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Diagnosis and Treatment of Gastrointestinal Parasites and their Influence on the Production of Honey, in LA Magdalena Parish, Cantón Chimbo

Fabricio Javier Averos Estrada[1], Vinicio Rolando Montalvo Silva[2], Vicente German Jacome Lopez[3], Wilson Fabian Montes Clavijo[4]

javieraveros12[at]hotmail.com; viniciomontalvo69[at]yahoo.com
Facultad de Ciencias Agropecuarias de la Universidad Estatal de Bolívar-Km1.1/2via a San Simón Telefax 032983211-2980716
fcca[at]ueb.edu.ec

Abstract: ***In the Panchigua Bajo area, belonging to the La Magdalena parish in the San José de Chimbo Canton of the Bolívar province, Ecuador, the investigation was carried out Diagnosis and treatment of gastrointestinal parasites of bees and their influence on the production of honey. evaluated the following specific objectives: Diagnose and treat gastrointestinal parasites and their influence on honey production, Identify the degree of gastrointestinal parasite incidence in adult bees, Evaluate hive production in the event of digestive parasites and Evaluate the best antiparasitic treatment against gastrointestinal parasitosis in bees. The trial was divided into 4 treatments with 3 repetitions adding a total of 12 hives, subjected to a Random Complete Block Design (DBCA) and Duncan's statistical tests and Analysis of Variance (ADEVA), the variables under study were: Weight and hive population, incidence and type of parasites as well as the effectiveness of the drug, having the following name: T1 (control did not provide antiparasitic), T2 (treatment with Secnidazole at a rate of 7.14mg / kg in medicated Candy), T3 (treatment with Tinidazole at a rate of 7.14mg / kg in medicated Candy) and T4 (treatment with Metronidazole at a rate of 7.14mg / kg in medicated Candy). The final results were: 1) there were no statistically significant differences (NS) between the means of the treatments in all the variables under study, 2) the diagnosed parasite was Malpighamoeba mellificae, commonly referred to as beekeeping amebiosis, the same one that was found in the 75% of the hives (9/12), and 3) the drugs that were 99.99% effective in the treatment of bee amebiosis at a rate of 7.14mg / kg, were T2 (Secnidazole) and T3 (Tinidazole), finally It can be concluded that the presence of Malpighamoeba mellificae did not affect the productivity of the hive in this case.***

Keywords: Gastrointestinal parasitosis, Amebiasis Honey production

1. Introduction

Beekeeping is an activity of considerable socio - economic importance. It is estimated that there are approximately 45 million bee hives in the world that produce around 1,016,000 tons of honey and about 25 million kg of wax annually (Salas, R. 2010).

Ecuador has 120,000 hives that produce 910,000 kilos of honey, with an average of 8 kg per hive / year; the price of a kilo of honey is $ 8.00 The number of associated beekeepers at the national level is 1,500 that form the FENADE (National Federation of Beekeepers of Ecuador), gathered in 10 provincial associations (Herrero, F. 2014).

Diseases of parasitic origin take on special importance in modern conventional beekeeping, since they cover around 30% of cases of infestations (Rodríguez, F. 2007).

A parasite is an organism that, in order to feed, reproduce or complete its life cycle, is housed in another being, organism or host, which can be an animal or plant species, either inside or outside of its body permanently or temporarily, causing apparent damage to him or to that organism that lives from another larger, more evolved organism, of a different species from which it feeds and that may or may not cause injury (Del Campillo, C. et al. 2009)

According to the OIE (World Organization for Animal Health, 2016) there are more than 20 known diseases of honey bees (Apis mellifera) recognized and systematically described as diseases of adult bees and young, these diseases can be parasitic, viral, bacterial and fungal.

Parasites in all species as well as in bees produce affection and depression of the immune system favoring the entry of other microorganisms that can affect honey production and even the loss of the hive in case of severe attacks (Mace, H. 2011).

The study of the presence of parasitosis in bees is an attractive and novel topic since it is a topic that has not been thoroughly studied on the existence of helminths or protozoa that can affect the productivity of the hive.

This research is an updated study of the parasitic diseases that affect the hives of Panchigua Bajo, La Magdalena parish in the Chimbo canton. In which the following objectives were raised:

Diagnose and treat gastrointestinal parasites and their influence on honey production, in La Magdalena parish, Chimbo canton.

Identify the degree of gastrointestinal parasite incidence in adult bees.

Evaluate the production of the hive before a digestive parasitosis.

To evaluate the best antiparasitic treatment against gastrointestinal parasitosis in bees.

2. Materials and Methods

Location of the experiment

Province	Bolivar
Canton	San José de chimbo
Parish	Magdalena (Pamchigua Bajo)

Geographical and climatic situation of the experiment site

Locality	Parameter
Altitude	2570msnm
Latitude	79º01`59"W
Longitude	1º40` 34"S
Temp. Media Anual	16ºC
Temp. Mínima	13ºC
Temp. Máxima	22ºC
Precipitación	371mm.
Heliofania	980h/l/año.
Humedad Relativa %	55

Fuente: GADP La Magdalena 2015

Life zone

According to the L. Holdrìdge life zone classification. The experimental site corresponds to the Lower Montane Forest formation. (BMB)

Experimental Material

Treatment	Description	T.U.E
T1	Witness	3
T2	Administration of Secnidazole in candy	3
T3	Administration of Tinidazole in Candy	3
T4	Metronidazole administration	3
Total		12

In the present investigation, 12 antiparasitic hives were used, which were three antiprotozoal agents (Tinidazole, Secnidazole and Metronidazole).

