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Monitoring Gastrointestinal Parasite Infestation (Cryptosporidium and Eimeria) On Sheep Farms in Northeastern Algeria

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ABSTRACT

Our study evaluates gastrointestinal parasite infestation in sheep farms in northeastern Algeria, focusing on Cryptosporidium and Eimeria.
Experimental research was conducted to examine various digestive tract parasites using parasitological techniques.
The results show that the flotation method is more sensitive (65% for ewes and 82.75% for lambs) compared to direct observation (40% for ewes and 44.82% for lambs), with at least one parasite detected in the animals. The study further confirms that sheep in the region are infested with gastrointestinal parasites across different age groups, particularly in autumn, with young animals exhibiting higher sensitivity.
These findings highlight the significance of gastrointestinal parasitism, which contributes to substantial production losses and occasional mortality on farms.

key words : infestation, sheep, flotation, gastrointestinal parasitism.

INTRODUCTION

Sheep farming is deeply rooted in Algerian tradition and remains a crucial sector for the country's agricultural economy (Dubeuf *et al*; 2016). It serves as the primary source of income for approximately one-third of the Algerian population (Iñiguez & Aw-Hassan., 2005).
For generations, sheep have been the preferred livestock for producing animal protein, particularly meat and milk, contributing to the dietary needs of rural and urban populations alike (Mottet *et al*; 2016). Sheep are also integral to Algeria's national economy, with an estimated 26 million sheep forming a significant portion of the country's overall livestock population, Their role extends beyond just food production, supporting livelihoods and fostering cultural practices related to animal husbandry. (Youcefi & Marouf, 2024)
In terms of geographical distribution, around 60% of the national sheep population is found in the steppe, which is currently facing a number of problems that are hindering its development, the most frequent of which is internal parasitism, which is responsible for a large proportion of production losses and sometimes for significant mortality on farms. (Khalil *et al*; 2023)
Internal parasitism is an obstacle to development, especially as health and zootechnical monitoring is inadequate. In this region, the parasites in question are mainly slaughterhouse findings, with *Cysticercus tenuicolis, Echinococcus granulosus, Coenurus cerebralis, Hémonchus contotus* and *Dictyocaulus filaria* having been identified, while little attention has been paid to other parasites of the respiratory and digestive systems (Pisseri *et al*; 2013; De Souza Silva *et al*; 2020).
Coccidiosis mainly affects lambs in sheepfolds. They are most infected between 3 and 8 weeks of age; before this period, they seem to be protected by the antibodies they receive from colostrum and, afterwards, they react by producing their own antibodies, which protects them from infestation (Meradi & Bentounsi, 2021).
The objective of our study is to evaluate gastrointestinal parasite infestation in sheep farms in northeastern Algeria, with a particular focus on Cryptosporidium and Eimeria.

MATERIALS AND METHODS

A total of 360 fecal samples will be collected from 239 adult ewes and 121 lambs (between 15 October 2022 and 16 December 2023), stored at 04°C and send to the laboratory.
In the laboratory, three distinct parasitological techniques were employed to evaluate the gastrointestinal parasite infestation in sheep. The first technique involved direct observation, which allowed for the visualization of a broad range of parasites, regardless of their prevalence in the samples. The second technique, flotation, was used to separate oocysts from the samples, making them easier to identify and assess under an optical microscope. The third technique, Ziehl-Neelsen staining, was specifically applied to stain Cryptosporidium oocysts, enabling their precise detection. This comprehensive approach was carried out on 121 lambs, with five samples taken from each lamb, ranging from 3 to 59 days old, to ensure a thorough examination of the parasite load across different age groups.

