SHORT COMMUNICATION

Relationship between number of adult rumen fluke (*Calicophoron microbothrioides*) and eggs per gram of feces in culled dairy cattle in Peru: A pilot study

Relación entre número de adultos de trematodos del rumen (Calicophoron microbothrioides) y huevos por gramo de heces en ganado lechero sacrificado en Perú: Estudio piloto

Relação entre o número de vermes adultos no rúmen (Calicophoron microbothrioides) e ovos por grama de fezes em bovinos leiteiros abatidos no Peru: Estudo piloto

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Abstract

Background: Paramphistomosis, a parasitic condition caused by *Calicophoron microbothrioides* in domestic ruminants, has garnered limited attention among cattle breeders in the Cajamarca Valley, Peru. Despite its status as an endemic affliction with considerable risk, scant investigations have been conducted on this parasite within this region. **Objective:** This study aimed to assess the correlation between the population of adult parasites (referred to as "number of adult parasites" or NAP) in rumen and reticulum of naturally infected cows and the number of eggs per gram of feces (EPG). **Methods:** A sample of twenty-two cattle harboring adult parasites in their rumen and reticulum was selected for analysis. Fecal samples were collected from these animals to establish a correlation between NAP and EPG. Data analysis included linear regression, the Shapiro-Wilk normality test, and the nonparametric Mann-Whitney test.

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Results: The study revealed a remarkably strong and directly proportional association between the number of adult parasites in rumen and reticulum and the coproparazitologic EPG (r = 0.971). **Conclusion:** According to the linear model (NAP = 60.381 ± 3.37; EPG - 22.979) increasing EPG by one lead to an increase by 60 NAP in rumen and reticulum (p<0.01).

Keywords: *bovines; Calicophoron microbothrioides; cattle; diagnosis; eggs; epidemiology; feces; paramphistomosis; parasite load; ruminants; rumen fluke; trematodes.*

Resumen

Antecedentes: La paramphistomosis, afección parasitaria causada por *Calicophoron microbothrioides* en rumiantes domésticos, ha despertado poco interés entre los ganaderos del valle de Cajamarca, Perú. A pesar de su condición de afección endémica con riesgo considerable, se han realizado escasas investigaciones sobre este parásito en esta región geográfica. **Objetivo:** El presente estudio tuvo como objetivo evaluar la correlación entre la población de parásitos adultos (denominada "número de parásitos adultos" o NPA) en rumen y retículo de vacas naturalmente infectadas, en relación con la cantidad de huevos por gramo de heces (HPG). **Métodos:** Para el análisis se seleccionó una muestra de veintidós bovinos que albergaban parásitos adultos en rumen y retículo. Se recogieron muestras fecales de estos animales para establecer una correlación entre la población de parásitos adultos y la HPG. Los datos se analizaron mediante regresión lineal, prueba de normalidad de Shapiro-Wilk, y prueba no paramétrica de Mann-Whitney. **Resultados:** El estudio reveló una asociación notablemente fuerte y directamente proporcional entre la población de parásitos adultos, tanto en el rumen como en el retículo, y la HPG determinada mediante coproparasitología (r = 0,971). **Conclusiones:** El modelo lineal (NAP = 60,381 ± 3,37; HPG - 22,979) indica que cada unidad de incremento del HPG se corresponde con un aumento de 60 en el NPA en rumen y reticulo (p<0,01).

Palabras clave: *bovinos; Calicophoron microbothrioides; carga parasitaria; diagnóstico; epidemiología; ganado; huevos; heces; paramphistomosis; rumiantes; trematodos; trematodo del rumen.*

Resumo

Antecedentes: A paramfistomose, uma condição parasitária causada por *Calicophoron microbothrioides* em ruminantes domésticos, tem recebido atenção limitada entre os criadores de gado no Vale de Cajamarca, Peru. Apesar de ser uma aflição endêmica com considerável risco, investigações escassas foram conduzidas sobre esse parasita nessa região geográfica. **Objetivo:** Este estudo teve como objetivo avaliar a correlação entre a população de parasitas adultos (referida como "número de parasitas adultos" ou NAP) no rúmen e no retículo de vacas naturalmente infectadas, em relação à quantidade de ovos por grama de fezes (OPG). **Métodos:** Uma amostra de vinte e dois bovinos portando parasitas adultos em seus rúmens e retículos foi selecionada para análise. Amostras fecais foram coletadas desses animais para estabelecer uma correlação entre a população de parasitas adultos e OPG. A análise dos dados envolveu a aplicação de regressão linear, o teste de normalidade de Shapiro-Wilk e o teste não paramétrico de Mann-Whitney. **Resultados:** O estudo revelou uma associação notavelmente forte e diretamente proporcional entre a população de parasitas adultos tanto no rúmen quanto no retículo, e o OPG determinado por meio da coproparasitologia (r = 0,971). **Conclusões:** O modelo linear (NAP = 60,381 ± 3,37; OPG - 22,979) indica que, com o aumento de um OPG, o NAP no rúmen e retículo aumenta em 60 (p<0,01).