Field materials

12 double-deck hives

Beekeeping materials

Lab's material

Conventional microscope

Photographic camera

Beakers

Pipettes

Slides and coverslips

Phenolated syrup

Lugol

Distilled water

Office supplies

Methods

Factors under study

The factor being studied in the research is to diagnose the presence of gastrointestinal parasitosis in bees and to establish treatment with three different antiparasitics. For this, four blocks were formed with three repetitions divided as follows: T1: control block was not applied, T2: antiparasitic administration of Secnidazole, T3: administration of Tinidazole and T4: administration of Metronidazole. These antiparasitics were applied using the medicated Candy technique.

Tratamientos

4 blocks or treatments were evaluated:

Scheme of the experiment

Mathematical model:

The statistical model that was used was:

YiJ = M + Ti + Bj + Eij.

Where:

Yij: Observations in the treatment block

M: Effect of the general mean

Ti: Treatment effect

Bj: Block effect

Eij: Associated experimental error

Type of Experimental Design

DBCA (Completely Random Block Design), with 3 treatments and 4 repetitions.

Process

Number of treatments	4
Number of experiment units	1
Size of the experimental unit	3
Number of hives per treatment	4
Total number of hives	12

The size of the experimental unit was 3 hives per treatment.

Statistical and functional analysis

For this research, the experimental results obtained have been subjected to the following statistical analyzes.

- Variance analysis. (ADEVA).
- Separation of means using the DUNCAN test (P <0.05) to compare factors under study and average of treatments.

Variance Analysis Scheme (ADEVA)

Sources of Variation	Degrees of Freedom
Total	11
Tratamientos	3
Repeticiones	2
Error Experimental	6

Experimental measurements

Weight of the hive (PC): Data that was taken in the field at the beginning of the experiment and every four weeks afterwards, a digital scale was used for this and its results are expressed in kg.

Hive population (P):

To determine the number of individuals per hive, we use the FARRAR method; for which we proceeded to weigh the hives with the bees inside it and then with the help of the Smoker removed them and the hives were weighed, obtaining a data by weight difference to which the following formula was applied:

FARRAR's formula to determine the population of bees tells us: 1 kg = 10,000 individuals.

Parasitic infestation (PI):
The bees were dissected, in order to extract the abdominal content, which was sent to the laboratory of the Faculty of Agricultural Sciences of the State University of Bolivar, (FAC-UEB) in order to To determine the presence of parasites and the positive ones to the analysis, the treatment was instituted

Types of parasites (TP):
The type of parasites present in the intestine of bees was determined through laboratory diagnosis, its classification is made according to the present species found. This value was expressed in eggs per gram (HPG).

Effectiveness in the treatment with Secnidazole (ETS), Tinidazole (ETT) and Metronidazole (ETM): in the laboratory analysis, once the presence of parasites was diagnosed, a treatment with Secnidazole, Tinidazole and Metronidazole was applied, at a rate of 7.14 mg / kg each and to measure their effectiveness, these results are expressed as a percentage (%).

Investigation procedure

Location of hives
The experiment begins with a visit to the apiary to make the selection of the hives and their location in a linear way separated at a distance of 1.5 m from each other according to the scheme of the experiment.

Selection of hives
We proceeded with the selection of the 12 double-decker hives as homogeneous as possible. They were later randomized and labeled according to the selected treatment.

ID
The hives were identified or rotated with their respective coding, the identification kardex that were stuck to one side of the hive for example. T2R1, T3R3, T4R1 by color sign

Dissection and collection of samples
After 12 days of the investigation, 20 bees were taken from each repetition (60 for each treatment) in order to take the abdominal content to examine microscopically to determine the presence of gastrointestinal parasitosis; With the help of a scalpel, the abdomen was cut and the intestinal content extracted, this content was deposited in containers for coproparasitic samples, the same ones that were identified and sent to the FAC-UEB diagnostic center in order to determine the parasitosis existing.

Preparation of the Candy with the Dewormer

The Candy was formulated as follows		
Pollen	10%	(25 g)
Honey	65%	(162.5 g)
Brown sugar	10%	(25 g)
Distilled water	15%	(37.5 g)
Sample weight	100%	250 g
Total:	500 mg/ portion	(25 g)

Candy placement
The antiparasitic in Candy was placed in disposable plates as corresponded to each treatment at the entrance of the gate and remained there for a period of 1 day; This was enough time for the worker bees to introduce the medicated Candy into the hive. This process was repeated 1 week later in order to complete the treatment.

Evaluation of the dewormer
After two weeks (14 days) of having finished with the medication, we proceeded to take 20 bees of each repetition (60 for each treatment) and with the help of a scalpel we proceeded to cut the abdomen and extract the intestinal content, this The content was deposited in containers for coproparasitic samples, which were identified and sent to the FCA-UEB veterinary diagnostic center in order to determine the effectiveness of the different antiparasitic agents used.

3. Results and Discussion

Hive Weight (PC)
The hives subjected to the diagnosis and treatment of gastrointestinal parasites in the La Magdalena parish of the San José de Chimbo Canton, presented the weights that are detailed below:

Analysis of Variance of the initial weights

Source of Variation	G.L	Sum of squares	Squares Media	F. Cal.	P
Total	11	3,065	1,5325		
Treatments	3	136,263	45,4208	0,79 ns	0,5416
Repetitions	2	344,415	57,4025	0,03 ns	0,9718
Error	6	483,743			
	C.V%	30.77	General average		24.625

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When the analysis of variance (ADEVA) of the weight of the hives (PC) was carried out, it did not present statistically significant differences (ns) between the initial weights, with the general average (x) being 24,625 kilograms (kg) and the coefficient of variation (CV) of 30.77%; This last value indicates that there was a great dispersion between the values of the sample under study.

