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Prevalence of gastrointestinal parasites in cattle in a dairy farm in Santa Clara municipality, Cuba

Lazo PL [1], González CJ [1], del Valle LD [2], de la Paz BRR [1], Álvarez VB [3], Acosta TL [1] and Fimia DR [4,*]

[1] *Department of Veterinary Medicine, Faculty of Agricultural Sciences, Universidad Central "Marta Abreu" de Las Villas, Cuba.*
[2] *Parasitology Department. Regional High Specialty Hospital (HARE), Dr. Juan Graham Casasús, México.*
[3] *Department of Zoonosis, Municipal Direction of Hygiene and Epidemiology. Santa Clara, Villa Clara, Cuba.*
[4] *Department of Hygiene and Epidemiology, Faculty of Health Technology and Nursing (FHTN), University of Medical Sciences of Villa Clara (UMS-VC), Cuba.*

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Abstract

Cattle are natural hosts of a wide variety of gastrointestinal parasites that affect their health and welfare, causing diarrhea, loss of appetite, mild to severe anemia, as well as low productivity, resulting in great economic losses. The objective of the study was to determine the prevalence of gastrointestinal parasites in cattle of the "Niña Bonita" dairy farm in Santa Clara municipality, Villa Clara province, Cuba. Fecal samples were taken and coprological diagnostic techniques of sedimentation and flotation were applied for the identification of parasites. Two genera of nematodes (*Strongyloides* and *Toxocara*) and protozoa of the genus *Eimeria* were identified. The prevalence of gastrointestinal parasites was high, as was the intensity of parasitic infection, which was dominated by moderate and mild infections. The risk of being severely and moderately infected was higher in Strongyloides parasitized animals. It is concluded that parasitic infections continue to be one of the main causes of disease and loss of productivity in livestock farms, so their control is absolutely necessary, where the knowledge of the prevalence of parasitosis, as well as the identification of these parasites, is vital for decision making and the establishment of much more rational and efficient programs.

Keywords: Coprology; Infection extent; Infection intensity; Gastrointestinal parasites; Villa Clara

1. Introduction

Despite the current knowledge on the epidemiology of gastrointestinal nematodes and the availability of modern broad-spectrum and highly effective antiparasitic, the reality shows that the prevention of economic losses and infection control in production practice, are becoming increasingly difficult (Borka et al., 2017; Boldbaatar, 2021; Sánchez et al., 2022). This is mainly due to the development of parasite resistance and changing production conditions where intensification of systems generates a high risk of parasite transmission (Borka et al., 2017; Boros et al., 2021; Sánchez et al., 2022).

The abuse and incorrect administration of antiparasitics, have resulted in the development of resistant parasite populations, which is already a serious problem for their control, not only in bovids (Colina et al., 2013; Cuong et al., 2018; Boldbaatar, 2021).

Parasitic diseases are considered one of the most prevalent pathologies around the world (Berhe et al., 2017; Boros et al., 2021; Sánchez et al., 2022). They are characterized for being one of the most important sanitary problems in cattle,

* Corresponding author: Rigoberto Fimia Duarte

causing a decrease in the productive capacity of the parasitized animals, which translates into economic losses (Pinilla et al., 2018; Boldbaatar, 2021; Sánchez et al., 2022). Gastrointestinal parasitism in cattle is caused by protozoa and helminths, and generally its presentation is multiethiological (Hiko and Seifu, 2018; Pinilla et al., 2018; Jerez et al., 2021). Clinical signs in gastrointestinal parasitism may vary depending on the parasitic load, the parasitic species and host immunity (Cuong et al., 2018; Hiko and Seifu, 2018; Sanchez, 2022).

Gastrointestinal parasitosis in cattle usually affects young animals and is produced by a variety of nematodes that lodge in the digestive tract generating lesions and functional disorders that impact livestock, causing growth retardation, decreased milk production, reproduction and poor feed conversion (Cuong et al., 2018; Figueroa et al., 2018; Sánchez, 2022).

The monitoring of coprological studies with a quarterly frequency has been lost in the current conditions of cattle raising in the province of Villa Clara (Lazo et al., 2018; Pinilla et al., 2018), so it is necessary to investigate the prevalence and severity of infections of gastrointestinal parasitosis in cattle, in order to improve the effectiveness of prevention and control plans of endoparasitosis.

