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SLAUGHTERHOUSE-BASED ASSESSMENT OF PARAMPHISTOMOSIS PREVALENCE AND MEAT SANITARY QUALITY IN SELECTED DISTRICTS OF WESTERN GEORGIA

Vasili Basiladze *, Demna Khelaia and Levan Makaradze

Faculty of Engineering, Agrarian and Natural Sciences, Samtskhe-Javakheti State University, Akhaltsikhe, Georgia.
* Corresponding Author

ORCID Details
Vasili Basiladze: https://orcid.org/0000-0001-9804-1473
Demna Khelaia: https://orcid.org/0000-0001-7056-7369
Levan Makaradze: https://orcid.org/0000-0002-2361-2398

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ABSTRACT

The objective of this study was to evaluate the prevalence of paramphistomosis in slaughtered cattle in selected districts of western Georgia and its effect on the veterinary-sanitary quality of beef. The study was carried out in two phases (November 2023–March 2024 and October 2025–May 2026), during which 481 and 967 cattle, respectively, were examined post-mortem at slaughterhouses. The rumen, reticulum, and gastrointestinal tract were dissected, paramphistomes were counted, and infestation was graded as low (<100 parasites), moderate (100–300), or high (>300). Meat samples from infested and non-infested (control) animals were subjected to organoleptic, physicochemical (pH, volatile fatty acids, moisture), qualitative biochemical (benzidine, copper sulfate and formol reactions), bacterioscopic, and proximate composition (protein, fat, ash) analyses. Paramphistomes were detected in 95 of 481 (19.8%) cattle in the first phase and in 202 of 967 (20.9%) cattle in the second phase, with municipal prevalence ranging from 18.8% to 22.2% and no significant differences between phases or municipalities. Low and moderate infestations did not noticeably change the organoleptic properties of meat. In contrast, high infestation was associated with dark red color, reduced firmness, incomplete dry-crust formation, and murky broth, together with increased pH (6.24–6.30 vs. 5.79–5.83 in controls) and volatile fatty acids, and decreased protein (18.92% vs. 21.31%), fat, and ash content. Meat from animals with low or moderate infestation can be released without restriction after standard veterinary-sanitary inspection, whereas meat from heavily infested animals requires additional bacteriological and biochemical testing before a decision on its use.

Keywords: Paramphistomosis; Cattle; Meat quality; Slaughterhouse; Prevalence; Western Georgia

1 INTRODUCTION

Livestock production constitutes a fundamental component of Georgia's agricultural sector, with its efficiency largely dependent on animal health, productivity, and the quality of animal-derived products [1]. Cattle production plays a

substantial role in both smallholder farming systems and the national economic framework, as the supply of high-quality meat, milk, and other livestock products is essential for food security and contributes significantly to national economic stability [2].

Despite favorable environmental conditions for livestock development in Georgia, the sector faces considerable constraints due to a wide range of diseases of diverse etiologies [3]. Among these, parasitic infections pose a major challenge, adversely affecting animal health and productivity, as well as the quality and quantity of slaughterhouse products, particularly meat [4]. The transmission, diagnosis, and control of parasitic diseases have therefore become major global concerns [5].

Among parasitic diseases, paramphistomosis, a trematode infection caused by *Paramphistomum* spp., is considered one of the most pathogenic conditions affecting domesticated animals and is responsible for substantial economic losses in the livestock industry [6,7]. The highest prevalence has been reported in tropical and subtropical regions, particularly in Africa, Asia, Australia, Eastern Europe, and Russia [6,8,9]. The epidemiology of *Paramphistomum* infection is influenced by multiple factors arising from the complex interaction between the parasite, the host, and the environment. One of the principal determinants of worm burden in animals is the intensity of infection acquired from pasture [10,11], which is further modulated by climatic conditions necessary for egg hatching, larval development, and the survival of infective stages on pasture [12].

Adult *Paramphistomum* spp. are primarily localized in the rumen and reticulum, whereas immature stages inhabit the duodenum [6,10,13]. Mature paramphistomes predominantly parasitize the forestomachs of ruminants, where heavy infections may impair digestive function and contribute to production losses [14].

The objective of this study was to evaluate the impact of paramphistomosis on the qualitative and quantitative properties of beef from infested animals in western Georgia, where the climate is humid subtropical [15].