The higher the value of the coefficient of variation, the greater the heterogeneity of the values of the variable; and the lower C.V., the greater homogeneity in the values of the variable. Suárez, M. (2011)

Levaratto, D. et al (2011) from the National University of La Plata at the 42nd international beekeeping congress in Argentina; presented a scoring grid for the evaluation of scientific journals and beekeeping techniques, establishing the following qualification model as a basis:

The coefficient of variation (CV = SD / mean) used in order to calculate the dispersion of the values of the evaluation grid with respect to their mean value was 12.4%, being able to confirm, in general terms, the homogeneity of the quality of the the magazines submitted and evaluated. Although it was possible to establish a low variability in the edition, layout and design (mean 18.63 ± 1.99 points) (CV = 10.7%) that allowed to confirm the quality, the main cause of score differences were the articles considered scientific (6.67 ± 10.39 points) belonging to sections and articles that showed a high coefficient of variation (154.4%) derived from the dispersion in the quantity and quality of their articles, being, in short, the preponderant causal factor that allowed to make a ranking of merits.

When comparing the coefficient of variation of the present investigation in the variable initial weight of the hive with the standards established by Levaratto, D. et al (2011) we can conclude that the present trial meets the criteria of an investigation within the field of beekeeping global.

Separation of means according to Duncan (P <0.05) of the initial weights.

Duncan	Medias	Treatments
A	29,400	Witness (Without medication) / (T1)
A	26,133	Administration of Tinidazole in Candy / (T3)
A	22,033	Administration of Secnidazole in Candy / (T2)
A	20,933	Administration of Metronidazole in Candy / (T4)

Likewise, when subjecting the initial weights of the hives to the separation of means according to Duncan (P <0.05), it is observed that the means of the mentioned treatments were not significant (equal letters), although the values were distributed between 20.93 (T4) and 29.40 (T1) kilograms (kg), which would indicate a range of 7.0367 kilograms between the extremes.

Salamanca, G. et al (2000) when carrying out a morphometric study in 116 hives in the department of Tolima determined that 5.1% turned out to be hybrids with a high percentage of European blood, while 68.2% of the sample were Africanized hybrids. the remaining 26.7% of the sample expressed its character in the range of hybrid with highly Africanized blood. The same report reveals the differences between the different variables under study (weights, number of individuals, aggressiveness, honey production, resistance to diseases); concluding that the variability of natural crosses produced (miscegenation) influence the productivity of the hive.

This data reveals the diversity of factors that intervene in the composition of the hive, hence the possibility of encountering weight variations such as those found in the present study.

Analysis of Variance of the weight of the hives in the fourth week of the test

Source of Variation	G.L	Sum of squares	Squares Media	F. Cal.	P
Total	11	3,065	1,5325		
Treatments	3	136,263	45,4208	0,79 ns	0,5416
Repetitions	2	344,415	57,4025	0,03 ns	0,9718
Error	6	483,743			
	C.V%	30.77	General average		24.625

Regarding the analysis of variance (ADEVA) for the weight of the hives (PC) in the fourth week of the test, they did not present statistically significant differences (ns) between the weights, being the general average (x), 24,625 kilograms (kg) and the coefficient of variation (CV) of 30.77%.

Salamanca, G. et al (2000) report that the size and weight of bees is influenced by the climate; Thus, in the premontane very humid forest association (bmh - PM), the largest bees were observed in reference to the characters that represented discrimination factors by zones, likewise they presented the highest percentages of European blood, while in the tropical dry forest consociation zone (bs –T) observed the smallest bees, which showed the highest degree of Africanization.

Keller et al (2006), estimated that bees use 125 to 140 mg of pollen to raise a new worker bee, which will subsequently consume an average of 3.4 to 4.3 mg of pollen daily, which makes them consume approximately 40 mg of pollen. pollen. In sum, 160 to 180 mg of pollen are required for the nutrition of a worker bee throughout its useful life. The same authors also assume that a healthy colony produces 100,000 to 200,000 bees per year, so 17 to 34 kg of pollen are required per colony annually.
The data reported by Salamanca, G. et al (2000) and Keller et al (2006) allow to show in greater depth the aspects that support beekeeping ethology, with the purpose of estimating the state or balance of the population of a beehive.

Separation of means according to Duncan (P <0.05) of the weights of the hives in the fourth week of experiment

Duncan	Half	Treatments
A	29,400	Witness (Without medication) / (T1)
A	26,133	Administration of Tinidazole in Candy / (T3)
A	22,033	Administration of Secnidazole in Candy / (T2)
A	20,933	Administration of Metronidazole in Candy / (T4)

In the separation of means according to Duncan (P <0.05), during the fourth week of the experiment, the results show that the means of the treatments in question were not significant (equal letters), although the values were distributed between 20.93 kg for treatment 4 and 29,400 kg, for the control T1 with a range of 7.0367 kilograms between the extreme values.

Monitoring the weight of the hive provides information regarding the honey content and the activity of the bees. Changes in this value indicate variations in the accumulation of honey that can be attributed to different factors, for example: the consumption of the reserves during the winter period, adverse weather conditions (rain or wind), changes in the nectar sources, the occurrence of swarms, etc. Another of its benefits is that through weight, you can determine the most appropriate time to harvest without having to open the hive previously. Valdés, P. (2014)

For the present study, when it was determined in the fourth week of the study, that the weights of the hives were invariable, it was decided not to carry out honey harvest in order to protect the hive population.

