



Health evaluation of Africanized *Apis mellifera* in the province of Pastaza

Evaluación sanitaria de *Apis mellifera* africanizada en la provincia de Pastaza

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Abstract

Approximately 35% of all food produced depends on biological pollination and 90% of this pollination involves *Apis mellifera*. This species is vital for agricultural pollination, but faces threats such as pests, diseases and climate change that have increased colony mortality. The objective of the work was to carry out a sanitary assessment of *A. mellifera* in the province of Pastaza. The methodological approach was observational and descriptive using stratified sampling in active hives to evaluate the presence of diseases such as american and european foulbrood, and the Varroa destructor mite. For *V. destructor*, the “jar test” and bacteriological cultures for the foulbrood were used. The results revealed that no cases of american or european foulbrood were detected in the hives, showing that environmental conditions in Pastaza could be less favorable for these pathogens. However, the prevalence of *V. destructor* was significant, affecting 34.21% of the evaluated hives, with some exceeding the critical threshold of 5% infestation, which represents a considerable risk to the health of the colonies. These results show the urgent need to implement integrated beekeeping management strategies to control *V. destructor* infestation and prevent colony collapse. In conclusion, the absence of foulbrood is a positive finding, and the high prevalence of *V. destructor* shows the importance of improving beekeeping management practices in Pastaza to ensure the long-term sustainability of beekeeping in the region.

Keywords: beekeeping, diseases, infestation, pollination, sustainability.

Resumen

Aproximadamente el 35% de todos los alimentos producidos depende de la polinización biológica y el 90% de esta polinización involucra a *Apis mellifera*. Esta especie es vital para la polinización agrícola, pero enfrenta amenazas como plagas, enfermedades y cambio climático que han aumentado la mortalidad de colonias. El objetivo del trabajo fue realizar una evaluación sanitaria de la *A. mellifera* en la provincia de Pastaza. El enfoque metodológico fue observacional y descriptivo empleando muestreo estratificado en colmenas activas para evaluar la presencia de enfermedades como loque americana y europea, y el ácaro Varroa destructor. Para *V. destructor*, se utilizó la “prueba de frasco” y cultivos bacteriológicos para las loques. Los resultados revelaron que no se detectaron casos de loque americano ni europeo en las colmenas, mostrando que las condiciones ambientales en Pastaza podrían ser menos favorables para estos patógenos. Sin embargo, la prevalencia de *V. destructor* fue significativa afectando al 34,21% de las colmenas evaluadas con algunas superando el umbral crítico del 5% de infestación, lo que representa un riesgo considerable para la salud de las colonias. Estos resultados muestran la necesidad urgente de implementar estrategias de manejo apícola integradas para controlar la infestación por *V. destructor* y prevenir el colapso de las colonias. En conclusión, la ausencia de loques es un hallazgo positivo, la alta prevalencia de *V. destructor* evidencia la importancia de mejorar las prácticas de manejo apícola en Pastaza para asegurar la sostenibilidad a largo plazo de la apicultura en la región.

Palabras clave: apicultura, enfermedades, infestación, polinización, sostenibilidad.



Introduction

Worldwide, the Western honeybee *Apis mellifera* is the primary pollinator in agriculture (Requier et al., 2019). Approximately 35% of all food produced depends on biological pollination, and 90% of that pollination involves *A. mellifera* (Chagas et al., 2019). Furthermore, the economic contribution of pollinators accounts for about 30% of the total annual agricultural income from pollination-dependent crops, equivalent to nearly 12 billion USD out of a total of approximately 45 billion USD (Giannini et al., 2015).

According to the Brazilian Institute of Geography and Statistics (IBGE), the state of Rio Grande do Sul (RS), located in southern Brazil, produced 6.42 thousand tons of honey in 2018 (Marcolin et al., 2021). In Ecuador, beekeeping productivity averages 10.2 kilograms of honey per hive per year. Moreover, in the provinces of Tungurahua and Chimborazo, experienced producers achieve an average of 25.08 kilograms per hive (Masaquiza-Moposita et al., 2023). In the Amazon region, particularly in Pastaza, honey production is growing, although exact data on current production volume is not yet available.

