Colegio de Postgraduados

Automated micro-injection system for *in-ovo* vaccination on day 18: design, validation, and comparison with conventional methods

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ABSTRACT

Objective: To evaluate an automatic *in ovo* injection device based on a low-cost Cartesian robot.

Design/Methodology/Approach: The prototype integrates a motor-driven pressurized syringe, a graphical user interface for selecting injection volumes, and a laser photo-interrupter sensor that ensures needle centering and height relative to the egg.

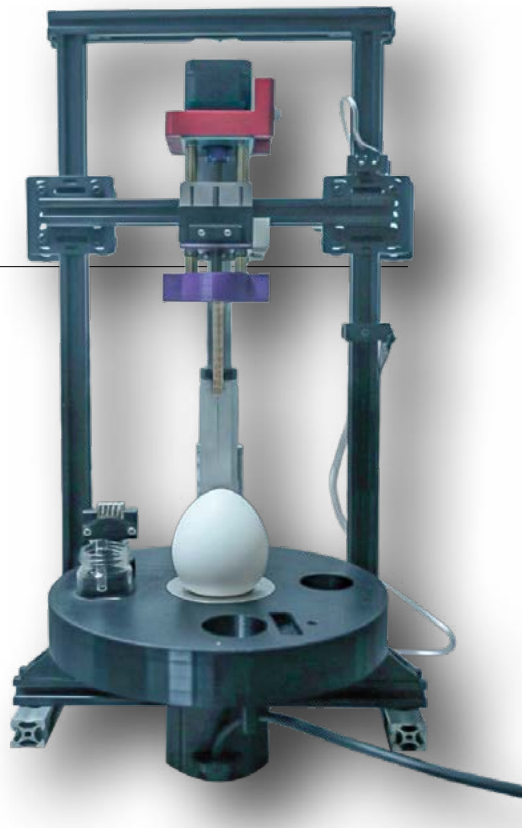
Results: The device underwent 50 injection trials using saline solution and was compared with manual techniques, showing a significant reduction in variability of the administered volume compared to manual methods, thereby improving injection accuracy, operational efficiency, and overall prototype performance.

Limitations/Implications: The device still requires validation under real farm conditions, testing in high-demand environments, and enhancements such as the incorporation of multiple syringes and integration with conveyor or incubator systems.

Findings/Conclusions: The automated device represents a significant advancement in the automation of *in ovo* vaccination, providing a viable and low-cost alternative for small- and medium-scale poultry producers. Although certain technical and validation challenges remain, the proposed system offers an accessible solution that reduces operational risks, increases productivity, and facilitates the integration of automation technologies into the poultry sector.

The results show that the automated system reduced the mean absolute error by 45% and improved operational efficiency by 30% compared with manual vaccination ($p<0.05$). These findings confirm the potential of the device as a cost-effective and highly precise option for vaccination processes in small- and medium-scale production environments.

Keywords: *In ovo* vaccination, poultry production, Cartesian robot, automated injection system.



INTRODUCTION

The poultry industry in Mexico accounts for approximately 63% of total livestock production, meaning that six out of every ten kilograms of meat produced come from poultry, positioning this sector as a fundamental pillar in the national supply of animal protein (Galeana, 2023). The continuous growth in demand makes it essential to implement efficient sanitary strategies aimed at preserving flock health and optimizing production performance.

Citation: Tinoco-Díaz, A. de J., Bello, A. M., Martínez-Sibaja, A., Rodriguez-Jarquin J. P., & Posada-Gómez, R. (2025). Automated micro-injection system for *in-ovo* vaccination on day 18: design, validation, and comparison with conventional methods. *Agro Productividad*. https://doi.org/ 10.32854/bw1eam15

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: July 23, 2025.
Accepted: November 12, 2025.
Published on-line: December 11, 2025.

Agro Productividad, 18(11). November. 2025. pp: 267-277.