Palavras-chave: *bovinos; Calicophoron microbothrioides; carga parasitária; diagnóstico; epidemiologia; feze; gado; ovos; paramfistomose; ruminantes; trematódeos; verme do rúmen.*

Introduction

Paramphistomosis is caused by rumen trematodes belonging to the family Paramphistomidae (Trematoda: Digenea; Eduardo, 1983). These trematodes affect all types of ruminants around the world, including domestic animals (Ferreras *et al.*, 2014; Ma *et al.*, 2015; Cauquil *et al.*, 2016), South American camelids (Millar *et al.*, 2017), as well as wild and feral ruminants (Munang'andu *et al.*, 2012; Pfukenyi *et al.*, 2018). These parasites use a freshwater snail as an intermediate host, and animals become infected upon ingestion of encysted metacercariae present in their feed or water (Fenemore *et al.*, 2021).

Regarding the lesions caused by these trematodes, variable outcomes are observed. For instance, *Calicophoron daubneyi* does not induce significant macroscopic pathologies in the rumen or reticulum, nor does it consistently trigger acute or chronic inflammatory changes in the duodenum (Busin *et al.*, 2023). On the other hand, in cases of acute parasitosis caused by *C. daubneyi*, the migration process during the juvenile phase from the small duodenum to the abomasum and rumen leads to pathological disorders, primarily characterized by enteritis, diarrhea, anemia with elevated morbidity, as well as emaciation and cachexia. Among the most susceptible are young ruminants in cases of extensive infections (O'Shaughnessy *et al.*, 2018). The attachment of adult *C. daubneyi* to the ruminal and reticular epithelium results in the detachment of ruminal papillae, ultimately causing local inflammation, fibrotic alterations, and the emergence of darkened areas (Fuertes *et al.*, 2015).

The parasitological technique involving observation of eggs in fecal sedimentation remains the sole viable approach for diagnosing rumen trematodes in live animals under field conditions. However, it is limited to identifying only patent infections (Sargison *et al.*, 2016). The detection of immature and adult trematodes can be accomplished through *post mortem* examinations of the reticulum and rumen (Toolan *et al.*, 2015; Cauquil *et al.*, 2016). While a coproantigen-based ELISA prototype has been developed (Huson *et al.*, 2021), its utility is not universally accessible across all geographical regions.

Presently, the Cajamarca Valley stands as an endemic region for paramphistomosis, a condition attributed to *Calicophoron microbothrioides* -which has been molecularly identified in cattle (Manrique *et al.*, 2013). In addition to the Cajamarca Valley, this parasite has also been detected in various districts within Cajamarca. It has been found in cattle -with prevalence ranging from 5.8 ± 2.4 to 38.5 ± 5.5% in coproparasitological diagnoses (Torrel *et al.*, 2023)- as well as in sheep -with 4.92% prevalence from 386 *post mortem* animals (Torrel-Pajares *et al.*, 2022). Despite the high presence of this parasite, several knowledge gaps persist due to its relatively recent emergence and limited research attention.

Given these circumstances, the present study aimed to establish a correlation between adult *C. microbothrioides* located in rumen-reticulum and the number of eggs per gram of feces in naturally infected slaughtered cattle. The main objective was to acquire a deeper comprehension of the parasite load and to establish the capacity to forecast the estimated count of adult parasites using coprological analysis within field conditions. This endeavor is intended to provide a foundational framework for subsequent investigations aimed at appraising the effectiveness of antiparasitic medications by considering parameters such as egg counts, parasite burden, and potential clinical manifestations.

Materials and Methods

Ethical considerations

Samples were initially obtained from live animals and, subsequently, from the same animals during post-slaughter -carried out at the Camal Municipal de Cajamarca. This slaughterhouse operates under the regulatory framework of Reglamento Sanitario del Faenado de Animales de Abasto del Perú (D.S. N° 015-2012-AG).

