiota's role in GLP-1 modulation, and developing dietary strategies to enhance endogenous GLP-1 secretion [2, 14, 15]. Understanding how early-life nutrition influences GLP-1 system development could have implications for programming metabolic health [12, 22]. Additionally, exploring GLP-1 modulation for improving feed efficiency and reducing environmental impacts of pig production represents an important sustainability consideration. The potential for GLP-1-based approaches to enhance both animal welfare and production efficiency warrants continued investigation.

## CONCLUSION

Glucagon-like peptide-1 is a hormone with diverse biological actions, playing a key role in glucose metabolism, digestive function, and appetite regulation in pigs. The physiological importance of GLP-1 in pigs largely parallels its role in other mammals, including humans, supporting the pig's utility as both an agricultural species and a translational model for metabolic diseases. Understanding GLP-1 mechanisms has practical implications for optimizing nutrition and management in pig farming. Continued research on GLP-1 physiology in pigs promises to gain insights relevant to both animal production and biomedical research, contributing to improved production efficiency and understanding of metabolic regulation across species.

### Ethical Statement

No ethical approval was necessary for this study.

### Conflict of Interest

The authors declare no conflicts of interest.

### Generative AI Statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing of this manuscript.


### Funding

This work is supported by the VEGA grant 1/0040/24: Research on the prevalence, etiological, and predisposing factors of gastric ulcerative changes in pigs.


### Authors' Contributions

**ĽČ –** Conceptualization, Methodology, Literature review, Writing—original draft, Writing—review and editing

**ZK –** Methodology, Literature review, Writing—review and editing, Supervision.

**KB –** Literature review, Writing—original draft, Visualization.

**JN –** Conceptualization, Investigation, Writing—review and editing, Supervision, Funding acquisition.

## REFERENCES


1. **Holst, J. J., 2007:** The physiology of glucagon-like peptide-1. *Physiol. Rev.*, 87, 4, 1409–1439. DOI: 10.1152/physrev.00034.2006
2. **Müller, T. D., Finan B., Bloom, S. R., D´Alessio, D., Drucker, D. J., Flatt, P. R., et al., 2019:** Glucagon-like peptide 1 (GLP-1). *Mol. Metab.*, 30, 72–130. DOI: 10.1016/j.molmet.2019.09.010
3. **Smits, M. M., van Raalte, D. H., Tonneijck, L., Muskiet, M. H., Kramer, M. H., Cahen, D. L., 2016:** GLP-1 based therapies: clinical implications for gastroenterologists. *Gut*, 65, 702–711. DOI: 10.1136/gutjnl-2015-310572
4. **Spurlock, M. E., Gabler, N. K., 2008:** The development of porcine models of obesity and the metabolic syndrome. *J. Nutr.*, 138, 2, 397–402. DOI: 10.1093/jn/138.2.397
5. **Koopmans, S. J., Mroz, Z., Dekker, R., Corbijn, H., Ackermans, M., Sauerwein, H., 2006:** Association of insulin resistance with hyperglycemia in streptozotocin-diabetic pigs: effects of metformin at isoenergetic feeding in a type 2-like diabetic pig model. *Metabolism*, 55, 7, 960–971. DOI: 10.1016/j.metabol.2006.03.004

6. **Renner, S., Blutke, A., Clauss, S., Deeg, C. A., Kemter, E., Merkus, D., et al., 2020:** Porcine models for studying complications and organ crosstalk in diabetes mellitus. *Cell Tissue Res.*, 380, 341–378. DOI: 10.1007/s00441-019-03158-9
7. **Baggio, L. L., Drucker, D. J., 2007:** Biology of incretins: GLP-1 and GIP. *Gastroenterology*, 132, 6, 2131–2157. DOI: 10.1053/j.gastro.2007.03.054
8. **Eissele, R., Göke, R., Willemer, S., Harthus, H. P., Vermeer, H., Arnold, R., et al., 1992:** Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur. J. Clin. Invest.*, 22, 4, 283–291. DOI: 10.1111/j.1365-2362.1992.tb01464.x
9. **Deacon, C. F., Pridal, L., Klarskov, L., Olesen, M., Holst, J. J., 1996:** Glucagon-like peptide-1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am. J. Physiol. Endocrinol. Metab.* .271, 3, E458–E464. DOI: 10.1152/ajpendo.1996.271.3.E458
10. **Hansen, L., Deacon, C. F., Orskov, C., Holst, J. J., 1999:** Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology*, 140, 11, 5356–5363. DOI: 10.1210/endo.140.11.7143
11. **Deacon, C. F., Hughes, T. E., Holst, J. J., 1998:** Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. *Diabetes*, 47, 5, 764–769. DOI: 10.2337/diabetes.47.5.764
12. **Hansen, C. F., Thymann, T., Andersen, A. D., Holst, J. J., Hartmann, B., Hilsted, L., et al., 2016:** Rapid gut growth but persistent delay in digestive function in the postnatal period of preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 310, 8, G550–G560. DOI: 10.1152/ajpgi.00221.2015
13. **Hansen, L., Hartmann, B., Mineo, H., Holst, J. J., 2004:** Glucagon-like peptide-1 secretion is influenced by perfusate glucose concentration and by a feedback mechanism involving somatostatin in isolated perfused porcine ileum. *Regul. Pept.*, 118, 1–2, 11–18. DOI: 10.1016/j.regpep.2003.10.021
14. **Hirasawa, A., Tsumaya, K., Awaji, T., Katsuma, S., Adachi, T., Yamada, M., et al., 2005:** Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.*, 11, 1, 90–94. DOI: 10.1038/nm1168
15. **Li, Z., Yi, C. X., Katiraei, S., Kooijman, S., Zhou, E., Chung, C. K., et al., 2018:** Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut*, 67, 1269–1279. DOI: 10.1136/gutjnl-2017-314050
16. **Hansen, L., Lampert, S., Mineo, H., Holst, J. J., 2004:** Neural regulation of glucagon-like peptide-1 secretion in pigs. *Am. J. Physiol. Endocrinol. Metab.,* 287, 5, E939–E947. DOI: 10.1152/ajpendo.00197.2004
17. **Liu, C., Liu, Y., Xin, Y., Wang, Y., 2022:** Circadian secretion rhythm of GLP-1 and its influencing factors. *Front. Endocrinol. (Lausanne)*, 13, 991397. DOI: 10.3389/fendo.2022.991397
18. **Simonsen, L., Holst, J. J., Deacon, C. F., 2006:** Exendin-4, but not glucagon-like peptide-1, is cleared exclusively by glomerular filtration in anaesthetised pigs. *Diabetologia*, 49, 706–712. DOI: 10.1007/s00125-005-0128-9
19. **Kreymann, B., Ghatei, M. A., Williams, G., Bloom, S. R., 1987:** Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet*, 330, 8571, 1300–1304. DOI: 10.1016/S0140-6736(87)91194-9
20. **Nauck, M. A., Quast, D. R., Wefers, J., Pfeiffer, A. F. H., 2021:** The evolving story of incretins (GIP and GLP-1) in metabolic and cardiovascular disease: A pathophysiological update. *Diabetes Obes. Metab.*, 23 (Suppl. 3), 5–29. DOI: 10.1111/dom.14496
21. **Nauck, M. A., Heimesaat, M. M., Orskov, C., Holst, J. J., Ebert, R., Creutzfeldt, W., 1993:** Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J. Clin. Invest.*, 91, 301–307. DOI: 10.1172/JCI116186
22. **Drucker, D. J., 2006:** The biology of incretin hormones. *Cell Metab.*, 3, 3, 153–165. DOI: 10.1016/j.cmet.2006.01.004
23. **Seino, S., Sugawara, K., Yokoi, N., Takahashi, H., 2017:** β-Cell signalling and insulin secretagogues: A path for improved diabetes therapy. *Diabetes Obes. Metab.*, 19 (Suppl. 1), 22–29. DOI: 10.1111/dom.12995
24. **Pedersen, J., Ugleholdt, R. K., Jørgensen, S. M., Windeløv, J. A., Grunddal, K. V., Schwartz, T. W, et al., 2013:** Glucose metabolism is altered after loss of L cells and α-cells but not influenced by loss of K cells. *Am. J. Physiol. Endocrinol. Metab.*, 304, 1, E60–E73. DOI: 10.1152/ajpendo.00547.2011
25. **Trahair, J. F., Sangild, P. T., 1997:** Systemic and luminal influences on the perinatal development of the gut. *Equine Vet. J.,* Suppl., 24, 40–50. DOI: 10.1111/j.2042-3306.1997.tb05077.x
26. **Schirra, J., Göke, B., 2005:** The physiological role of GLP-1 in human: incretin, ileal brake or more? *Regul. Pept.*, 128, 2, 109–115. DOI: 10.1016/j.regpep.2004.06.018