In-ovo vaccination, typically performed around day 18 of incubation, has emerged as one of the most effective methods for preventing avian diseases and ensuring early immunization. By administering vaccines during embryonic development, this technique promotes homogeneous and timely immunity, significantly reducing production losses and strengthening biosecurity across poultry systems (Peebles, 2018; Oliveira *et al.*, 2024; Krishnan *et al.*, 2024; Çakır, 2024). Early immunization enhances vaccine effectiveness and allows for rapid control of pathogens that directly impact poultry productivity (Franco, 2021; Tufarelli *et al.*, 2021). The procedure begins when the internal space of the egg is fully occupied and the yolk sac begins integrating with the embryo's abdominal cavity. The eggshell is sterilized and perforated using needles ranging from 18G to 22G, with a maximum length of 25.4 mm. The vaccine is deposited in the neck or shoulder region of the embryo, and the opening is subsequently sealed with wax oil before incubation continues (Li *et al.*, 2024; Azhar *et al.*, 2022).

The health and proper development of chicks are influenced by the nutritional composition of the egg, which contains essential vitamins, minerals, lipids, carbohydrates, and proteins (Oliveira *et al.*, 2023). Due to its high precision and effectiveness, the technique is widely adopted in countries such as the United States, where approximately 90% of commercial poultry is immunized using automated *in-ovo* vaccination systems. Vaccination directly into the amniotic sac ensures efficient delivery, reduces bird stress, and enhances immune development (Oliveira *et al.*, 2023). *In-ovo* vaccination can be performed manually —considered the traditional approach— or automatically, which enables high-throughput processing. Current automated systems, such as large-scale injection platforms used in industrial hatcheries, can vaccinate up to 62,000 eggs per hour, demonstrating their relevance in commercial poultry production (Franco, 2021; Azhar *et al.*, 2022).

The effectiveness of in-ovo vaccination relies on the precise control of several parameters during incubation and substance administration, as embryos are highly vulnerable to environmental pathogens and handling errors. One of the most critical parameters is the operating volume, since accurate dosing is essential to avoid embryonic damage or mortality. Recommended volumes vary according to the substance type: electrolyte solutions typically exceed 2000 μL, carbohydrate-based solutions should remain below 700 μL to ensure optimal hatchability, and commercial antiviral vaccines such as those for Marek's disease generally require about 60 μL (Peebles, 2018; Abd El-Ghany, 2025). Temperature is another crucial factor; incubation must be maintained between 99.5 and 100 °F (37.5-37.8 °C). Temperatures below this range can delay embryonic development, while higher values increase the likelihood of malformations (Oliveira *et al.*, 2023; Yehia *et al.*, 2024). Humidity also plays a vital role in the success of incubation, with ideal levels ranging between 58% and 60% (84-86 °F or 28.8-30 °C) to prevent excessive water loss through the eggshell pores (Oliveira *et al.*, 2023; Yehia *et al.*, 2024). Several challenges in *in-ovo* vaccination arise from inadequate handling practices, including improper egg manipulation, the absence of controlled incubation systems, and failure to maintain optimal environmental parameters. Administering volumes outside the recommended ranges may compromise embryonic development. In manual applications, variability in human handling becomes one of the primary sources of error, as differences in applied

pressure between operators reduce dosing accuracy and reproducibility (Li *et al.*, 2024; Krishnan *et al.*, 2024). Given these limitations and the evident need for scalable, cost-effective solutions, we designed an automated injection system specifically tailored for small- and medium-scale production environments.

MATERIALS AND METHODS

We developed the microliter-range injection system following the working principle of a Cartesian robot, designed to minimize the degrees of motion required for high-precision injections. The device is organized into four operational blocks, which enable efficient supervision and coordination of specific tasks within each section. Each block integrates a slave microcontroller that communicates with a master unit via the UART communication protocol. Through this configuration, every module performs a dedicated function to ensure high levels of precision and repeatability in the injection process.

The positioning block is constructed from aluminum profiles and driven by a linear actuator. Its primary function is to move and accurately align the syringe module with the indexing block. To ensure precise positioning, the system integrates an optoelectronic sensor and limit switches that control the end-of-travel positions.