In recent years, mortality rates in *A. mellifera* colonies have increased, affecting wild populations and essential plants for ecosystems, mainly plagues, climate change, habitat degradation, agrochemicals and nutritional issues (Akongte et al., 2023). Moreover, they faced pathogens such as viruses, parasites, bacteria, fungi and lack of quality foods. The main threat for bee colonies is colony collapse disorder (CCD), which is aggravated by a deficient management *Varroa destructor* infections, alongside diseases like the American foulbrood caused by the *Paenibacillus larvae*, and its European counterpart caused by the *Melissococcus plutonius* (Osterman et al., 2021). These pathogens are easily transmitted due to the social behavior of bees in large colonies, shortening their life-spans and causing a gradual colony collapse (Nekoei et al., 2023). The contagion may be vertical, from the queen to her descendants, or horizontal, between bees and trophallaxis, which accelerates disease transmission (Hernández-Fuentes et al., 2021).

The sanitary evaluation of Africanized *A. mellifera* in the province of Pastaza is essential due to its critical role in the

plant-pollinator relations, promoting biodiversity in the land. Bee health is crucial for preventing colony loss caused by disease and parasites like *V. destructor*, and, in turn, ensuring productivity. Currently, there are no breeding programs in the region that promote resistance to parasites, which is essential for maintaining healthy colonies and minimizing reliance on chemical treatments. Therefore, the implementation of selection methods for resistance traits is necessary (Büchler et al., 2020).

The reproductive health of queens and the viability of drones were affected by environmental factors and internal hive conditions, highlighting the need to understand and mitigate these factors to ensure colony stability and productivity (Rangel and Fisher, 2019).

Varroa mite control was addressed through a combination of mechanical, chemical, and biological methods. Authors such as Jack and Ellis (2021) described three mechanical techniques. The first involved drone brood removal, which consists of removing brood frames before emergence to reduce mite populations. The second method used screened bottom boards that allow mites to fall to the ground, preventing reinfestation. Lastly, the powdered sugar dusting technique stimulates grooming behavior in bees, causing mites to fall to the bottom of the hive. While these methods can be effective, they must be combined with others to achieve comprehensive control.

In the chemical domain, Devi et al. (2019) highlighted the use of mild acids, such as formic acid, which is effective against reproducing mites due to its ability to penetrate wax cells, and oxalic acid, which is useful during broodless periods, although it does not penetrate sealed cells. The use of essential oils—especially thyme oil—is also popular, although its efficacy is variable and often needs to be complemented with other treatments (Warner et al., 2024). Finally, Reinbacher et al. (2018) emphasized biological methods, such as the use of entomopathogenic fungi, which have shown high effectiveness against the mite, and natural predators like pseudoscorpions, although the latter are still in the experimental phase.

Based on the above premises, the objective was to carry out a sanitary evaluation of *A. mellifera* in the province of Pastaza.

This analysis focused on identifying the infestation level of *V. destructor* and the presence of *P. larvae* and in the region's beekeeping production.

Materials and methods

Location

The province of Pastaza, located in the Amazon region of Ecuador, covers an area of 29,773 km², with Puyo as its capital. Characterized by a humid tropical climate, the average annual temperature ranges between 23 and 26 °C, with annual rainfall exceeding 3,000 mm (Franco et al., 2024). This region, rich in biodiversity and vast tropical forests, provides ideal conditions for beekeeping. The study focused on the province of Pastaza and its surrounding areas, evaluating environmental conditions and their impact on bee health (figure 1).