27. **Ranganath, L. R., Beety, J. M., Morgan, L. M., Wright, J. W., Howland, R., Marks, V., 1996:** Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut*, 38, 916–919. DOI: 10.1136/gut.38.6.916
28. **Vrang, N., Larsen, P. J., 2010:** Preproglucagon derived peptides GLP-1, GLP-2 and oxyntomodulin in the CNS: role of peripherally secreted and centrally produced peptides. *Prog. Neurobiol.*, 92, 3, 442–462. DOI: 10.1016/j.pneurobio.2010.07.003
29. **Nauck, M. A., Meier, J. J., 2018:** Incretin hormones: Their role in health and disease. *Diabetes Obes. Metab.*, 20 (Suppl. 1), 5–21. DOI: 10.1111/dom.13129
30. **Moeser, A. J., Pohl, C. S., Rajput, M., 2017:** Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. *Anim. Nutr.*, 3, 4, 313–321. DOI: 10.1016/j.aninu.2017.06.003
31. **Krepelková, Z., Novotný, J., Bárdová, K., Link, R., Csörgö, A., 2024:** Gastric ulcers in pigs – a review. *Folia Vet.*, 68, 2, 33–42. DOI: 10.2478/fv-2024-0015
32. **Lee, Y. S., Jun, H. S., 2016:** Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. *Mediators Inflamm.*, 2016, 3094642. DOI: 10.1155/2016/3094642
33. **Wong, C. K., Drucker, D. J., 2025:** Antiinflammatory actions of glucagon-like peptide-1–based therapies beyond metabolic benefits. *J. Clin. Invest.*, 135, 21, e194751. DOI: 10.1172/jci194751
34. **Sheikh, A., 2013:** Direct cardiovascular effects of glucagon like peptide-1. *Diabetol. Metab. Syndr.*, 5, 47. DOI: 10.1186/1758-5996-5-47
35. **Delgado-Aros, S., Kim, D. Y., Burton, D. D., Thomforde, G. M., Stephens, D., Brinkmann, B. H., et al., 2002:** Effect of GLP-1 on gastric volume, emptying, maximum volume ingested, and postprandial symptoms in humans. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 282, 3, G424–G431. DOI: 10.1152/ajpgi.2002.282.3.G424
36. **Wettergren, A., Wøjdemann, M., Holst, J. J., 1998:** Glucagon-like peptide-1 inhibits gastropancreatic function by inhibiting central parasympathetic outflow. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 275, 5, G984–G992. DOI: 10.1152/ajpgi.1998.275.5.G984
37. **Andrews, C. N., Bharucha, A. E., Camilleri, M., Low, P. A., Seide, B., Burton, D., et al., 2007:** Nitrergic contribution to gastric relaxation induced by glucagon-like peptide-1 (GLP-1) in healthy adults. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 292, 5, G1359–1365. DOI: 10.1152/ajpgi.00403.2006
38. **Holst, J. J., Andersen, D. B., Grunddal, K. V., 2022:** Actions of glucagon-like peptide-1 receptor ligands in the gut. *Br. J. Pharmacol.*, 179, 4, 727–742. DOI: 10.1111/bph.15611
39. **Colwill, M., Povlsen, S., Pollok, R., Patel, K., Goodhand, J., Ahmad, T., et al., 2025:** Glucagon-like peptide-1 (GLP-1) receptor agonists in inflammatory bowel disease: mechanisms, clinical implications, and therapeutic potential. *J. Crohns Colitis*, 19, 9, jjaf167. DOI: 10.1093/ecco-jcc/jjaf167
40. **Tang-Christensen, M., Vrang, N., Larsen, P. J., 1998:** Glucagon-like peptide 1(7-36) amide's central inhibition of feeding and peripheral inhibition of drinking are abolished by neonatal monosodium glutamate treatment. *Diabetes*, 47, 4, 530–537. DOI: 10.2337/diabetes.47.4.530
41. **Lee, J., Hong, S. W., Rhee, E. J., Lee, W. Y., 2012:** GLP-1 receptor agonist and non-alcoholic fatty liver disease. *Diabetes Metab. J.*, 36, 4, 262–267. DOI: 10.4093/dmj.2012.36.4.262
42. **Villanueva-Peñacarrillo, M. L., Márquez, L., González, N., Díaz-Miguel, M., Valverde, I., 2001:** Effect of GLP-1 on lipid metabolism in human adipocytes. *Horm. Metab. Res.*, 33, 2, 73–77. DOI: 10.1055/s-2001-12428
43. **Theodorakis, M. J., Carlson, O., Michopoulos, S., Doyle, M. E., Juhaszova, M., Petraki, K., et al., 2006:** Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. *Am. J. Physiol. Endocrinol. Metab.*, 290, 3, E550–E559. DOI: 10.1152/ajpendo.00326.2004
44. **Yoder, S. M., Yang, Q., Kindel, T. L., Tso, P., 2010:** Differential responses of the incretin hormones GIP and GLP-1 to increasing doses of dietary carbohydrate but not dietary protein in lean rats. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 299, 2, G476–G485. DOI: 10.1152/ajpgi.00432.2009
45. **Deacon, C. F., 2019:** Physiology and pharmacology of DPP-4 in glucose homeostasis and the ttreatment of type 2 diabetes. *Front. Endocrinol. (Lausanne)*, 10, 80. DOI: 10.3389/fendo.2019.00080
46. **Alturki, M. S., Alkhodier, R. A., Gomaa, M. S., Hussein, D. A., Tawfeeq, N., Al Khzem, A. H., et al., 2025:** Structure-based discovery of orthosteric non-peptide GLP-1R agonists via integrated virtual screening and molecular dynamics. *Int. J. Mol. Sci.*, 26, 13, 6131. DOI: 10.3390/ijms26136131

DOI: 10.2478/fv-2026-0019

FOLIA VETERINARIA, 70, 2: 75—80, 2026

ORIGINAL ARTICLE

# ANALYSIS OF FACTORS INFLUENCING SOMATIC CELL COUNT IN DAIRY COW MILK

**Petra Timkovičová Lacková*, Iveta Maskaľová, Tomáš Mihok, Zuzana Farkašová, Michaela Harčárová**

Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

OPEN ACCESS

***Correspondence:** petra.timkovicova@uvlf.sk

**Citation:** Timkovičová Lacková, P., Maskaľová, I., Mihok, T., Farkašová, Z., Harčárová, M., 2026: Analysis of factors influencing somatic cell count in dairy cow milk. Folia Veterinaria, 70, 2, 75–80.

**Received:** January 12, 2026

**Accepted:** April 9, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**The aim of this study was to evaluate the effects of parity, days in milk (DIM), and season on somatic cell count (SCC) in milk of Holstein dairy cows. The study was conducted on a single dairy farm in eastern Slovakia on 600 cows with an average annual milk yield of 10,317 ± 653 kg, fed a total mixed ration. Cows were grouped according to parity, DIM, and season. Milk composition (protein, fat, lactose) was determined using near-infrared spectrophotometry (MilkoScan FT+), and SCC was measured using Fossomatic FC and Bentley FCM. Parity significantly affected SCC, with the lowest values observed in primiparous cows and a significant increase ($P < 0.001$) with advancing lactation number; the highest somatic cell count was recorded in the 4$^{th}$ and higher lactations. The effect of days in milk showed increased somatic cell count during early (0–30 days) and peak lactation (31–100 days), followed by the lowest values ($P < 0.05$). As lactation progressed and milk yield declined, somatic cell count increased, reaching maximum levels after 305 days. Seasonal variation was also significant, with the highest somatic cell count observed in summer ($P < 0.001$) and the lowest in winter months.**

**Keywords: cow milk; days in milk; mastitis; parity; season**

## INTRODUCTION

The somatic cell count (SCC) in milk is one of the most important indicators of hygienic quality and mammary gland health in dairy cows. Somatic cells (SC) are a natural component of cow's milk and consist mainly of leukocytes (white blood cells) and epithelial cells originating from the lining of the mammary gland. Their primary function is to provide immune protection against microbial infections in the udder. An increased SCC is generally associated with inflammatory processes in the mammary gland, particularly mastitis, which is one of the most common and economically significant diseases in dairy cows [1, 2].