Winters are generally mild in the lowland and coastal zones, with mean temperatures typically remaining above 0 °C. Snowfall is infrequent at lower elevations but becomes more common and persistent in mountainous areas due to orographic effects. Summers are warm and humid, with average temperatures ranging from 25 °C to 30 °C in the plains and coastal regions [15].

The combination of high precipitation, persistent humidity, and moderate temperatures creates favorable conditions for the development of moist pastures, which are necessary for egg hatching, larval development, and the survival of infective stages of paramphistomosis on pasture [16,17].

Overall, western Georgia exhibits a humid, precipitation-rich climatic regime with pronounced vertical zonation driven by topography.

To assess the degree of *Paramphistomum* infestation in cattle, we categorized infestations into three levels based on parasite burden observed during post-mortem examination [18]: low, moderate, and high.

Cattle farming is a cornerstone of high-quality meat and food production. However, intensive development of this sector is often constrained by various etiological factors—particularly invasive diseases—that can significantly reduce productivity, including weight gain and recovery rates. These infections also compromise meat quality, frequently leading to spoilage and the disposal or destruction of affected organs.

Paramphistomosis is an acute and chronic parasitic disease affecting cattle, small ruminants, and wild ungulates. Trematodes of the family Paramphistomatidae cause it [19]. The acute phase, characteristic of the larval stage, primarily affects young animals when larvae inhabit the small intestine and caecum, often resulting in high mortality. As the larvae mature, they migrate to the rumen and reticulum, where the disease transitions to its chronic form. In the rumen, adult paramphistomes feed on infusoria and bacteria, which are essential for breaking down ingested food. This consumption markedly reduces microbial populations, disrupting rumen digestion [20,21] and impairing protein synthesis by rumen microorganisms, thereby depriving affected animals of a critical source of high-quality protein [22].

Despite extensive research on the epizootiology, prevention, and treatment of paramphistomosis, there is limited information on its impact on animal-derived products—-—particularly meat quality. No such studies have been conducted in the Republic of Georgia. Given this gap, the present study provides relevant insights into the disease's spread and its implications for ensuring the safety and quality of animal products for consumers. Because paramphistomosis causes pathological changes in the rumen and small intestine that disrupt digestive processes, its potential adverse effects on the veterinary-sanitary quality of meat and other slaughter products cannot be overlooked. Similar findings in other parasitic diseases such as fascioliasis, dicrocoeliasis, and sarcosporidiosis support this concern [23–25]. The economic impact of paramphistomosis includes animal mortality or forced culling, reduced productivity,

developmental delays in young animals, increased per-unit production costs, and expenditures for medical and preventive interventions [4,5].

By assessing prevalence, infestation intensity, and associated biochemical and organoleptic changes in beef from western Georgia, this study establishes a crucial evidence base linking parasitic burden to veterinary-sanitary outcomes. These findings are expected to guide farmers, meat producers, veterinarians, policymakers, and especially slaughterhouse meat inspectors during post-mortem meat safety and quality inspections. Accordingly, this study evaluates how different levels of paramphistome infestation affect the qualitative and quantitative properties of beef and situates these results within the broader regional epidemiological context.

2 MATERIALS AND METHODS

2.1 Study area and study design

The study was conducted in two experimental phases. The first phase was carried out between November and December 2023 and February and March 2024, during which post-mortem examinations were performed on 481 slaughtered cattle at slaughterhouses in Abasha, Zugdidi, and Poti (western Georgia). Comprehensive pathological examinations of internal organs, including the rumen, reticulum, and gastrointestinal tract, were conducted to identify the presence of *Paramphistomum* spp. The second phase was carried out between October and December 2025 and February and May 2026, when 967 slaughtered cattle from slaughterhouses in western Georgia (Abasha, Khobi and Samtredia) underwent the same post-mortem examinations. Meat samples from infested animals and from clinically healthy, non-infested cattle (control group: 10 animals in the first phase and 20 in the second phase) were collected for laboratory analysis.

2.2 Post-mortem examination and grading of infestation

To determine the impact of parasite infestation on meat quality, we categorized infestation levels. We conducted post-mortem diagnostics and helminthological examinations of animals and individual organs during both experimental phases. The rumen and reticulum were specifically examined. We performed detailed helminthological dissection of individual organs to accurately determine helminth localization and assess infection intensity [26].