The objective of this study was to determine the prevalence of gastrointestinal parasites in cattle of a dairy farm in the municipality of Santa Clara with a semi-intensive production system.

2. Material and methods

2.1. Geographic location and topo-geodetic characterization of the area where the study was carried out

The "Pretty girl" dairy farm is located seven kilometers northeast of the city of Santa Clara, between 22º 25' 35" and 22º 26' 10" north latitude and 79 º53' 10" and 79 º53'40" west longitude. The magnetic declination is 9º west. The orientation of the longitudinal axis of the area is: azimuth- 162º and bearing- 18º SE. The physical limits are: to the north, the road to Camajuaní; to the south, the agricultural development zone "La Movida"; to the east, the "Callejón de los Patos" community; and to the west, the "Ochoa" river. The area has an irregular relief, framed between contour lines 75 and 102, according to the "SIBONEY" elevation system. The maximum and average slope is 3 and 1.5 percent, respectively. It is located at 95 meters above sea level. The variation in precipitation between the driest and wettest months is 129 mm. The approximate precipitation is 983 mm. The average annual temperature is 24.4 °C. Throughout the year, temperatures vary by 5.7 °C. The highest relative humidity is measured in September (81.93 %). The lowest in April (65.81 %) (World Climate Data-Climate-Data.org, 2023).

2.2. Study design for the coprological analysis

An observational, longitudinal and prospective epidemiological study was carried out from March 2022 to January 2023 and from September to November 2023. The animals used were of the zootechnical categories cows, calves, yearlings and of the crossbred genotype by Siboney.

2.3. Universe and sampling

From a universe of 97 cattle as population size (59 cows, 24 calves and 14 yearlings; two steers and one bull were not included because they were not in direct contact with the population to be studied), 51 fecal samples were taken using a simple random design. The sample size to be taken in each zootechnical category was estimated by applying the formula for finite populations according to the expected prevalence (Thrusfield, 2018).

$$n = (t^2 * p * (1-p)) / (d^2 / (N-1) + (t^2 * p * (1-p)).$$

Legend: n= sample size, t = value corresponding to the Gaussian distribution = 1.96, SD standard deviation, p= expected prevalence of the parameter to be assessed, 1- p = proportion of the population expected to be affected, L = 0.05 allowed error.

The expected prevalence included in the formula was 46.43%, for cows, based on a previous study conducted in a bovine population in Cienfuegos, Cuba, by Lazo et al. (2018). Of 65.3% for calves and 73.8.3% for yearlings, based on previous studies conducted in Colombia by Pinilla et al. (2018). The confidence level was 95 % so the absolute error accepted was 5 %.

2.4. Procedure for sample collection

Samples of bovine feces were obtained directly from the rectum (using gloves) or from the central portion of the fecal bolus (using a spatula), immediately after defecation. To obtain the samples from the rectum of the animals, the following protocol was followed:

With the help of a gynecological glove in the hand was introduced into the rectum of the cows taking about 50 grams of feces. Once the glove was removed, it was turned inside out and closed with a knot to prevent the entry of air into the glove and the hatching of gastrointestinal parasite eggs. With the help of a marker, each sample was identified with data of the earring, name of the animal and date.

All samples were sealed in polyethylene bags, identified and refrigerated. They were then transferred to the Parasitology Laboratory of the Veterinary Clinic of the Faculty of Agricultural Sciences at the Central University "Marta Abreu" of Las Villas.

The coproparasitological diagnosis was performed using the sedimentation-flotation tests, to determine the parasite load, considered reference tests for the diagnosis of parasites in feces, whose performance was evaluated by Bosco (2014), De Castro (2017), Noel (2017), Chuchuca (2019) and Casado (2020).

The data were collected in a Data Collection Model developed by the authors. The 51 bovine fecal samples were subjected to three replicates of the chosen coprological technique, for a total of three counts per sample, choosing the one with the highest result of eggs per gram of feces (hgh) for each sample.

2.5. Variable individual percentage of gastrointestinal parasites

Fecal samples from each cattle were analyzed to determine whether or not the animal had parasites. If the results were positive to any of the techniques, the animal was considered to be infected. The extent of infection or prevalence was calculated as the ratio of the number of parasitized animals (with positive samples) to the number of animals investigated and was expressed as a percentage.