According to Regulation (EC) No. 853/2004, European food legislation has established limit values for the average somatic cell count (SCC) in raw cow's milk of 400,000 cells.ml$^{-1}$ in European Union countries [3]. In the United States, the maximum permissible SCC limit

for raw milk is 750,000 cells.ml$^{-1}$ [4]. Milk from healthy cows typically contains a low SCC (< 200,000 cells.ml$^{-1}$), whereas elevated values indicate an immune response associated with inflammation or the presence of subclinical mastitis [5].

In practice, the SCC is widely used as an important tool for monitoring herd health status, enabling early detection of subclinical mastitis, and evaluating the effectiveness of management measures in dairy herds. In addition to its relevance to animal health, SCC has a substantial impact on the technological properties of milk. Elevated somatic cell counts adversely affect milk composition, particularly protein, fat, and lactose contents, impair milk processing characteristics, and may reduce both the yield and quality of dairy products, especially cheese [6, 7].

Factors influencing the SCC in the milk of dairy cows include the stage of lactation, parity, season, time of milk sampling, milk yield, body condition, herd management, genetics, and the health status of the mammary gland [8].

Parity, days in milk (DIM), and season are major biological and environmental determinants of SCC variability. Parity reflects cumulative exposure to pathogens and physiological aging of the mammary gland, DIM represents changes associated with different stages of lactation and metabolic status, and season reflects environmental influences such as temperature stress. These factors are among the most frequently reported determinants of SCC in dairy production systems.

The aim of this study was to evaluate the effects of parity, days in milk, and season on the SCC in the milk of dairy cows. We hypothesized that parity, DIM, and season significantly influence SCC, with higher SCC expected in cows of higher parity, at the end of lactation, and during the summer season due to environmental and physiological stress.

## MATERIALS AND METHODS

### Data collection

The study was conducted on a single dairy farm in the east of Slovakia, which is situated at 48°34′ north latitude and 20°53′ east latitude, an analysis of Holstein dairy cows (n = 600), whose annual milk production was 10,317 ± 653 kg and which were fed a total mixed ration (TMR) formed from corn, clover, and grass silage supplemented with a concentrate component based on corn, wheat, barley, oats, soybean, and rapeseed extracted meal.

Dairy cows were fed uniformly in groups of primiparous and multiparous cows at the beginning of lactation and in the 2$^{nd}$ and 3$^{rd}$ lactation phases. The cows were fed separately in the 1$^{st}$ lactation phase (31 – 100 DIM). The influence of parity, DIM, and season on the SCC in milk was evaluated as average, with milk samples divided into the following groups according to:

- lactation order: primiparous cows (number of lactations = 1), multiparous cows (number of lactations 2, 3, and 4+)
- days in milk (DIM): 0 – 30, 31 – 100, 101 – 200, 201 – 305, > 305
- seasons: spring (March – May), summer (June – August), autumn (September – November), winter (December – February)

The cows were maintained in free-stall housing. The dairy cow beds were designed as mattress areas covered with a thin layer of straw. The beds were regularly groomed and supplemented with bedding as needed, with occasional liming used to reduce microbial growth and odors. The dairy cows were fed a total mixed ration (TMR) *ad libitum,* provided in the feed trough. The TMR was formed monthly, depending on the nutrient requirements according to the milk yield and capacity of dry matter intake (DMI). The average daily DMI was 21.75 ± 2.27 kg and did not differ significantly between groups. The samples of the TMR were taken from the feed manager on the control day and were analysed for dry matter (DM), which consisted of crude protein (CP), fat, acid, and neutral detergent fibre (ADF, NDF), and starch analysed by conventional methods [9]. The DM was determined by weight upon drying the sample at 105 °C under the prescribed conditions. The CP content was determined by the Kjeldahl method using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Hoganas, Sweden). The fat was determined by the device Det-Gras (JP SELECTA, Spain). The ADF and NDF were determined using a Dosi-Fibre Analyser (JP SELECTA, Spain), and the content of starch was determined by polarimetry. The net energy of lactation (NEL) and non-fibre carbohydrates (NFC) were calculated using regression equations according to the National Research Council [10].

The cows were milked twice a day at 06:00 a.m. and 04:00 p.m. in a parallel milking parlour (BouMatic, Sweden), and individual milk samples were analysed once per

month. On the farm, a basic milking hygiene program was implemented. After milking, teats and udder were treated with a disinfectant solution, typically iodine-based. The samples were cooled to 4 °C and immediately transported to the Central Analytical Laboratory of Milk with accreditation under the registration number 096/5878/2015/2 in collaboration with The Breeding Services of Slovakia, using the breeding information system. A total of approximately 4,000 milk samples were included in the analysis.

Milk samples were analysed for milk protein, fat, and lactose by a near-infrared spectrophotometric assay using MilkoScan FT+ (Foss Electric, Hillerød, Denmark) according to STN 57 0536 [11], and SCC was analysed using a Fossomatic FC (Foss Electric, Hillerød, Denmark) and Bentley FCM (Bentley Instruments Inc., Chaska, USA) according to STN EN ISO 13366-2 [12].

### Statistical analysis

The results were processed using the statistical program GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) and expressed as mean (x), standard deviation (SD), minimum and maximum values. To evaluate differences in SCC depending on parity, DIM, and season, we used one-way analysis of variance (ANOVA) and Tukey's test with a 95% confidence interval for multiple comparisons of means. Only animals that remained in the milk recording system throughout the entire lactation were selected for the final statistical dataset, ensuring consistency of observations across the study period. A total of 600 unique dairy cows were included in the analysis, each contributing on average 7 test-day measurements. Since individual cows contributed observations to multiple DIM categories and seasonal groups, a linear mixed-effects model with cow ID as a random effect was employed to appropriately account for the dependence of repeated measurements.

## RESULTS

The average nutrient concentration in TMR and production indicators for the farm are shown in Table 1.

The SCC, depending on the parity, is shown in Table 2. The lowest SCC was confirmed in the 1st lactation (110.96 ± 52.33 x $10^3$.$ml^{-1}$), which increased significantly ($P < 0.001$) with increasing lactation number (in the 2nd lactation, 213.56 x $10^3$.$ml^{-1}$ and in the 3rd lactation, 313.22 x $10^3$.$ml^{-1}$), while the highest SCC was found in dairy cows in the 4th and higher lactations (417.95 ± 97.85 x $10^3$.$ml^{-1}$).

The SCC, depending on DIM, is shown in Fig. 1. The detected SCC fluctuated significantly with increasing lactation. In 0 – 30 DIM, an increased SCC (210.01 x $10^3$.$ml^{-1}$) was detected, which subsequently decreased with increasing milk production and days in milk; when at the peak of lactation (1st phase of lactation), it reached the lowest values (140.94 x $10^3$.$ml^{-1}$). With a gradual decrease in milk production from the peak of lactation, SCC

**Table 1. Analysed nutrient content of TMR and milk composition**

| Nutrients in TMR (% DM) | x | SD | Minimum | Maximum |
|---|---|---|---|---|
| DM (%) | 44.12 | 3.54 | 34.7 | 51.4 |
| CP (g.$kg^{-1}$ DM) | 153.7 | 11.0 | 105.0 | 174.0 |
| NEL (MJ.$kg^{-1}$ DM) | 6.44 | 0.2 | 5.7 | 6.7 |
| NDF (g.$kg^{-1}$ DM) | 373.1 | 40.0 | 280.0 | 450.0 |
| ADF (g.$kg^{-1}$ DM) | 229.9 | 28.0 | 187.0 | 342.0 |
| Starch (g.$kg^{-1}$ DM) | 229.3 | 50.0 | 103.0 | 301.0 |
| NFC (g.$kg^{-1}$ DM) | 358.0 | 37.0 | 206.0 | 409.0 |
| Fat (g.$kg^{-1}$ DM) | 41.8 | 5.0 | 31.0 | 60.0 |
| **Milk production and composition** | | | | |
| Milk yield (kg.$d^{-1}$) | 34.00 | 10.0 | 10.00 | 79.13 |
| Fat (%) | 3.72 | 0.7 | 1.17 | 8.17 |
| Protein (%) | 3.28 | 0.4 | 1.40 | 5.70 |
| Lactose (%) | 4.86 | 0.2 | 2.43 | 5.40 |
| SCC ($10^3$.$ml^{-1}$) | 266.94 | 82.53 | 2.00 | 485.22 |

DM – dry matter; CP – crude protein; NEL – net energy of lactation; NDF – neutral detergent fibre; ADF – acid detergent fibre; NFC – non-fibre carbohydrates; TMR – total mixed ration; d – day; SCC – somatic cell count

Table 2. SCC ($10^3$.ml$^{-1}$) in milk depending on parity

| Parity | x | SD | Minimum | Maximum |
|---|---|---|---|---|
| 1st lactation | 110.96[a] | 52.33 | 3.00 | 150.85 |
| 2nd lactation | 213.56[b] | 78.59 | 2.00 | 270.52 |
| 3rd lactation | 313.22[c] | 95.78 | 2.00 | 420.89 |
| 4th and ↑ lactation | 417.95[d] | 97.85 | 2.00 | 659.87 |

Means with different superscripts are significantly different (P < 0.001)

increased, reaching the highest values (464.43 x $10^3$.ml$^{-1}$) after the 305th DIM.