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Ziehl-Neelsen Staining is technique is designed to stain Cryptosporidium oocysts, which are acid-fast and require a modified procedure to enhance their visibility under a microscope. (Henriksen & Pohlenz, 1981)

a. Reagents:

1. **Carbol Fuchsin Stain (Primary Stain)**:
- 1 g of **Basic Fuchsin** dissolved in 100 mL of 95% ethanol
- 5 mL of **Phenol crystals**
- 95 mL of **Distilled water**
- 20 mL of **Concentrated hydrochloric acid** (HCl)

2. **Acid-alcohol Solution (Decolorizer)**:
- 95% **Ethanol**: 90 mL
- **Concentrated Hydrochloric Acid** (HCl): 10 mL

3. **Methylene Blue (Counterstain)**:
- 0.5 g of **Methylene Blue**
- 100 mL of **Distilled water**

b. Procedure:

1. **Preparation of Smear**:
- Prepare a smear from the fecal sample on a clean glass slide. Let it air dry and then heat-fix by passing it over a flame 2-3 times.

2. **Application of Primary Stain**:
- Flood the smear with **Carbol Fuchsin Stain** and heat gently for about 5 minutes. Do not boil, but keep the stain hot (near boiling).
- After heating, allow the slide to cool for 2 minutes.

3. **Decolorization**:
- Rinse the slide with **acid-alcohol solution** (95% ethanol and HCl), until the excess stain is removed. This step helps in removing non-acid-fast organisms while leaving Cryptosporidium oocysts stained.

4. **Counterstaining**:
- Rinse the slide with **distilled water** to remove the decolorizing solution.
- Counterstain with **Methylene Blue** for 1-2 minutes.
- Rinse again with distilled water and blot dry gently with a clean tissue.

5. **Microscopic Examination**:
- Examine the prepared slide under the microscope using **oil immersion** (100x objective). Cryptosporidium oocysts will appear **bright red** against a **blue background**.

This protocol is effective for detecting **Cryptosporidium** oocysts in fecal samples, as they exhibit strong acid-fast staining properties, making them easily distinguishable from other microorganisms present in the sample.

The statistical analysis

The statistical analysis of the results in this section was conducted to ensure a comprehensive understanding of the outcomes. The data were presented in a clear and accessible format to facilitate interpretation. To assess the significance of the observed differences, we employed a **Student's t-test** for comparisons between groups, allowing us to determine whether the variations in parasite infestation rates were statistically significant. Additionally, to visualize the distribution and variability of the data, a **box plot** was used, which effectively highlighted the median, quartiles, and any potential outliers in the results. These statistical tools provided a robust framework for analyzing and presenting the data in a scientifically rigorous manner. (Berghiche *et al*; 2022)

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Results

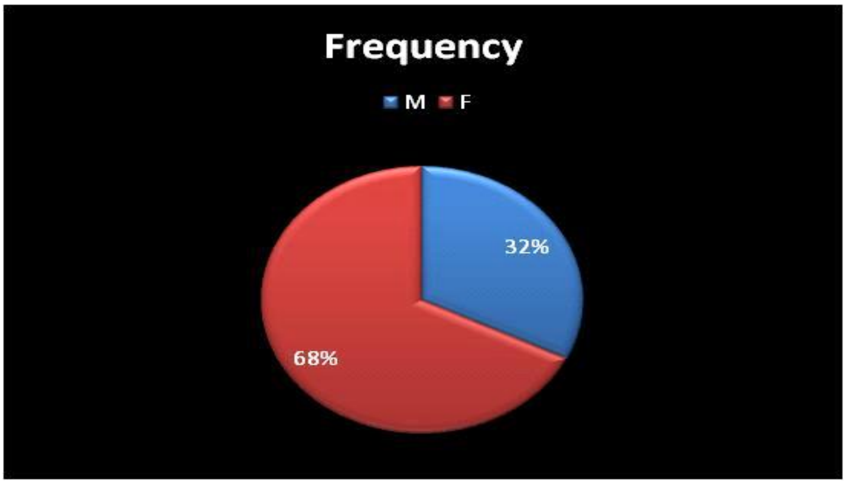

Figure 1. frequency of intestinal parasitoses in relation to animal gender.

