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### **Subclinical gastrointestinal nematode infections in sheep from San Pedro: A One Health approach for parasite control and conceptual management analysis**

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#### **ABSTRACT**

Gastrointestinal nematode (GIN) infections continue to be one of the main health constraints in small ruminant production systems, potentially affecting productivity, compromising reproductive efficiency, and increasing production costs. Recently, a new challenge has emerged: the potential zoonotic risk. The objective of this study was to provide field evidence on the presence, prevalence, and intensity of GIN infections in clinically asymptomatic sheep and to integrate these findings into a critical review of their productive and sanitary relevance, serving as a basis for the design of control strategies. A descriptive cross-sectional study was conducted on a farm in the department of San Pedro, Paraguay. Fecal samples were collected directly from the rectum of 59 sheep and analyzed using qualitative flotation techniques and the McMaster method to determine eggs per gram (EPG) of feces. GIN eggs were detected in 78% (46/59) of the animals, with identification achieved only at the family level. The mean infection intensity was 268.87 EPG, indicating mild infections at the flock level. The high prevalence combined with low intensity demonstrates the presence of subclinical parasitism in apparently healthy sheep. These findings highlight the importance of routine monitoring to identify asymptomatic carriers, which contribute to pasture contamination and represent a health risk. In line with current literature, the relevance of subclinical GIN infections for animal health, productivity, and the development of sustainable parasite control strategies in small ruminant systems with potential zoonotic risk is highlighted.

**Keywords:** GIN, prevalence, parasitology.

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## Infecciones subclínicas por nematodos gastrointestinales en ovinos de San Pedro: un enfoque de Una Salud para el control de parásitos y análisis conceptual de manejo

### RESUMEN

Las infecciones por nematodos gastrointestinales (NGI) continúan siendo una de las principales limitantes sanitarias en los sistemas de producción de pequeños rumiantes pudiendo afectar la productividad, comprometer la eficiencia reproductiva y aumentar los costos de producción. Recientemente, se ha agregado un nuevo desafío: el potencial riesgo zoonótico. El objetivo de este estudio fue presentar evidencia de campo sobre la presencia, prevalencia e intensidad de infecciones por NGI en ovinos clínicamente asintomáticos e integrarla en una revisión crítica de su relevancia productiva y sanitaria, sirviendo como base para el diseño de estrategias de control. Se realizó un estudio descriptivo de corte transversal en una finca del departamento de San Pedro, Paraguay. Se recolectaron muestras fecales directamente del recto de 59 ovinos y se analizaron mediante técnicas de flotación cualitativa y el método de McMaster para determinar la presencia huevos por gramo de heces (HPG). Se detectaron huevos de NGI en el 78% (46/59) de los animales llegándose solo a la identificación a nivel de familia. La intensidad media de infección fue de 268.87 HPG, indicando infecciones leves a nivel de rebaño. La elevada prevalencia junto con baja intensidad evidencia la presencia de parasitismo subclínico en ovinos aparentemente sanos. Estos hallazgos resaltan la importancia del monitoreo rutinario para identificar portadores asintomáticos que contribuyen a la contaminación de pasturas y representan un riesgo sanitario. A la luz de la literatura actual, se subraya la relevancia de las infecciones subclínicas por NGI para la salud animal, la productividad y el desarrollo de estrategias sostenibles de control parasitario en sistemas de pequeños rumiantes con potencial riesgo zoonótico.

**Palabras clave:** NGI, prevalencia, parasitología.

### INTRODUCTION

Gastrointestinal nematode (GIN) infections remain one of the most significant health constraints affecting livestock production worldwide<sup>(1)</sup>, these infections result in substantial economic losses associated with reduced milk and meat production, as well as increased costs related to treatment and control strategies<sup>(2)</sup>.

In recent years, extensive literature has focused on the prevalence, epidemiology and anthelmintic resistance of GIN worldwide but beyond their economic impact GIN infections also represent an emerging concern for public health<sup>(3-5)</sup>. The most important and prevalent nematode genera worldwide belong to the families *Trichuridae*, *Trichostrongylidae*, *Ancylostomidae*, *Ascarididae* and *Strongyloididae* (6). It is well known that grazing animals can be infected with one or more genera of GIN. However the intensity of the infection and the clinical signs associated with the disease can vary considerably. The severity of the disease is influenced by factors such as the species of parasite present, the number of worms present in the gastrointestinal tract, the general health and immune system of the host, age, physiological status and environmental factors such as climate and type of pasture, stress, stocking rate, management and/or diet<sup>(7,8)</sup>. Nevertheless, it is well-known that a small proportion of animals within a flock is responsible for most of the pasture contamination, highlighting the importance of targeted monitoring strategies<sup>(9)</sup>. In this context, descriptive field studies remain valuable tools for identifying clinical and subclinical infections and supporting farm-level decision-making<sup>(10)</sup>.