Means with different superscripts are statistically significant (P < 0.05)

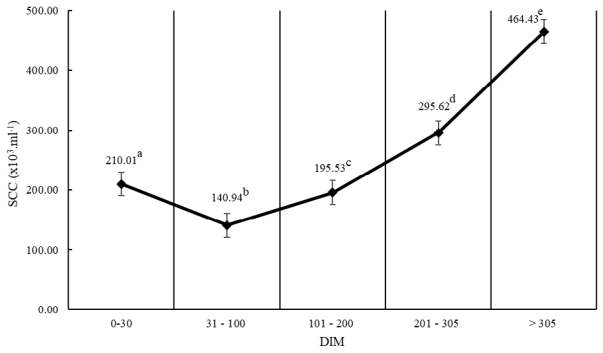


**Fig 1. SCC in milk ($10^3$.ml$^{-1}$) depending on DIM**

The SCC, depending on the season, is shown in Fig. 2. The highest SCC was confirmed in the summer (387.29 x $10^3$.ml$^{-1}$) and the lowest in the winter (218.88 x $10^3$.ml$^{-1}$) months, with confirmed statistical significance (P < 0.05).

Means with different superscripts are significantly significant (P < 0.05)

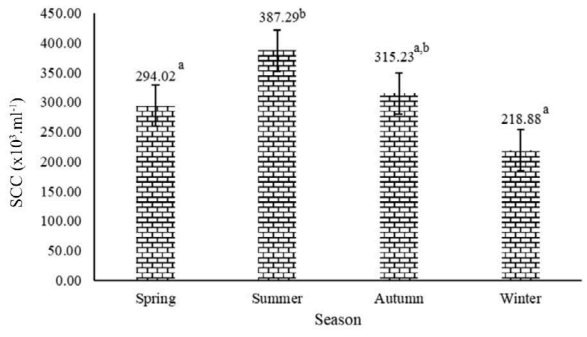


**Fig. 2. SCC in milk ($10^3$.ml$^{-1}$) depending on season**

## DISCUSSION

Fluctuations in production parameters in milk indicate different composition and nutrient content of TMR for individual groups of dairy cows due to different genetic potential of the herd, order, and lactation phase of dairy cows. Nutrition, particularly the composition and balance of the TMR, plays an important role in modulating immune function and SCC levels. Adequate energy and protein supply supports immune competence, while imbalances (negative energy balance, insufficient fibre, or excess rapidly fermentable carbohydrates) may predispose cows to metabolic stress and increased susceptibility to mastitis, which is reflected in elevated SCC. Although the TMR in this study met recommended nutrient requirements, variations in nutrient intake across lactation phases may have contributed to fluctuations in SCC.

Evaluation of SCC depending on the parity, we confirmed the lowest SCC in the 1st lactation, which increased significantly with increasing lactation number as a result of shorter exposure to pathogens and less wear and tear of the mammary gland tissue. The mammary gland of primiparous cows is less damaged by previous inflammatory processes and therefore responds more effectively to infectious stimuli [13]. Older dairy cows in higher lactation (4th and ↑) have a statistically significantly higher average SCC as a result of a more frequent occurrence of subclinical and chronic mastitis, cumulative damage to the mammary gland, and, due to age, a reduced effectiveness of the immune response [14]. In addition, older cows are often under production stress, and their physiological tissue regeneration capabilities are weakened. According to Olde Riekerink et al. (2008), SCC in multiparous cows can be 1.5 to 2 times higher compared to primiparous cows. These differences are important not only for herd management but also for decisions on selection and culling of animals [15].

By evaluating SCC depending on the DIM, we confirmed that immediately after calving, SCC increases due to physiological adaptation, metabolic stress after calving as a result of negative energy balance, and a weakened immune system [16]. SCC stabilizes and reaches its lowest values at the peak of lactation, with SCC often < 200 x $10^3$.

$ml^{-1}$ in healthy cows as an indicator of good health [17]. As lactation progresses (> 101 DIM), SCC increases as a result of decreased milk production, cell accumulation, increased permeability of the mammary gland epithelium, and a higher susceptibility to subclinical mastitis [18]. In > 305 DIM, values of SCC can exceed 300 – 600 x $10^3$. $ml^{-1}$ even in clinically healthy dairy cows [7]. According to Sordillo et al. (2002), the increase in SCC in the late phase of lactation is associated with poorer tissue regeneration and repeated inflammation of the mammary gland [19].

Evaluation of SCC in milk across different seasons revealed the highest values during summer, likely as a consequence of heat stress. High summer temperatures negatively affect the physiological functions of the animals, alter mammary gland cells at the molecular level, cells responsible for the synthesis of milk components, and increase the activity of certain microorganisms associated with mammary gland infections. These changes reduce the mammary gland's defense capacity and promote bacterial colonization [20, 21, 22]. When evaluating individual factors affecting SCC, we found that approximately 15% of cows exceeded the recommended limit of 400,000 cells. $ml^{-1}$.

## CONCLUSIONS

Evaluation of SC in dairy cow milk serves as an important indicator of mammary gland health, particularly in subclinical forms of mastitis that do not present visible symptoms. Elevated SCC indicates inflammation and negatively affects milk quality, reducing its technological value, yield, and hygienic safety. Regular monitoring of SCC helps to minimize economic losses in milk production, optimize herd management, and ensure compliance with legislative milk quality standards. The results of our study confirmed that SCC in dairy cow milk is significantly influenced by parity, DIM, and season. These findings highlight the importance of targeted herd management that takes into account the physiological state of the animals, age, lactation, and climatic conditions to reduce the incidence of mastitis and maintain high hygienic quality of milk.

### Ethical Statement

In this study, we used data obtained with the consent of the farm in collaboration with the Breeding Services of Slovakia, using the Breeding Information System.

### Data Availability Statement

Data are contained within the article.

### Conflict of Interest

The authors declare no conflicts of interest.


### Funding

This study was supported by the Ministry of Education, Science, Research, and Sport of the Slovak Republic, Project VEGA No. 1/0608/24 and KEGA No. 011UVLF-4/2024.


### Generative AI Statement

No generative AI and AI-assisted technologies were used in writing the manuscript.

### Authors' Contributions

Conceptualization, P. T. L.; methodology, P. T. L., Z. F., I. M.; investigation, P. T. L., I. M.; data curation, P. T. L., M. H., T. M.; writing—original draft preparation, P. T. L.; writing—review and editing, M. H., T. M., I. M., Z. F.; funding acquisition, I. M., Z. F.

All authors have read and agreed to the published version of the manuscript.