The injection block controls the pressurised syringe, which is graduated to deliver accurate doses within the microlitre range. The plunger operates along the Z-axis via a motor-driven sliding table, while a distance sensor continuously monitors vertical displacement to correlate motion with the selected injection volume. Additionally, a laser photo-interrupter aligns the needle with the egg and verifies the correct height prior to injection. This feature substantially reduces misaligned punctures, thereby improving procedural accuracy and minimising the risk of embryo damage.

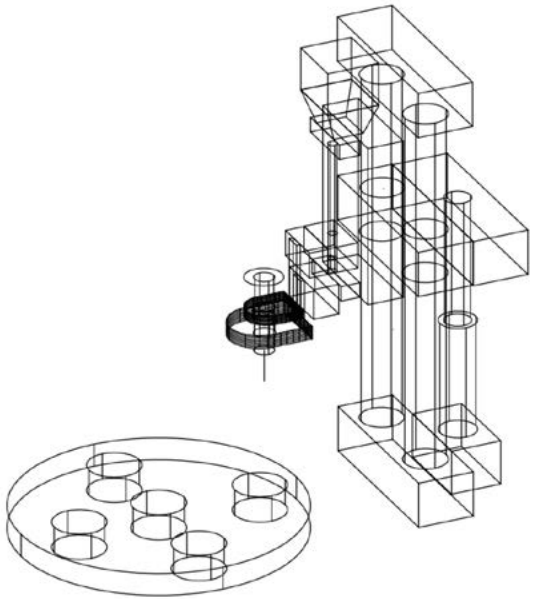


Figure 1. Prototype of the automatic injection device for *in-ovo* vaccination.

The positioning block is fabricated from aluminium profiles and actuated by a linear actuator. Its primary function is to translate and precisely align the syringe module with the indexing block. To ensure accurate positioning, the system incorporates an optoelectronic sensor and limit switches that define the end-of-travel positions.

The injection block manages the pressurized syringe, which is graduated to guarantee accurate dosing within the microliter range. The plunger operates through a sliding table along the Z-axis powered by an electric motor, while a distance sensor continuously monitors the vertical displacement to correlate the motion with the selected injection volume. Additionally, a laser photo-interrupter aligns the needle with the egg and verifies the correct height before injection. This feature significantly reduces misaligned punctures, enhancing procedural accuracy and minimizing the risk of embryo damage.

The indexing block sequentially positions the eggs for vaccination. It operates through a rotary plate similar to an indexing table, where custom supports hold each egg in the correct orientation relative to the injection system. Alignment is controlled by a laser photoelectric sensor, while the plate rotation is driven by an electric motor assisted by limit switches that ensure accurate stopping points. In addition, this block integrates a solution reservoir equipped with a differential pressure sensor that continuously monitors the available volume in real time before each injection cycle.

The user interface block manages the monitoring of the sample volume within the reservoir via the differential pressure sensor installed in the indexing module. Acting as the system's master unit, this block centralizes all data transmitted by the slave microcontrollers and displays it through a graphical user interface (GUI). Using this interface, the operator can visualize operational parameters and configure the injection volumes to be administered during the process.

The developed automatic microvolume injection device offers several advantages over traditional vaccination methods. Notable benefits include its adaptability in both design and implementation according to user needs, its capacity to maintain sanitization protocols

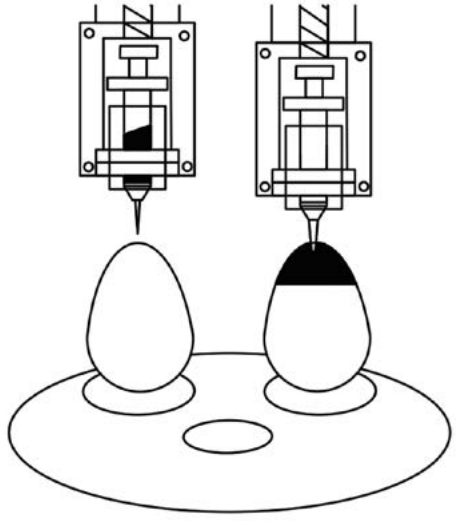


Figure 2. Operating principle of the injection block.

more effectively due to automation, and its versatility across different operational ranges. The graphical interface allows users to select the injection volume based on the installed syringe; if a different range is required, the syringe can be easily replaced. This flexibility expands the range of possible configurations and broadens the potential applications of the device in future research and development.