2.6. Degree of individual infection or intensity of infection of gastrointestinal parasites

The degree of infection was assessed according to Astudillo (2016), (modified by the authors of the present investigation; because there is a value that is repeated in two of the series and because the term infestation is replaced by infection when dealing with endoparasites). The degree of parasitism was classified as: not parasitized, mild, moderate, severe and very severe.

Table 1 Interpretation of the degree of infection (modified from Astudillo, 2016)

	Signs of infection	
Eggs per field	**Symbology**	**Interpretation**
0	-	Unparasitized
1-3	+	Slight
4-7	++	Moderate
8-9	+++	Severe
10	++++	Very severe

2.7. Results processing

Descriptive statistics: In the case of qualitative variables, frequency distributions expressed in absolute and relative values (number and percentage) were made.

Analytical statistics: To explore the association between variables, the test of independence based on the Chi-square distribution ($\chi 2$) was used. When more than 20 percent of the expected frequencies less than five were obtained, the probability associated with the exact tests available in the program was considered.

According to the p value, the difference was classified as:

- Very significant: If p < 0.01.
- Significant: If p >= 0.01 and p < 0.05.
- Not significant: If p >= 0.05.

The prevalence ratio and the relative risk (RR) were estimated through the conformation of 2x2 contingency tables (Thrusfield, 2018), applying a retrospective cross-sectional observational analytical study, to determine if there was an association between the degree of intensity of the infection and the parasite found with greater occurrence.

2.8. Ethical Aspects

The research was subject to ethical norms that made it possible to minimize the possible harm to the animals included in the study, as well as to the technical personnel of the dairy farm that was involved in the collection of feces samples, in order to generate new knowledge without violating the ethical principles established for these cases. On the other hand, all authors involved in the research, publication and dissemination of the results are responsible for the reliability and accuracy of the results shown (Declaration of Helsinki WMA, 2013).

3. Results

Table 2 shows the occurrence of positive and negative cases of gastrointestinal parasites. The prevalence of endoparasites was 80.39 %. The highest prevalence was observed in calves, with 85.71 %, followed by the cow category, with 84.61 %, although statistically the difference was not significant according to Pearson's Chi-square test 5.7422, with p=0.0566.

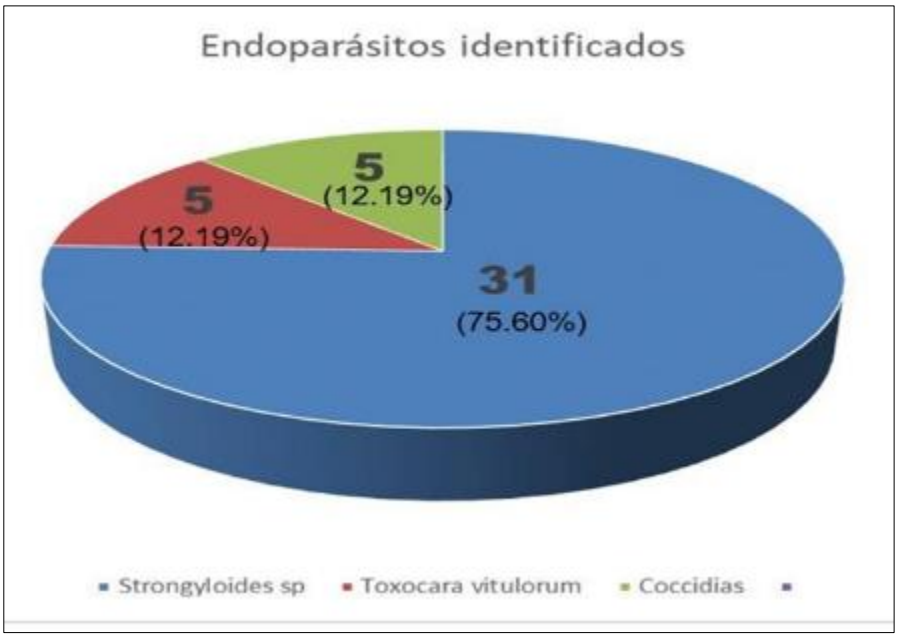
Table 2 Prevalence of gastrointestinal parasitosis in cattle at the "Pretty girl" dairy farm