## REFERENCES


1. **Watson, C. J., Oliver, C. H., Khaled, W. T., 2011:** Cytokine signalling in mammary gland development. *J. Repro. Immunol.,* 88, 2, 124–129. DOI: 10.1016/j.jri.2010.11.006
2. **Alhussien, M. N., Panda, B. S. K., Dang, A. K., 2021:** A comparative study on changes in total and differential milk cell counts, activity, and expression of milk phagocytes of healthy and mastitic indigenous sahiwal cows. *Front. Vet. Sci.,* 8, 670811. DOI: 10.3389/fvets.2021.670811

3. **Commission Regulation (EC) No. 853/2004, 2004:** *Laying down specific hygiene rules for food of animal origin.* Official Journal of the European Union, L 139.
4. **USDA: United States Department of Agriculture, 2002:** General Specifications for Dairy Plants Approved for USDA Inspection and Grading Service.
5. **Schukken, Y. H., Wilson, D. J., Welcome, F., Garrison-Tikofsky, L., Gonzalez, R. N., 2003:** Monitoring udder health and milk quality using somatic cell counts. *Vet. Res.,* 34, 5, 579–596. DOI: 10.1051/vetres:2003028
6. **Walstra, P., Wouters, J. T. M., Geurts, T. J., 2005:** *Dairy Science and Technology.* 2nd ed., CRC Press, Boca Raton, USA, 763. DOI: 10.1201/9781420028010
7. **Ruegg, P. L., 2017:** A 100-Year Review: Mastitis detection, management, and prevention. *J. Dairy Sci.,* 100, 12, 10381–10397. DOI: 10.3168/jds.2017-13023
8. **Stocco, G., Cipolat-Gotet, C., Stefanon, B., Zecconi, A., Francescutti, M., Mountricha, M., Summer, A., 2023:** Herd and animal factors affect the variability of total and differential somatic cell count in bovine milk. *J. Anim. Sci.,* 101, 1–10. DOI: 10.1093/jas/skac406
9. **Commission Regulation (EC) No. 691/2013, 2013:** Amending Regulation (EC) No. 152/2009 as regards methods of sampling and analysis. OJEU, L197/1–L197/12.
10. **NRC, 2021:** Nutrient Requirements of Dairy Cattle: 8th ed. National Academy Press, Washington, DC.
11. **STN 57 0536, 1995:** Milk – Determination of composition by infrared spectrophotometry. Bratislava: Slovak Office of Standards, Metrology and Testing.
12. **STN EN ISO 13366-2, 2007:** Milk — Enumeration of somatic cells — Part 2: Guidance on the operation of fluoro-opto-electronic counters. Bratislava: Slovak Office of Standards, Metrology and Testing.
13. **Alhussien, M. N., Dang, A. K., 2018:** Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. *Vet. World,* 11, 5, 562–577. DOI: 10.14202/vetworld.2018.562-577
14. **Koivula, M., Mäntysaari, E. A., Negussie, E., Serenius, T., 2005:** Genetic and phenotypic relationships among milk yield and somatic cell count before and after clinical mastitis. *J. Dairy Sci.,* 88, 2, 827–833. DOI: 10.3168/jds.S0022-0302(05)72747-8
15. **Olde Riekerink, R. G. M., Barkema, H. W., Kelton, D. F., Scholl, D. T., 2008:** Incidence rate of clinical mastitis on Canadian dairy farms. *J. Dairy Sci.,* 91, 4, 1366–1377. DOI: 10.3168/jds.2007-0757
16. **Bach, K. D., Barbano, D. M., McArt, J. A. A., 2021:** The relationship of excessive energy deficit with milk somatic cell score and clinical mastitis. *J. Dairy Sci.*, 104, 1, 715–727. DOI: 10.3168/jds.2020-18432
17. **Mondini, S., Gislon, G., Zucali, M., Sandrucci, A., Tamburini, A., Bava, L., 2025:** Factors influencing somatic cell count and leukocyte composition in cow milk: A field study. *J. Dairy Sci.,* 108, 3, 2721–2733. DOI: 10.3168/jds.2024-25357
18. **Hagnestam-Nielsen, C., Emanuelson, U., Berglund, B., Strandberg, E., 2009:** Relationship between somatic cell count and milk yield in different stages of lactation. *J. Dairy Sci.,* 92, 7, 3124–3133. DOI: 10.3168/jds.2008-1719
19. **Sordillo, L. M., Streicher, K. L., 2002:** Mammary gland immunity and mastitis susceptibility. *J. Mammary Gland Biol. Neoplasia,* 7, 2, 135–146. DOI: 10.1023/a:1020347818725
20. **Gao, S. T., Ma, L., Zhou, Z., Zhou, Z. K., Baumgard, L. H., Jiang, D., et al., 2019:** Heat stress negatively affects the transcriptome related to overall metabolism and milk protein synthesis in mammary tissue of midlactating dairy cows. *Physiol. Genomics*, 51, 8, 400-409. DOI: 10.1152/physiolgenomics.00039.2019
21. **Park, T., Ma, L., Gao, S., Bu, D., Yu, Z., 2022:** Heat stress impacts the multi-domain ruminal microbiota and some of the functional features independent of its effect on feed intake in lactating dairy cows. *J. Anim. Sci. Biotechnol.,* 13, 71, 1–15. DOI: 10.1186/s40104-022-00717-z
22. **Lemal, P., May, K., König, S., Schroyen, M., Gengler, N., 2023:** Invited review: From heat stress to disease - Immune response and candidate genes involved in cattle thermotolerance. *J. Dairy Sci.,* 106, 7, 4471–4488. DOI: 10.3168/jds.2022-22727

DOI: 10.2478/fv-2026-0020

FOLIA VETERINARIA, 70, 2: 81—87, 2026

ORIGINAL ARTICLE

# EXPRESSION PATTERNS OF COPPER/ZINC SUPEROXIDE DISMUTASE (SOD1) IN QUAIL EMBRYONIC TISSUES UNDER *IN OVO* AND *EX OVO* CONDITIONS

**Bronislava Pokorná[1]*, Slavomíra Štefancová[1], Helena Ivanić[1], Eva Petrovová[2], Lenka Luptáková[1]**

[1]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; [2]Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

OPEN ACCESS

***Correspondence:** bronislava.pokorna@student.uvlf.sk

**Citation:** Pokorná, B., Štefancová, S., Ivanić, H., Petrovová E., Luptáková, L., 2026: Expression patterns of copper/zinc superoxide dismutase (SOD1) in quail embryonic tissues under *in ovo* and *ex ovo* conditions. Folia Veterinaria, 70, 2, 81–87.

**Received:** February 1, 2026

**Accepted:** April 21, 2026

**Published:** June 15, 2026



**Ethical considerations:** When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

## ABSTRACT

**Temperature fluctuations can act as stressors affecting embryonic development and cellular homeostasis. This study examined the effects of preincubation at 22°C (P22) or 30°C (P30) on quail embryos under *in ovo* and *ex ovo* conditions, focusing on the expression of the antioxidant enzyme superoxide dismutase (*SOD1*) in selected tissues. Under *in ovo* incubation, *SOD1* expression was significantly upregulated in both liver and heart at P22 and P30, with the highest expression observed in the liver at P30. In contrast, under *ex ovo* conditions, *SOD1* expression was significantly reduced in the liver at P22 but partially restored at P30. In the heart, it was not significantly increased at P22 but slightly decreased at P30. These findings demonstrate tissue- and condition-specific regulation of *SOD1* and suggest that *in ovo* preincubation supports robust antioxidant defence, whereas *ex ovo* culture imposes complex stress responses.**

**Keywords: *ex ovo*; *in ovo*; preincubation; quail embryo; SOD1**

## INTRODUCTION

Various factors, such as egg storage period, age, breed, preincubation conditions, lighting, nutritional status, and mating practices, are known to influence fertility in birds [1]. In addition, the process of embryonic development, hatching, and survival of quail (*Coturnix japonica*) can be influenced by multiple environmental and management conditions, including the egg storage conditions, egg rotation, and relative humidity. It has been determined that temperature has a significant influence on these processes. These environmental and management factors can act as stressors, leading to an increased production of reactive oxygen species (ROS) within the developing embryos [2].

To cope with such oxidative challenges, living organisms have evolved specific antioxidant mechanisms. These natural antioxidant systems are essential for survival in oxygen-rich environments, protecting cells from damage caused by ROS. The first line of defence involves preventing free radical formation by removing their precursors or

deactivating catalytic metal ions, primarily through metal-binding proteins. The second line of defence consists of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), which detoxify ROS once they are formed. SOD, which converts superoxide radicals, is considered a crucial component of this first defence level, as superoxide represents the primary free radical generated in cells [3]. SOD catalyses the disproportionation of the superoxide anion ($O_2^{\bullet-}$), converting it through the reaction $2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$. This process contributes to the reduction of oxidative stress in cells [4].

