Biosecurity Control Systems in Two Phases for Poultry Farms

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Abstract**—**In this work was developed and implemented a thermal fogging disinfection system to counteract pathogens from poultry feces in agribusiness farms, to reduce mortality rates and increase biosafety in them. The control system consists of two phases for the conditioning of the farm during the sanitary break. In the first phase, viral and bacterial inactivation was performed by treating the stool dry cleaning, along with the development of a specialized product that foster the generation of temperatures above 55 °C in less than 24 hr, for virus inactivation. In the second phase, a process for disinfection by fogging was implemented, along with the development of a specialized disinfectant that guarantee no risk for the operators' health or birds. As a result of this process, it was possible to minimize the level of mortality of chickens on farms from 12% to 5.49%, representing a reduction of 6.51% in the death rate, through the formula applied to the treatment of poultry litter based on oxidising agents used as antiseptics, hydrogen peroxide solutions, glacial acetic acid and EDTA in order to act on bacteria, viruses, micro bacteria and spores

Keywords**—**Innovation, triple-helix, innovation, poultry farms.

I. Introduction

IN MEXICO, there are 19,167 units (farms) for production of chicken and hens, with a population of 605'241,520 birds [1], being the seventh largest producer of chicken meat in the world. At the end of 2014, the poultry industry produced 5'574,554 tons of food, of which 2'994,254 tons are for chicken production and 2'572,300 tons are for eggs for dishes, with a monetary value of 133.026 million of pesos generating one million 154 thousand jobs in the country [2]. However, the poultry industry is affected by the incidence of diseases caused by various pathogenic agents that can cause a high mortality rate. According to studies of the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA), the most common diseases affecting the birds in Mexico are: chronic respiratory disease, coryza and fowl cholera (caused by bacteria); Newcastle disease, fowl pox, infectious bronchitis and Marek's disease (caused by virus); Coccidiosis, Teniasis, lice and mites (caused by parasites). The presence of these diseases is very variable and depends on the area where the birds [3] are raised. To prevent the spread of these pathogenic agents, a biosafety system is necessary which represents a determining factor for achieving health for these foods. Biosecurity is a set of practices to prevent entry and transmission of pathogens that can affect the health of the birds [5].

Biosecurity is related to the establishment and implementation of procedures to minimize the risk in the use, handling and spread of pathogens, either of animals, plants or humans, and that relates to the activities of the facility, focused on minimize the exposure and/or release of infectious biological materials. Biosecurity also covers biocontainment, linked to factors of infrastructure (both construction and equipment) and focuses on the physical and administrative means (notarized) to ensure the control of biological material and information (data and people) that could endanger public health or cause economic losses [6].

Biosecurity encompasses both programs and health measures and labor standards that focus on reducing input and/ or output of diseases on a poultry farm, in order to prevent its spread. That's why biosecurity covers all actions involving prevention, protection and healthcare of birds of any infectious agent, of any kind, be it bacterial or parasitic among others [7].

The objectives of the biosecurity program are [7]:

- Reduce the risk of exposure of the birds to infectious agents.
- Reduce the possibility of contamination (through the environment, food, water, the staff working on the farm, vermin, contaminated vaccines, etc.) from the poultry farm.
- Provide an atmosphere of comfort as clean as possible for birds to develop their full genetic potential for better productive results.

As for the regulations in the country, the Mexican Official Standards are:

> *"Mandatory regulation issued by the competent agencies, which establishes rules, specifications, attributes, guidelines, characteristics or requirements for a product, process, installation, system activity, service or method of production or operation, as well as those relating to terminology, symbols, packaging, marking or labeling and relating to its enforcement or implementation "* [8]

Some of the standards related to security in poultry farms are given in Table I. In addition, a legal framework has been set in the Federal Law on Animal Health, in which the Secretary of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA) will determine the measures on Good Livestock Production Practices to be applied in primary production, to reduce pollutants or animal health risks that may be present in these [9].