Building on this framework, our study aims to support producers and veterinarians in implementing tailored preventive veterinary practices that incorporate farm-specific monitoring and strategic parasite control measures. This approach is consistent with an integrative preventive veterinary model within a One Health perspective, addressing both animal health and potential zoonotic risks. Accordingly, the objective of this study was to provide field evidence on the presence, prevalence, and intensity of gastrointestinal nematode infections in asymptomatic sheep and to contextualize these findings within a critical narrative emphasizing their impact on productivity and broader sanitary significance.

## MATERIALS AND METHODS

### *Study area*

The study was conducted in Cañada Lourdes, Department of San Pedro, Paraguay (24°40'39.2" S, 56°38'40.0" W). The area is characterized by a humid subtropical climate, with an annual average temperature of approximately 23 °C and average annual precipitation of 1.324 mm.

### *Farm Selection and Animal Inclusion Criteria*

A single sheep farm was selected through convenience sampling based on owner consent and accessibility for field sampling. All animals present on the premises at the time of the visit were included in the study to avoid selection bias within the flock. According to the owner's declaration, no animals exhibited clinical signs of disease at the time of sampling. Additionally, no anthelmintic treatments had been administered for at least two months prior to sample collection, allowing the evaluation of naturally occurring gastrointestinal nematode infections under field conditions. Therefore, all sampled animals were considered clinically asymptomatic at the time of inclusion.

### *Study design and sample collection*

A descriptive cross-sectional study was conducted using a convenience sampling approach. A total of 59 sheep, ranging in age from 2 months to 10 years, were included in the study. Animals were classified into two age groups: lambs (<1 year) and adult sheep (≥1 year). Each animal was sampled once. Fecal samples were collected directly from the rectum using clean plastic containers and transported to the laboratory for processing. Sampling was conducted in October 2024 as a pilot study.

### *Parasitological analysis*

Macroscopic examination of fecal samples was performed to assess color, consistency and the presence of adult parasites, when applicable<sup>(11)</sup>. Qualitative detection of parasite eggs was conducted using a flotation technique. Quantitative analysis was performed using the McMaster method to estimate eggs per gram of feces (EPG)<sup>(12)</sup>. Eggs were identified to family level based on morphological characteristics under light microscopy at 10× and 40× magnification<sup>(13,14)</sup>.

### *Statistical analysis*

The prevalence of infection was calculated as the proportion of positive samples relative to the total number of samples examined. Infection intensity was expressed as mean EPG. Descriptive statistics were used to summarize EPG values by age group and sex. Statistical analyses were performed using XLSTAT 2022.

## RESULTS

### *Composition of the study sample by sex and age category with prevalence of gastrointestinal nematode infections*

Gastrointestinal nematode infections were detected in 46 of the 59 animals, yielding an overall prevalence of 78%. Infection was observed across all sexes and age groups, indicating widespread exposure within the flock. The composition of the study sample and prevalence within each subgroup is summarized in (Table 1).

**Table 1.** Prevalence of gastrointestinal parasites in sheep stratified by age and sex in San Pedro.

| Sex    | Total n (%) | Prevalence total (%) | Lamb n (%) | Prevalence Lamb (%) | Adult sheep n (%) | Prevalence Adult Sheep (%) |
|--------|-------------|----------------------|------------|---------------------|-------------------|----------------------------|
| Male   | 20 (34%)    | 85% (17/20)          | 7 (12%)    | 100% (7/7)          | 13 (22%)          | 77% (10/13)                |
| Female | 39 (66%)    | 74% (29/39)          | 14 (24%)   | 100% (14/14)        | 25 (48%)          | 60% (15/25)                |
| Total  | 59 (100%)   | 78% (46/59)          | 21 (35%)   | 100% (21/21)        | 38 (64%)          | 66% (25/38)                |

Lambs of both sexes exhibited a prevalence of 100%, whereas adult males and females showed lower prevalence (77% and 60%, respectively).

*Morphological identification of nematode eggs*

Eggs with morphological characteristics consistent with strongylid-type nematodes were detected in 28 samples. The remaining positive samples contained eggs morphologically consistent with *Ascarid*-type nematodes (Ascarididae) and *Capillaria* type.

Ascarid eggs can be distinguished from strongylid eggs by their larger size, thick smooth shell, and more rounded shape, while *Capillaria* eggs are recognized by their elongated barrel shape with bipolar plugs.