Location and sample size estimation

The study took place in the city of Cajamarca, Peru. Guided by a prior investigation by Torrel (2009), the sample size was established considering a population of 30 animals already confirmed as positive for *Calicophoron microbothrioides*. Consideing an $\alpha = 0.01$ significance level, a sample size of 22 positive cattle from various age groups, breeds, and both genders was defined.

Sampling and sample processing

To establish a correlation between the recorded number of adult parasites (NAP) in the rumen and reticulum and the number of eggs per gram of feces (EPG), samples were procured from 22 cattle confirmed as positive for Paramphistomidae. These cattle were subjected to slaughter at Camal Municipal de Cajamarca. The animals were identified one day prior to slaughter, and fecal samples (approximately 100 g) were directly collected from the rectum using veterinary obstetric gloves.

Collected samples were then placed in polyethylene bags, each labeled with the corresponding animal identification and sequential numbers. These bags were subsequently deposited into an expanded polystyrene container for safe transportation to Laboratorio de Parasitología Veterinaria of Facultad de Ciencias Veterinarias at Universidad Nacional de Cajamarca (Peru). Once there, the samples underwent thorough analysis using the Natural Sedimentation technique (Foreyt *et al.*, 2001) for egg detection and EPG count.

Fecal samples from animals in which adult paramphistomids were not detected in rumen and reticulum during *post mortem* examination were excluded from the study. In addition, fecal samples from animals displaying *Fasciola hepatica* in their bile ducts during *post mortem* liver inspection were also eliminated from consideration.

Through macroscopic observation, the contents of positive rumen and reticulum were gathered in labeled polyethylene bags. Subsequently, these contents were sifted by incorporating clean water through a metal mesh with 0.5 cm^2 opening between threads. This process facilitated separation of free adult parasites, which remained on the sieve.

Concurrently, a thorough examination of rumen and reticular mucosa of the animals was conducted. Parasites adhering to the mucosa were precisely detached using surgical forceps and subsequently placed into a glass container. These collected parasites were transported to the laboratory for in-depth enumeration and assessment.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics 25.0 (IBM Corp. Armonk, NY, USA). Initially, a linear regression analysis was conducted to establish the relationship between egg count and the count of adult parasites. This analysis was accompanied by its corresponding analysis of variance. Furthermore, normality of all variables under investigation was assessed with the Shapiro-Wilk statistical test.

Variables demonstrating normal distribution underwent a completely randomized analysis of variance using the model $Y_{ij} = \mu + \tau_i + \xi_{ij}$, where Y_{ij} represents the observed data, μ denotes the overall mean, τi signifies the treatment effect, and ξij symbolizes the error term associated with each observation.

Regarding correlation between EPG and count of adult *C. microbothrioides*, along with the presentation of data through Whisker Box plots, the nonparametric Mann-Whitney test was used to assess statistical significance.

Results

Figure 1 displays the adult parasites of *C. microbothrioides* affixed to the rumen, as well as a depiction of a characteristic egg from this trematode.

The most frequently occurring (mode) EPG value was 150, while for NAP, it was 9500. Half of the cattle (n = 11) displayed EPG levels below the median of 106 and NAP levels of 5810 in their rumen and reticulum. Conversely, the highest quantity of EPG and NAP were observed in the first quartile, spanning from 4 to 8.75 (with a range of 4.75 for EPG) and 235 to 646.25 (with a range of 411.25 for NAP), respectively. Additionally, the third quartile showcased EPG values ranging from 106 to 156.25 (a range 50.25 for EPG), and NAP ranging from 5810 to 9500 (with a range of 3690 for NAP). These findings indicate reduced variability within the first quartile (Table 1 and Figure 2).

Table 1. Eggs per gram (EPG) of feces and number of adult *Calicophoron microbothrioides* (NAP) in rumen and reticulum.

Parameter		EPG	NAP
Mean		95.77	5,759.86
Median		106.00	5,810.00
Mode		150.00	9,500.00
Standard deviation		77.947	4,848.09
Range		213.00	15,285.00
Minimum		4.00	235.00
Maximum		217.00	15,520.00
Percentiles	25	8.75	646.25
	50	106.00	5,810.00
	75	156.25	9,500.00