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According to the manual "Good Practices in the Broiler Production " in their primary production units (poultry farms) published by SAGARPA, the requirements to be met in the unit of poultry production are established to ensure safety during aging, growing and finalization in the production of broiler chickens, as the location of the production unit; design and construction; the handling in the production unit; training and staff hygiene; the nutrition; the water; the health; the biosecurity and animal welfare [7].

TABLE I
MEXICAN NORM FOR POULTRY FARMS [8]

RULE	CONCEPT
NOM-005-1993	Campaign against Avian Salmonellosis.
NOM-013-ZOO-1994	National Campaign against Newcastle Disease Velogenic presentation.
NOM-044-ZOO-1995	National Campaign Against Avian Influenza.
NOM-022-ZOO-1995	Specific characteristics for animal health for facilities, equipment and operation of establishments that sell chemical, pharmaceutical, biological and food products for animal use or consumption.
NOM-024-ZOO-1995	Specifications and characteristics for animal health for the transport of animals, their products and by-products, chemical, pharmaceutical, biological and food products for animal use or consumption.
NOM-025-ZOO-1995	Specifications and characteristics for animal health, equipment and operation of establishments that manufacture food products for animal use or consumption.
NOM-026-ZOO-1994	Specifications and characteristics for animal health, equipment and operation of establishments manufacturing chemical, pharmaceutical and biological products for use with animals.
NOM-051-ZOO-1995	Human treatment in the animal movement.
NOM-052-ZOO-1995	Minimum requirements for vaccines used in the prevention and control of Newcastle disease.
NOM-054-ZOO-1996	Establishment of Quarantines for animals and their products.
NOM-055-ZOO-1995	Minimum requirements for the development of emulsified vaccines inactivated against H5N2 avian influenza.
NOM-061-ZOO-1999	Animal health specifications of foodstuffs for animal consumption.

In the section of biosafety are established guidelines for the program for cleaning and disinfection of the production unit; phases of the cleaning process; disinfection; vermin control; manure management; management of the wastewater program and organic and inorganic waste [7].

One of the most important points in the biosecurity is the cleaning procedure (removal of all dust, waste food, bed and all kinds of organic matter) and disinfection (removal of microorganisms and a disinfectant is an agent or substance that when applied to objects destroys or inactivates microorganisms) [7].

The cleaning and disinfection procedure includes:

A) Elimination of poultry manure (dry cleaning): The poultry manure is the excreta of broilers mixed with the material used as bedding for chickens, sawdust, rice hulls or soy, corn cob ground corn or other. [10]
B) Wash planters.
C) Washing machine.
D) Rinsing planters.
E) Disinfection.
F) Sanitizer choice.

The selection of a disinfectant is the very important step in a biosecurity program, using the conceptual biosafety which refers to the physical location of the farm and the structural biosafety acting on the farm design and health systems. The points to be considered for choosing the products are [11]:

- Effectiveness: The biocide should be of a broad spectrum for effective control of all pathogenic microorganisms that affect the species in question.
- Security: It must be a manageable and safe product for the operator, for the animals and the environment and should validate that this is not corrosive.
- Cost: The cost-benefit ratio should be favorable because with this data afterwards economic benefits should be reflected directly in the corporate profits.

The article presents the process and the results carried out for the development and implementation of a new disinfectant-sanitizer formulation for the use in poultry farms.

II. EXPLANATORY FRAMEWORK.

A Mexican company (Grupo Rosmar) dedicated to develop and implement health processes in the food industry, in 2013 developed studies to determine the causes of mortality in poultry farms. The result of this study showed that the main cause of bird mortality was related to the treatment of poultry litter in the planters [4].

By identifying the problem, the company began an investigation to improve the processes of washing and disinfection of the house through a process for cleaning and sanitization of agro-industrial production areas, through a system that includes an autonomous mobile unit comprising a production unit foam to apply a detergent formulation specialized livestock of livestock use and an unit for auto cleaning with water under pressure to pre-rinse and rinse, as well as the process for cleaning and sanitization, using the system including the autonomous unit, achieving increased efficiency and cleaning productivity and reduce bird mortality for reasons inherent from ineffective health. This resulted in the registration of a patent with registration number PCT/ MX2013/000195 "Process and System Cleaning and Sanitizing of Agroindustrial Farms", this innovation had a direct impact on the process of conditioning of the farms in the period of sanitary rest. Therefore, the company took the initiative to continue to innovate and submitted in 2014 a project to research and development of a control system for biosafety for the birds. The project was approved in 2015 to be financed by the National Council of Science and Technology (CONACYT) under the Program of Incentives for Innovation. Under this project was developed a disinfectant formulation for thermofogging for the poultry litter and stands in the poultry farms.