		Cows		**Calves**			**Yearlings**				**Total**
Results											
	No.	**%***	**%****	**No.**	**%***	**%****	**No.**	**%***	**%****		**%****
Negative	6	15.40	60.0	60	14.29	10	3	60	30	10	19.61
Positive	33	84.61	84.61	6	85.71	14.63	2	40	4.88	41	80.39
Total	39	100	100	7	100	-	5	100	-	51	100

Legend: *percent based on category, **percent based on result, ***percent based on total; Chi Cuadrado de Pearson 5.7422 p=0.0566

The authors consider that the high prevalence observed in the present investigation is related to the deficit of antiparasitic drugs in the country, which generates the non-compliance of preventive deworming schemes once every three months in 100% of the mass. In addition, in the productive scenario where the study was carried out, it was observed that the animals are not given the 72-hour waiting period in the deworming room, once the treatment has been administered, which favors reinfection with the infective stages and dispersion of eggs in the environment that constitute sources of infection. On the other hand, the use of a small arsenal of nematicides has been abused and they are frequently administered in inadequate doses, which favors the development of drug resistance.

Figure 1 shows the occurrence of the parasites identified in the samples analyzed.

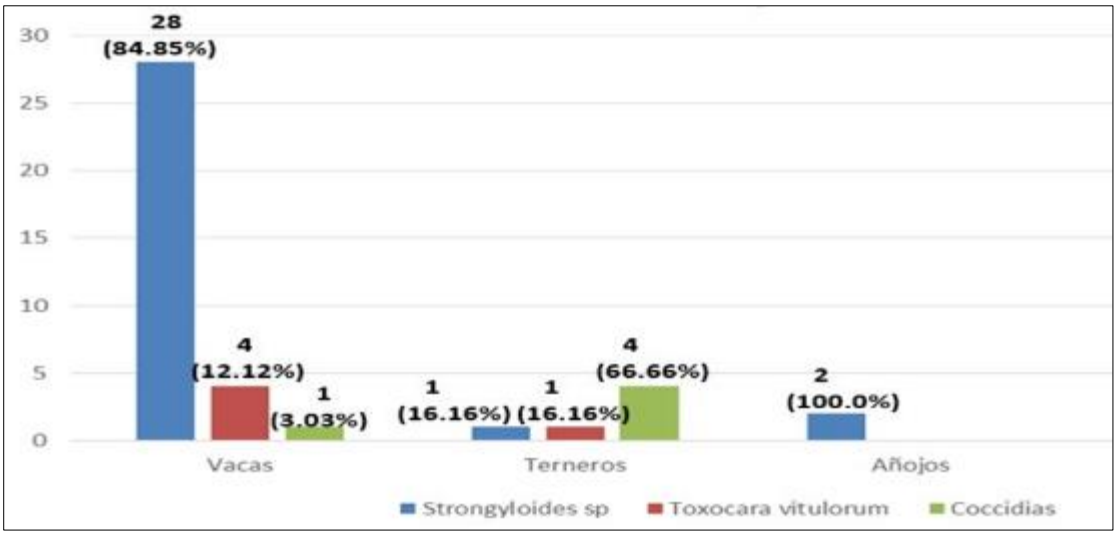


Percent based on total positives.

Figure 1 Occurrence of endoparasites identified in samples

Two genera of nematodes (*Strongyloides* and *Toxocara*) and one genus of protozoa of the genus *Eimeria* were identified in the present investigation. The authors of the present investigation consider that the results of the study are related to the fact that *Strongyloides* infective larvae are very active, penetrate through the intact skin or hair follicles of their hosts or can be ingested. The usual route of entry is cutaneous, and a high percentage of larvae that penetrate through the skin reach sexual maturation earlier than those that are ingested.

Figure 2 shows the occurrence of endoparasites according to the zootechnical category of cattle.



Percent based on zootechnical category.

Figure 2 Occurrence of endoparasites according to cattle category

Legend: X axis: Distribution of the occurrence of endoparasites in cows, calves and yearlings. Y axis: Ranges according to number of endoparasites by genus.

The highest occurrence of parasite infection by *Strongyloides* sp was observed in cows and yearlings and by Coccidia in calves, with significant statistical differences (Chi-square 20.7445 and p value=0.0004).

Table 3 shows the severity of infection in cows with positive results of the coprological analysis, based on the intensity of infection, expressed in eggs per field.