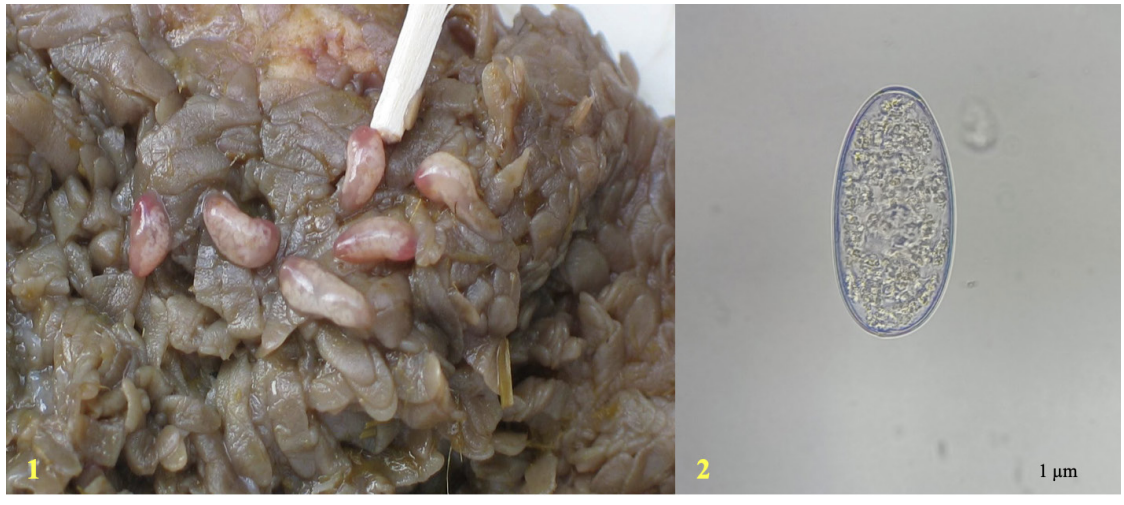

Figure 1. Adult *Calicophoron microbothrioides* within the rumen 1.6X (1) and egg with 40X view (2).

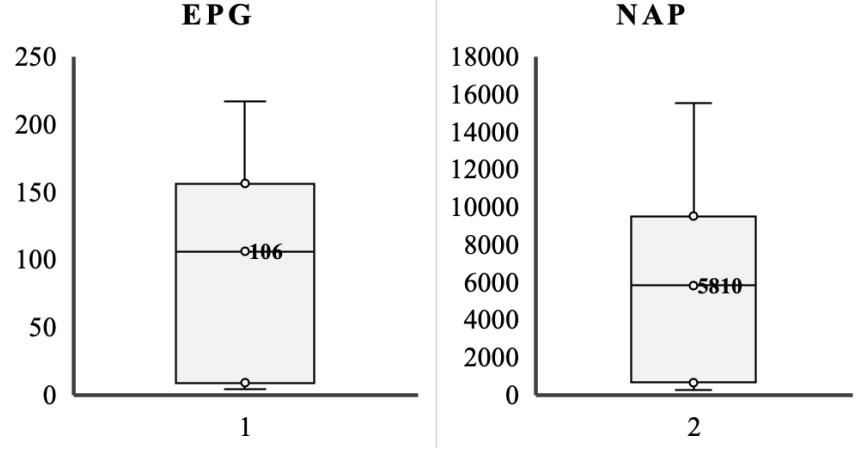

Figure 2. Box-and-whisker plot illustrating estimated eggs per gram (EPG; 1) and number of adult *Calicophoron microbothrioides* (NAP; 2) in rumen and reticulum showing lower dispersion in the first quartile.

Linear model validation for establishing relationship between EPG and NAP

To ascertain the reliability of the model, regression analysis was used. The linear model generated the following equation: NAP = 60.381 EPG - 22.979. Statistical significance was confirmed with $p<0.01$. The correlation coefficient (r) was computed to be 0.971, demonstrating a strong and positive relationship. Furthermore, the coefficient of determination (R^2) was determined to be 0.939 (corrected).

Within the linear model, the significance of the coefficient (-22.979) was evaluated and found to be non-significant ($p>0.05$). The estimated coefficient for EPG was 60.381 with a 95% confidence interval of ±3.337. This result is particularly noteworthy; for each additional gram of eggs per gram of feces, the parasite count increases by approximately 60.381 (equivalent to 60 parasites). This coefficient was highly significant ($p<0.0001$), meaning that it is distinct from zero and thereby confirming the validation of the model. The correlation coefficient (r = 0.971) underscores a robust and positive correlation ($p<0.01$).

Normality of residuals. The evaluation of the normal distribution of residuals was conducted through graphical examination, as depicted in Figure 3. Upon observation, it becomes evident that the scatter plot of points closely aligns with the reference straight line, indicating a strong fit to the linear regression model. Consequently, it is reasonable to affirm that the residuals exhibit a normal distribution.