III. METHODOLOGY

The project implementation was carried out during 2015 in the area of Laguna in Coahuila/Durango as a strategic sector in the poultry sector. The representative sample was of 8 poultry farms owned by sharecroppers. These 8 farms, provide a total of 84 booths of between 1,800 and 2,200 m ^ 2 and cover a total population of approximately 2,380,000 birds per cycle, which represents 8% of fattening farms in the region working with this producer.

The current process of cleaning of the stands requires meaningful investments in equipment and in the staff in order to do it productively and with good results. Currently the production company has 5 integrated teams distributed throughout the region to clean farms (around 20 daily booths). Each integrated team consists of the following elements:

Investment in infrastructure support:

a. Pressure water system with tank, pump and 4 autonomous guns.
b. System 1 for disinfectant spray.
c. Tractor to move washing or sprinkling systems.
d. Platforms and tractors to move systems between farms.
e. Tractor trailers to move the platforms.
f. Trucks to move the people who clean.
g. Waterpipes (not to stop the process).

Staff:

a. Supervisor.
b. Tractor drivers.
c. Driver of tractor-trailer.
d. Driver pipe.
e. Sprayer staff.
f. Support staff in cleaning.

The methodology was designed in four stages:

Stage I: Development of a Technological Plan of Chemical and Microbiologist Evaluation

Within the development of the technology plan were taken into account factors affecting the destruction of microorganisms, highlighting the factors of concentration of the agent, exposure time, the medium pH and temperature.

In the development of the disinfectant formula for the treatment of poultry litter, were taken into account various oxidizing agents (products that release oxygen) and its operation consists in the inactivation of enzyme proteins, seeking that its reaction could be on vegetative bacteria, virus, micro bacteria and spores.

Stage II: Sampling for Microbiological Analysis (Bacteria and Viruses)

Sampling chicken manure.

- Receipt of bed: Receipt of the extended bed in the house (no stacking). Measuring the necessary temperature and the data obtained are recorded.
- Sprinkling Peroxirros: The Peroxirros solution is applied by spraying and chicken manure is gathered.
- Chicken manure pile up: After being sprinkled across the bed, the solution of Peroxirros of chicken manure is being piled up as normally done and covered with plastic.
- Data collection: for 24 hours temperature measurements are performed to observe its behavior.

Stage III: Cleansing or Washing Booths (Wet Cleaning Only)

Several tests were performed to verify the benefits of houses washing process during 2015. To demonstrate that the results were consistent when the process was implemented, it has continued to perform steadily in subsequent cycles.

System of developed products: According to the innovation project several prototypes were made in order to be tested during several cycles in the representative sample, within them were included: Prototypes of detergents, disinfectants for cleaning and descaling booths. Foaming prototype system and autonomous hydro cleaning. Process used: the process was changed using processes, times, products and application equipment recommended by our company, derived from innovation project.

Stage IV: Thermospray

Several tests were performed to verify the benefits of current thermofogging process during 2015. System of developed products.

According to the innovation project several prototypes were made in order to be tested during several cycles in the representative sample, within them were included: Prototypes of disinfectant thermofogging, prototype of the application system.

Process used: the process was changed using processes, times, products and application equipment recommended by our company, derived from the innovation project.

TABLE II
FORMULATION I

Chemical	*Concentrations %*			
Raw material	***A***	***B***	***C***	***D***
50% Hydrogen peroxide	25	30	15	20
Glacial acetic acid	25	20	35	30
EDTA	2	5	3	1
Water	48	45	47	49

IV. RESULTS

In the formula shown in Table II, the compounds that were used as antiseptics to neutralize bacteria and viruses that may be present in chicken manure, are solutions of hydrogen peroxide, glacial acetic acid and EDTA, in order to determine the amounts to be used in this formula, various tests and analysis were conducted.

