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EVALUATION OF CLEANING AND DISINFECTION OF LEAN BROILER SLAUGHTERHOUSE USING HYDROGEN PEROXIDE IN NORTH BAHRI LOCALITY, SUDAN

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ABSTRACT

This descriptive and experimental study was conducted between January and Aril, 2020 in Lean slaughterhouse, Alkadro, Khartoum North, Sudan using a regular swab sampling on a weekly basis for a period of 3 weeks with the objective to evaluate cleaning and disinfection procedures against some microbial isolates in the slaughterhouse. Routine cleaning and disinfection procedures which included walls, floors and equipment using hydrogen peroxide solution in 0.01 concentration were investigated. Both environmental swab samples and standardized and structured checklist were applied to assess the status of some of the Prerequisites Programs (PRPs). The study revealed that the location and structure of the slaughterhouse and staff training were found satisfactory while water supply had failed adequacy rule. According to adequacy rule used in this study all the parameters of sanitation program operational procedures scored satisfactory (100%). While the average of averages of coliform count in the floor, wall, equipment and water was found 2733.3±540.6 cfu during the three weeks period with significant statistical differences in the count between the three weeks with $p \leq 0.05$. The average of Total Bacterial Count for the sample sites during the three weeks period was 2250±680.6 cfu, with statistical differences in the count between the three weeks with $p \leq 0.05$.The average of *E. coli* count was 341.6±85.6 cfu for the sample sites during the three weeks period with statistical differences in the count between the three weeks with $p \leq 0.05$. The multiple comparison test of least significant differences showed no significant differences in the Salmonella count between floor, wall, and equipment with $p \leq 0.05$. The mean TBC differences was found to be significant between wall and floor and wall and equipment with $p \leq 0.05$; also the mean differences of E. Coli count was found to be significant between wall and equipment from one side and floor and equipment from the other side with $p \leq 0.05$, and the mean Coliform count differences was found to be significant between the wall and equipment with $p \leq 0.05$. The study concluded that the overall evaluation of the PRPs and sanitation program operational procedures were satisfactory.

KEYNOTES: Cleaning and disinfection, Poultry slaughterhouse, Hydrogen peroxide.

INTRODUCTION

The process of cleaning comprises both dry and wet phases. While dry cleaning refers to physical removal of foreign material from a surface, wet cleaning is performed with water under high pressure to remove loose organic matter.[1] Wet cleaning consists of factors such as time, mechanical action, chemistry and temperature.[2] Soaking with detergent is preferably performed from the floor towards the ceiling, while washing is performed vice versa to avoid contaminating already cleaned surfaces.[3]

The disinfectants act on microorganisms at several target sites resulting in membrane disruption, metabolic inhibition, and lysis of the bacterial cell.[4]

Some micro-organisms as E. coli and Salmonella are still able to survive and detected in environmental samples of the slaughterhouse and in carcass samples.[5] It has been described that holes in floors and walls make it difficult for the penetration of disinfectant solutions and what is more, the bioflms created by Salmonella can make the action of the disinfectants more difficult.[6] This will contribute to the contamination of the carcass directly or indirectly through the workers, equipment and air.

Inefficient sanitation and improper disinfection program in a slaughterhouse can contribute to bacterial contamination of carcasses.[7] Fecal matter is a major source of contamination and could reach carcasses through direct deposition, as well as indirect contact with contaminated equipment, workers, installations and air.[8] Hence, proper cleaning and perfect sanitation procedures will lead to effective reduction to bacterial count in a slaughterhouse and against the recovery of environmentally-robust zoonotic enteric pathogens such as E. coli and Salmonella.[8]

Slaughterhouses sanitation procedures can be applied using different chemical agents among which hydrogen peroxide H2O2 is widely used as it is strong oxidizing and disinfecting agent.[9]

Abdalla et al.[10,11] evaluated bacterial contamination of carcasses in Khartoum State and stated that inefficient sanitation and improper disinfection program in a slaughterhouse can contribute to bacterial contamination of carcasses. Hence, the objective of this study was to evaluate cleaning and disinfection procedures adopted in Lean slaughterhouse.