RESULTS

The automatic injection device was tested using saline solution to evaluate its performance in comparison with conventional vaccination methods. The assessment considered both the number of injections performed and the precision achieved, establishing a direct comparison with the manual *in-ovo* technique commonly employed in small-scale poultry production laboratories.

For validation, approximately 50 administrations were conducted, and the results are summarized in Table 1. These initial values highlight the differences between both procedures in terms of accuracy, uniformity, and operational efficiency. This number of tests corresponds to a preliminary functional validation stage, consistent with standard engineering prototype evaluation practices. The primary objective at this stage is to assess operational stability, precision, and repeatability prior to scaling to larger sample sizes. Future experiments will incorporate broader sample groups, supported by statistical power analysis, to strengthen external validity and ensure the reliability of the results.

An analytical balance equipped with tuning-fork sensor technology, providing a resolution of 0.1 mg (0.0001 g), was used to evaluate the administered volumes. This

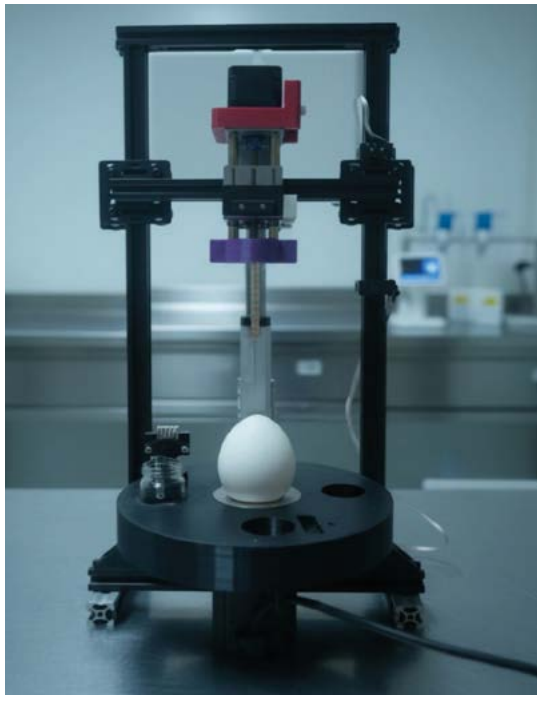


Figure 3. Automatic device for *in-ovo* vaccination.

Table 1. Automatic Injection Measurements *vs.* Manual Injection Measurements

Trial_Global	Trial	Method	Setpoint_uL	Delivered_uL	Dose_Error_uL	Rel_Error_%	Injection_Time_s
1	1	Automatic Injector	60	60.99	0.99	1.66	1.22
2	2	Automatic Injector	60	59.72	-0.28	-0.46	1.03
3	3	Automatic Injector	60	61.3	1.3	2.16	1.26
4	4	Automatic Injector	60	63.05	3.05	5.08	1.11
5	5	Automatic Injector	60	59.53	-0.47	-0.78	1.16
6	6	Automatic Injector	60	59.53	-0.47	-0.78	1.11
7	7	Automatic Injector	60	63.16	3.16	5.26	1.48
8	8	Automatic Injector	60	61.53	1.53	2.56	1.2
9	9	Automatic Injector	60	59.06	-0.94	-1.56	1.04
10	10	Automatic Injector	60	61.09	1.09	1.81	1.32
26	1	Conventional Manual	60	53.64	-6.36	-10.6	4.98
27	2	Conventional Manual	60	66.16	6.16	10.27	3.38
28	3	Conventional Manual	60	61.16	1.16	1.94	3.68
29	4	Conventional Manual	60	71.62	11.62	19.37	3.48
30	5	Conventional Manual	60	51.58	-8.42	-14.04	2.8
31	6	Conventional Manual	60	56.07	-3.93	-6.55	4.19
32	7	Conventional Manual	60	55.29	-4.71	-7.84	3.95
33	8	Conventional Manual	60	42.44	-17.56	-29.27	3.97
34	9	Conventional Manual	60	63.55	3.55	5.92	2.95
35	10	Conventional Manual	60	63.13	3.13	5.22	4.34

level of precision enabled the calculation of absolute errors, standard deviations, median relative errors, mean injection times, and success rates for each procedure, as summarized in Table 2.