The antioxidant protection of avian embryos is subject to change over time and between individual tissues during development. The sensitivity of tissues to damage caused by ROS is dependent on the composition of their lipids, resulting in differences between tissues. In avian species, the predominant source of antioxidants consists of substances of maternal origin, which are stored in the yolk. During embryonic development, yolk antioxidants are gradually transported into the embryo, where they are distributed unevenly among different tissues [5].

Embryonic development in birds is highly sensitive to incubation temperature, particularly during early developmental stages. Exposure to continuous or intermittent deviations from optimal incubation temperature has been shown to impair hatchability and alter physiological and hormonal responses [6]. Experimental manipulation during incubation has been used to influence the antioxidant capacity of avian embryos. *In ovo* administration of antioxidant trace elements, such as nano-selenium or nano-zinc oxide, has been shown to enhance antioxidant enzyme activity and reduce oxidative stress under challenging incubation conditions. These findings highlight the importance of controlled experimental approaches for studying embryonic development and redox balance [7].

Most studies utilise the windowing technique (*in ovo*) to observe developmental changes after chemical exposure, as it facilitates long-term experimentation with minimal abnormalities and permits natural hatching [8, 9]. The *ex ovo* method, a widely applied technique in the fields of embryology, genetic manipulation, toxicology [8], and cancer research [10, 11], provides excellent visualisation and unrestricted access to the chorioallantoic membrane (CAM, [12]), thereby enabling direct monitoring of the effects of pathogens or drugs on embryonic vasculature [9]. However, the success of *ex ovo* culture is contingent upon meticulous regulation of oxygen, moisture, and calcium levels to mitigate the risks of dehydration and infection, which, in the absence of such regulation, can result in elevated mortality rates [13].

In summary, this study focuses on gene expression of *SOD1* as a key component of the antioxidant defence system in quail embryos incubated under different preincubation temperatures and incubation methods. By comparing *in ovo* and *ex ovo* conditions, this work aims to clarify how incubation environment and thermal preconditioning influence antioxidant defence during early embryonic development and to contribute to the optimization of *ex ovo* cultivation strategies.

## MATERIALS AND METHODS

### Preincubation and Incubation

Fertilized quail eggs (*Coturnix coturnix japonica*, n = 144) were purchased from the quail farm (Mala Ida, Kosice, Slovakia), no older than three days after laying. The eggs were disinfected with 70% ethanol and divided into two groups: *in ovo* incubation (65 eggs) and *ex ovo* incubation (79 eggs).

In both the *in ovo* and *ex ovo* incubation groups, the fertilized eggs were divided into three groups according to the incubation conditions (Table 1). During the 12-hour preincubation period, control embryos were stored under standard cold storage conditions at 14 °C in a temperature-controlled wine cooler. Subsequently, this first group of embryos (22 *in ovo*; 27 *ex ovo*) was incubated under standard conditions at 38.2 °C and 60% relative humidity. The second group (21 *in ovo*; 29 *ex ovo*) was preincubated at controlled room temperature (approximately 22 °C; P22), and the third group (22 *in ovo*; 23 *ex ovo*) was preincubated at 30 °C (P30). The embryos were maintained in a closed, climate-controlled, low-disturbance environment during the preincubation period, minimizing potential temperature fluctuations. Preincubation in the P22 and P30 groups lasted 12 hours, after which all eggs were transferred to standard incubation conditions. For the *ex ovo* method, eggs were stored vertically during the preincubation period and placed horizontally during incubation. After 56 hours, the contents of the eggs, including the embryo, were carefully transferred under sterile conditions into a 6-well culture plate. *Ex ovo* incubation required

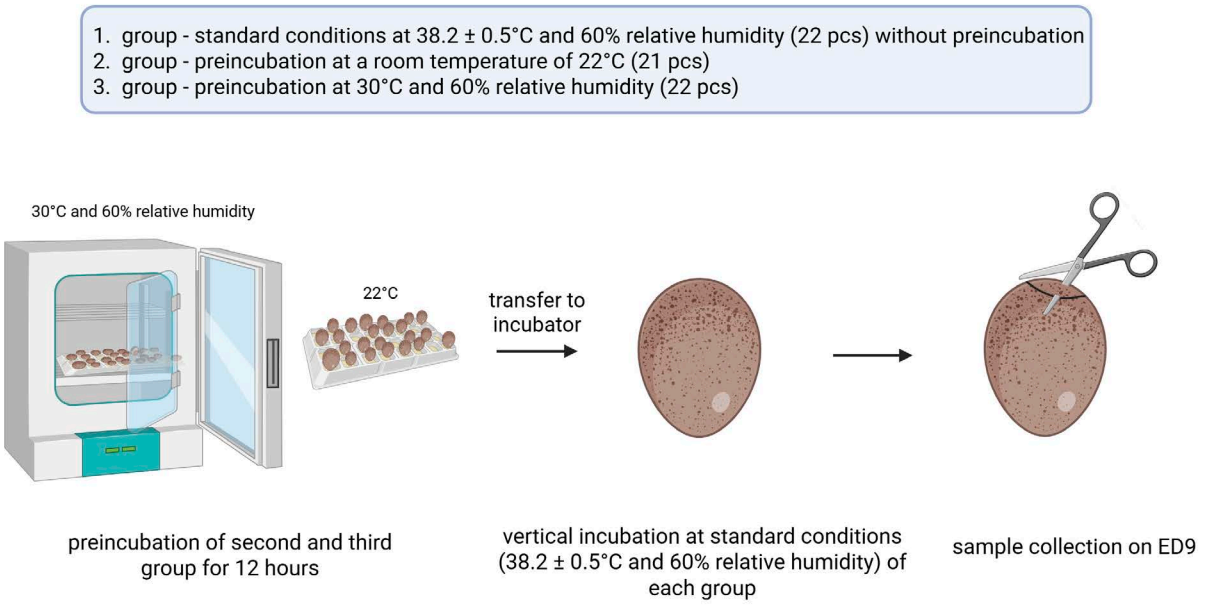


**Fig. 1. *In ovo* method**

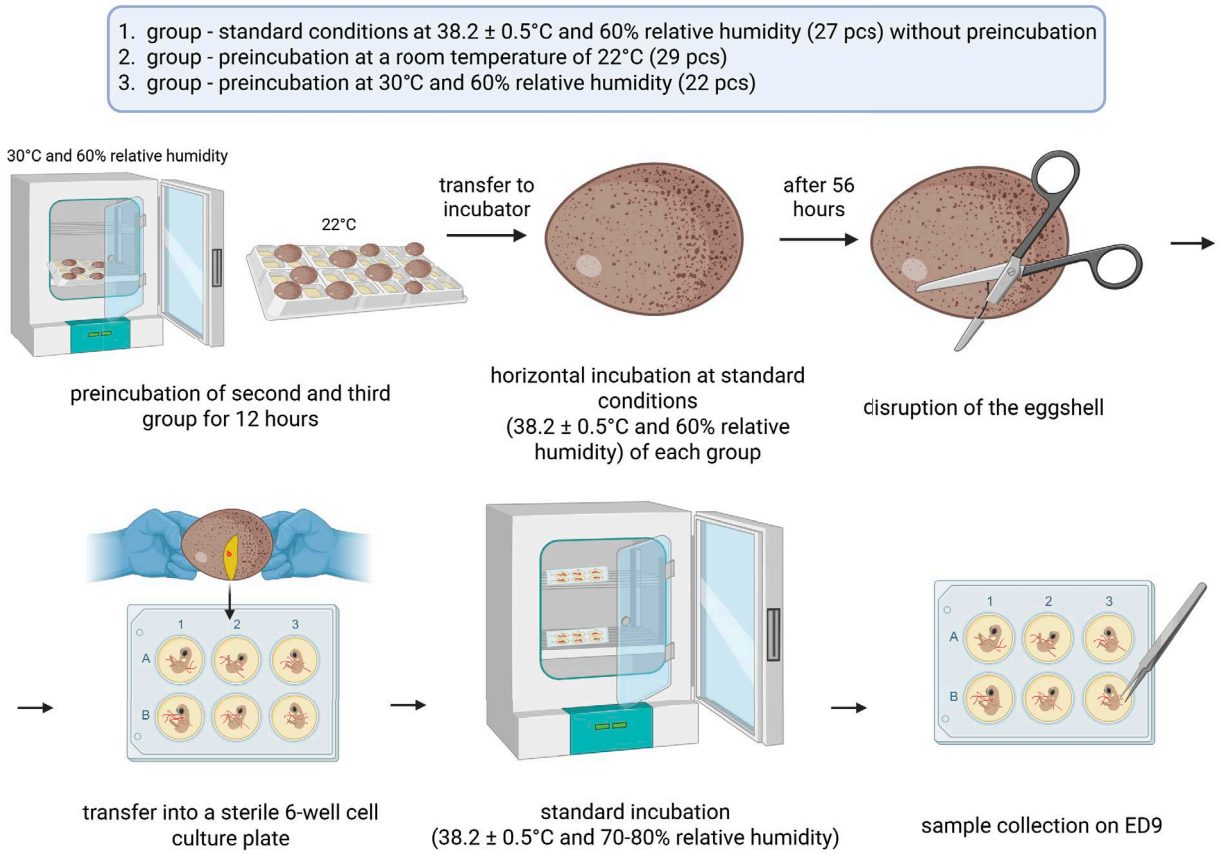


**Fig. 2. *Ex ovo* method**

higher relative humidity (70–80%), which was applied after transferring embryos to culture plates.