Analysis of Variance of the weight of the hives in the eighth week of the test

Source of Variation	G.L	Sum of squares	Squares Media	F. Cal.	P
Total	11	13,087	6,5433		
Treatments	3	203,333	67,7778	1,73 ns	0,2593
Repetitions	2	234,727	39,1211	0,13 ns	0,8759
Error	6	451,147			
	C.V.%	22,08	General average		28.333

The analysis of variance (ADEVA), for the weight of the hives (PC), did not present statistically significant differences (ns) between the weights during the eighth week of the experiment. The general average (x) was 28,333 kilograms (kg) and the coefficient of variation (C.V.) was 22.08%.

On a global scale, various investigations are carried out on the behavior of the hive and its influence on productivity in what is called "precision beekeeping". The main topics of analysis focused on optimizing productivity are traceability, georeferencing for foraging areas. Internal monitoring of the hive, geographic information systems, etc.

Oskman, M (2009) mentions that the state of a hive (strong or weak) is measured by the activity that it presents in a certain period of time, said author affirms that when opening a hive if its liner is stuck to the frames or racks it could be considered as strong.

When comparing the average weights at eight weeks with respect to the previous measurements (beginning and week 4), it can be seen that there was an increase in weight, which allows us to speculate on the effect that the medication had on the productive activity of the colonies.

Separation of means according to Duncan (P <0.05) of the weights of the hives during the eighth week of the experiment

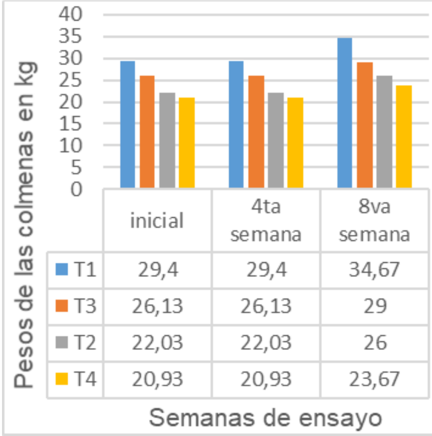
Duncan	Medias	Treatments
A	34.667	Witness (Without medication) / (T1)
A	29.000	Administration of Tinidazole in Candy / (T3)
A	26.000	Administration of Secnidazole in Candy / (T2)
A	23.667	Administration of Metronidazole in Candy / (T4)

The separation of means according to Duncan (P <0.05), during the eighth week of the experiment, shows that the means of the mentioned treatments were not significant (equal letters), although the values were distributed between 23,667 kg, (treatment 4) and 34,337 kg, (treatment 1) with a range of 11.0 kilograms (kg) between the extreme values, the range was much higher than the initial one whose value was 7.0367 (kg).

Keller et al (2006) observed a different behavior in the population growth of hives, subjected to different areas of pecoreo, thus determining shrub species with high attractiveness and fidelity, estimating that there are certain tree species that present a greater periodicity in their flowering but that the acceptance of bees is lower even in times of scarcity of nectars; This being a preponderant factor in the productivity of the hive.

In Ecuador, honey activity is mainly concentrated in the hands of small beekeepers. According to data provided by the Ecuadorian Institute of Statistics and Census through the National Agricultural Survey (INEC - Espac) in "Beekeeping Production: Semiannual Report to 2014", 67% of beekeepers have less than 20 hives; This reveals little access to information, which is essential for the development of any type of agricultural activity, including beekeeping. Knowledge of bees, their environment and the climate are relevant for productive success

Weight distribution of hives during the experiment



In graph 1, the behavior of the weight of the hives (PC) can be observed during the weeks of experiment, in this way we can synthesize that the treatments (T1, T2, T3 and T4) started with a weight of 29.4, 22.03, 26.13 and 20.93 kilograms (kg) respectively; these weights were maintained until the fourth week; Finally, 34.67, 29.0, 26.0 and 23.67 kilograms (kg) were obtained in the same order at the end of the test. Said weights were numerically different, but statistically no significant differences (ns) were found between the treatment and block means.

The weights registered in the present investigation agree with those indicated by (Jean-Prost, P. 2013), who states that the weight of a Langstroth-type hive is the sum of three basic elements, these being: hive, bees and provisions. It is interesting to know what each of these three elements contributes to the weight of the hive. These authors mention that the weight of an empty hive averages 15 kilograms (kg).

In this same aspect, in 1937 the American beekeeper entomologist, Dr. Clarence L. Farrar, determined that if a full brood chamber has 10,000 bees, it is known that 10,000 bees weigh approximately 1 kg. (Le Conte, Y. 2013).

The last element of the hive corresponds to provisions (pollen, honey, propolis, Jelly, etc); this is calculated based on the difference of the two previous events with the total weight of the hive. Example, if a hive weighs a total of 25 kg and we know that the empty hive weighs 15 kg, and we were able to determine the presence of 3 kg of mass of individuals (30,000 bees) the difference would give as such 7 kg of forecasts. (Jean-Prost, P. 2013).

Population of the Hive (P)

Analysis of Variance for the hive population at the beginning of the trial

Source of Variation	G.L	Sum of squares	Squares Media	F. Cal.	P
Total	11	2.789			
Treatments	3	8.158	2.719	1,42 ns	0,3255
Repetitions	2	8.267	4.133	1,90 ns	0,2056
Error	6	1.146			
	C.V.%	22.91	General average		19083

The analysis of variance (ADEVA) for the population of the hives (P), did not present statistically significant differences (ns) between the hives at the beginning of the experiment. The general average (x) was 19083 individuals / hive and the coefficient of variation (C.V.) was 22.91%.