Table 3 Severity of infection in cows. “Pretty girl" cow farm

Grade of infection	***Strongyloides* sp.**		***Toxocara vitulorum***		***Coccidias***			
	Frequency	**Percent***	**Frequency**	**Percent***	**Frequency**	**Percent***	**Frequency**	**%****
Severe	2	7.14	-	-	-	-	2	6.06
Moderate	21	75.00	2	50.0	-	-	23	69.69
Leve	5	17.85	2	50.0	1	10	8	24.24
Total	28	100.0*	4	100.0*	1	100*	83	100.0**

* Percentage based on total positive samples for each parasite (*Strongyloides* sp. 28, *Toxocara vitulorum* 4 and *Coccidias* 1). ** Percentage based on the total number of positive samples for category (33) Pearson's $\chi 2$ =5.3228 p=0.2558.

Moderate infection (++) predominated, with a total of 23 cases, which constituted 69.69 % of the total infected cases for the category; followed by mildly infected (+) with a total of eight cases, which constituted 24.24 %. It was shown that there was no significant statistical relationship between the variables degree of infection and parasite identified (Pearson's $\chi 2$ = 5.3228 and p value=0.2558).

Table 4 Severity of infection in calves. “Pretty girl" dairy farm

Grade of infection	***Strongyloides* sp.**		***Toxocara vitulorum***		***Coccidias***			
	Frequency	**Percent***	**Frequency**	**Percent***	**Frequency**	**Percent***	**Frequency**	**%****
Severe	-	-	-	-	4	100	4	66.66
Moderate	1	10	-	-	-	-	1	16.66
Leve	-	-	1	100	-	-	1	16.66
Total	1	100	1	100	4	100	6	100

Severe infection (+++) predominated, with a total of four cases, which constituted 66.66 % of the total infected cases for the category; followed by mild (+) and moderate (++) with one case each grade, for 16.66 %. A significant statistical relationship was shown to exist between the variables Grade of infection and parasite identified (Coccidia) in calves ($\chi 2$ = 12, 000 p-value=0.0174).

Table 5 show the severity of infection in yearlings with positive results of the coprological analysis, based on the intensity of infection, expressed in eggs per field.

Table 5 Severity of infection in yearlings. Cowshop "Pretty girl”

Grade of infection	***Strongyloides* sp.**		***Toxocara vitulorum***		***Coccidias***		**Total**	
	Frequency	**Percent***	**Frequency**	**Percent***	**Frequency**	**Percent***	**Frequency**	**%****
Severe	-	-	-	-	-	-	-	-
Moderate	2	100	-	-	-	-	2	100
Leve	-	-	-	-	-	-	-	-
Total	2	100	-	-	-	-	2	100

Moderate (++) infection predominated, with a total of two cases, which constituted 100% of the total infected cases for the category.

As the number of non-zero rows and columns is not greater than 2, statistical tests were not performed. However, it was clinically significant that 100 % of those infected in the yearling category were moderately infected with *Strongyloides*.

In the risk analysis carried out to evaluate if there was an association between the *Strongyloides* sp. positivity factor and the degree of infection (severe and moderate), it was found that the risk of being severely and moderately infected was 1.5 times higher (prevalence ratio 1.48) in animals parasitized by *Strongyloides* sp, than in animals parasitized by *Toxocara vitulorum* and *Coccidia* (Table 6), but this association was not significant (p= 0.07), nor statistical CI: 95% (0.89-2.61).

Table 6 Risk analysis and association between *Strongyloides* positivity and degree of infection (severe and moderate)

	Association		**Statistical significance**		**Impact measures**			
Group	RP	IC:(95%)	χ2	Value of p	FAE	IC: 95%	FAP	IC: 95%
Severe and moderate Infestation	1.48	0.89-2.61	3.06	0.07	0.72	-0.21-0.93	0.58	-0.08-0.89

Legend: PR: prevalence ratio, CI: Confidence Interval, FAE: Fraction Attributable in Exposed, FAP: Fraction Attributable in the population.

Strongyloides sp. positivity was attributed to 72% of the animals with a severe and moderate level of infection, and 58% of the entire population of animals positive for these endoparasites.

It was also estimated that animals infected with *Strongyloides* sp. were affected four times more with a severe and moderate degree of infection than those infected with *Toxocara vitulorum* and *Coccidia* (odds ratio 3.61 CI: 95% (0.86-15.28) (Table 7).