MATERIAL AND METHODS

Study area

This study was conducted in Lean slaughterhouse, Alkadro, Khartoum North, Sudan between January and Aril, 2020.

Study design

This descriptive and experimental study was conducted in Lean slaughterhouse using a regular sampling on a weekly basis for a period of 3 weeks. Routine cleaning and disinfection procedures which included walls, floors and equipment using hydrogen peroxide solution in 0.01 concentration were investigated.

Data collection

Checklist preparation

A standardized and structured checklist was developed and applied to assess the status of some of the Prerequisites Programs (PRPs) and other related activities that could adversely affect meat safety.

The checklist was divided into 10 elements which were further assigned to a series of parameters to accurately describe the status of each assessed element.

Scoring system

Each parameter under assessment was scored and points were assigned. A score of zero (0%) was assigned when the parameter posed a very high degree of risk for meat safety, whereas full marks (100%) were given when there was no risk for meat safety.

Evaluation of the PRPs

The total score of each meat process was split into two categories depending on the risk of contamination and the possibility of cross-contamination. These were: *Satisfactory* which covered assessment scores of 75% or more and *Unsatisfactory* which covered assessment scores below 75%.

Sampling

A total of **90** environmental swab samples were collected every week for a period of 3 weeks from slaughterhouse walls, floors, and equipment (each 10) in addition to 10 water samples.

The samples were collected after disinfecting and before starting slaughtering process using sterile swabs previously moistened in peptone buffered water (PBW). Each swab sample was obtained by swabbing five points of 25 cm × 25 cm, except for equipment.

Ten water samples were collected before cleaning and disinfection procedures from the main water sources inside the slaughterhouse using sterile plastic bottles.

Sample preparation

Environmental samples were prepared according to the technique recommended by APHA.[12]

After preparation one mL of the original dilution of all samples (wall and floor swabs, water samples) were transferred aseptically to a test tube containing 9 mL sterile 0.1% buffered peptone water (w/v) to prepare a dilution of 10-2. Tenfold decimal serial dilution up to 10-6 were prepared to cover the expected range of sample contamination which could be easily counted.

Bacteriological examination

Total Bacterial Count (TBC), total coliform Count (TCC), E. coli count and Salmonella count were applied using drop plate technique as described by.[13,14]

Isolation of Salmonella and E. coli spp

All samples were pre enriched in peptone buffer water and incubated at 37°C for 8h. Then 0.1 mL of pre-enriched sample was transferred to 10 mL pre-warmed tetrathionate broth, and incubated at 42 °C for 24h. A loop from tetrathionate broth was streaked onto CHROM agar plates and incubated at 37°C for 24 h. Typical Salmonella spp. colonies will then show pink colour, while E. coli colonies will show blue colour.[14]

Data analysis

The obtained data were coded and analyzed using Statistical Packaging for the Social Sciences (SPSS/PC version 21 for windows). Data were analyzed for Descriptive Statistical Analysis. Chi-square was also used.

RESULTS

Table (1) revealed that the adequacy of the location and structure of the slaughterhouse were satisfactory i.e. scored 100%, while maintenance had failed adequacy and scored only 33.33%. Environment and water supply

had also failed adequacy rule and scored 40% and 66.66%, respectively. Both drainage and staff training were satisfactory and had passed the adequacy rule i.e. scored 100%.

According to adequacy rule used in this study the overall evaluation of the PRPs parameters in this table was satisfactory (77.14%).

Table 1: The overall evaluation of some prerequisites programs (PRPs) in the slaughterhouse.