Farrar's rule, known to beekeepers for many years, says that the more the population of a hive increases, the greater the individual production of each bee. This amounts to saying that it increases productivity and is known as a principle of synergy. This is because as the number of bees in a hive increases, the proportion of foragers also increases, according to the following table Valdés, P. (2014).

Total de obreras	10.000	20.000	30.000	40.000	50.000	60.000
Pecoreadoras	2.000	5.000	10.000	20.000	30.000	39.000
Porcentaje pecoreadoras	20 %	25 %	30 %	50 %	60 %	65 %
Peso de la población	1 kg	2 kg	3 kg	4 kg	5 kg	6 kg
Rendimiento miel	1 kg	4 kg	9 kg	16 kg	25 kg	36 kg

By doing a mathematical calculation by which knowing the population of bees in a hive, the production of this can be estimated approximately. We say that the production capacity is equal to the square of the weight of the population. If a full brood chamber has 10,000 bees and we know that 10,000 bees weigh about 1 kg. A hive that has 50,000 bees will be able to produce 5 squared, which means 25 kg of honey (Le Conte, Y. 2013).

Estimation of honey production based on the Farrar method

Experimental units	Population weight (kg)	8th week # of scavengers	According to farrar Honey yield (kg)
T1R1	2	5000	4
T1R2	3.3	9900	10.98
T1R3	3.8	11400	14.4
T2R1	2	5000	4
T2R2	2	5000	4
T2R3	2	5000	4
T3R1	3	9000	9
T3R2	2	5000	4
T3R3	4	20000	16
T4R1	2	5000	4
T4R2	2	5000	4
T4R3	2	5000	4

You can see the calculation of the possible honey production of the hives studied based on the method established by Farrar in 1937, which indicates that the honey production of a hive is equal to the square of the weight of its population

Separation of means according to Duncan (P <0.05) of the population of the hives during the beginning of the experiment

Duncan	Medias	Treatments
A	23000	(T2) Administration of Secnidazole in Candy
A	19000	(T3) Administration of Tinidazole in Candy
A	18667	(T4) Administration of Metronidazole in Candy
A	15667	(T1) Witness (Without medication)

When analyzing the previous table, the separation of means according to Duncan can be seen, for the hive population variable at the beginning of the experiment, in this it is shown that the means of the mentioned treatments were not significant (equal letters), although the values are distributed between 23,000 and 15667 individuals / hive, with a range of 7,333 individuals / hive between the extreme values.

Gil, S. (2016) indicates, the population of bees, health, management and production, form a set of factors in which all are interrelated with the rest. Thus, high populations of adult bees are going to positively influence health and production, and undoubtedly by maintaining healthy hives in which we carry out good management, we will be creating the basic conditions so that the colonies can develop correctly and reach their full potential. . To all this, we must add the environmental conditions, which will influence in one way or another on the rest of the factors.

These values allow us to establish that the population factor of the hive is not directly proportional to the weight of the hive.

Analysis of Variance for the population of the hive in the fourth week of the test

Source of Variation	G.L	Sum of squares	Squares Media	F. Cal.	P
Total	11	2.789			
Treatments	3	8.158	4.133	1.42 ns	0.3255
Repetitions	2	8.267	2.719	1,90 ns	0,2056
Error	6	1.146	1.911		
C.V.%		22.91	General average		19083

The analysis of variance (ADEVA) for the population of the hives (P), did not present statistically significant differences (ns) between the weights during the fourth week of the experiment. The general average (x) was 19083 individuals / hive and the coefficient of variation (C.V.) was 22.91%.

In this sense, it can be as important to carry out a good control of the diseases that affect bees, as having well-located apiaries, in order to maintain a positive population balance (population increase) in whose surroundings we find a wide variety of blooms and a mild climate. However, getting these locations is not always easy, and will depend on the resources that each beekeeper can count on, while in relation to disease control we do have greater decision-making capacity. Gil, S. (2016)

In relation to what was stated by Gil, S. (2016) and comparing with the data obtained regarding the population of the hives up to the fourth week of testing, it can be noted that the activity of the hives during this period did not show an increase or decrease in activity, that is, said factors cited by this author did not appear.

Separation of means according to Duncan (P <0.05) for the population of the hive in the fourth week of the test

Duncan	Medias	Treatments
A	23000	(T2) Administration of Secnidazole in Candy
A	19000	(T3) Administration of Tinidazole in Candy
A	18667	(T4) Administration of Metronidazole in Candy
A	15667	(T1) Witness (Without medication)

In the previous table you can see the separation of means according to Duncan, for the hive population variable during the fourth of the experiment, in this it is shown that the means of the mentioned treatments were not significant (ns), although the values were distributed between 23,000 and 15667 individuals / hive, with a range of 7,333 individuals / hive between extreme values.

There are various investigations that have managed to determine parameters that provide useful information on beekeeping production, which can be monitored and recorded in an automated system that indicates the occurrence of significant changes, both inside and outside the hive: the quantification of honey production , the determination of the health status, the activity level of the bees, the monitoring of available food sources, the distance between apiaries and the distribution of diseases. Valdés, P. (2014).