Infestation intensity was graded as follows:

- Low infestation— – fewer than 100 parasites;
- Moderate infestation— – 100 to 300 parasites;
- High infestation— – more than 300 parasites.

2.3 Laboratory analyses

To assess the impact of infestation on meat quality, we conducted organoleptic evaluation, physicochemical tests, qualitative biochemical reactions, bacterioscopy, and proximate composition analysis (Table 1).

Table 1: Laboratory tests used to assess meat quality

Category	Tests
Physicochemical analysis	pH, Volatile fatty acids, Moisture content
Qualitative biochemical reactions	Benzidine reaction, Copper sulfate reaction, Formol reaction
Proximate composition analysis	Protein, Fat, Ash, Moisture
Organoleptic evaluation	Color, odor, consistency, boiling (broth) test, bone marrow condition, dry-crust formation
Bacterioscopy	Microscopic examination of smears from muscle tissue

All analyses were conducted 24 hours post-sampling, during the meat maturation period. Meat sampling and subsequent analyses were carried out in compliance with the following standards: [27–29]

- GOST 7269-2015: Meat. Methods for sampling and organoleptic determination of freshness.
- GOST 23392-2016: Meat. Methods for chemical and microscopic assessment of freshness.
- GOST 25011-2017: Meat and meat products. Methods for determining protein content.

The pH of meat was measured potentiometrically with a pH meter in an aqueous extract of minced muscle tissue. Volatile fatty acids (VFA) were determined according to GOST 23392-2016 [28]: 25 g of minced meat was acidified with sulfuric acid, the volatile fatty acids were distilled with steam, and the distillate was titrated with 0.1 N potassium hydroxide (KOH) solution in the presence of phenolphthalein. VFA content was expressed as mg KOH per 25 g of meat; values up to 4.0 mg KOH/25 g correspond to fresh meat, 4.1–9.0 mg KOH/25 g to meat of questionable freshness, and values above 9.0 mg KOH/25 g to stale meat. Bacterioscopy and the benzidine (peroxidase) reaction were performed according to the same standard, while the formol reaction and the copper sulfate reaction in broth were carried out according to the rules for veterinary-sanitary examination of meat [18]. Protein content was determined according to GOST 25011-2017 [29], and moisture, fat and ash contents were determined by standard gravimetric methods (oven drying, solvent extraction and incineration, respectively).

2.4 Data presentation and statistical analysis

Quantitative results are presented as mean ± standard deviation (SD) for each infestation group and for the control group. Prevalence is given with 95% confidence intervals calculated by the Wilson score method. Differences among the infestation groups and the control group were assessed by one-way analysis of variance (ANOVA), and each infestation group was compared with the control group using Welch's t-test with Bonferroni correction. Differences in prevalence between the study phases and among municipalities were tested with the chi-square test. A p-value < 0.05 was considered statistically significant.

3 RESULTS

3.1 Prevalence and intensity of paramphistomosis

In the first phase of the study, we conducted a comprehensive pathological examination of internal organs, including the rumen, reticulum, and gastrointestinal tract, on 481 slaughtered cattle between November and December 2023 and February and March 2024. Of these animals, 95 (19.8%) were infected with paramphistomes. (Figure 1).

The distribution of infection by slaughterhouse location was as follows:

- Abasha Municipality: 213 cattle examined, of which 40 (18.8%) were infected;
- Zugdidi Municipality: 159 cattle examined, with 32 (20.1%) infected;
- Poti City: 109 cattle examined, of which 23 (21.1%) were infected.