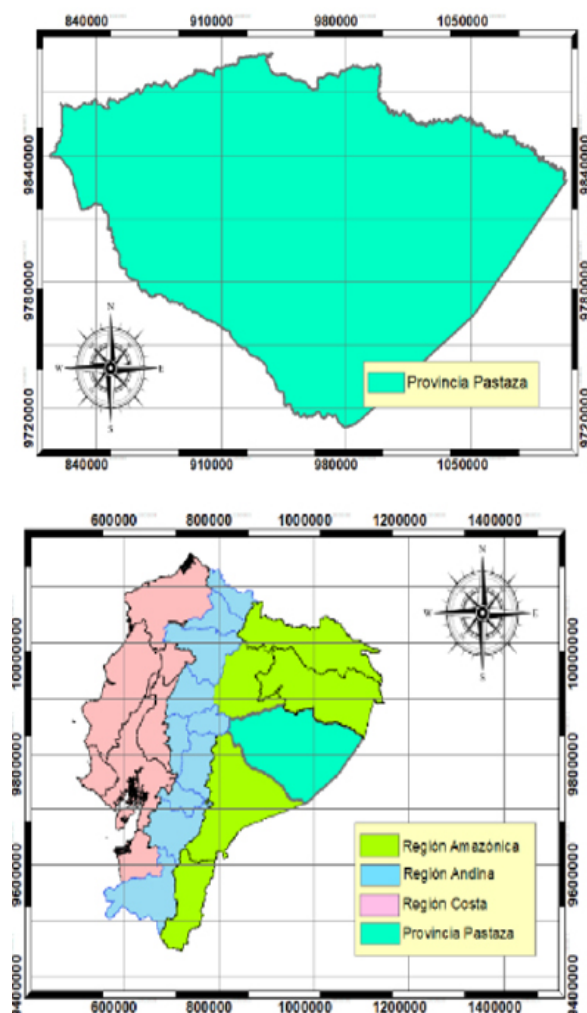


Figure 1. Geography location of the study.

Research approach

This study employed an observational and descriptive design with a non-experimental, cross-sectional approach to assess the health of *A. mellifera* colonies in Pastaza. Using stratified sampling and data collection at a single point in time, the prevalence of diseases and parasites was determined without direct manipulation of variables. The quantitative approach enabled objective measurement of pathogen and ectoparasite incidence within the hives.

Sampling methodology

To carry out the sampling, a database of *A. mellifera* hives in the province of Pastaza was used, compiled from official records and direct field observations. Upon initiating the search for hives, it was found that some were inactive, which led to focusing the study exclusively on those that were active and relevant to the research. Among the selected hives were those certified by the Agency for Phytosanitary and Zoonosanitary Regulation and Control (AGROCALIDAD), as well as others that, although not certified, presented appropriate conditions for sanitary evaluation.

These hives, located in the province of Pastaza and its surrounding areas, belong to local beekeepers who maintain *A. mellifera* colonies, providing a broader perspective on the prevalence of diseases such as American foulbrood, European foulbrood, and *V. destructor* infestation.

To assess *V. destructor*, the “jar test” method proposed by De Jong et al. (1982) was used. This method involved sliding a wide-mouthed jar beneath brood frames to collect approximately 200 bee specimens. Then, 70% alcohol was added to the jar, which was sealed and shaken for 5 minutes. The contents were subsequently poured through a double-sieve system: the upper sieve retained the bees, while the lower sieve captured the mites.

In cases where signs or symptoms suggestive of American foulbrood were observed—such as dead brood with a fishy odor—all suspected indicators mentioned by Alvarado et al. (2012) were recorded. Standard bacteriological methods were used, including the collection of suspicious larvae in a sterile transport medium, followed by culturing on selective media such as blood agar for the identification of *P. larvae*, the causative agent of the disease. Identification was further supported by biochemical and serological tests to confirm the presence of the bacterium (Kušar et al., 2021).

In the case of European foulbrood, a similar procedure was followed by collecting samples of suspicious larvae from affected hives. These samples were cultured on blood agar to

identify the presence of *M. plutonius*, the etiological agent. As with American foulbrood, biochemical and molecular tests were conducted to ensure accurate identification of the pathogen (Grossar et al., 2023).