Valverde, C, (2013) when evaluating the introduction of queens of high genetic value "Italian bee" (Apis melífera) through three methods (transport boxes of queens, smeared in honey, dusted in flour) for genetic improvement in the Laguacoto II sector, Bolívar province; In the variable population of the hive, it reports statistically significant differences between the different methods used, being the transport box (T1) and dusted in flour (T2) methods the ones that obtained the largest population at the end of the trial with an average weight of the population of 4 kilograms.

The data reported by Valverde, C (2013) are higher than those found in the present study, this is possibly due to the genetic material introduced.

Analysis of Variance for the hive population in the eighth week of the test

Source of Variation	G.L	Sum of squares	Squares Media	F. Cal.	P
Total	11	6.289			
Treatments	3	3.103	1.034	2.44 ns	0.1625
Repetitions	2	1.181	5.908	0,94 ns	0,4253
Error	6	2.545	4.242		
	C.V.%	25.96	Average General		25083

In the analysis of variance (ADEVA) for the population of the hives (P), it did not present statistically significant differences (ns) between the weights in the eighth week of the experiment. The general average (x) was 25083 individuals / hive and the coefficient of variation (C.V.) was 25.96%.

Valverde, C (2013) when evaluating three methods of introduction of queens of high genetic value "Italian bee" (Apis melífera) concludes, at the beginning of the experiment the population decreased but with the introduction of the new queens of high genetic value began the position and therefore the recovery of the hives, according to the following order: T1 (transport box), T3 (dusted with flour) with 4 Kg, in relation to T2 (smeared with honey) and T4 (control) with 3 Kg of workers.

When comparing the data obtained in the present study with those reported by Valverde, C (2013) it can be deduced that, the production of honey and eggs go hand in hand with the existing flowering around the apiary; with greater flowering, more posture, therefore, greater birth of workers, greater production of honey within the hives.

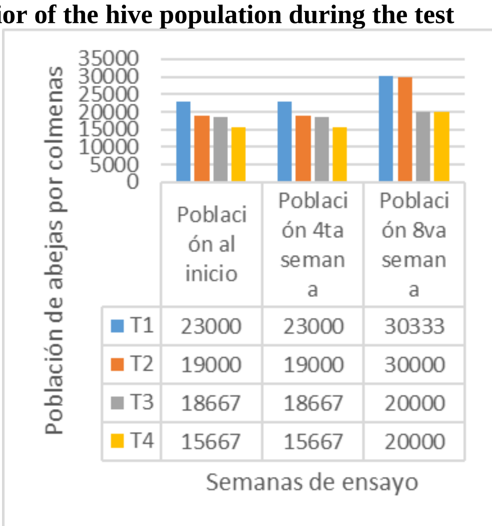
Separation of means according to Duncan (P <0.05) for the population of the hive in the eighth week of the test

Duncan	Medias	Treatment
A	30333	Witness (Without medication) / (T1)
A	30000	Administration of Tinidazole in Candy / (T3)
A	20000	Administration of Secnidazole in Candy / (T2)
A	20000	Administration of Metronidazole in Candy / (T4)

According to the previous table, the separation of means according to Duncan can be seen, for the hive population variable during the eighth of the experiment, which shows that the means of the mentioned treatments were not significant (ns), although the values were distributed between 30333 and 20000 individuals / hive, with a range of 10333 individuals / hive between the extreme values.

Valverde, C (2013) when performing the analysis of variance and Duncan's test (5%) with regard to the population of the hive found a total relationship between the rest of variables such as egg production, larvae, capped cells, production This means that the higher the production of eggs, the higher the births and consequently the higher the population.

Behavior of the hive population during the test



Graph 2 shows the values of the means obtained according to Duncan during the test; being (T1) the one that presented the best population averages with an average of 23,000 to

30,333 individuals / hive during the trial, followed by T2 and T3 respectively the treatments with average values and finally T4 the treatment with lower population / hive averages .

The values found in the present research are closely related to what was indicated by Mace, H. (2011), who proposes that the relationship between the hive population and honey production is closely related.

This author mentions that approximately a hive with 30,000 individuals (3kg) would be capable of producing 9 kilograms of honey. In conclusion, it is estimated that arithmetically the production of honey is exponential based on the population of the hive.

In this same aspect Reyes, C. (2011) states that honey production and the population of the hive grows based on the availability of food, said author states that a foraging worker bee visits an average of 90 flowers daily.

Ruttner, F. (2012) maintains that the ratio of the number of adult bees to the number of offspring decreases with the increase in the size of the colony population. A large hive can have a ratio of 1 adult bee per larva, while a small hive has a ratio of 2 larvae per adult bee.

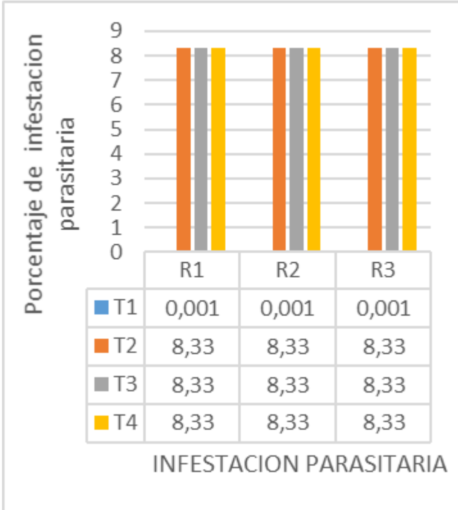
In this case we can infer that the growing hive behaves as Strategist R, once it reaches population equilibrium it behaves as Strategist K. This type of selection through which a hive transits in the season is the explanation for the high rate of reproduction or swarming of Africanized Bees that constantly maintain their swarms / hives in a juvenile state.