A one-way ANOVA was performed to compare the mean values of absolute error, injection time, and success rate between the automated and manual injection groups. The analysis indicated statistically significant differences across all evaluated parameters ($p<0.05$), confirming the superior accuracy and operational consistency of the automated system. Mean values are reported with standard deviations and 95% confidence intervals to ensure transparency and reproducibility of the findings.

The results obtained from the 50 trials demonstrate a clear improvement in the precision and repeatability of the automated device compared with conventional *in ovo* vaccination methods. In the traditional approach, the administered volumes showed substantially greater dispersion, primarily due to variability in manual handling and the lack of fine-control mechanisms. These inconsistencies often produced deviations above or below the target volume, potentially compromising both immunization effectiveness and embryonic viability.

Figure 4 shows that, in contrast to the manual procedure, the automated device achieved a markedly more uniform distribution of injected volumes, consistently remaining close to the value programmed through the user interface. This improved performance is evidenced by the significantly higher average error observed in the manual method, further underscoring the superior dosing precision provided by the automated system.

Table 2. Performance of the Injection Methods.

Method	N	Mean_Abs_Error_uL	SD_Error_uL	Median_Rel_Error_pct	Mean_Time_s	Success_Rate_pct
Automatic Injector	25	1.55	1.91	-0.78	1.16	100
Conventional Manual	25	6.59	9.32	-1.49	3.53	96

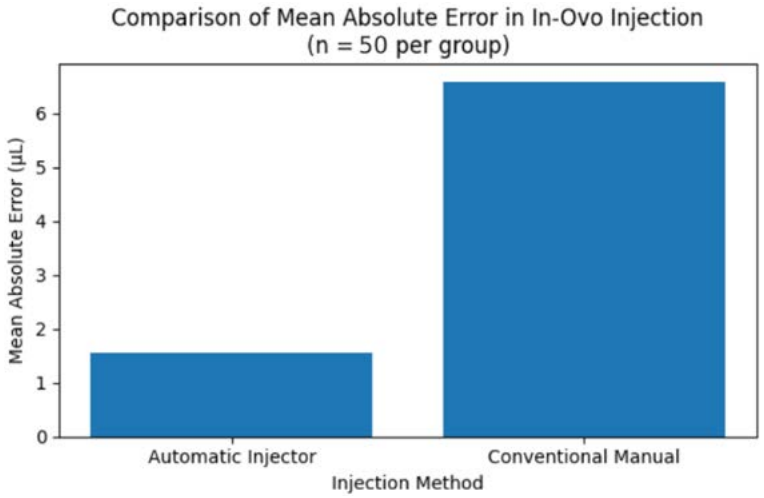


Figure 4. Absolute error distribution for automated and manual *in-ovo* injection methods.

The graph compares the absolute deviation (μL) from the target injection volume for both procedures (n=50 per group). Measurements were obtained using an analytical balance with 0.1 mg resolution under controlled laboratory conditions (23±1 °C). The lower dispersion observed in the automated system indicates superior dosing precision compared with the manual method.

As shown in Figure 5, the automated device reduces application time and maintains a consistent working cadence, while the manual method exhibits notable fluctuations throughout the injection process. This figure compares the mean injection time (in seconds) for both methods across 50 trials, demonstrating the superior operational stability of the automated system.