Sample collection

For sample collection, following the procedure of De Jong et al. (1982), approximately 200 bees were captured per sample, focusing on the central frames containing open brood. These frames were particularly important, as they are the primary site for the development of ectoparasites such as *V. destructor* and the bacteria responsible for American and European foulbrood. Moreover, this area is where nurse bees are more vulnerable to infestations due to their involvement in brood care.

In the case of American and European foulbrood, no sampling was performed since the colonies showed no signs or symptoms consistent with these diseases, as outlined by Alvarado et al. (2012).

The sampling process began with the identification and labeling of hives using visible tags, recording the location, hive number, and corresponding sample for each. In addition, a representative sample was taken based on hive location, as shown in table 1. This information was essential to ensure proper tracking of samples throughout the analysis process

Table 1. Research zones of hives.

Beekeeper	Number of hives	Samples	Location
1	10	6	Unión Base
2	1	1	Vía Puyo-Tena km 102
3	30	17	Madre Tierra. Shell.
4	6	6	Tarqui. Madre Tierra.
5	4	3	Tarqui.
6	6	5	Madre Tierra. Shell.

To calm the bees, a smoker was used, applying smoke at the hive entrance for about 2 minutes (figure 2). The hive lid was then removed using a J-type hive tool, and additional smoke was applied to minimize bee flight. A frame lifter was used to extract a corner frame first, facilitating access to the central frames.

Once the central frames were identified, they were removed one by one and held in a vertical position. A wide-mouthed jar was gently slid from the top to the bottom of each frame to capture bees inside the container. This procedure was

repeated on both sides of each frame and similarly applied to the remaining four central frames.



Figure 2. Sample collection in the study area.

Sample processing

The processing of the collected samples was carried out using a double-sieve system. First, each jar containing the bees along with potential ectoparasites and pathogens such as *V. destructor* and the agents responsible for American and European foulbrood was carefully opened, and its contents were poured into the sieves. To ensure that all ectoparasites and bacteria were dislodged from the bees, 70% alcohol was poured over the bees in the sieves multiple times.

The upper sieve retained the bees, while the finer lower sieve captured ectoparasites and other pathogens. These parasites and bacteria were then transferred to separate jars, each properly labeled to identify the hive and its location of origin. A magnifying lens was subsequently used for a preliminary examination of the ectoparasites, and bacteriological cultures were performed to identify *P. larvae* (American foulbrood) and *M. plutonius* (European foulbrood).

The entire process was carried out under strict safety measures, including the use of gloves and a clean, disinfected environment. Once the ectoparasites and bacteria were separated, identified, and labeled, a detailed analysis was conducted.

Finally, the percentage of *Varroa* infestation in adult bees (%VIA) was calculated for each selected hive using the formula proposed by De Jong et al. (1982), which can also be applied to calculate infestation rates of other parasites. A %VIA equal to or greater than 5% was considered positive for varroosis, while a percentage below 5% was considered negative (Gregorc et al., 2018). To conclude this process, the prevalence of ectoparasite-induced diseases diagnosed in the apiaries was calculated using the following formula (2) (Vargas Hidalgo et al., 2024).

$$\%IVA = \left(\frac{\text{Number of mites}}{\text{Number of bees in the sample}} \right) * 100 \quad (1)$$

$$\text{Average infestation per beekeeper} = \left(\frac{\sum \%IVA}{\text{Number of hives}} \right) \quad (2)$$

Results and discussion

American foulbrood in hives in Pastaza

No presence of American foulbrood (*P. larvae*) was detected in any of the hives evaluated in the province of Pastaza. This finding is particularly significant given that American foulbrood is one of the most devastating diseases that can affect *A. mellifera* colonies.