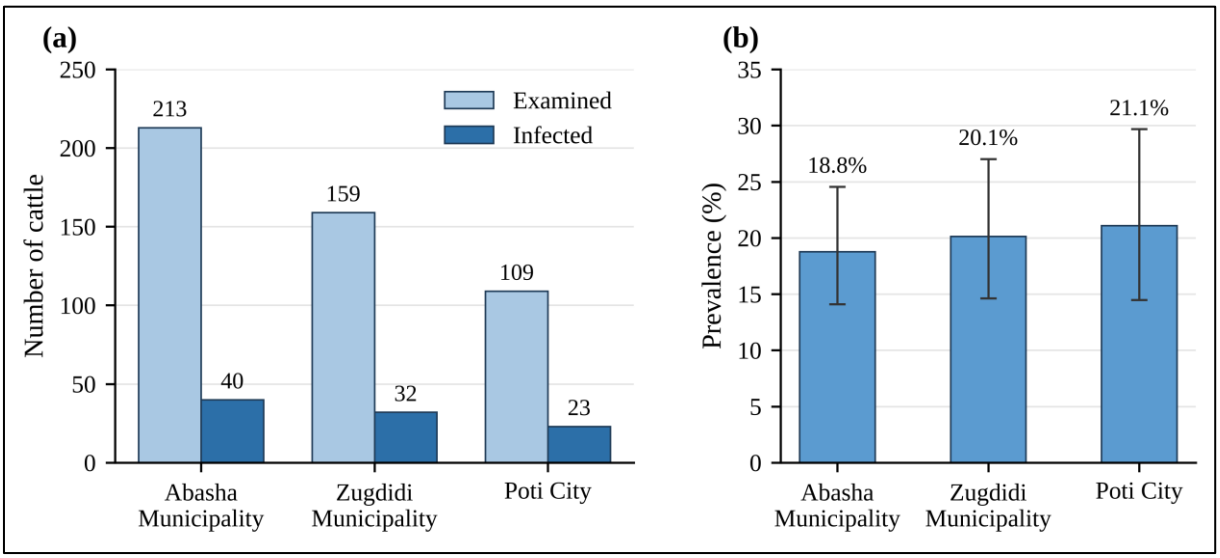


Figure 1: Paramphistomosis in cattle examined at slaughterhouses in the first phase (2023–2024) by location: (a) number of examined and infected animals; (b) prevalence with 95% confidence intervals (Wilson method)

In the first phase of the study, we included 10 meat samples from non-infected cattle as a control group and analyzed them using the same laboratory procedures.

The second phase of the study was conducted between October and December 2025 and February and May 2026, during which we performed similar post-mortem examinations on 967 slaughtered cattle. The comprehensive pathological assessments also included evaluation of the rumen, reticulum, and gastrointestinal tract. In this phase, researchers

detected *Paramphistomum* infection in 202 animals, corresponding to a prevalence of 20.9%. Prevalence did not differ significantly between the two phases (χ^2 = 0.26, p = 0.61); the overall prevalence for both phases was 20.5% (95% CI 18.5–22.7%).

The overall prevalence was similar to that of the first phase, and the distribution of infection by municipality was as follows (Figure 2):

- Khobi: 297 cattle examined, of which 66 (22.2%) were infected;
- Samtredia: 329 cattle examined, with 69 (21.0%) infected;
- Abasha: 341 cattle examined, of which 67 (19.6%) were infected.

