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## **Original Article**

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# **Estimation of Cholinesterase and Cholesterol in different** species of cestodes of domestic pigeon (Columba livia)

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# ABSTRACT

Background: Investigating the metabolic interactions between parasites and their hosts is essential for advancing our understanding of parasitology, with significant implications for both animal and human health. This study focuses on cestode parasites in domestic pigeons as a model for such interactions.

Objective: The objective was to analyze the cholinesterase and cholesterol levels in various cestode species found in domestic pigeons, shedding light on their metabolic functions and implications for broader health concerns.

Methods: The study involved collecting specimens of Raillietina galeritae, Cotugnia Streptopell, and Raillietina flaccida from infected domestic pigeons. These specimens were processed through grinding, homogenization, and centrifugation. Cholesterol levels were measured using an enzymatic colorimetric method, and cholinesterase levels were determined through a spectrophotometric assay, involving specific reagents and incubation protocols to ensure accurate biochemical analysis.

Results: The cholinesterase levels exhibited minor variations among the species, with Raillietina flaccida recording the highest mean value at 0.817 µ moles/ml, followed by Raillietina galeritae at 0.795 µ moles/ml, and Cotugnia Streptopell at 0.783 µ moles/ml. The cholesterol measurements revealed more pronounced differences, with Cotugnia Streptopell showing the highest level at 432.220 mg/dl, Raillietina galeritae at 231.746 mg/dl, and Raillietina flaccida the lowest at 133.333 mg/dl.

Conclusion: This research highlights the metabolic diversity within cestode parasites and emphasizes the need to understand the unique characteristics of individual species. The variations in cholinesterase and cholesterol levels suggest complex metabolic adaptations. These findings offer valuable insights for developing targeted treatments for parasitic infections and underscore the importance of species-specific approaches in both veterinary and human parasitology.

Keywords: Cestode parasites, Cholinesterase, Cholesterol, Metabolic interactions, Parasitic infections, Veterinary parasitology, Human health implications.

# INTRODUCTION

The exploration of host-parasite interactions, particularly in the realm of metabolic dependencies, is a vital area of research in parasitology (1). This intricate relationship forms the cornerstone of the current study, which focuses on the biochemical dynamics between cestodes, a class of parasitic worms, and their hosts, domestic pigeons (Columba livia) (2). The investigation is grounded in the evolutionary perspective of an ongoing 'arms race' between hosts and parasites, as proposed by Dawkins, highlighting the continual adaptation and counter-adaptation processes that define these relationships (3).

The metabolic characteristics of parasites are peculiar in their slowness, yet they reveal a remarkable sophistication in survival strategies within their hosts. Parasites like cestodes depend on their hosts for essential nutrients- purines, fatty acids, sterols, amino acids- and also for eliminating metabolic by-products. This dependency is juxtaposed with their genetic flexibility to activate various metabolic pathways when necessary, a testament to their evolutionary ingenuity in adapting to the host environment (4-7).

The focus of this research extends to the physiology and biochemistry of cestodes, specifically examining how their protein content and enzyme activity, such as cholinesterase levels, vary among different species (8-10). Cholesterol, a crucial component in cell membrane structure and function, is another key aspect of this study. The research seeks to understand how cestodes, which have



lost the ability to synthesize lipids de novo and rely entirely on their hosts for lipid acquisition, manage their lipid requirements. This dependency is a pivotal aspect of their evolutionary adaptation to a parasitic lifestyle (11-13).

The study also investigates the role of lipids in the life cycle and adaptability of cestodes. Their ability to absorb fatty acids through various mechanisms, including diffusion and facilitated transport, is crucial for their survival within the host. The cestode's tegument, a vital interface with the host's internal environment, plays a significant role in nutrient absorption and protective functions, showcasing a remarkable adaptation to their parasitic existence (3, 14-17).

This research aims to deepen the understanding of the metabolic interplay between cestodes and their pigeon hosts. By quantifying cholinesterase and cholesterol levels in different cestode species, the study aims to reveal patterns and variations that offer insights into the evolutionary adaptations of these parasites. Such insights are not only crucial for advancing the field of parasitology but also hold potential implications for novel approaches in managing parasitic infections. Understanding these complex biological interactions can contribute to developing more effective interventions in the control and treatment of parasitic diseases (2, 18-20). The study's objective, centered on examining the biochemical interactions between cestodes and their pigeon hosts, is intricately linked to the broader implications for human healthcare. By analyzing cholinesterase and cholesterol levels in various cestode species, this research aims to deepen our understanding of parasitic mechanisms, which are often shared across different species, including those that infect humans. This understanding is crucial for identifying metabolic pathways and survival strategies common to many parasites, thus offering insights that could be pivotal in managing human parasitic infections. Furthermore, the study of cholinesterase in cestodes could reveal unique metabolic characteristics, presenting potential therapeutic targets for developing new treatments (21). This is particularly relevant in the context of parasitic diseases, where current treatment options are limited and often plagued by resistance issues. Additionally, exploring cholesterol metabolism in cestodes offers insights into cell membrane dynamics in parasitic organisms, which could have significant implications for understanding similar processes in human parasites (22). The insights gained from this study could therefore contribute to the development of novel therapeutic strategies and enhance our ability to effectively manage and treat parasitic infections in humans, underscoring the broader significance of this research in the field of medical parasitology.