- The hydrogen peroxide acts as an oxidizing agent, in a solution of 30%, is used in this formula as it can sterilize the stands where the manure is stored.
- The Glacial Acetic Acid is used as an antiseptic, as it works acidifying the environment where it is applied, which provides antibacterial and fungicides properties. Its activity depends on the concentration in which it is being used.
- EDTA is an antibacterial on certain specific bacterial species such as *Streptococcus aureus* and *Staphylococucus alfahemolíticos* and has a high antifungal effect. Basing on these compounds were

determined different concentrations thereof to make the solution.

Based on the different concentrations it was decided that D was the most suitable for use in poultry manure.

TABLE III
AVERAGE MICROBIOLOGICAL RESULTS WITH THE CURRENT PROCESS (WITHOUT CHEMICAL TREATMENT)

C-3 without product	Sampling time	Aerobic mesophilic (UFC/g)	%	Total coliforms (UFC/g)	%	Mold (UFC/g)	%	yeasts (UFC/g)	%
Chicken manure without product	0	11,000,000	100	23000	100	160	100	40	100
Chicken manure without product	24	1,500,000	14	<10	0.0	80	50	60	6000
Chicken manure without product	48	21,000000	191	8500	37	10	6	<10	25

TABLE IV
MICROBIOLOGICAL RESULTS WITH AVERAGE CHEMICAL TREATMENT (CHEMICAL TECHNOLOGY AND PROCESS DEVELOPED IN THE PROJECT OF INNOVATION)

C-3 without product	Sampling time	Aerobic mesophilic (UFC/g)	%	Total coliforms (UFC/g)	%	Mold (UFC/g)	%	yeasts (UFC/g)	%
Chicken manure with product	0	74,000,000	100	38 000	100	70	100	20	100
Chicken manure with product	24	250,000	0	40	0	10	140	<10	50
Chicken manure with product	48	4,500, 000	6	<10	0	10	14	<10	50