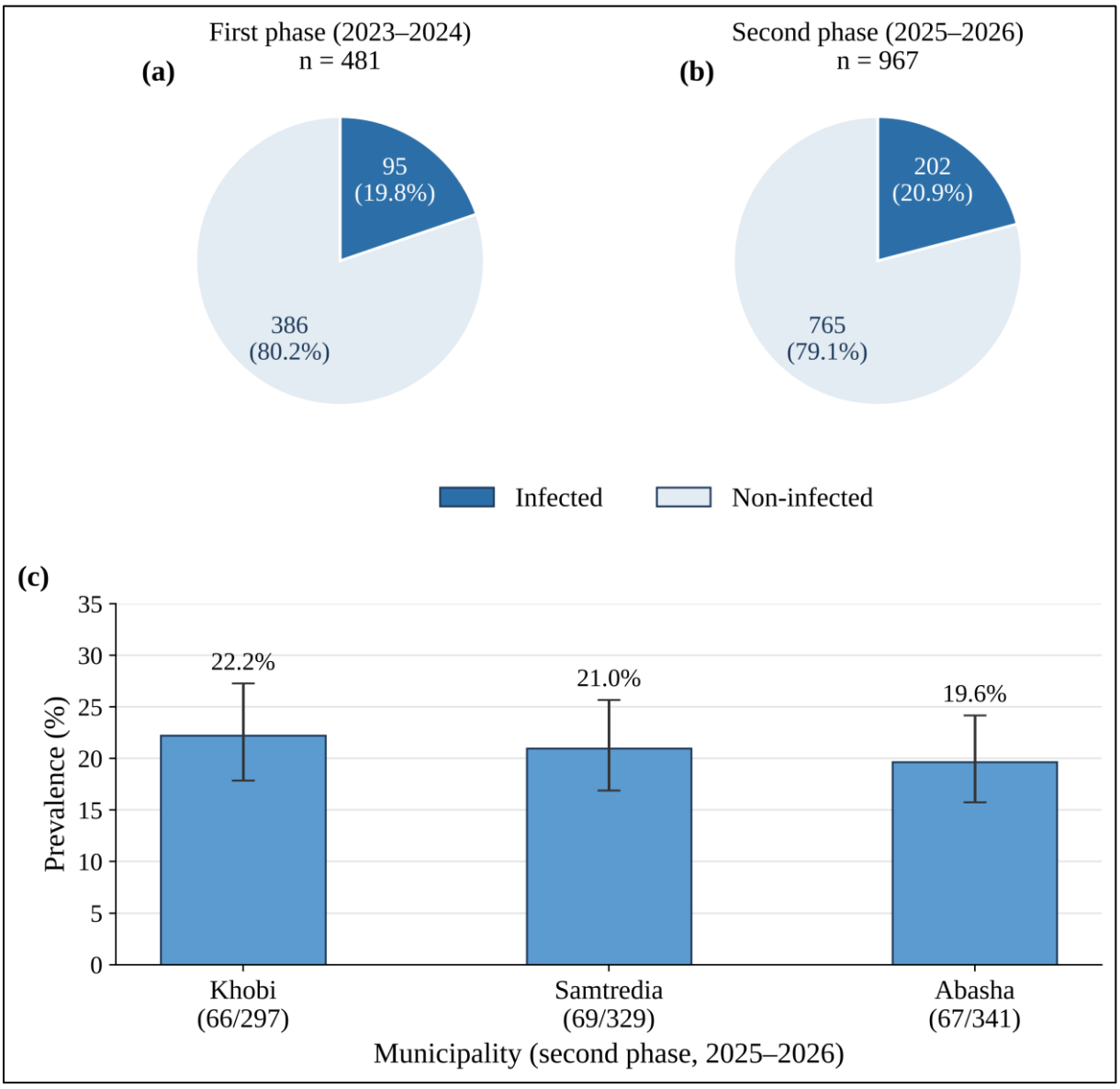


Figure 2: Paramphistomosis in slaughtered cattle: proportion of infected and non-infected animals in (a) the first phase (2023–2024; n = 481) and (b) the second phase (2025–2026; n = 967); (c) prevalence by municipality in the second phase with 95% confidence intervals (Wilson method); numbers in parentheses = infected/examined

Differences in prevalence among municipalities were not statistically significant in either phase (first phase: χ^2 = 0.27, p = 0.88; second phase: χ^2 = 0.64, p = 0.73).

In the second phase of the study, we included 20 meat samples from non-infected cattle as a control group and analyzed them using the same laboratory procedures.

Paramphistome infestation intensity varied from fewer than 100 to more than 400 parasites per animal. In the first phase, six cases from Abasha Municipality exceeded 400 parasites; in the second phase, 21 cases from Abasha Municipality exceeded 300 parasites. Based on these findings, infestation levels were categorized for subsequent studies (organoleptic, biochemical, and bacteriological) as follows:

- Low infestation–– fewer than 100 parasites;
- Moderate infestation–– 100 to 300 parasites;
- High infestation–– more than 300 parasites.

3.2 Organoleptic evaluation

Organoleptic evaluation included assessments of meat color, odor, consistency, boiling-test results, bone marrow condition, and the presence of the so-called "dry crust." No noticeable differences were observed between the control group and cattle with low-to-moderate infestations.

Carcasses from animals with low to moderate infestation exhibited: good muscular cohesion; satisfactory exsanguination; presence of a dry crust; firm meat consistency; light red meat color; visible subcutaneous fat deposits, especially from the ribs to the gluteal region.

In contrast, highly infested carcasses showed: only satisfactory muscular cohesion; drops of blood visible on cut meat surfaces; incomplete coverage by the dry crust; dark red meat coloration; reduced firmness (with slow rebound from finger pressure); and occasional small deposits of subcutaneous fat. (Figure 3).