The aim is to examine whether gender influences the frequency of intestinal parasitic infections in animals, the data shows that **Males** account for 32 % of cases of intestinal parasitoses and **Females** account for 68 % cases of intestinal parasitoses, From the numbers provided, females appear to have a higher frequency of intestinal parasitic infections than males.

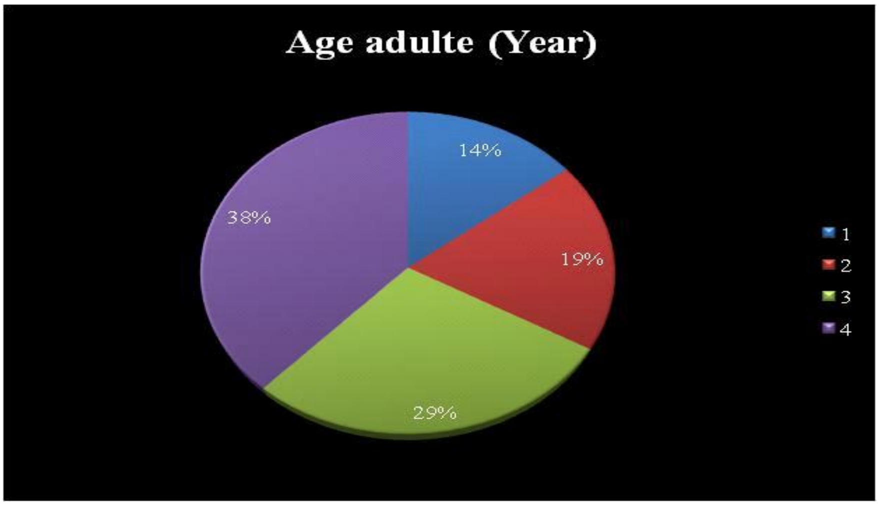

Figure 2 . frequency of sheep's age.

To analyze the age distribution of sheep in the study population the ages of the sheep are categorized in years and the frequency of individuals in each age category is recorded. The data would typically be presented in chart of frequency showing how many sheep fall into each age group from one to four years with 14%, 19% , 29% and 38 % respectively. Understanding the age distribution is essential for herd management, as it affects productivity, reproduction rates, and disease susceptibility for example, younger sheep may be more susceptible to certain diseases, while older sheep might have declining productivity.

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Comparison between flotation and direct observation

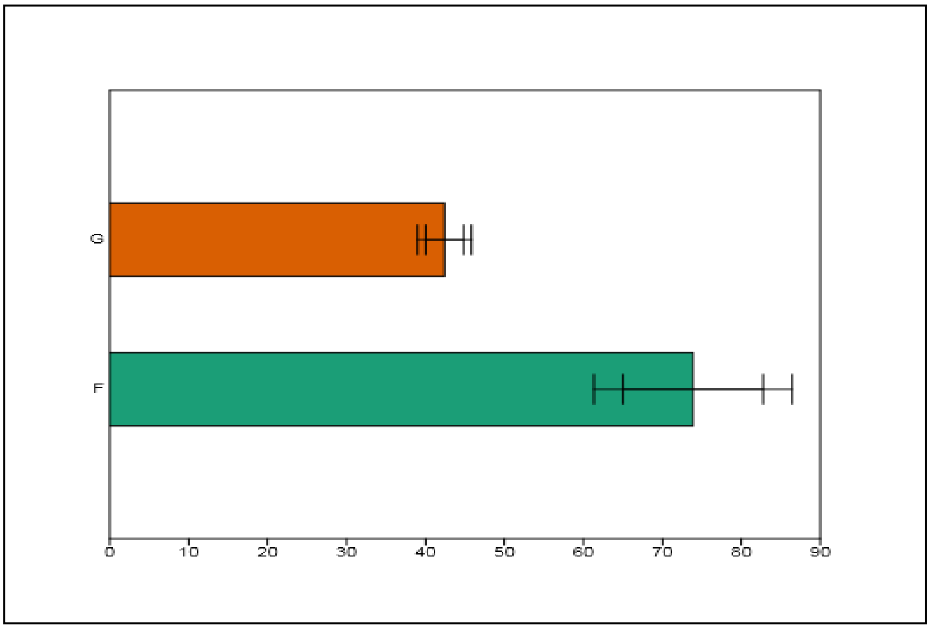

Figure 3 . Box plot of cases percentage according to techniques.