*Intensity of infection or Parasite charge*

The intensity of infection, expressed as eggs per gram of feces (EPG), yielded a mean of 268.87 (SD = 305.48), reflecting substantial overdispersion and a characteristically skewed distribution. The distribution was characterized by a predominant group of individuals with low-level infections; specifically, 69.5% of the animals (n=41) presented parasite charges  $\leq 100$  EPG. In contrast, a small proportion of the population concentrated the highest parasite burdens, with only 10.2% of the individuals (n=6) exceeding 500 EPG. The remaining animals were distributed across intermediate ranges: 8.5% (n=5) between 101-200 EPG, 5.1% (n=3) between 201-300 EPG, and 6.8% (n=4) between 301-500 EPG. This high variance-to-mean ratio underscores significant individual heterogeneity, with a predominant trend toward low-intensity infections at the flock level.

*Conceptual framework and decision options about GIN traitement*

To summarize the epidemiological and productive implications of these findings, a conceptual framework of asymptomatic GIN infections and decision options was developed (Table 2). This framework integrates field observations and literature, illustrating the role of asymptomatic sheep as reservoirs, the overdispersion phenomenon in egg shedding, pasture contamination risks, and potential impacts on animal productivity and public health. It also highlights practical decision options for producers, veterinarians, and public health professionals, including monitoring, identification of high shedders, targeted selective treatment, and management adjustments.

**Tabla 2.** Conceptual Framework of asymptomatic GIN infections in sheep and decision options for different stakeholders

| Component                          | Finding                                                                          | Implication / Risk                                                     | Decision Options / Stakeholders                                                                                                                                                                                                                                                                | References |
|------------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>Infection status</b>            | Sheep are clinically asymptomatic but GIN-positive                               | Hidden reservoirs that can contaminate pastures and transmit parasites | <b>Producers:</b> Implement routine fecal egg count (FEC) monitoring; identify high-shedding individuals.<br><b>Veterinarians:</b> Advise on monitoring protocols and identify priority animals for treatment.<br><b>Public health personnel:</b> Provide guidance on zoonotic risk awareness. | (1,7)      |
| <b>Prevalence &amp; intensity</b>  | High prevalence (78%), low mean intensity (~269 EPG)                             | Even low-intensity infections contribute to epidemiological risk       | <b>Producers:</b> Apply targeted selective treatment (TST) rather than blanket deworming.<br><b>Veterinarians:</b> Develop herd-specific treatment plans and monitor efficacy.<br><b>Public health personnel:</b> Monitor for zoonotic potential in farms with high prevalence.                | (15,16)    |
| <b>Egg shedding pattern</b>        | Overdispersion: few animals shed most eggs                                       | A small number of animals disproportionately contaminate pastures      | <b>Producers:</b> Focus treatment on high shedders to reduce pasture contamination.<br><b>Veterinarians:</b> Identify and confirm high-shedding individuals through lab analysis.<br><b>Public health personnel:</b> Assess potential environmental contamination risks.                       | (9,17–19)  |
| <b>Productivity impact</b>         | Potential subclinical effects: reduced growth, milk yield, and immune competence | Economic losses accumulate even without clinical signs                 | <b>Producers:</b> Integrate parasite monitoring with herd productivity records.<br><b>Veterinarians:</b> Correlate parasitism with production metrics and advise interventions.<br><b>Public health personnel:</b> Not directly involved but can support awareness of occupational risk.       | (20–28)    |
| <b>Zoonotic/public health risk</b> | Some GIN species may be zoonotic                                                 | Risk to farm workers and veterinarians                                 | <b>Producers:</b> Enforce hygiene practices and educate workers.<br><b>Veterinarians:</b> Train staff on zoonotic transmission prevention.<br><b>Public health personnel:</b> Conduct outreach and monitor zoonotic disease risk.                                                              | (29)       |
| <b>Management factors</b>          | Grazing, stocking density, and pasture rotation affect infection                 | Environmental conditions facilitate parasite survival and transmission | <b>Producers:</b> Adjust grazing practices; rotate pastures; reduce stocking density.<br><b>Veterinarians:</b> Recommend herd management strategies to reduce infection pressure.<br><b>Public health personnel:</b> Advise on environmental sanitation and zoonotic mitigation.               | (30)       |
| <b>Strategic framework</b>         | Integration of data and literature                                               | Guides decision-making for sustainable control                         | <b>Producers:</b> Use framework to plan interventions on the farm.<br><b>Veterinarians:</b> Apply framework to design herd-specific control programs.<br><b>Public health personnel:</b> Utilize framework for farm-level risk assessments and guidance.                                       | (31–37)    |

## DISCUSSION

The present study shows a high prevalence of gastrointestinal nematode infections in clinically asymptomatic sheep, coupled with a generally low intensity of infection in San Pedro, Paraguay. This pattern is characteristic of subclinical parasitism, which often remains undetected under routine farm conditions yet may exert cumulative negative effects on animal productivity, subclinical GIN infections have been associated with reduced weight gain, impaired feed efficiency and altered immune responses, even in the absence of overt clinical signs <sup>(38,39)</sup>. In the present study, sheep from San Pedro exhibited no observable alterations in body condition, clinical signs, or productive performance.