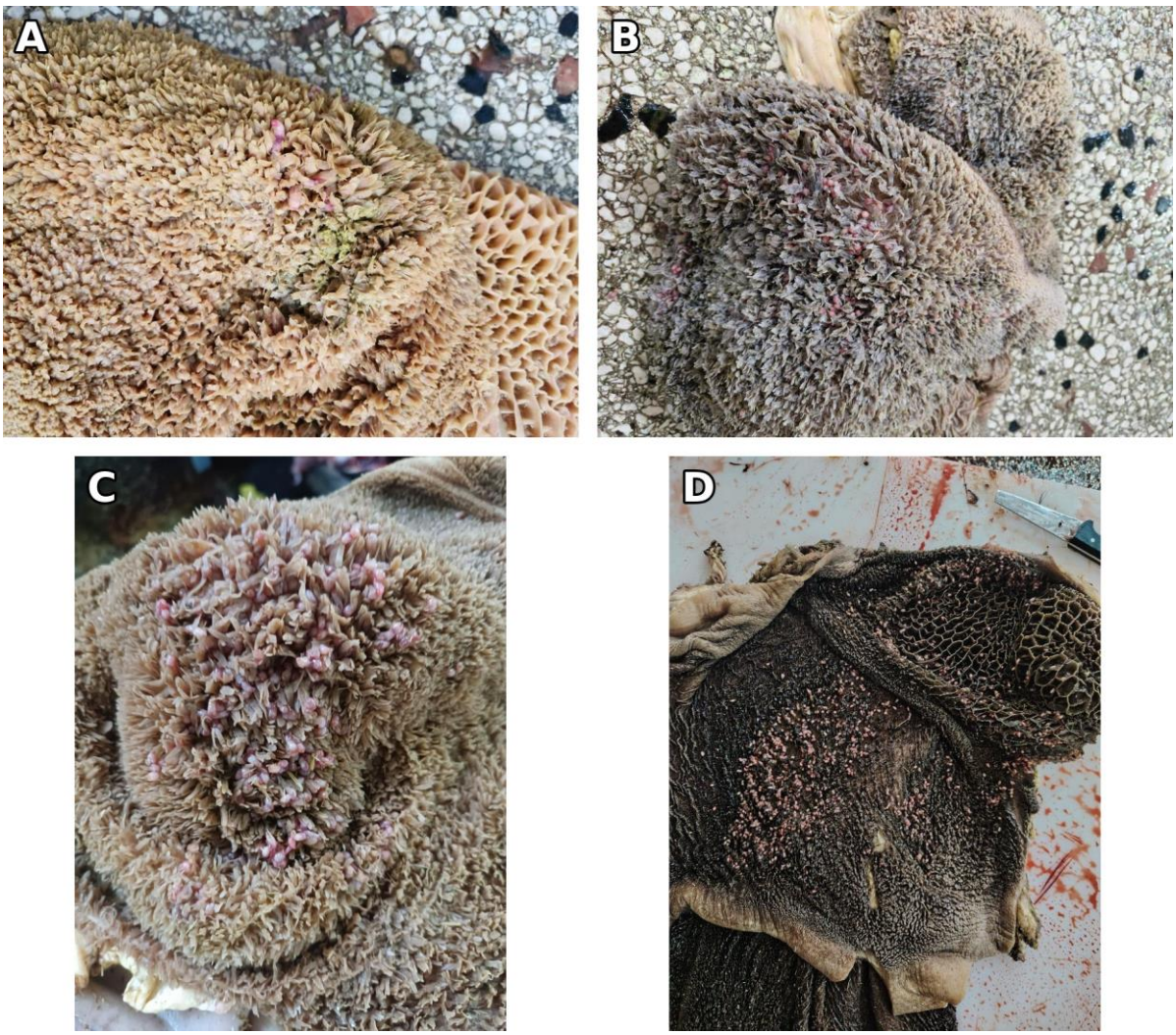


Figure 3: Rumen and reticulum of slaughtered cattle with low, moderate, and high paramphistomosis infestation

A– Low paramphistomosis infestation; B –– Moderate paramphistomosis infestation; C–, D –– High paramphistomosis infestation.

3.3 Biochemical and microbiological indicators of meat

Biochemical analysis of the meat was conducted in two directions:

- Assessment of meat freshness;
- Comparison with biochemical profiles typical of meat from sick or euthanized animals.