The interpretation of the box plot based on our data: **Floating Technique for Ewe** with a percentage of 65% , this technique shows a moderate percentage for ewes compared to the other techniques, the **floating Technique for Lamb** percentage is 82.75% the highest percentage among all techniques and categories, indicating that the floating technique for lambs is the most effective or most frequently used. **Direct Observation Technique for ewe** and **lamb** percentages are 44.82% and 40% respectively, this technique shows a lower percentage for ewes compared to the floating technique and is slightly higher than the percentage for lambs under direct observation, this is the lowest percentage among all techniques, suggesting it may be the least effective or least frequently used method.
Overall Observations, the **Floating Technique** is generally associated with higher percentages for both ewes and lambs, with lambs having the highest percentage and the **direct Observation Technique** shows lower percentages for both ewes and lambs, with lambs having the lowest percentage of all.
The results suggest that the floating technique might be more reliable or effective for detecting or managing cases in both ewes and lambs and further investigation could explore why the direct observation technique has lower percentages and whether these differences are statistically significant. If this data were expanded to include a larger sample size, box plots would better showcase variability and distribution.

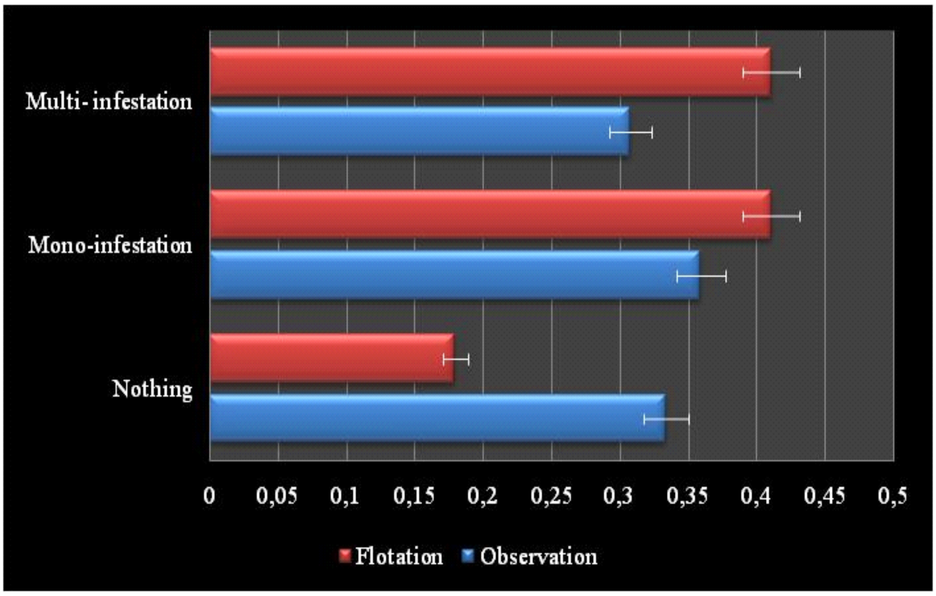

Figure 4 . Comparison of Detection Rates Between Observation and Flotation Techniques Across Infestation Categories