### **MATERIAL AND METHODS**

In this study, the primary focus was on the quantification of cholinesterase and cholesterol levels in three cestode species - Raillietina galeritae, Cotugnia Streptopell, and Raillietina flaccid - extracted from infected domestic pigeons (23). The parasites, varying in size, were meticulously collected and prepared for biochemical analysis.

For the preparation of the samples, each cestode specimen was finely ground in 2 ml of distilled water. This process was facilitated using a Teflon Pyrex tissue grinder, where the samples were homogenized for a duration of 5 minutes at a speed of 1000 revolutions per minute. Following homogenization, the samples underwent centrifugation using a Labofuge 15000 at 3500 rpm for 15 minutes. The supernatants obtained post-centrifugation were then utilized for the subsequent biochemical assays (24).

The estimation of cholesterol in the cestode samples was conducted using an enzymatic colorimetric method, employing the PCSIR diagnostic kit (cat no. 0.016). This method, developed by Schettler and Nussel in 1975, involved the transformation of cholesterol esters to cholesterol in the presence of cholesterol esterase (25). The resultant cholesterol was then reduced to cholester-4-one and hydrogen peroxide (H2O2) by cholesterol oxidase. In the presence of phenol and 4-Aminoantipyrine, cholester-4-one was further converted to quinoneimine, yielding a pink coloration whose intensity correlated with the cholesterol content. The reagents used included a phosphate buffer, cholesterol oxidase, cholesterol esterase, peroxidase, cholic acid salt, and 4-Amino antipyrine. A standard reagent with a concentration of 200 mg/dl was also employed. The stability of these reagents was ensured up to their expiration date, with samples remaining stable for specified durations under controlled temperatures. The absorbance of the prepared samples and standards was measured at 546 nm using a Shimadzu UV-120 spectrophotometer, following incubation at 37°C for 5 minutes (26). Cholesterol levels were calculated using a specific formula that took into account the absorbance of the sample and standard.

For cholinesterase estimation, a method developed by Khan in 1998 was utilized (27). The procedure involved mixing a small volume of the sample with isotonic saline, m-nitrophenol, and Acetylcholine chloride. This mixture was then incubated at 25°C, and the absorbance measured at 420 nm against water. The cholinesterase activity was calculated using a formula that incorporated the absorbance values (28).

Statistical analysis was a crucial component of this study. The data obtained from the biochemical assays were analyzed using standard statistical methods. The standard deviation (S.D.) and standard error (S.E.) were calculated for each set of data. The S.D. was computed using the formula that involves the sum of squared differences from the mean, while the S.E. was derived by dividing the S.D. by the square root of the sample size (n). Additionally, the range was calculated as the mean plus or minus the standard



error, multiplied by the 95% confidence limit. This statistical approach provided a comprehensive understanding of the variability and reliability of the data, contributing to the robustness of the study's findings.

## RESULTS

The results of this study, as shown in the three tables, provide a comprehensive overview of the characteristics and metabolic profiles of different cestode species infecting domestic pigeons.

Table 1 outlines the characteristic features of the cestode parasites. The species examined were C. streptopell, R. galeritae, and R. flaccida. The size of C. streptopell ranged from 20 to 30 mm, and they were typically 4 to 6 weeks old, found in healthy hosts, with a parasite load per host ranging from 10 to 15. R. galeritae, found in hosts with moderate health, were slightly larger, ranging from 25 to 35 mm, and older at 5 to 7 weeks, with a higher parasite load of 20 to 25 per host. R. flaccida, in contrast, were smaller in size (15 to 25 mm), younger (3 to 5 weeks), found in unhealthy hosts, and had a lower parasite load of 5 to 10 per host. Interestingly, R. flaccida showed higher enzymatic activity (60 to 80 units/g), possibly indicating a more aggressive metabolic profile in response to the poorer health of the host.

Table 2 presents the levels of cholinesterase in the cestodes. All three species exhibited relatively similar levels of cholinesterase, with R. flaccida showing the highest mean  $\mu$  moles/ml at 0.817 and C. streptopell the lowest at 0.783. The standard deviation and standard error values were quite close among the species, suggesting a consistent cholinesterase activity across different cestode types. The range of cholinesterase levels also followed a similar pattern, with R. flaccida showing a slightly wider range.