To assess freshness, we conducted the following tests: quantitative determination of pH and volatile fatty acids; bacterioscopy; qualitative reaction with copper (II) sulfate in broth; peroxidase enzyme test (benzidine test); and formol reaction (to evaluate meat from sick animals). The detailed test results are presented in Tables 2 and 3. As infestation intensity increased, the meat's protein content decreased. In the second phase, the protein content of meat from the control group was 21.31 ± 1.43%, whereas in moderate and high infestation it declined to 19.84 ± 1.21% and 18.92 ± 1.16%, respectively (Table 3).

In the first phase, meat samples for laboratory analysis were obtained from the following groups:

- 47 samples from cattle with low infestations
- 36 samples from cattle with moderate infestations
- 12 samples from cattle with high infestations

In addition, 10 uninfected cattle samples served as the control group.

Meat samples from animals with varying infestation intensities (low, moderate, and high) were subsequently subjected to biochemical and organoleptic analyses. In addition, meat samples from a control group of 10 clinically healthy, non-infested cattle were analyzed under the same laboratory parameters to provide a baseline for comparison. The results are presented in Table 2.

Table 2: Biochemical and microbiological indicators of meat samples from cattle with varying levels of paramphistome infestation in the first phase (2023–2024)

Indicators	Experimental Group I Low Infestation	Experimental Group II Moderate Infestation	Experimental Group III High Infestation	Control Group
pH Volatile Fatty Acids (mg KOH/25 g)	5.95 ± 0.15* 3.45 ± 0.3*	6.23 ± 0.13* 4.2 ± 0.15*	6.30 ± 0.07* 5.7 ± 0.3*	5.83 ± 0.07 2.86 ± 0.25
Bacterioscopy	Up to 10 Microbes	10-–20 Microbes	10-–30 Microbes	Up to 10 Microbes
Reaction With $CuSO_4$ in Broth	Positive	Positive	Positive	Positive
Benzidine Test	Positive	Suspicious	Suspicious	Positive
Formol Reaction	Negative	Negative	Negative	Negative

Values are mean ± SD. * Significantly different from the control group (Welch's t-test with Bonferroni correction, $p < 0.05$). All quantitative indicators differed significantly among the four groups (one-way ANOVA, $p < 0.001$).

In the first phase, increased parasite burden was associated with changes in meat quality compared with the control group: higher pH values, increased volatile fatty acid concentrations, higher microbial counts on bacterioscopy, and suspicious benzidine reactions in the moderate and high infestation groups, whereas the formol reaction remained negative in all groups.

In the second phase, meat samples were obtained from the following groups:

- 108 samples from cattle with low infestations
- 73 samples from cattle with moderate infestations
- 21 samples from cattle with high infestations

In addition, 20 uninfected cattle samples served as the control group.

While the first phase evaluated meat samples primarily for volatile fatty acids, pH, benzidine reaction, copper sulfate reaction in broth, and formol reaction, the second phase used a broader set of biochemical indicators to provide a more comprehensive evaluation of meat quality. The second phase also analyzed moisture content, protein concentration, fat content, and ash content. The results are presented in Table 3.

Table 3: Biochemical and microbiological indicators of meat samples from cattle with varying levels of paramphistome infestation in the second phase (2025–2026)

Indicators	Experimental Group I Low Infestation	Experimental Group II Moderate Infestation	Experimental Group III High Infestation	Control Group
pH Volatile Fatty Acids (mg KOH/25 g)	5.92 ± 0.12* 3.18 ± 0.18	6.09 ± 0.16* 4.14 ± 0.21*	6.24 ± 0.11* 5.22 ± 0.28*	5.79 ± 0.08 3.11 ± 0.12
Bacterioscopy	Up to 10 Microbes	10-–20 Microbes	10-–30 Microbes	Up to 10 Microbes
Reaction With $CuSO_4$ in Broth	Positive	Positive	Suspicious	Positive
Benzidine Test	Positive	Positive	Suspicious	Positive
Formol Reaction	Negative	Negative	Suspicious/Positive	Negative
Moisture ϵContent	72.4 ± 2.3%	74.3 ± 3.1%*	78.9 ± 4.3%*	71.9 ± 2.8%
Protein Concentration	20.91 ± 1.22%	19.84 ± 1.21%*	18.92 ± 1.16%*	21.31 ± 1.43%
Fat Content	3.24 ± 0.21%*	3.07 ± 0.12%*	2.16 ± 0.17%*	3.66 ± 0.15%
Ash Content	1.21 ± 0.08%	1.02 ± 0.05%*	0.85 ± 0.09%*	1.24 ± 0.07%

Values are mean ± SD. * Significantly different from the control group (Welch's t-test with Bonferroni correction, $p < 0.05$). All quantitative indicators differed significantly among the four groups (one-way ANOVA, $p < 0.001$).