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This bar chart compares the percentages of co-infection detected by two techniques, **Observation** (blue bars) and **Flotation** (red bars), across three categories: **Nothing**, **Mono-infestation**, and **Multi-infestation**. The error bars on each bar indicate the level of variability or uncertainty in the measurements.**"Nothing" (No infestation) observation** the Higher percentage (~33%) compared to Flotation (~18%). this suggests that the observation technique identifies a greater proportion of cases without any infestation than the flotation technique. **"Mono-infestation"**: **Flotation**: Detects a higher percentage (~41%) compared to Observation (~36%), this implies that the flotation technique may be better at identifying single infestations. and **"Multi-infestation"** for **Flotation**: Again detects a higher percentage (~41%) compared to Observation (~31%), this indicates that flotation is more effective or sensitive for detecting multiple infestations.
Key Observations firstly the **Flotation technique** generally detects higher percentages of infestation (mono- and multi-infestation categories) than the observation technique. **secondly** The **Observation technique** detects a higher percentage in the "Nothing" category, which may indicate it is less sensitive for detecting infestations compared to flotation. **Flotation** appears to be the more sensitive technique for identifying infestations (both mono- and multi-infestation). **Observation** might be underestimating the presence of infestations, as it shows a higher percentage of "Nothing." Flotation should be prioritized when precise identification of infestations is critical. The results may benefit from further statistical analysis to confirm the significance of these differences.

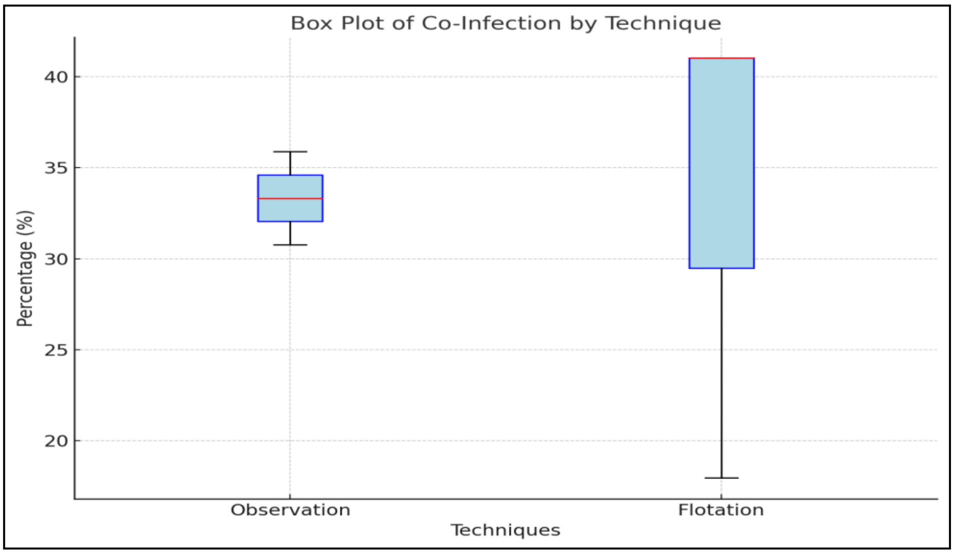

Figure 5 . Box plot of co infection.

This box plot will compare the distribution of co-infection frequencies between two techniques (Observation and Flotation) across three categories: "Nothing" (no infection), "Mono-infestation" (single infection), and "Multi-infestation" (multiple infections). The central tendencies (median values) for each category in both techniques will be compared. Variability within categories and techniques can be evaluated if multiple data points are present for each category.
This box plot visualizes the percentages of co-infection by the two techniques (**Observation** and **Flotation**) across the three categories ("Nothing," "Mono-infestation," and "Multi-infestation").
Interpretation of observation technique: The range of percentages is narrow, indicating more consistent values. **with a** Median: ~33–34% the data for "Nothing," "Mono-infestation," and "Multi-infestation" lies close together and for **flotation Technique** the range of percentages is wider, reflecting higher variability with a Median: ~41%. Higher percentages are observed for "Mono-infestation" and "Multi-infestation," while the "Nothing" category shows a lower percentage (~18%). The **Flotation technique** appears to detect higher percentages of mono- and multi-infestations than the observation technique, which suggests it may be more sensitive for these cases. The **Observation technique** shows less variability, possibly indicating more uniform performance across categories. The "Nothing" category shows a much lower percentage for Flotation, implying this technique may be better at identifying infections compared to observation.
Ziehl Neelson staining modified by Henriksen and Pohlenz
The absence of Cryptosporidium parvum oocysts was found in lambs aged less than 60 days, so this group of lambs is free from Cryptosporidiosis.
In the study, no Cryptosporidium parvum oocysts were detected in lambs aged less than 60 days. This absence suggests that lambs within this age group are not infected with Cryptosporidium parvum, indicating that they are free from Cryptosporidiosis. This finding may be attributed to factors such as maternal immunity, lower environmental exposure, or reduced susceptibility during the early stages of life. However, further investigations are needed to confirm these hypotheses and to determine whether older age groups are at a higher risk of infection due to increased exposure or waning maternal immunity