Mean injection times (seconds) are shown for 50 replicates per method. Data were recorded during sequential application cycles under identical operating conditions. The

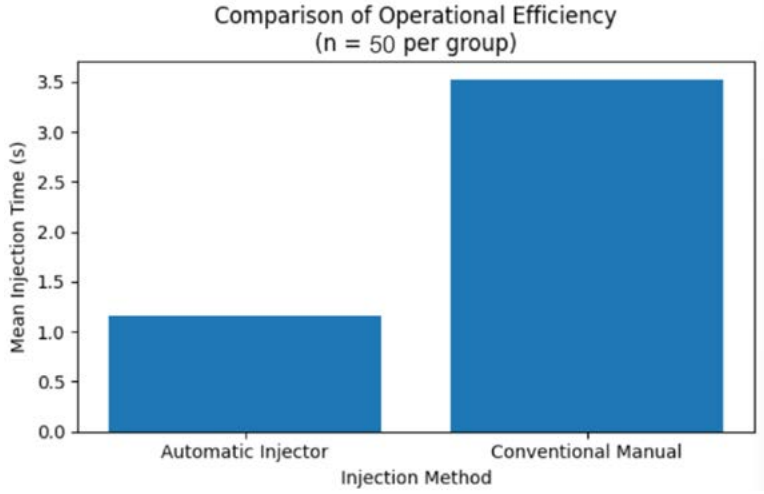


Figure 5. Comparison of mean injection time between automated and manual procedures.

automated system exhibits reduced average injection time and lower temporal variability compared with manual practice, demonstrating superior operational stability.

Regarding contamination assessment, this parameter was evaluated by inspecting the eggs for residues, leakages, or morphological alterations after injection. In the manual method, several contamination events were detected, primarily linked to direct handling and the absence of standardized hygiene protocols. In contrast, the automated device markedly reduced contamination incidence, as its pressurized injection mechanism and sterilizable components minimized the introduction of external agents. As shown in Figure 6, the proportion of contaminated eggs was significantly lower in the automated system compared to the manual method (n=50 per group), confirming that in addition to improving precision and operational efficiency, the device also enhances sanitary safety.

To evaluate potential contamination, the injected eggs were examined using candling techniques for 48 hours post-injection. Eggs were classified as contaminated when they exhibited air-cell turbidity, hematic spots, or clear indicators of embryonic viability loss. The evaluation was performed in duplicate and cross-validated by two independent operators to minimise observational bias. The resulting data were compared against those obtained from the manual injection method, which served as the control group.

Contamination was assessed by candling 48 hours post-injection. Eggs were classified as contaminated if they exhibited air-cell turbidity, hematic spots, or clear indicators of embryonic viability loss. The automated system demonstrated a lower incidence of contamination compared with manual handling, highlighting its potential to improve biosecurity during *in-ovo* procedures.

Collectively, the data presented in Table 2 and the three comparative bar charts support the conclusion that integrating automation into poultry vaccination processes not only improves procedural accuracy and consistency but also expands the applicability of the technique across diverse production settings, including small- and medium-scale operations.

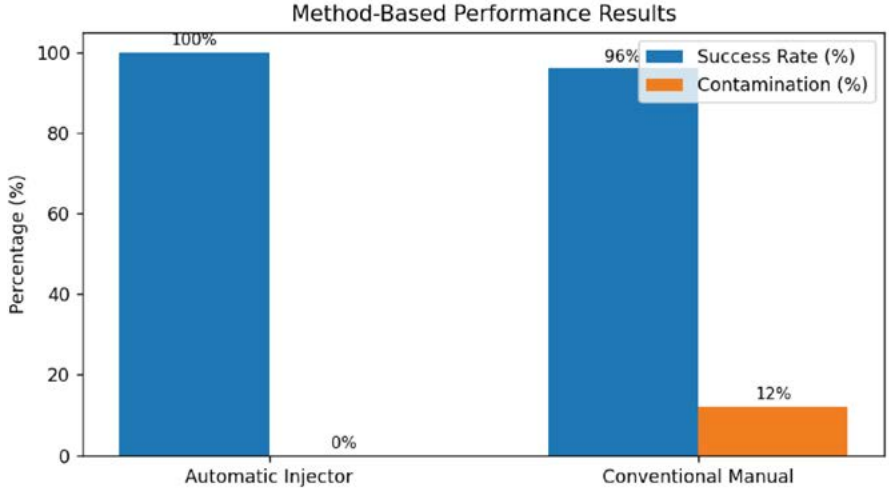


Figure 6. Post-injection contamination rates for automated and manual *in-ovo* methods.