One of the main reasons for the absence of American foulbrood is the province's environmental context. Pastaza, characterized by its humid tropical climate and high biodiversity, provides a less favorable environment for the spread of this pathogen compared to more temperate regions. Environmental conditions such as high humidity and the abundance of diverse flora create natural barriers that limit the proliferation of *P. larvae* (Wilhelm et al., 2023). This setting, in contrast to areas where American foulbrood is more common, highlights the influence of ecological factors on the occurrence of the disease.

Moreover, this result aligns with studies showing that the incidence of American foulbrood can vary significantly depending on region and climate. Authors such as Rowland et al. (2021) demonstrated that in areas with more temperate climates and lower floral diversity—such as certain regions of Europe and North America—the disease was more prevalent.

European foulbrood in hives in Pastaza

No presence of European foulbrood (*M. plutonius*) was detected in the hives evaluated in the province of Pastaza. Although this disease is less devastating than American foulbrood, it can still cause serious problems in colonies if not properly managed, primarily affecting larvae and causing their death before completing development.

The absence of European foulbrood in Pastaza was associated with similar factors to those influencing the absence of American foulbrood, such as the region's humid tropical climate and high biodiversity. These conditions may not be favorable for the proliferation of *M. plutonius*, as this bacterium tends to thrive in more temperate climates and under specific beekeeping management conditions that do not appear to be prevalent in Pastaza.

Studies have shown that the prevalence of European foulbrood is higher in regions with temperate climates, where conditions for the development of the disease are more optimal (Alburaki et al., 2024).

In addition, hive health management in the region may be contributing to the prevention of this disease. It is possible that the beekeeping practices implemented by apiculturists in Pastaza—such as regular frame replacement and the removal of contaminated materials—are helping to maintain colony health and prevent the emergence of this disease.

Varroa destructor in hives in Pastaza

In the hives evaluated from Beekeeper 1, hive number 6 showed a *V. destructor* infestation rate of 7.33%, exceeding the critical threshold of 5%, which indicates a significant risk to colony health (Table 2). This level of infestation highlights the need for urgent intervention to prevent the colony's deterioration. The remaining hives exhibited lower infestation levels, ranging from 0 to 4.17%, suggesting that while immediate action is not required, close monitoring is essential. These results underscore the importance of implementing beekeeping management strategies to control *V. destructor*, as even low-level infestations can have cumulative negative effects over time. When compared to global studies, it becomes evident that proper management of *V. destructor* is crucial for maintaining hive health and productivity (Büchler et al., 2020).

Table 2. *Varroa* infestation in Beekeeper 1.

Beekeeper 1				
Number of hives	Number of analyzed bees	Number of parasites	% of infestation	Infestation
1	284	9	3.16	None
2	160	3	1.87	None
3	106	2	1.88	None
4	407	17	4.17	None
5	106	0	0.00	None
6	300	22	7.33	Yes

In the hive evaluated from Beekeeper 2, where 225 bees were analyzed, only one *V. destructor* parasite was identified, indicating an extremely low infestation rate of 0.44%. This level of infestation was negligible and did not pose an immediate risk to colony health. The absence of significant infestation suggested effective beekeeping management or environmental conditions that did not favor the proliferation of the parasite. Compared to generally accepted standards, an infestation level below 1% is considered safe and does not require intervention. However, it is important to maintain regular monitoring to ensure that this low infestation rate remains stable over time, as any increase could compromise the health of the colony if left unchecked.

In the hives evaluated from Beekeeper 3, a significant variability in *V. destructor* infestation levels was observed. All 17 hives



analyzed exhibited infestation rates above the critical threshold of 5%, indicating a considerable risk to colony health. Notably, Hive 2, with an infestation rate of 9.63%, and Hive 9, with 8.00%, were in particularly serious condition and required immediate intervention (table 3).

Table 3. *V. destructor* in Beekeeper 3.