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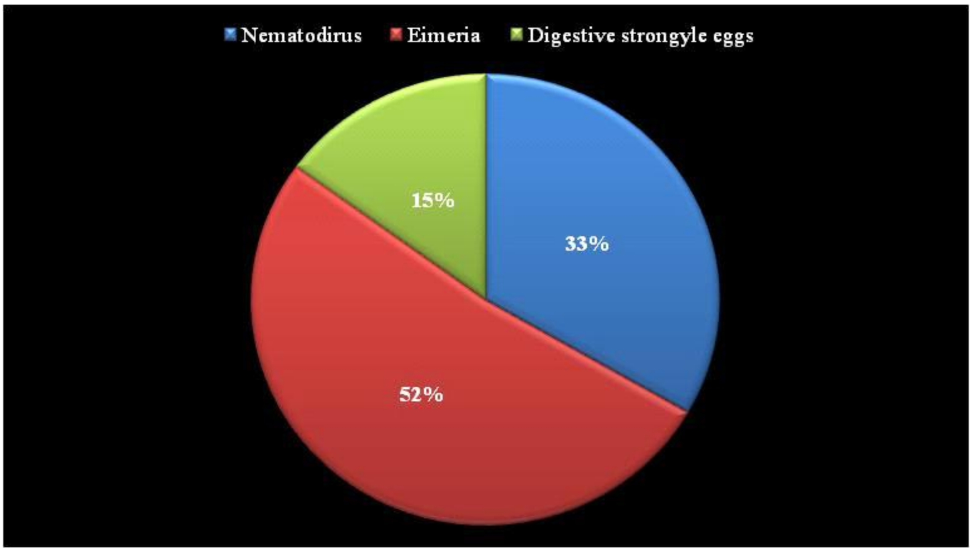

Figure 6 . Prevalence of gastrointestinal parasites

Coprological examinations showed a more or less significant excretion of digestive strongyles eggs by the two age classes on this farm (adults and lambs). Among the nematodes of the digestive tract, the presence of *Nematodirus* was not negligible. In addition, the presence of other digestive strongyles was reported, indicating that these animals are highly parasitised. These results are explained by the type of farming, which is extensive; the animals are always grazing, which allows them to be infested, and in addition the animals do not receive any preventive treatment against digestive parasites. On the other hand, coccidial parasitism was relatively higher in lambs than in ewes.
The statistical analysis gives us a statistically significant description of the pathogenicity in sheep, which can be described as follows (Table 1): All age groups can be affected, particularly young animals, the son-in-law no longer of risk, the animals less. In terms of epidemiology, coinfestations are the most notable characteristics compared to the presence or co-presence of both parasitoses at the same time**.**

***Table 1.* table summarizes the t-test results for each factor influencing parasite infestation.**

Infestation	**Factors**	**t**	**p**
Season	**Summer**	4,85	**0,**046**
	Winter		
Age	**Adult**	4,97	**0,**043**
	Lamb		
Gender	**Male**	2,81	0,2
	Female		
Co-infection	**Nothing**	4,33	**0,**049**
	Mono		
	Multi		