Table 7 Distribution of independent variables associated with *Strongyloides* sp positivity

Variable	**Level of exposure**	**Positives / Total/ (%)**	**OR**	**IC: (95%)**	**Value of p**
Grade of infection	Moderate and severe	26* / 32 / (81,25) 6** / 11/ (54,54)	3, 61	0,86-15,28	0,07

Legend: * positive for *Strongyloides* sp. ** positive for *Toxocara vitulorum* and Coccidia.

81.25% of the animals with severe and moderate degree of infection were infected with *Strongyloides* sp. while only 54.54% of the animals infected with *Toxocara vitulorum* and *Coccidia* manifested severe and moderate degree of infection. But this association is not significant (p = 0.07), nor statistically CI: 95% (0.86-15.28).

The research showed a high prevalence of intestinal parasitosis in the cattle of the "Pretty girl" dairy farm. Two genera of nematodes and one of protozoa were identified. *Strongyloides* sp was identified in most of the positive cases. Moderate infection predominated, followed by mild infection.

4. Discussion

Regarding the prevalence found in this study (Table 2), we agree with Astudillo, (2016), who points out that the inadequate and empirical use of dewormers, due to over or under dosage, has produced parasitic resistance, so it is necessary before applying them to have a support of coproparasitic tests to identify the parasitic families and have an accurate diagnosis to suggest an appropriate treatment, all this aimed at reducing the parasitic load and increase milk production, reduce the incidence of sick animals and improve productive efficiency.

This aspect was also analyzed by Boldbaatar (2021) and Jerez et al. (2021) who referred to the low availability of antiparasitic drugs in a rearing farm in Cuba for several years, where Labiozol (albendazole) and Labiomec (ivermectin) were used sporadically, without following a calendar or criteria based on laboratory results, but on the physical condition of the animals.

These results agree with Paredes (2014) who states that gastrointestinal parasitosis affects cattle in all production systems and produce high morbidity in ruminants.

The prevalence values found in this study are higher than those obtained by Lazo et al. (2018) in a diagnostic survey carried out to determine the extent of parasitic invasion in extensive animal production systems in farms of the cooperative sector in the province of Cienfuegos, Cuba, where they obtained a prevalence of 46.43% and found that in cattle, parasites of the Strongilidae family predominated. The researchers attributed these results to the fact that, in spite of the deworming that was carried out periodically in cattle, they did not give them the waiting period in deworming rooms, and therefore the animals were re-infested, on the other hand, they assured that most of the units or farms did not have manure dumps for the correct deactivation of the manure.

In other geographical latitudes, values of prevalence of endoparasites in ruminants similar to those found in this study have been obtained (Guayllas, 2015), who observed a prevalence of 81% in the canton of Yantzaza, Zamora Chinchipe, Peru. Also, Cueva (2015), in a study conducted in the Calvas-Loja canton, Peru, determined a prevalence of 88.33%.

However, Romero and Valverde (2015), sampled 94 female and male cattle in that country, and obtained a prevalence of 100% of gastrointestinal parasites by sedimentation technique, which differs from the results obtained in this research.

Different results were obtained by Rodríguez and Juela (2016), in a study conducted in the canton of Cuenca, Peru, where a prevalence of 69.4% was determined, and by Armijos (2013), who analyzed 266 fecal samples of cattle in Santa Isabel, Peru, where he found a prevalence of 51.13%, which, according to the aforementioned researcher, could be due to the dry warm climate of the area, which reduces the appropriate conditions for parasitic development.

In a study carried out in Mexico, 119 cattle and 101 goats from local ranches were analyzed. In cattle, a prevalence of 96.6% of gastrointestinal parasites was determined (Figueroa et al., 2018).

In relation to the parasites identified in the fecal samples from the cattle investigated (Figure 1), the presence of two genera of nematodes and protozoa of the family Coccidiae was demonstrated. These results are lower than those obtained by Castro (2014), who analyzed fecal samples from Holstein Friesian breed cattle and identified several species of parasites in two seasons of the year. In winter they collected 50 samples and the results obtained were *Moniezia* sp (56%), *Eimeria bovis* (32%), *Trichuris* sp (26%), *Trichostrongylus* sp (20%), *Capillaria* sp (18%), *Fasciola hepatica* and *Strongyloides* sp. (16%), Cooperia and Nematodurios (4%). Another 50 samples were collected in summer, and the results obtained were *Eimeria bovis* (48%), *Strongyloides* (40%), *Trichostrongylus* sp (24%), *Moniezia* sp (22%), *Fasciola hepatica* and *Trichuris* sp (12%).