Beekeeper 3				
Number of hives	Number of analyzed bees	Number of parasites	% of infestation	Infestation
1	175	3	1.71	None
2	249	24	9.63	Yes
3	155	10	6.45	Yes
4	226	11	4.86	None
5	186	5	2.68	None
6	203	7	3.44	None
7	277	7	2.52	None
8	170	9	5.29	Yes
9	250	20	8.00	Yes
10	279	12	4.30	None
11	159	8	5.03	Yes
12	216	13	6.01	Yes
13	137	9	6.56	Yes
14	187	12	6.41	Yes
15	203	7	3.44	None
16	179	12	6.70	Yes
17	286	7	2.44	None

Studies have shown that infestation levels above 5% can lead to a significant decline in bee populations, negatively impacting their productivity and increasing their susceptibility to other diseases (Guichard et al., 2020).

When compared with previous research, these findings reinforce the importance of implementing proper beekeeping management and preventive treatments to keep infestation levels under control. Effective control of *V. destructor* is essential to maintaining the long-term health and viability of colonies, as highlighted by recent studies (Mondet et al., 2020).

In the hives of Beekeeper 4, all colonies exhibited *V. destructor* infestation levels below the critical threshold of 5%, indicating good colony health. With infestation rates ranging from 0 to 3.44%, no immediate risks to the bees were identified, demonstrating that the beekeeping management practices were

effective in keeping the parasite's proliferation under control (table 4). Specifically, two hives showed 0% infestation, reflecting successful prevention of *V. destructor*. The presence of minimal infestation in the other hives, while not critical, highlighted the need for continued regular monitoring to ensure these levels do not increase (DeGrandi-Hoffman et al., 2016).

Table 4. *V. destructor* infestation in Beekeeper 4.

Beekeeper 4				
Number of hives	Number of analyzed bees	Number of parasites	% of infestation	Infestation
1	139	1	0.71	None
3	145	5	3.44	None
4	202	0	0.00	None
4	115	2	1.73	None
5	193	1	0.51	None
6	175	0	0.00	None

In the hives of Beekeeper 5, two colonies exhibited critical levels of *Varroa destructor* infestation. Hive 1 showed a high infestation rate of 10.06%, while Hive 3 reached 5.30%, both indicating an urgent need for intervention to prevent colony loss. These figures revealed that the parasite had seriously impacted these hives, threatening their long-term viability. In contrast, Hive 2 presented an infestation level of 4.81%, slightly below the critical threshold. Although it did not require immediate intervention, it should be closely monitored to prevent a rise in infestation levels (table 5). Authors such as Alburaki et al. (2024) have emphasized the need for effective control strategies to manage *V. destructor* and ensure the continued health of bee colonies.

Table 5. *Varroa* infestation in Beekeeper 5.

Beekeeper 5				
Number of hives	Number of analyzed bees	Number of parasites	% of infestation	Infestation
1	159	16	10.06	Yes
2	166	8	4.81	None
3	226	12	5.30	Yes

In the hives evaluated from Beekeeper 6, only one colony showed a *V. destructor* infestation level above the critical threshold of 5%, with a rate of 6.66%, indicating a significant risk to the health of that colony. This hive required immediate attention to

prevent further deterioration. The remaining hives exhibited low infestation levels, ranging from 0.56% to 4.22%, demonstrating that the management practices in place were effective in keeping the parasite under control (table 6). However, the case of Hive 4 highlighted the importance of continuous monitoring and timely intervention when dangerous infestation levels are detected. These findings are consistent with studies that emphasize the need for constant surveillance to prevent infestations from rising above critical levels that could compromise colony viability (Wagoner et al., 2019).

Tabla 6. *Varroa* infestation in Beekeeper 6.

Beekeeper 6				
Number of hives	Number of analyzed bees	Number of parasites	% of infestation	Infestation
1	142	6	4.22	None
3	176	1	0.56	None
4	90	6	6.66	Yes
4	146	2	1.36	None
5	140	4	2.85	None

Infestation level of *Varroa destructor*

The average *V. destructor* infestation across the hives of the different beekeepers showed clear variability, reflecting differences in management practices and the environmental conditions of each apiary. Notably, Beekeeper 5 faced a critical situation, with an average infestation rate of 6.73%, indicating that a significant proportion of their colonies were severely affected, as shown in figure 3. This high infestation level suggested potential shortcomings in parasite control and highlighted that the current management practices were not sufficiently effective, as noted by Leclercq et al. (2018).