Interpretation of Statistical Results:

1. **Age Factor**: **t-value: 4.97, p-value: 0.043**

The analysis reveals a statistically significant difference between adult and lamb sheep concerning gastrointestinal parasite infestation. Specifically, the lower p-value (**0.043**) indicates that age plays a significant role in susceptibility to infestation. Lambs are more prone to infection compared to adult sheep, which is consistent with many studies suggesting that younger animals often exhibit higher levels of parasitic infections due to their developing immune systems.

2. **Gender Factor**: **t-value: 2.81, p-value: 0.2**

In this case, the p-value of **0.2** indicates that there is **no significant difference** in parasite infestation rates between male and female sheep. While the t-value suggests a slight difference, the p-value is greater than the commonly accepted threshold of 0.05, meaning gender does not appear to influence the rate of infestation in this study. This suggests that both male and female sheep in the studied population are equally susceptible to gastrointestinal parasites.

3. **Co-infection Factor**: **t-value: 4.33, p-value: 0.049**

The results show a **statistically significant difference** between sheep with no co-infection ("Nothing") and those infected with either mono- or multi-parasites. With a p-value of **0.049**, the presence of additional infections (either mono- or multi-infections) seems to correlate with a higher infestation rate. This suggests that sheep with multiple types of parasites or a

single infection combined with another pathogen are more likely to have a higher burden of gastrointestinal parasites than those without any co-infections. Co-infections could possibly exacerbate the overall health impact on the sheep, making them more vulnerable to parasitic infestations.
Age is the most significant factor influencing gastrointestinal parasite infestation, with lambs showing a higher infestation rate than adults; **Gender** does not significantly influence parasite infestation rates, indicating that both male and female sheep are equally affected finally **Co-infection** with other pathogens or multiple parasitic species increases the likelihood of higher parasite loads, with sheep infected with only one parasite being less affected than those with additional infections.
These results highlight the need for targeted interventions, particularly focusing on younger sheep (lambs) and those potentially suffering from co-infections, to reduce the impact of gastrointestinal parasites on farm productivity.
we concluded the **Age**: There was a statistically significant difference in infestation between adults and lambs (**p = 0.043**), with lambs showing a higher infestation rate. **Gender**: No significant difference was found between male and female sheep (**p = 0.2**) and **Co-infection**: Sheep with no co-infection (nothing) exhibited significantly different infestation rates compared to those with mono- or multi-infections (**p = 0.049**).
Further Considerations for this analysis that Total sample size and relative proportions of males and females should be considered to avoid biased interpretations. contextual factors such as diet, living conditions, and hormonal or physiological differences should also be explored.