Embryos from both methods were incubated until embryonic day 9 (ED9), when sampling was performed. All incubation was carried out in an automatic incubator (COVINA ET 49, Italy). For the *in ovo* method, eggs were opened at the blunt end on ED9, and embryos were removed. *Ex ovo* embryos were collected from the culture plates in the same manner. After weighing, samples of the heart and liver were stored at −80 °C for RNA extraction.

Figure 1 and Figure 2 show methodological procedures for *in ovo* and *ex ovo* incubation.

**Table 1. Number of eggs in experimental groups (*in ovo* and *ex ovo*) at the start of the study**

| | *in ovo* | *ex ovo* |
|---|---|---|
| **Standard incubation** | 22 | 27 |
| **Preincubation at 22°C** | 21 | 29 |
| **Preincubation at 30°C** | 22 | 23 |

### Gene Expression Analysis

Total RNA was isolated from liver and heart using the QIAshredder and RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA purity and concentration were assessed with a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Gene expression was analysed using a two-step RT-qPCR protocol. First, cDNA was synthesized from 1 µg of total RNA in a 20 µL reaction using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, Waltham, MA, USA) according to the manufacturer's instructions. In the second step, qPCR was performed using SYBR Green Master Mix (Applied Biosystems™, Waltham, MA, USA) and specific primers for *SOD1*. Each 10 µL reaction contained 300 nM of each primer. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the endogenous control, and relative expression was calculated using the $2^{-\Delta\Delta CT}$ method [14]. Amplification conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All samples were run in triplicate and melt curve analysis confirmed specificity. Gene expression levels were normalized to the control group without preincubation. Melting temperatures ranged from 77.22–78 °C for *SOD1*.

### Statistical Analysis

Statistical analysis was performed with the GraphPad Prism program (version 9.3, USA) using a two-way ANOVA test with Tukey's post hoc test. The value of statistical significance was considered as $p < 0.0001$. Data were evaluated as mean ± standard deviation (SD).

## RESULTS

### Gene Expression Analysis of *In Ovo* and *Ex Ovo* Incubation

#### Liver

The expression of *SOD1* in the liver was found to be significantly affected by both preincubation temperature and culture system. *In ovo*, *SOD1* expression increased markedly with rising preincubation temperature, showing a significant upregulation ($p < 0.0001$) at P22 compared with the control temperature, and reaching the highest expression level ($p < 0.0001$) at P30.

In contrast, under *ex ovo* conditions, P22 led to a significant decrease ($p < 0.0001$) in *SOD1* expression in comparison with the control group. The P30 demonstrated an increase compared to the control group and P22 group; however, this increase was not statistically significant (Figure 3).

#### Heart

During the observation of *SOD1* expression in the heart, a significant upregulation ($p < 0.0001$) was observed in both groups with preincubation (P22 or P30) in *in ovo* conditions.

The *ex ovo* conditions demonstrated a range of outcomes. P22 demonstrated a non-significant upregulation in comparison with the control group. P30 demonstrated a marginal yet non-statistically significant reduction in *SOD1* expression when compared to the control group (Figure 3).

To summarize, the *in ovo* incubation approach resulted in a significant upregulation ($p < 0.0001$) of *SOD1* expression in all studied tissues (liver and heart) under both preincubation conditions (P22 and P30). The highest levels of *SOD1* expression were observed in the liver, followed by the heart, particularly when preincubation was conducted at 30°C. This upregulation of *SOD1* may reflect an adaptive increase in antioxidant defence capacity rather than directly indicating elevated oxidative stress levels.

In contrast, the *ex ovo* method exhibited differential effects contingent on tissue and temperature. In the liver, P22 resulted in a significant downregulation of *SOD1* ($p < 0.0001$) in comparison with the control group. In the heart, P30 led to a marginal yet non-significant reduction in *SOD1* expression. The observed patterns may indicate that excessive stress under *ex ovo* conditions is associated with impaired antioxidant responses. The overall expression trends are summarised in Table 2.

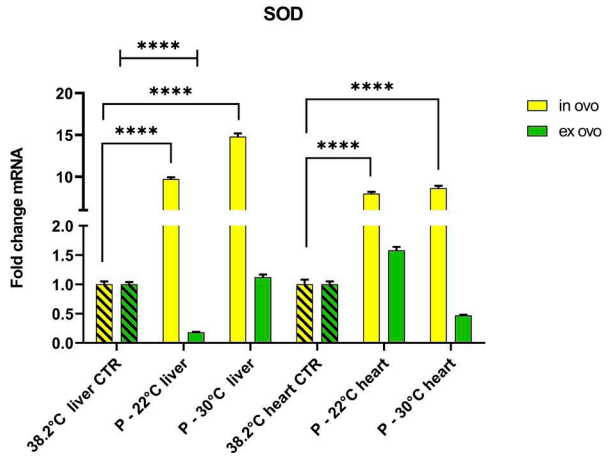


**Fig. 3. Gene expression of *SOD1* in *in ovo* and *ex ovo* groups with preincubation (**** indicates statistical significance at $p < 0.0001$)**

**Table 2. Gene expression of *SOD1* in *in ovo* and *ex ovo* groups with preincubation**

| | | SOD1 | |
|---|---|---|---|
| | | P22[a] | P30[b] |
| **liver** | *in ovo* | +[c] | +[c] |
| | *ex ovo* | -[d] | +ns[e] |
| **heart** | *in ovo* | +[c] | +[c] |
| | *ex ovo* | +ns[e] | -ns[f] |

P22[a] – Preincubation at 22°C; P30[b] – Preincubation at 30°C; +[c] – Significant upregulation ($p < 0.0001$); -[d] – Significant downregulation ($p < 0.0001$); +ns[e] – Nonsignificant upregulation; -ns[f] – Nonsignificant downregulation

## DISCUSSION

The level of oxidative stress in embryos may be influenced by multiple factors, including the interval between laying and storage, the duration of storage, fluctuations in incubation conditions, and the length of the hatch window [15]. During embryogenesis, antioxidants derived from the yolk are distributed unevenly among the developing tissues. Previous studies have demonstrated tissue-specific differences in lipid oxidation and antioxidant enzyme activity. The brain has been reported to exhibit the highest concentrations of malondialdehyde (MDA), a marker of lipid peroxidation, followed by the liver, kidney, and thigh. Antioxidant enzymes such as SOD and CAT have been detected across multiple tissues, including the brain, liver, kidney, and muscle, with their activity varying in a tissue-dependent manner. Among these tissues, SOD activity is tissue-dependent, with the highest levels observed in the heart, followed by muscle, yolk sac membrane, kidney, lung, and liver [16]. However, these findings are based on enzyme activity measurements and may not directly correspond to gene expression levels.

Our results demonstrated condition-dependent regulation of *SOD1* expression, with tissue-specific differences observed only under *ex ovo* incubation conditions. In the context of *in ovo* incubation, a significant upregulation of *SOD1* was observed in both the liver and heart at both preincubation temperatures (P22 and P30). The highest expression levels were detected in the liver, followed by the heart, particularly at P30. A moderate yet significant increase in *SOD1* was observed at P22 in both organs, suggesting that embryos can mount an adaptive antioxidant response to mild oxidative stress within the stable *in ovo* environment.