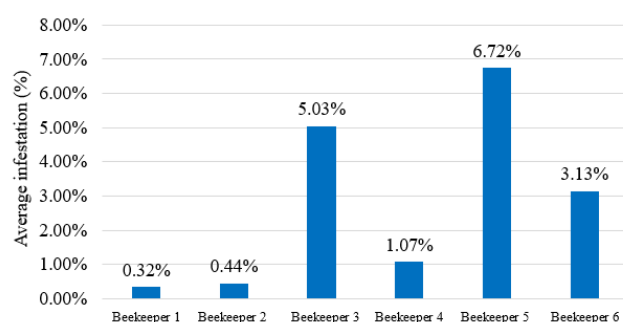


Figure 2. Level of infestation in hives.

In comparison, Beekeepers 1 and 2, registered infestation averages of 0.32% y 0.44%, due to a more controlled beekeeping process and being in an environment less prone for *V. destructor* proliferation. The low level of infestation in the hives could be related with the implementation of more rigorous prevention

techniques, for example, constant monitoring and timely techniques.

Beekeeper 3, with an infestation level of 5.03%, was in an area with considerable hive infestation, which is certainly a worrying situation. This result indicated that, although certain practices may be ongoing, they were not strong enough to control the spread of the parasite. Beekeeper 4, on the other hand, showed a low infestation level of 1.06% and relatively more efficient process.

Finally, Beekeeper 6, with an average infestation rate of 3.13%, managed to keep infestation levels within a moderate range but still needs to improve control strategies to prevent further spread. According to Büchler et al. (2020), these results underscore the importance of a proactive approach to hive management, where prevention and regular monitoring are essential to ensuring the long-term health and productivity of colonies.

A total of 38 hives were identified in the province of Pastaza, of which 13 showed signs of *V. destructor* infestation. This resulted in an overall average infestation rate of 2.75% for the province, a value that indicates moderate colony health in the region. According to Traynor et al. (2020), high infestation prevalence can lead to a significant decrease in hive productivity and an increased risk of colony collapse, particularly in regions where beekeepers face environmental and management challenges.

According to Rosenkranz et al. (2010), the implementation of integrated control methods—including the strategic use of acaricides, biological control techniques, and improvements in management practices—was crucial for reducing *V. destructor* infestation and enhancing overall hive health. Moreover, constant monitoring and early detection of infestations were essential to prevent prevalence levels from rising to critical thresholds that could endanger colony viability. The adoption of a more proactive and multifaceted approach to *V. destructor* management contributed to the reduction of observed prevalence and ensured the long-term sustainability of beekeeping in the Pastaza region.

Conclusion

The health assessment of *Apis mellifera* in the province of Pastaza reveals mixed results for local beekeeping. On the one hand, no cases of American or European foulbrood were detected in the hives, suggesting that environmental conditions and beekeeping practices in the region are helping to prevent these devastating diseases. On the other hand, the situation regarding *V. destructor* is concerning. In some hives, infestation levels alarmingly exceeded the critical threshold of 5%, reaching up to 10.06% in certain cases. This high infestation rate indicates a significant risk of colony collapse if urgent interventions are not implemented. Additionally, the overall average infestation rate in the province was 2.75%, a figure that indicates moderate sanitary conditions in the region.

Conflict of interest

The authors declare no conflicts of interest at any stage of the present publication.

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Declaration of contributio according to CRediT taxonomy

Jaime Andrés Chamba Tivan: study conceptualization, methodological procedures, writing-original draft. **Danilo Reni Vinocunga-Pillajo:** formal analysis, writing-original draft, writing-review and editing.