Discussion

In our work, the results indicate that the flotation technique is more sensitive than the observation technique; ***40%*** and ***44.82%*** ewes and lambs successively reported affected by at least one parasite species using the direct observation technique, compared with ***65%*** and ***82.75%*** ewes and lambs successively reported affected by at least one parasite species using the flotation technique.
This study showed that sheep in the study region, whatever their age group, are infested by gastrointestinal parasites, particularly digestive strongyles and coccidia, especially in Winter but youngsters are more susceptible. (Mahlehla, 2017 ; Baker & Gray, 2004).
The results of our work show that the first excretion of oocysts begins at 17 days of age. These parasites must have played a role in the many cases of mortality observed in lambs in the region following diarrhoeal syndromes, hence the need for appropriate treatment. (Chappell *et al*., 1996; Navarre & Pugh, 2009).
Contact with the parasite has allowed an acquired immunity to develop over time, which explains the absence of clinical coccidiosis in ewes. Even if they are infested, the oocysts eliminated in faeces are very low. As a result, coccidiosis is less important in adults than in strongylosis (Wakelin, 1996).
This study showed that sheep rearing on grassland in the Nord-east of Algeria, which is a steppe area, is exposed to multiple infestations by gastrointestinal parasites, of which digestive strongyles and coccidia play a very important role. (Sharma *et al*., 2020).
The results obtained from the ewes show moderate infestation by digestive strongyles and low infestation by coccidia, indicating that these animals have good immunity; in other words, the ewes were generally in balance with these parasites, due to the geoclimatic and/or zootechnical conditions in the region. (Hwang *et al*., 2012).
However, they remained exposed to an imbalance in favour of the parasites on certain occasions (food shortage or parturition periods). On the other hand, the results obtained from the lambs show a high level of infestation, particularly with coccidia (Eales, *et al*., 2008).
The risk of infestation is omnipresent throughout the year, due to the permanent use of pastures and favourable climatic conditions. The fight against these parasites requires the implementation of a strategic treatment programme based on precise knowledge of existing parasite species and the seasonal evolution of infestations (Biswas & Das, 2024 ; Reddy & Reddy, 2015).
Autumn and spring remain the seasons with the highest parasite load, when animals must be systematically treated to relieve them of their high parasitism (Skuce, *et al*., 2013).
Young animals and pregnant or lactating females most often excrete a higher number of parasites (The essential source of parasites); L1 and L2 larvae are not very resistant in the external environment, which explains the very high mortality rate. (Góralska & Blaszkowska, 2015)
Climatic factors have a direct beneficial effect on the biology of parasites, leading to an increase in the number of L3 eggs and larvae in the outdoor environment. The most important of these are rainfall. They provide excess moisture, which helps the parasites to thrive and survive longer. There are also early high temperatures in early spring, which accelerate exogenous evolution, potentially leading to the development of additional generations of worms in animals (Bowman *et al*., 2021; Nansen & Roepstorff, 1999).
The level of infestation depends not only on the number of infesting larvae available but also on the quantity of grass, All ages of sheep can be infected by *Emiera*, the main source of oocysts is ewes, particularly around the peri-partum period when the ewe's immune status is lower, adult sheep are usually resistant to the disease but act as carriers (Fthenakis *al*., 2015).

Conclusion

This study provides a valuable contribution to the growing body of research on sheep infections caused by gastrointestinal parasites, with a particular focus on intestinal strongylosis. It presents comprehensive epidemiological data on the prevalence of this infection and identifies key risk factors associated with its occurrence in different age groups of sheep in northeastern Algeria. The findings highlight that the rearing system plays a critical role in the susceptibility of sheep to gastrointestinal parasites, with more intensively managed flocks exhibiting higher infection rates. This is likely due to factors such as higher animal density, increased exposure to contaminated feed or water, and potentially lower immune responses in intensively farmed sheep.

The study emphasizes the importance of understanding how various environmental and management factors, such as farming practices, age, and co-infection, contribute to the spread of gastrointestinal parasites. Further investigations are necessary to clarify the role of these factors in the geographic and temporal distribution of the disease, which will be pivotal for developing more targeted control strategies.

Prophylaxis against gastrointestinal parasite infections is essential for maintaining both the health of the sheep and the productivity of the farm. Early detection through microscopic examination of fecal samples for the identification of oocysts is a key diagnostic tool. This method can help identify infected animals before they contribute to further contamination, allowing for timely treatment and intervention. Additionally, measures such as isolating infected sheep, controlling water sources to prevent contamination, and maintaining high standards of hygiene are critical for reducing the risk of outbreaks. Furthermore, adherence to proper farming conditions—such as rotational grazing, adequate pasture management, and minimizing overcrowding—can further help mitigate infestations.

In conclusion, a multifaceted approach involving early detection, strict biosecurity measures, and improved management practices is essential for controlling gastrointestinal parasitic infections in sheep. This research underscores the need for ongoing efforts to enhance prophylactic strategies, thereby improving animal welfare and reducing the economic losses associated with parasitic diseases in livestock farming.

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