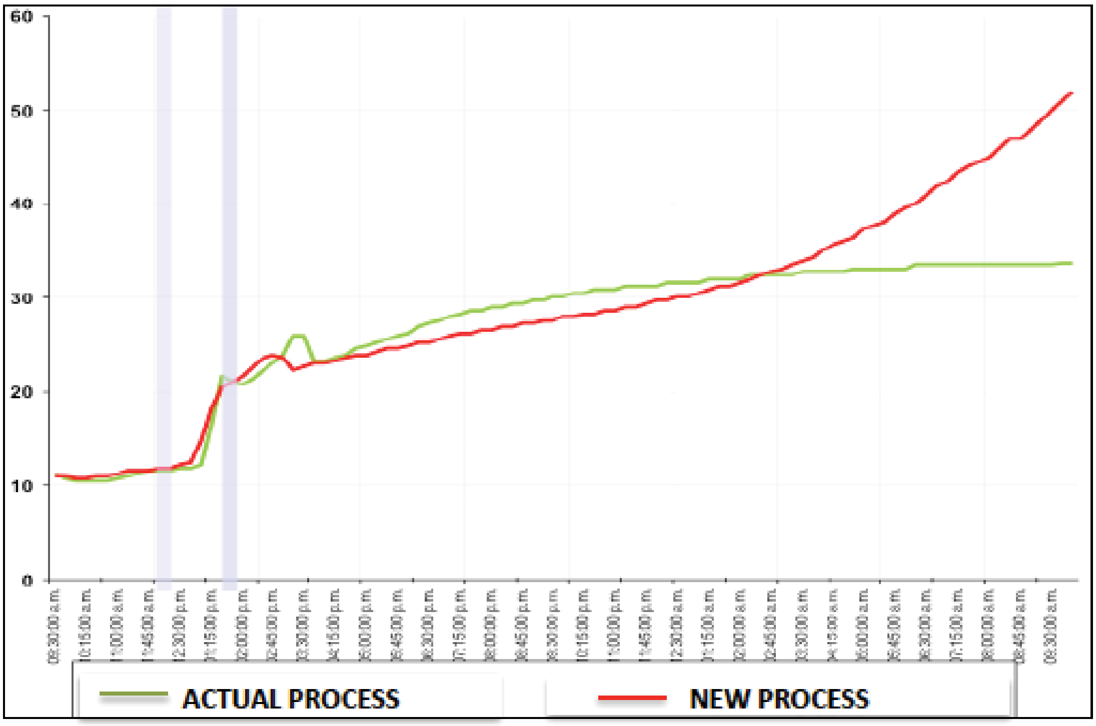

Fig. 1 Results of temperature increase every 15 minutes starting 09:30am

A thermofogging process was used for the sanitization of the planters, as this process allows substances to be spread more quickly and effectively throughout the planters, because the liquid substances are vaporized during the process and aerosols are formed, the substance that was developed considering the tertiary amine (COCO-DIMETI) and Glycerin USP, as the main asset of the process.

- Tertiary amine (coco dimethyl amine): The compounds of quaternary ammonium penetrate in the membranes of microorganisms thanks to carbon chains (hydrophobic). The biocide action of tertiary amines is also due to its interaction with the plasma membrane.
- The USP Glycerin is a product of excellent physical and chemical properties; it is available for cosmetic, pharmaceutical and food applications with a concentration of glycerol at levels of 96%, 99.0% and 99.5%, which is why the development of a formulation directed to sanitation is required with the above characteristics.

Using these two compounds were developed concentrations and that helped to choose which concentration was the most suitable for this process of thermofogging.

The most suitable concentration for use in this process of thermofogging in the planters is C.

Results of Stage I: Sampling for Microbiological Analysis (Bacteria and Viruses).

To corroborate the efficacy of these compounds, different samples were taken, at the beginning were performed measurements over 24 hours, in which the solutions to the booths neither to the chicken manure were not applied. PCR analysis were also performed during the chemical treatment to detect Avian Influenza and New Castle, where in both cases the results were negative against a low % of untreated poultry manure which were positive. Additionally, the developed product was certified in a lab test by the Mexican Accreditation B.C. (EMA) and the result was as follows:

- Avian Influenza: N7N3 99.99% of efficiency.
- New Castle: 99.99% of efficiency.
- Increase of temperature: Concerning the temperature increase, it was found that the average product performance is as shown in Fig. 1 depicting an increase of 5 to 10 Centigrade to additional current process.

Results of Stage II: Cleansing or Washing Booths (Wet Cleaning Only)

The microbiological results after the current process of cleaning and disinfection, microbiological sampling were conducted by certified laboratories to the EMA where they reached the indicative results for microorganisms.

TABLE V
RESULT OF INDICATIVE MICROORGANISMS BEFORE IMPLEMENTING THE PROCESS

Installation	Aerobic mesophilic bacteria UFC/cm^2	Total coliforms UFC/cm^2	Fungi UFC/cm^2	Yeasts UFC/cm^2
Section 8 stand 1				
Waterer	35000	1000	900	90
Place to eat	20000	7000	880	100
Floor	7000	5500	1600	150
Section 8 stand 2				
Waterer	30000	850	800	80
Place to eat	18000	8250	750	60
Floor	6250	4500	1000	110
Section 8 stand 7				
Waterer	28000	900	750	75
Place to eat	21000	6800	800	90
Floor	7500	5000	1100	130

TABLE VI
RESULTS OF PRODUCTIVITY BEFORE IMPLEMENTING THE PROCESS

Activity	Stand 1 time in minutes	Stand 2 times in minutes	Stand 2 times in minutes
Soaping of the roof, wall and equipment	70	70	80
Washing of the floor	30	40	35
Sanitization of the equipment	10	10	10
Sanitization of the floor	10	10	10
Total Time in Minutes	***120***	***130***	***135***

In the results above, it can be seen that in a time of 8 hours with a lunch break, 30 minutes to change and bath on arrival and 30 minutes to shower and change to go, 6 effective hours a day, only 2 to 3 booths per day maximum can be made.