No.	PRPs	Satisfactory	Unsatisfactory	Adequacy %
1.	**Location**: (premise located in an area which is appropriate for a poultry meat slaughterhouse, and which does not pose a risk of contaminating the meat)	X		100.00
2.	**Structure**: the buildings and surroundings designed, constructed and maintained in a manner which: • Easy cleaning and sanitation • Ensure appropriate product and personnel flows • Separation between clean and dirty areas	 X X X		100.00
3.	**Maintenance** preventative maintenance programs available • Walls properly maintained • Floors properly maintained • Equipment properly maintained	 X	 X X	33.33
4.	**Environment** • waterproof flooring • walls durable and impermeable with a light-colored, washable coating • material (equipment) rot-proof and odorless • adequate ventilation and good steam extraction • ceiling clean and easily cleaned	 X X	 X X X	40.00
5.	**Water supply** Source of water: a. Municipal **b. Well** c. Other(specify) • adequate supply of potable water available • Available supplies for both hot and cold water • water storage tank covered • an inspection hatch available and is it lockable • microbiological analysis carried out on samples of water taken at various sites around the plant (monthly)	 X X X X	 X X	66.66
6.	**Drainage** • suitable floor drainage • floors sloped uniformly • water directed to grated drains • water directed to grated drains • water drain trapped inside and outside the building • drainage lines from toilets separate from other drainage lines?	 X X X X X X		100.00
7.	**Staff training** • documented training program available • written process for washing hands policy, including the use of sanitizer and/or gloves available • staff trained and supervised to ensure that they: • Wear their protective clothing, footwear, hair covering, gloves etc. in the appropriate manner • Keep their own personal equipment such as aprons, knives and steels clean and tidy	 X X X X		100.00
	Overall evaluation of the PRPs			**77.14**

Table (2) shows the sanitation program operational procedures. The type of disinfectant used in the slaughterhouse was hydrogen peroxide (H2O2), which was usually applied after each work shift with more than one hour contact time according to the company specifications. Microbiological swabbing was usually conducted monthly to determine the effectiveness of sanitizer used. According to adequacy rule used in this study all the parameters of sanitation program operational procedures scored satisfactory (100%). According to adequacy rule used in this study the overall evaluation of the PRPs parameters in this table was satisfactory (77.14%).

Table 2: Sanitation program operational procedures.

No.	Parameter	Satisfactory	Unsatisfactory	Adequacy %
1.	**Sanitation program** • a written sanitation program for the plant available • cleaning staff trained to apply sanitation program as directed • all areas of the plant and equipment visually examined before production to ensure the cleaning procedures have been effective cleaning phases include: a. Dry cleaning only b. **Dry and wet cleaning** • equipment cleaning includes dismantling of equipment parts	X X X X		100.00
2.	**Sanitizers (disinfectants) and their usage in sanitation program** What disinfectants used: **a. H2O2** b. Chlorine c. Other (specify) Disinfectant preparation is done by: **a. Supervisor (Veterinarian)** b. cleaning staff c. Other (specify) Disinfectant applied: **a. After each work shift** b. Every second day c. Every week Disinfectant contact time: a. Less than one hour b. One hour c. More than one hour d. **As per company specifications**	X X X X		100.00
3.	**Microbiological swabbing** microbiological swabbing conducted to determine the effectiveness of sanitizers use Time duration between each swabbing: a. Every week b. Every two weeks **c. Monthly**	X X		100.00
	Overall evaluation of sanitation program operational procedures			100.00

The average count of during the three weeks period was 100 ± 57.7 cfu, 200± 57.7 cfu, 100±10 cfu and 0.00 in walls, floors, equipment and water, respectively. The differences during the three weeks and between different sites were found to be insignificant with $p \leq 0.05$ (Table 3) and (Table 4).

Table (3) also displays the average of averages of coliform count in the floor, wall, equipment and water. It was 2733.3±540.6 cfu during the three weeks period with significant statistical differences in the count between the three weeks with $p \leq 0.05$.

The average of Total bacterial Count for the sample sites during the three weeks period was 2250±680.6 cfu. There was statistical differences in the count between the three weeks with $p \leq 0.05$ (Table 3).