Parasitic Infestation (PI)

Experimental results for the variable parasitic infestation.

Treatments	Repetitions	Infested hive	%
1	1	-	0.00
2	1	+	8.33
3	1	+	8.33
4	1	+	8.33
1	2	-	0.00
2	2	+	8.33
3	2	+	8.33
4	2	+	8.33
1	3	-	0.00
2	3	+	8.33
3	3	+	8.33
4	3	+	8.33
Total	12	9	75.00

When analyzing the previous table, we can observe the distribution of the parasite infestation obtained in the investigation, these results were obtained by means of laboratory diagnosis. The same ones that were carried out in the analysis laboratory of the FCA-UEB.



Graph 3: Distribution of the parasitic infestation

Graph 3 shows the percentages of parasitic infestation according to each repetition, the percentage being similar for T2, T3 and T4 with 25%; while T1 did not show any infestation.

Bees, like all animals including man, are sensitive to bacteria, viruses and parasites. Their resistance to adverse factors is greater if they are in optimal health and nutrition. Environmental challenges, including chemicals used to protect crops from insects and weeds, can have detrimental effects on the health of bees, particularly if they host pathogens (OIE, 2016).

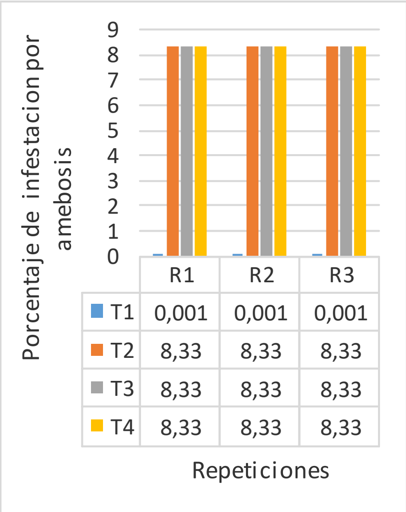
Type of Parasite (TP)

Experimental results for the variable Type of parasite.

Treatments	Repetitions	TP	%
1	1	Nn	0.00
2	1	Amebas	8.33
3	1	Amebas	8.33
4	1	Amebas	8.33
1	2	Nn	0.00
2	2	Amebas	8.33
3	2	Amebas	8.33
4	2	Amebas	8.33
1	3	Nn	0.00
2	3	Amebas	8.33
3	3	Amebas	8.33
4	3	Amebas	8.33
Total	12	9	75.00

In the table that precedes, we can observe the distribution of the infestation by amoebae (amoebiasis) obtained in the investigation, these results were obtained by means of laboratory diagnosis. The same ones that were carried out in the analysis laboratory of the FCA-UEB.

At the laboratory level, the bees were dissected to obtain the abdominal content, which once collected was directly observed under the microscope in order to determine the presence or not of gastrointestinal parasites.



Graph 4: Distribution of amebiosis

The results were positive for the infestation of the gastrointestinal parasite Malpighamoeba mellificae, observing cysts through the walls of the Malpighi tubules with a 40X light microscope. This infestation represented to be in 75% of the hives studied (T2, T3 and T4).

Mace, H. (2011) indicates, Amebiasis or Amebosis, is a parasitic disease of the Malpighi tubules of adult bees, caused by the protozoan Malpighamoeba mellificae Prell. The disease is contagious and its severity is still debated; most authors do not consider it important. Maassen in 1916 in Germany was the first to observe the parasite. In 1926 Prell described and classified the protozoan.

Malpighamoeba mellifícae Prell, is a microscopic parasite of the Phylum of Protozoa and of the order of Sardines that is characterized by the formation of cysts as resistance stages. The parasites are extracellular and feed on pseudopods, although it seems that they also have flagella that help them reach the Malpighi tubules. The cysts are round in shape and are 5 to 8 microns in diameter. The cysts survive for more than 6 months in the feces of bees on the combs, but are susceptible to common disinfectants. (Herrero, F. 2014)

The disease is widely spread in Europe, Oceania, and America. Amebiasis is almost exclusive to worker bees, since it is very difficult for the queen and the drones to become infected. The source of contagion and the transmission mechanisms, as well as the factors that favor the development of the disease, are virtually the same as those of Nosemiasis (Le Conte, Y. 2013).

The life cycle of Malpighamoeba mellificae lasts between 22 and 24 days and its initial and final stages are made up of its form of resistance and dissemination, which is the cyst. Once ingested, the cysts reach the ventricle of the bee, where the gastric juices favor their germination and release of the vegetative form, which occurs at the level of the pylorus where a lot of solid matter from food accumulates. This solid matter acts as a "plug", causing the parasites to migrate into the Malpighi tubules which flow into the pylorus. Once in the Malpighi tubules, the protozoa acquire their amoeboid shape, attach to the epithelium, and begin to feed with the help of their pseudopods (Mace, H. 2011).

The parasites multiply by binary fission and after 3 to 4 weeks, many epithelial cells in the tubules have already been destroyed and have released the parasite cysts. The cysts can infect other cells or pass into the intestine and then into the rectum to be excreted with the stool. (Álvarez, J. 2007)

These authors systematically describe the disease of beekeeping amebiasis, but it should be noted that the presence of the disease has not yet been described in the country or there are no technical reports that evidence the attack of the disease until the present study.