The coexistence of high prevalence and low EPG values observed in this study suggests a state of host-parasite equilibrium, in which animals exhibit varying degrees of resilience. Notably, the presence of individual animals with high egg excretion highlights the disproportionate role of a small subset of hosts in pasture contamination, a phenomenon widely described in small ruminant production systems<sup>(9,40,41)</sup>. Although coprological methods based on egg counts remain practical and cost-effective tools for monitoring parasitism, they present inherent limitations, particularly regarding species-level identification and temporal variability in egg shedding, because female nematodes lay eggs intermittently and EPG values may fluctuate according to physiological and environmental factors <sup>(42,43)</sup> underscoring the need for repeated monitoring and integrated control strategies.

From a sanitary and productive perspective, identifying asymptomatic carriers is essential for targeted parasite control<sup>(38)</sup>. Animals with high egg excretion, despite the absence of clinical signs, may act as key sources of infection for the entire flock<sup>(9)</sup>. Therefore, regular parasitological monitoring, combined with selective treatment approaches, represents a rational strategy to reduce parasite burdens while mitigating the development of anthelmintic resistance.

The conceptual framework provides a structured approach to understanding the implications of these subclinical infections. It emphasizes the importance of identifying high-shedding individuals, implementing routine monitoring, and applying targeted selective treatments<sup>(2,20,44,45)</sup>. Furthermore, the framework links infection dynamics with animal productivity and zoonotic risk, offering decision options for producers, veterinarians, and public health professionals to design effective, sustainable control strategies. By presenting this framework, the study not only reports field prevalence and infection intensity in asymptomatic animals but also provides a practical tool for informed decision-making in small ruminant management and parasite control. It is important to note that we do not propose a generic parasite control plan, as our approach encourages each farm to develop its own strategy according to its management conditions. Producers are urged to conduct routine coproculture monitoring even in the absence of clinical signs, thereby reducing the risk of parasitism in animals and humans and supporting proactive, farm-specific control measures.

The absence of coproculture and larval identification represents a limitation of this study. These procedures were not included due to time constraints and because the primary objective was to estimate prevalence and infection intensity rather than species-level identification. Future studies incorporating longitudinal sampling and molecular or larval identification methods would provide a more detailed characterization of GIN populations in this region.

## CONCLUSION

To our knowledge, no studies in the country have focused on personalized preventive veterinary medicine aimed at detecting subclinical infections. While previous studies have reported prevalence and frequency of gastrointestinal nematodes, our study highlights the potential risk posed by subclinical infections, particularly regarding zoonotic diseases transmissible through contact. Therefore, we encourage producers and veterinarians to implement routine fecal examinations and strategic control measures, complemented by laboratory analyses, to support preventive veterinary practices tailored to each farm. These findings underscore the

importance of routine parasitological monitoring, identification of high-shedding individuals, and implementation of targeted parasite control strategies to mitigate infection risks. In conclusion, subclinical gastrointestinal nematode infections in sheep represent a significant epidemiological and productive threat, even at low infection intensities.

### **Ethical considerations**

All ethical considerations were observed during fecal sample collection. Prior to sampling, the objectives of the study were explained to the animal owner, and informed consent was obtained. The work protocol was approved by the CEMIT Ethics Committee (CE-00006). The laboratory where the samples were processed follows the NP-ISO/IEC 17025:2018 Quality Management System standard of CEMIT. All procedures were conducted in accordance with the guiding principles for biomedical research involving animals established by the Council for International Organizations of Medical Sciences (CIOMS) and the International Council for Laboratory Animal Science (ICLAS) (CIOMS/ICLAS Guiding Principles, 2012). Animals found positive for gastrointestinal nematode (GIN) infections were treated with anthelmintics following laboratory confirmation.

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### **Author contributions**

Griselda Asunción Meza Ocampos (GAMO): conceptualization, study design, field sampling, data interpretation, writing – original draft preparation.

Ismael Llano Obregon (ILLO): technical support, laboratory analysis, data collection, methodology implementation.

Nilsa González Britéz (NGB): data analysis, literature review, interpretation of results, manuscript preparation.

Edith Alba Segovia Corrales (EASC): critical review and editing of the manuscript, provision of materials and laboratory resources, supervision of experimental procedures.

Approval of the final version: Griselda Asunción Meza-Ocampos, Ismael Llano Obregon, Nilsa Elizabeth González-Brítez and Edith Alba Segovia-Corrales.

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### **Conflicts of interest**

The authors declare that they have no conflicts of interest.

### **Data availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Peer Review**

This article underwent a double-blind peer review process, in accordance with the journal's editorial transparency policy. Reviewer identities and comments are not available for this publication. The authors considered the reviewers' comments and suggestions and made the necessary revisions to produce the final published version. This process is intended to ensure the scientific integrity of the article.

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