The average of *E. coli* count was 341.6±85.6 cfu for the sample sites during the three weeks period. There was

statistical differences in the count between the three weeks with p ≤0.05 (Table 3).

Table 3: The average counts of different bacteria during the three weeks interval and their relative significance values.

Organism	Sample site	Averages	Standard Error	F value	Significance
Salmonella	wall	100.00	57.735	1.600	.264
	floor	200.00	57.735		
	equipment	100.00	10.000		
	water	.00	.000		
	Total	100.00	34.816		
Coliform count	wall	4966.67	548.736		
	floor	3166.67	166.667	72.364	.000
	equipment	2700.00	208.167		
	water	100.00	57.735		
	Total	**2733.33**	**540.669**		
TBC	wall	6000.00	577.350		
	floor	1500.00	100.000		
	equipment	1266.67	133.333		.
	water	233.33	33.333		
	Total	**2250.00**	**680.630**	42.998	.000
E. coli	wall	233.3333	33.33333		
	floor	400.0000	57.73503		
	machine	733.3333	120.18504		
	water	.0000	.00000		
	Total	**341.6667**	**85.68647**	20.137	.000

As appears in Table (4) using the multiple comparison test of least significant differences there was no significant differences in the Salmonella count between floor, wall, and equipment with p ≤0.05. Same like, no significant difference was found between equipment and floor. No significant difference was observed in the TBC between the floor and the equipment with p ≤0.05. While, the mean differences in the E. coli and Coliform counts between the wall and floor was not significant with p ≤0.05, the same result can be observed in the coliform count between the equipment and floor which was also insignificant with p ≤0.05. The mean TBC differences was found to be significant between wall and floor and wall and equipment with p ≤0.05, also the mean differences of E. Coli count was found to be significant between wall and equipment from one side and floor and equipment from the other side with p ≤0.05, and the mean Coliform count differences was found to be significant between the wall and equipment with p ≤0.05.

Table 4: Multiple comparison between the averages of different sites of the slaughter house using the Least Significant Difference (LSD).

Organism	Sample site	Sample site	Significance
Salmonella	Wall	Floor	.305
	Wall	Equipment	.305
	Equipment	Floor	.060
TBC	Wall	Floor	.000
	Wall	Equipment	.000
	floor	Equipment	.598
E. coli	Wall	Floor	.125
	Wall	equipment	.001
	Floor	equipment	.009
Coliform	Wall	Floor	.305
	Wall	Equipment	.004
	equipment	Floor	.080

DISCUSSION

The objective of this study was to evaluate cleaning and disinfection procedures adopted in Lean slaughterhouse using a regular sampling on a weekly basis for a period of 3 weeks. The results of this study is in line with Vangroenweghe et al.[15] who reported that evaluation of cleaning and disinfection protocols should be performed more frequently in broiler slaughterhouse facilities to monitor the efficacy of protocols and products.

According to adequacy rule used in this study the overall evaluation of the PRPs parameters was satisfactory. This finding was supported by the statement of,[16] who reported that the design and construction of slaughterhouses should ensure suitable product and personnel flow and facilitate separation between clean and dirty operations to forestall carcass contamination.

In some abattoirs the surfaces of equipment may develop numerous fissures, interstices and corrosion points. Therefore, cleaning must be performed thoroughly to dissolve all organic matter.[17] Equipment in this study was found to be properly maintained. This finding is supported by.[18] who reported that dirty equipment and utensils can affect the dressed carcasses by increasing their microbial load which leads to reduced storage quality and safety.

The slaughterhouse in this study used water from the farm well without any chemical treatment. Therefore, it was assessed as unsatisfactory. Nonetheless, water bacterial load in this study was found at acceptable level (Salmonella 00. ± 000; Coliform count 100.00±57.735; TBC 233.33± 33.333; E. coli 00. ± 000). This finding is in line with that recorded by ABU-RUWAIDA et al.[18] who found that the processing water (main water tank) was nearly free of contamination, showing only negligible aerobic bacteria counts (log 2.2 to 2.9 and 2.1 to2.5 CFU/ml in samples collected on days 1 and 2, respectively.