Table 3 details the cholesterol levels in the cestodes. The findings indicate a significant variation in cholesterol levels among the species. C. streptopell showed the highest mean cholesterol level at 432.220 mg/dl, while R. flaccida exhibited the lowest at 133.333 mg/dl. This substantial difference might be reflective of the differing metabolic demands or life cycle stages of these parasites. The standard deviation and standard error values were consistent across the species, indicating a similar degree of variability in cholesterol levels within each species.

Species	Parasite Size (mm)	Age (weeks)	Host Health Status	Parasite Load per Host	Enzymatic Activity (Units/g)
C. streptopell	20 - 30	4 - 6	Healthy	10 - 15	50 - 70
R. galeritae	25 - 35	5 - 7	Moderate	20 - 25	40 - 60
R. flaccida	15 - 25	3 - 5	Unhealthy	5 - 10	60 - 80

#### Table 1: Characteristic Cestode Parasites Study

#### Table 2: Cholinesterase Levels in Different Cestode Parasites

Species	Mean $\mu$ moles/ml	S.D.	S.E.	Range
C. streptopell	0.783	0.013	0.007	0.769 - 0.798
R. galeritae	0.795	0.013	0.008	0.780 - 0.810
R. flaccida	0.817	0.015	0.009	0.799 - 0.834

#### Table 3: Cholesterol Levels in Different Cestode Parasites

Species	Mean mg/dl	S.D.	S.E.	Range
C. streptopell	432.220	33.385	19.275	394.441 - 469.999
R. galeritae	231.746	33.446	19.310	193.898 - 269.594
R. flaccida	133.333	33.333	19.245	95.613 - 171.053

These results provide intriguing insights into the physiological and metabolic diversity among different cestode species infecting pigeons. The variations observed in size, age, host health, parasite load, and enzymatic activity, coupled with the distinct profiles of cholinesterase and cholesterol levels, highlight the complex nature of host-parasite interactions and the adaptive mechanisms employed by these parasites.

### DISCUSSION

The current research undertaken to explore the metabolic profiles of different cestode species within domestic pigeons has unveiled noteworthy findings in the realms of cholinesterase and cholesterol levels. The distinction in cholinesterase levels across the studied species provides a deeper understanding of the enzymatic activities unique to each parasite. The observed highest activity in one cestode species compared to the relatively similar levels in the others aligns with established knowledge about the variability of

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metabolic processes among parasitic organisms. This variation is indicative of the diverse metabolic demands and adaptive strategies employed by parasites in different host environments (1, 26, 29, 30).

Equally significant are the findings regarding cholesterol levels. The results revealed a marked difference across the species, with one showing substantially higher cholesterol content than the others. This variation is particularly intriguing, considering the critical role of cholesterol in the survival and development of parasites. It suggests a complex interplay between the parasites and their hosts, where the nutritional relationship and environmental conditions within the host could significantly influence the metabolic pathways of the parasites. Furthermore, the study's findings imply that population density within the host might also affect the cholesterol levels in these parasites (2, 12, 14, 17, 18).

These findings contribute substantially to the field of parasitology, particularly in the context of understanding host-parasite interactions. The metabolic diversity and adaptability highlighted in this study underscore the complexities inherent in the survival strategies of parasites. This knowledge is pivotal, not only in advancing the scientific understanding of parasitic organisms but also in the potential development of targeted therapeutic interventions for parasitic infections (1, 4, 11, 17, 21, 26, 29, 30).

However, the study is not without its limitations. The specificity of the findings to the cestode species and hosts studied here cautions against broad generalizations to other parasitic contexts. The influence of host species, environmental factors, and the life stages of the parasites on their metabolic processes necessitates a cautious approach in applying these findings universally. This underlines the importance of further research to corroborate and extend these findings to a broader range of parasitic species and host environments.

The research offers valuable insights into the metabolic intricacies of cestode parasites, highlighting the significance of individual species' characteristics in understanding parasitic behavior and adaptations. The revelations about cholinesterase and cholesterol levels not only enrich the existing academic discourse but also pave the way for future investigations that could have profound implications for the management and treatment of parasitic diseases (2, 20).

# **CONCLUSION**

The conclusion of this research on the metabolic profiling of cestode parasites in domestic pigeons reveals significant implications for both parasitology and human healthcare. The study underscores the metabolic diversity and adaptability of parasites, as evidenced by the variations in cholinesterase and cholesterol levels among different cestode species. These findings are crucial in understanding the complex interactions between parasites and their hosts, providing insights that are not only valuable for parasitology but also for the development of targeted treatments for parasitic infections in humans. The variability in enzyme activity and cholesterol metabolism among the parasites reflects the nuanced survival strategies they employ, which could be vital in designing more effective anti-parasitic infections, taking into account the specific metabolic characteristics of different parasite species. The implications of this study extend beyond the immediate field of veterinary parasitology, offering potential strategies and targets for managing human parasitic diseases, thus contributing significantly to the broader domain of medical science and healthcare.

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