Table VII shows the results of this stage once the process is implemented.

TABLE VII
MICROBIOLOGICAL RESULTS AFTER IMPLEMENTING THE PROCESS

Installation	Aerobic mesophilic bacteria UFC/cm^2	Total coliforms UFC/cm^2	Fungi UFC/cm^2	Yeasts UFC/cm^2
Stand 7 Before Cleaning and sanitization				
Waterer	32000	1050	850	85
Place to eat	19000	7500	800	80
Wall	12000	8000	800	180
Floor	6800	5800	1500	100
Stand 7 Before Cleaning and sanitization				
Waterer	280	8	0	0
Place to eat	380	60	1	14
Wall	385	40	1	5
Floor	370	160	1	8

The results of productivity after implementing the new process were significant, taking into consideration the same number of people with the products, processes and machinery, but with better standard of health which was used, increased from 2.5 stands at 6 per day with the same number of people, which means an increase of 140%.

Results of Stage III: Thermospray

a) Microbiological Results: The percentage reduction in pathogens with certified laboratories to the EMA.

1. Avian Influenza 99.99%
2. New Castle 99.99%

TABLE VIII
RESULT OF SURFACES AFTER FOGGING

Total count	97.2% average within specification.
Total coliforms	98.8% average within specification.
Fungi	97.5% average within specification.
Yeasts	97.7% average within specification.

b) Results of the increase in productivity: Productivity results were significant, as the number of persons was reduced from 4 to 1 that made the application and in a time in the process of 30 minutes was reduced to 10 minutes each booth.

V. CONCLUSIONS

Biosecurity in the poultry farms is of national significance because it is the main food of animal origin in the Mexican population and its production is a source of employment and income to poultry farms.

The reduction of the death rate in these farms is of a big importance not only for the economic benefits they generate, but also for the good use of resources and implementing new healthcare systems. The development of this sanitizing formula helps to reduce this rate, helps the prevention of different diseases such as HPAI (H7N3) influenza in birds.

TABLE IX
MEASUREMENTS OF THERMAL FOGGING WITHOUT PRODUCT

Farm without Product	Stand	Aerobic Mesophilic Bacteria (UFC/g)	Total Coliforms (UFC/cm^2 surface)	Molds (UFC/cm^2 surface)	Yeasts (UFC/cm^2 surface)
A	1	122,689,000	4,705,882	15,406,100	5,063,200
	2	890,000	8,800	4,700	2,800
B	3	87,389,620	1,382,280	2,531,600	1,465,876
	4	1,200,000	77,000	5,200	17,000
C	5	107,906,900	1,343,020	377,906	218,023
	6	90,765,000	987,435	459,756	312,345

TABLE X
MEASUREMENTS OF THERMAL FOGGING WITH PRODUCT

Farm with Product	Stand	Aerobic Mesophilic Bacteria (UFC/g)	Total Coliforms (UFC/cm^2 surface)	Molds (UFC/cm^2 surface)	Yeasts (UFC/cm^2 surface)
A	9	8,870,330	143,251	1,621,100	794,464
	10	200,000	1,700	300	400
B	11	7,116,871	47,073	249,180	357,130
	12	397,000	33,000	1,400	1,100
C	13	836,012	4,019	54,308	40,771
	14	587,980	34,500	178,578	98,070

Using the developed formula in this project, it can be showed that:

a. The project at this stage proved to be an effective process to control Avian Influenza virus and New Castle.
b. A stand with treatment guarantees the reduction of the microbiological charge present in manure comparatively against the one which does not contain it.
c. Also it demonstrated that innovation is an improvement in farm productivity because:
d. The treatment guarantees the removal of the chicken manure in 24 hours, saving 24 hours for the conditioning process and rest of the farm.
e. The treatment guarantees protection to the staff of the farm.

The benefits and impacts of this new formulation in the domestic poultry sector helps in order that the farms comply with biosafety standards more efficiently and effectively, promoting a healthy and qualitative sector.

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