Fewer species of parasites were found than in a study conducted by Ribeiro *et al*. (2014) who analyzed 473 fecal samples of cattle, corresponding to 456 Dutch breeds and 17 Gyrholando breeds. The study was conducted from October 2010 to May 2011, where they determined the prevalence of Strongylid nematodes, whose value resulted in cows 34.2% and heifers 14.4%. The nematodes identified in cows were *Haemonchus* sp (66.7%), *Cooperia* sp (11.8%), Ostertagia sp (27.5%), *Trichostrongylus* sp (41.2%) and *Oesophagostomum* sp (5.9%); while, in heifers, *Haemonchus* sp (67.7%), *Cooperia* sp (35.5%), *Ostertagia* sp (35.5%) and *Trichostrongylus* sp (22.6%).

The findings of the occurrence of endoparasites according to the zootechnical category of the cattle investigated (Figure 2) demonstrated the presence of gastrointestinal parasites.

The results of the *Strongyloides* genus in calves were similar with those obtained by Pinilla et al. (2018), with a prevalence of 20.4%, being lower in yearlings (5.7%). For *Toxocara vitulorum*, the prevalence in calves was lower (3.7%), coinciding with the results in yearlings.

Results obtained by Colina *et al*. (2013) show a high percentage of coccidia infestation (84.9%) with a higher prevalence in animals younger than three years old, as well as Braae *et al*. (2018) who found a prevalence of 63 and 65% in animals younger than 24 months, and a lower prevalence in animals older than 24 months.

Tables 3, 4 and 5 show the severity of parasitic infection in cows, calves and yearlings, respectively. These results are higher than those obtained by Chuchuca (2019) who conducted an investigation that yielded an infection intensity of 3.41 % with severe (17.42%), moderate and mild (28.41%) infestation. In a study by Armijos (2013), mild parasitism predominated in 45.49% of his sample, which was higher than the results of the present investigation.

Colina et al. (2013) determined that the intensity of infection in cattle in Pacanga district, Peru, was found to be mild. While Rodriguez and Juela (2016), in their study conducted in Cuenca, Ecuador, presented mild degree for all parasites and mild and moderate degrees of infection for *Eimeria bovis* and *Ostertagia* spp.

In general, we agree with Hiko and Seifu (2018) who point out that parasites have been, are and will be in pastoral production systems. The current challenge for control incorporates the concept that, in pastoral production, it is impossible to eradicate the disease from livestock fields, forcing them to coexist in a biological and economic balance that makes the production system sustainable (Verweij et al., 2009; Berhe et al., 2017; Braae et al., 2018).

5. Conclusion

Parasitic infections continue to be one of the main causes of disease and loss of productivity in livestock farms, so their control is absolutely necessary. The knowledge of the prevalence of parasitosis in cattle, as well as the identification of these parasites, will help in decision making and in the establishment of more rational treatment and control programs, taking into account the characteristics of each facility, and based on a rational system that allows managing in an integrated way the different existing mechanisms and tools for the control of intestinal parasitosis.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exists among the Authors.

Statement of ethical approval

The research was subject to ethical norms that made it possible to minimize the possible harm to the animals included in the study, as well as to the technical personnel of the dairy farm that was involved in the collection of feces samples, in order to generate new knowledge without violating the ethical principles established for these cases.

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Authors short biography

Lic. Rigoberto Fimia Duarte, MSc., Ph.D.; Born in 1966 in the current province of Sancti Spíritus, Cuba. Graduated in 1989 in Biology Science. Professor and Researcher at the Central University "Marta Abreu" of Las Villas. Currently works at the University of Medical Sciences of Villa Clara (UCM-VC), Cuba. He has to his credit, 569 scientific results/publications, of which, he is the author of 389 scientific articles in specialized journals of recognized prestige and impact, both in Cuba and abroad, many of them indexed in group 1 and Web of Science (WoS) databases, as well as 31 books. ORCID Code: https://orcid.org/0000-0001-5237-0810; ID Scopus: 23472337200. Web of Science Research ID: ACB-4904-2022.