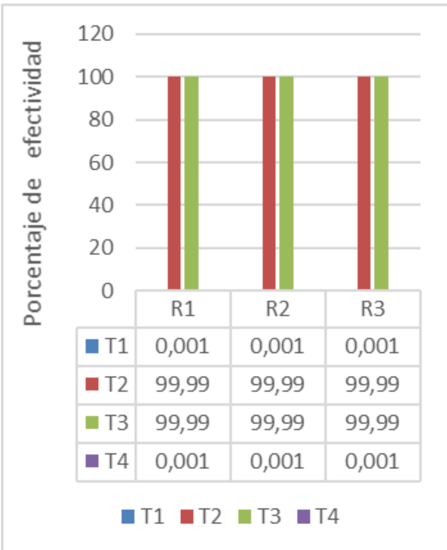
Effectiveness of Treatment (ETT)

Experimental results of treatment effectiveness.

Treatments	Repetitions	Effectiveness	Drugs	Percentage (%)
1	1	Nn	S.F	0
2	1	Cash	Secnidazole	99.99
3	1	Cash	Tinidazol	99.99
4	1	No Cash	Metronidazol	0
1	2	Nn	S.F	0
2	2	Cash	Senidazol	99.99
3	2	Cash	Tinidazol	99.99
4	2	No Cash	metronidazol	0
1	3	Nn	S.F	0
2	3	Cash	Secnidazole	99.99
3	3	Cash	Tinidazol	99.99
4	3	No Cash	Metronidazol	0
Total	12			

When analyzing the previous table we can say that the effectiveness of the different drugs used in the investigation for the control of amoebiasis, these results were obtained by means of laboratory diagnosis. The same ones that were carried out in the analysis laboratory of the FCA-UEB.

Melgar, O. (2012) when evaluating the antiparasitic effect of three infusions of jacaranda flower (Jacaranda mimosifolia Prell) in the control of amoebas and nosemas that affect beekeeping in Chiquimullilla, Santa Rosa - Guatemala; I determine that the infusion at a rate of 25 grams / liter of water managed to control amoebiosis 100%.



Graph 5: Effectiveness of the different treatments

Graph 5 shows the effectiveness of the drugs used in the trial to control bee amoebosis T2 (Secnidazole 7.14 mg / kg), T3 (Tinidazole 7.14 mg / kg) and T4 (Metronidazole 7.14 mg / kg). Resulting effective in 99.99% Secnidazole and Tinidazole; while metronidazole was ineffective in controlling amoeba infestation.

Jean-Prost, P. (2013) mentions, there are no chemicals to treat amoebiasis, but sulfa drugs have a certain action on the parasite. The use of acetic acid fumigations as in Nosemiasis, has proven to be very effective in the decontamination of combs.

Secnidazole is structurally related to other nitroimidazoles such as metronidazole and tinidazole. These drugs share a common spectrum of activity against anaerobic microorganisms, it seems that Secnidazole is especially effective in the treatment of

Amoebiasis, giardiasis, trichomoniasis and bacterial vaginosis. (Genfar Laboratories, 2015)

Tinidazole is a drug derived from nitroimidazole used as an antiparasitic agent, approved for protozoal infections such as trichomoniasis, amebiasis and giardiasis. It has also been used to treat or prevent a variety of bacterial infections, including Helicobacter pylori. Tinidazole is widely distributed in Europe and developing countries because of its similarity to metronidazole, a drug used as the first line of treatment for amoebiasis although with unpleasant side effects. (Mensa, J. 2008)

Melgar, O. (2012) concludes that the best treatment for the control of amebiasis and nosemiasis in bees (Apis mellifera) was the infusion of 25 grams of dehydrated flower in one liter of water. Under the conditions in which the research was carried out and with the use of dehydrated jacaranda flower infusions, the population of amoebae and nosemas is totally controlled (100%) in the bee hive (Apis mellifera).

These authors systematically describe the antiprotozoal drugs used in the control of amebiasis, but there are no studies on the use of these drugs in the control of amebiasis in bees.

4. Discussion

The average weight of the hives (PC) at the beginning and the fourth week of the trial were 24,625 kilograms, while, said average weight at the eighth of investigation amounted to 28,333 kilograms, these values did not report statistically significant differences (ns) between the treatments for the separation of means according to Duncan ($P < 0.05\%$) although numerically there was an increase of around 4 kg in the weeks after the pharmacological treatment established to control amoebiasis, which allows to conclude that said parasitosis produced the stagnation of the population from the hive at the start.

Regarding the population of the hive (P) established by the Farrar method, it was determined that both the number of individuals / initial hive and after four weeks of experiment had a similar number of individuals, the average being 19083 individuals / hive on average; while in the eighth week the average was 25083 individuals / hive; None of these values reported statistically significant differences (ns) between the treatments for the separation of means according to Duncan ($P < 0.05\%$), but, as happened with the variable PC, the latter was also favored once the hives were treated against amebiasis.

When carrying out a projection of the honey harvest, applying Farrar's rule, it was possible to determine that at the eighth week of the experiment a honey production of 6.86 kg of honey is expected on average, with a total of honey collected of 82.38 kg.

Parasitic infestation (PI) in the trial had a percentage equivalent to 75%, with T2, T3 and T4 being the treatments that showed a degree of parasite infestation.

Regarding the type of parasite (TP), it was possible to diagnose the presence of the unicellular parasite (Malpighamoebamellifiae) known as bee amebiasis, managing to be found in T2, T3 and T4.

Regarding the inherent effectiveness of the drugs used in the treatment of amebiasis (ETT), it was found that the Metronidazole (T4) in medicated Candy was not effective in the treatment of bee amebiasis; while Tinidazole (T3) and Secnidazole (T2) were 99.9% effective to control amebiasis in bees.

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