Cooking trials revealed that in the first phase, broth from moderately and highly infested carcasses was murky in 9 of 36 (25.0%) and 7 of 12 (58.3%) cases, respectively, with small fat particles present on the surface.

4 DISCUSSION

The findings of this study reveal a consistently high prevalence of paramphistomosis in slaughtered cattle in western Georgia (19.8% and 20.9% in the two phases; 18.8–22.2% across municipalities), with no significant differences between phases or among municipalities, which indicates that the infection is evenly distributed across the study area. The overall prevalence (20.5%) was more than twice that reported in cattle slaughtered in Van Province, eastern Turkey (8.95%) [8], but lower than that recorded in cattle slaughtered in eastern Ethiopia (40.6%) [30]; such differences probably reflect regional differences in climate, pasture moisture and grazing management. Although environmental and animal-movement data were not collected in this study, the spread of the disease is likely influenced by ecological factors, such as marshy pastures and the presence of natural water sources, and possibly by the movement and introduction of cattle from other regions. This pattern aligns with previous research on the ecological distribution of paramphistomes in wet and marshy environments [19,20]. The veterinary-sanitary evaluation indicated that low to moderate paramphistomosis infestations did not noticeably affect the organoleptic properties of meat. No notable changes in color, consistency, or bleeding were observed at slaughter. However, in cases of high infestation, both biochemical and sensory characteristics were markedly altered: pH levels rose, volatile fatty acid concentrations increased, protein content decreased, and bacterioscopic abnormalities were observed. In the second phase, protein, ash and moisture content differed significantly from the control group only in the moderate and high infestation groups, while small but significant changes in pH and fat content were already detectable at low infestation. These findings are consistent with earlier studies on parasitic infections, which have shown that heavy parasite loads can markedly reduce meat quality [23,25].

The formol reaction, which was suspicious or positive only in the high-infestation group of the second phase, indicated the accumulation of protein degradation products, while the suspicious benzidine (peroxidase) reactions indicated reduced freshness of meat from heavily infested animals. These biochemical indicators suggest that meat from highly

infested animals is unsuitable for unrestricted consumption. In contrast, meat from animals with low to moderate infestation may be considered safe, provided it undergoes rigorous veterinary-sanitary inspection and control.

5 CONCLUSION

Paramphistomosis was widespread in slaughtered cattle in western Georgia, with a prevalence of 19.8% and 20.9% in the two study phases and no significant differences among municipalities. Carcasses from animals with low to moderate infestations showed no noticeable differences in organoleptic properties compared to those of the control (healthy) group. However, in cases of high infestation, several alterations were observed: blood drops on the cut surface of the meat, incomplete formation of the dry crust, dark red muscle coloration, and reduced muscle firmness, as evidenced by the slow rebound of depressions formed by finger pressure.

Increasing intensity of paramphistomosis infestation is associated with alterations in the biochemical properties of meat. In highly infested cases, parameters such as pH, volatile fatty acid content, and bacterioscopic findings reflect traits associated with questionable freshness.

In high infestations, even during the animal's lifetime, there is a considerable accumulation of intermediate and final products of protein breakdown (polypeptides, peptides, amino acids, etc.) in muscle tissue. The formol reaction detects these products by precipitating them with formaldehyde, using a 1:1 water extract of meat.

Meat from animals with low or moderate paramphistomosis typically shows no marked morphological or biochemical abnormalities and may be released for market without restriction, provided all sanitary standards are met. In contrast, meat from highly infested animals should not be allowed into unrestricted trade. Final decisions on its use must be based on thorough bacteriological and biochemical assessments.

Taken together, these findings underscore the importance of considering infestation intensity when evaluating the sanitary quality of meat from animals affected by paramphistomosis. A differentiated, case-by-case approach is essential to ensure both food safety and public health.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The present research work does not contain any studies performed on live animals by any of the authors. All samples were obtained post-mortem from cattle slaughtered for commercial purposes under routine veterinary inspection. Therefore, approval from an institutional animal ethics committee was not required for this study.

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