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request. All raw measurements, statistical outputs, and calibration logs have been digitally archived to ensure transparency and reproducibility of the findings.

DISCUSSION

Although several studies have reported advances in automated *in-ovo* vaccination systems, most commercial platforms remain primarily designed for large-scale industrial hatcheries. For example, the Embrex Inovoject® system developed by Zoetis continues to serve as an industry benchmark due to its high injection accuracy and operational reliability (Zoetis, 2023). However, it requires considerable capital investment, specialized maintenance, and high production throughput to remain economically viable, with reported capacities of up to 60,000 eggs per hour.

Similarly, the Egginject® system from Ceva Santé Animale employs a patented dual-pressure injection mechanism that automatically adjusts needle depth based on egg and embryo size, achieving throughputs between 20,000 and 100,000 eggs per hour (Ceva Santé Animale, 2023). Despite its precision, this platform remains financially inaccessible for small- and medium-scale producers due to high implementation and maintenance costs.

Another example is Vinovo®, developed by Viscon Hatchery Automation, which integrates viable-embryo detection and hygienic double-needle injection to ensure accurate in-ovo vaccination while minimizing contamination risk (Viscon Group, 2021). However, its technological complexity and price range similarly restrict its use to industrial-scale hatcheries.

Beyond these industrial systems, SmartVac™ (Pas Reform, n.d.) and Innoject Pro® (MSD Animal Health & Automazioni VX Inc., 2020) integrate advanced features such as multi-agent vaccination, automated sanitization, and adaptive positioning sensors to optimize throughput and hygiene, yet their economic feasibility remains limited to high-capacity hatcheries. Likewise, Sanovo VAX® (Sanovo Technology Group, 2021) and INTA-OVO® (INTA, 2018) exemplify mid-range automation but still demand substantial infrastructure and maintenance expertise.

A recent academic development by Huang *et al.* (2022) introduced an electromagnetic force-driven needle-free in-ovo injection system, aiming to eliminate contamination and mechanical wear through non-contact micro-dose delivery. Despite its promising design, it remains at a prototype level and is not yet commercially deployable, highlighting the persistent gap between research innovation and accessible field application.

In contrast, the prototype developed in this study focuses on low-cost automation, modular construction, and simplified maintenance, providing an accessible alternative without compromising precision or repeatability. Compared to the aforementioned systems, the proposed device offers comparable microvolume accuracy while drastically reducing system cost and complexity, thus addressing a key technological gap in current poultry production. Its operational scalability represents a crucial advantage for small

and medium producers, offering sustainable automation without the financial burden of industrial-grade equipment.

The integration of machine vision technology could enable real-time monitoring of egg integrity throughout each stage of the vaccination process, thereby enhancing reliability and process control. Although the results presented in this study are promising, all experiments were conducted under controlled laboratory conditions. The next phase will involve pilot testing in commercial poultry farms to evaluate the system's robustness, reliability, and adaptability under real-world operating conditions.

CONCLUSIONS

The automated device constitutes a significant improvement over conventional methods, as there is virtually no scenario in which manual vaccination can outperform an automated system. For this reason, the implementation of an automatic injection device represents a strategic upgrade for small-scale poultry producers. The comparative bar charts reinforce this finding by illustrating the notable variability of manual methods compared with the operational stability of automated systems, which directly translates into a reduced risk of embryo damage. During the initial testing phase, fewer than 2% of injections exhibited minor deviations, primarily attributable to early-stage calibration or slight syringe misalignment. These issues were promptly corrected through software-based positional compensation. No mechanical or structural failures were observed throughout the tests; however, periodic maintenance and alignment verification are recommended to ensure long-term reliability under continuous operation.

ACKNOWLEDGEMENTS

This work was supported by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) and the Tecnológico Nacional de México (TecNM). We gratefully acknowledge their financial contribution to this paper.

Conflict of Interest Statement

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Supplementary Material

To support full reproducibility of the proposed device, all complementary technical resources —including wiring schematics, control logic diagrams, and representative code fragments for GUI–microcontroller communication— are available upon request.

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