The equation's analysis indicates absence of autocorrelation among residuals, as evidenced by the Durbin-Watson test result of DW = 1.806. In addition, coefficient of determination (R^2) within the linear equation signifies that 94.2% of the variability in adult parasites can be elucidated by the number of eggs per gram in fecal samples. Given the findings and the establishment of optimal fitting linear equation, the rationale for using linear equation in the current study becomes evident.

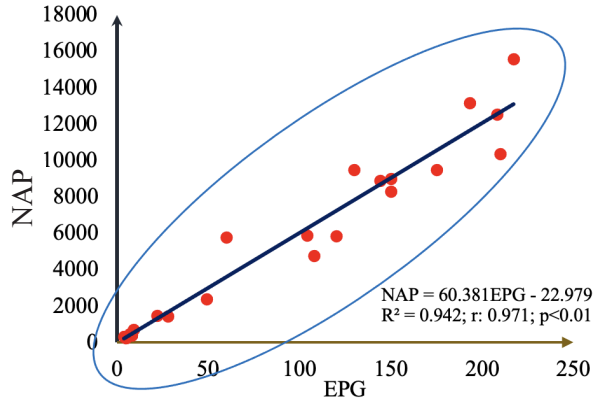

Figure 3. Relationship between eggs per gram (EPG) and number of adult *Calicophoron microbothrioides* (NAP) of cattle infected with *C. microbothrioides*. Where, R^2: Coefficient of determination; r: Correlation; $p<0.01$ (significance at 99%).

Discussion

The relationship found between NAP in rumen-reticulum and EPG determined through coproparasitology exhibited a strong positive relationship (r = 0.971) that is directly proportional. Specifically, the derived model obtained (NAP = 60.381 ± 3.37; EPG -22.979) demonstrates that for each increase in one EPG, NAP in rumen-reticulum increases by 60. This trend is similar to another study involving Limousin bulls infected with *Paramphistomum daubneyi*, where a direct proportional correlation was identified: for every 1 to 12 paramphistomes, the average EPG ranged from 7 to 15. Furthermore, animals harboring an average of 215 (29 to 620) adult parasites displayed an average EPG of 41.5 (Mage and Dorchies, 1998).

The findings from the present study surpass those reported by Fuertes *et al.* (2015), who recorded a total animal parasite burden of 19,487 adult *C. daubneyi*, with variations ranging from 8 to 8005 parasites per animal (median 144). In a separate investigation, a significant correlation was established between EPG count and adult parasite burden, where an EPG count exceeding 100 indicated the presence of 100 adult *C. daubneyi* in the rumen or reticulum; however, their correlation between both variables was not direct (Rieu *et al.*, 2007). In other words, a higher

EPG count wouldn't necessarily be obtained even if the number of parasites in the rumen increased. This phenomenon stems from the fact that parasite overpopulation tends to decrease the rate of egg laying (Horak, 1971). Contrastingly, none of the various coproparasitological methods currently available exhibit 100% sensitivity and specificity (Rieu *et al.*, 2007; Malrait *et al.*, 2015).

To the authors' best knowledge, no studies have been conducted on cattle, sheep, or other ruminants in Peru concerning rumen fluke and their correlation to EPG. The greater quantity of eggs detected in heavily infected cattle could be linked to intrinsic host variability, where progression of parasitic infection can manifest as slow or rapid due to varying immunological resistance of the animal. Such resistance is influenced by factors such as age, reinfection, and nutritional status (Atcheson *et al.*, 2020; Hajipour *et al.*, 2021; Torrel-Pajares *et al.*, 2022). Discrepancies between these findings and those of other researchers might be attributed to specific parasitic load stemming from site-specific conditions, such as pasture attributes (including the abundance of infected intermediate hosts), seasonal and climatic variations, and characteristics of surface water and soil. Additionally, the age of the definitive host in relation to acquired immunity might play a role. Further research is warranted to ascertain the risk factors associated with *C. microbothrioides* in the Cajamarca region.

Considering the objective and outcomes, the correlation between NAP and eggs per gram of feces was highly significant ($r = 0.971$; $p<0.01$). For each one EPG increase in feces, the number of adult parasites in both the rumen and reticulum increases by 60 ($p<0.01$).

Declarations

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Conflict of interest

The authors declare they have no financial interests or personal relationships that could have influenced the results presented in this article.

Author contributions

TTP and JFC conceptualized, designed the methodology, supervised, and managed the study. JFC executed and carried out field and laboratory work. LV-R and JFC contributed to software, validation, data curation and writing-preparation of the original draft. All authors collaborated in the visualization, writing-revising and editing of the manuscript. All authors approved the final manuscript and accept responsibility for its content.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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