In contrast, *ex ovo* conditions elicited more variable tissue-specific responses. The liver showed a significant decrease in *SOD1* expression after P22, while P30 led to a partial restoration of expression, although not to a significant extent. Conversely, the heart exhibited a divergent response: *SOD1* expression was non-significantly increased at P22, suggesting a degree of adaptability. However, a slight, also non-significant decrease in *SOD1* expression was observed after preincubation at P30. These findings suggest that both *ex ovo* culture conditions and preincubation temperature may contribute to the observed tissue-specific differences in *SOD1* expression, likely through combined effects on the embryonic microenvironment.

Several physiological limitations of *ex ovo* incubation, such as the absence of the protective eggshell, increased risk of dehydration, nutrient loss, and impaired calcium availability, may elevate cellular stress and contribute to the reduced antioxidant response [13]. Optimization of humidity, airflow, calcium supplementation, and egg handling has been proposed to improve embryonic viability under *ex ovo* conditions [11, 13].

Consistent with our observations, Amevor et al. reported that extended egg storage periods (7 and 14 days) resulted in increased oxidative stress, reflected by elevated MDA levels and decreased antioxidant indicators, including total SOD (T-SOD), in the liver, heart, and breast muscle [1]. Furthermore, incubation temperature is a critical factor influencing embryonic development, as low temperatures slow development and increase mortality, whereas temperatures within the physiological range accelerate development and improve hatching success [17].

## CONCLUSION

Several environmental factors, including temperature fluctuations and modified oxygen supply, can alter avian embryonic development. One of the key cellular responses to such changes is the regulation of genes related to antioxidants, particularly SOD1. This gene encodes an enzyme that plays a key role in the body's initial defence against ROS.

Our results indicate that *in ovo* incubation supports a controlled upregulation of *SOD1* gene expression, reflecting a regulated antioxidant response under physiologically stable conditions. In contrast, *ex ovo* incubation exposes embryos to altered environmental conditions, which is associated with reduced *SOD1* gene expression, suggesting differences in the regulation of antioxidant defence-related genes. In addition, preincubation temperature may have contributed to the observed variation in expression patterns, particularly under *ex ovo* conditions.

As this was a pilot study, analysis of other markers of oxidative damage or enzyme activity was not performed. Therefore, the observed changes in *SOD1* expression should be interpreted as an indication of antioxidant capacity.

### Data Availability Statement

The data is publicly available.

### Ethical Statement

This research study used avian embryos as experimental *ex ovo* and *in ovo* models, which do not fall under the legislation for protecting animals used for scientific purposes (2010/63/EU). No approval of research ethics committees was required to accomplish the goals of this study.

### Conflict of Interest

The authors declare that there is no conflict of interest.


### Funding

This research was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V05-00017 (IGA-ESGD/04/2024).


### Generative AI Statement

The authors used AI-based language tools (DeepL Write and Grammarly) solely for improving grammar and style. No generative AI tools were used to create or modify the scientific content of this work.

### Authors' Contributions

Bronislava Pokorna: Conceptualization, Formal analysis, Resources, Writing – Original draft. Slavomira Stefancova: Data curation. Helena Ivanic: Conceptualization, Visualization, Investigation, Methodology. Eva Petrovova: Funding acquisition, Project administration, Supervision, Validation, Writing – Reviewing and Editing. Lenka Luptakova: Funding acquisition, Validation, Writing – Reviewing and Editing.

## REFERENCES


1. **Amevor, F. K., Cui, Z., Du, X., Ning, Z., Deng, X., Xu, D., et al., 2022:** Synergy between dietary quercetin and vitamin E supplementation in aged hen's diet improves hatching traits, embryo quality, and antioxidant capacity of chicks hatched from eggs subjected to prolonged storage. *Front. Physiol*., 13, 873551. DOI: 10.3389/fphys.2022.873551
2. **Surai, P. F., 2020:** Antioxidants in poultry nutrition and reproduction: An update. *Antioxid.*, 9, 2, 105. DOI: 10.3390/antiox9020105
3. **Surai, P. F., 2015:** Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. *Antioxid*., 4, 1, 204–247. DOI: 10.3390/antiox4010204
4. **Li, K., Jiang, L., Wang, J., Xia, L., Zhao, R., Cai, C., et al., 2020:** Maternal dietary supplementation with different sources of selenium on antioxidant status and mortality of chicken embryo in a model of diquat-induced acute oxidative stress. *Anim. Feed Sci. Technol.*, 261, 114369. DOI: 10.1016/j.anifeedsci.2019.114369
5. **Tsunekage, T., Ricklefs, R. E., 2015:** Increased lipid peroxidation occurs during development in Japanese quail (*Coturnix japonica*) embryos. *Br. Poult. Sci.*, 56, 2, 262–266. DOI: 10.1080/00071668.2014.994592
6. **Abuoghaba, A. A. K., Ali, F., Ismail, I. I., Saleh, M., 2021:** Impact of acute short-term high thermal stress during early embryogenesis on hatchability, physiological body reaction,

and ovarian follicles development of quails. *Poult. Sci.*, 100, 2, 1213–1220. DOI: 10.1016/j.psj.2020.11.019

7. **Shokraneh, M., Sadeghi, A. A., Mousavi, S. N., Esmaeilkhanian, S., Chamani, M., 2020:** Effects of in ovo injection of nano-selenium and nano-zinc oxide and high eggshell temperature during late incubation on antioxidant activity, thyroid and glucocorticoid hormones and some blood metabolites in broiler hatchlings. *Acta Sci. Anim. Sci.*, 42, e46029. DOI: 10.4025/actascianimsci.v42i1.46029
8. **Tahara, Y., Obara, K., 2021:** *Ex ovo* culture system for avian embryos and its application. *J. Poult. Sci.,* 58, 1, 1–4. DOI: 10.2141/jpsa.0200016
9. **Kundeková, B., Máčajová, M., Meta, M., Čavarga, I., Bilčík, B., 2021:** Chorioallantoic membrane models of various avian species: Differences and applications. *Biology (Basel),* 10, 4, 301. DOI: 10.3390/biology10040301
10. **Lenkavska, L., Blascakova, L., Jurasekova, Z., Macajova, M., Bilcik, B., Cavarga, I., et al., 2019:** Benefits of hypericin transport and delivery by low- and high-density lipoproteins to cancer cells: From *in vitro* to *ex ovo*. *Photodiagnosis Photodyn. Ther*., 25, 214–224. DOI: 10.1016/j.pdpdt.2018.12.013
11. **Rasmussen, S. V., Berlow, N. E., Price, L. H., Mansoor, A., Cairo, S., Rugonyi, S., Keller, C., 2021:** Preclinical therapeutics *ex ovo* quail eggs as a biomimetic automation-ready xenograft platform. *Sci. Rep.,* 11, 23302. DOI: 10.1038/s41598-021-02509-3
12. **Chen, L., Wang, S., Feng, Y., Zhang, J., Du, Y., Zhang, J., et al., 2021:** Utilisation of chick embryo chorioallantoic membrane as a model platform for imaging-navigated biomedical research. *Cells (Basel)*, 10, 2, 463. DOI: 10.3390/cells10020463
13. **Sukparangsi, W., Thongphakdee, A., Intarapat, S., 2022:** Avian embryonic culture: A perspective of *in ovo* to *ex ovo* and *in vitro* studies. *Front. Physiol.,* 13, 903491. DOI: 10.3389/fphys.2022.903491
14. **Rao, X., Huang, X., Zhou, Z., Lin, X., 2013:** An improvement of the 2ˆ (–delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostat. Bioinforma. Biomath*, 3, 3, 71–85.
15. **Roza, L. F., de Oliveira, E. M., Staub, L., Dornelas, T. C. E., Pintro, P. T. M., Hermoso, D. A. M., et al., 2023:** Supplementation of Japanese quail (*Coturnix coturnix japonica*) breeders with *Tagetes erecta* flower extract and vitamin E improves the oxidative status of embryos and chicks. *Poult.*, 2, 4, 449–462. DOI: 10.3390/poultry2040034
16. **Truong, L., King, A. J., 2023:** Lipid oxidation and antioxidant capacity in multigenerational heat stressed Japanese quail (*Coturnix coturnix japonica*). *Poult. Sci*., 102, 11, 103005. DOI: 10.1016/j.psj.2023.103005
17. **Thoral, E., Correia, M., Chamkha, I., Elmér, E., Nord, A., 2025:** Incubation temperature shapes growth and mitochondrial metabolism across embryonic development in Japanese quail. *Proceedings B*, 292, 2054, 20251752. DOI: 10.1098/rspb.2025.1752