Staff training in this study was found to be satisfactory. This result complies with that stated by.[19] who reported that inspection and monitoring of all hygienic operations on poultry production premises should be appointed for an official control veterinarian.

The aim of disinfection is to eliminate the microorganisms still present on surfaces and adhering to anchorage points. Bacteria can be found attached on the surface or produce substances (biofilm) that is difficult to break down.[20] According to adequacy rule used in this study all the parameters of sanitation program operational procedures were found satisfactory.

In this study the main disinfection used was H2O2 and it was considered satisfactory. This is proven true as the characteristics of H2O2 disinfection has the largest effect on Gram +ve and Gram –ve bacteria and spores and Fungi.[21]

This study showed that the sanitation program applied was satisfactory. This finding is supported by the statement of Vangroenweghe et al.[15] who reported that the routine cleaning and disinfection protocols differ from farm to farm but generally consisted of dry, wet cleaning, flushing and disinfection.

Mahato.[22] analyzed microbial isolates of meat samples against sanitation parameters and found that microbial load was higher in cemented outlets, improperly washed slaughter area and chopping box, meat handlers wearing unwashed apron, improper drainage facility, and poorly sanitized meat. This study also aimed at evaluating cleaning and disinfection procedures adopted in Lean slaughterhouse against some microbial isolates.

In this study the average count of Salmonella during the three weeks period was 100 ± 57.7 cfu, 200± 57.7 cfu, 100±10 cfu and 0.00 in walls, floors, equipment and water, respectively. The highest was observed in floors. These mean differences of count was found to be not significant during the three weeks and between sample sites with $p \leq 0.05$. This finding is in line to that reported by.[23] who found that *Salmonella* spp. was present in improperly washed slaughterhouse. The higher count found in floor may be due to inadequate cleaning or maintenance of the floor.

This study also displays that the average of Coliform count in the floor, wall, equipment and water was 2733.3±540.6 cfu during the three weeks period with statistical differences in the count between them with $p \leq 0.05$. The highest count was observed in the wall 4966.67 cfu followed by floor 3166.67 cfu. This count was found lower by two logs when compared to the Coliform count records performed in the slaughterhouse before applying the disinfection protocol.

Total Bacterial Count is a broadly accepted measure of the general degree of microbial contamination and hygienic conditions of processing plants or outlets.[24] This study found that the average of TBC for the sample sites during the three weeks period was 2250±680.6 cfu with statistical differences in the count between the three weeks sampling interval with $p \leq 0.05$. The highest count was detected in the walls of the slaughterhouse 6000.00 cfu, followed by the floor 1500 cfu then the equipment 1266 cfu. This count was found lower with three logs when compared to the TBC records performed in the slaughterhouse before introducing the disinfection protocol.

It is stated that E. coli can contaminate broiler meat at any stage during processing particularly evisceration.[25] Besides being used as an indicator organism of sanitary quality, E. coli is also used as an index organism of pathogens.[26]

This study also revealed that the average of *E. coli* count was 341.6±85.6 cfu for the sample sites during the three weeks period with statistical differences in the count between the three weeks with $p \leq 0.05$. The highest mean count of E. coli was detected in the equipment 733.3333 cfu, followed by the floor 400.0000 cfu then the wall 233.3333cfu and the mean difference was statistically significant between the wall and equipment and the floor and equipment. This higher count in the equipment may be due to the effect of the evisceration process.[25] which mainly affect the equipment and the floor with high

number of E. coli. The result is also supported by.[22] who stated that E. coli is the foremost bacteria found in most of the sanitation parameters such as improperly washed slaughterhouses.

CONCLUSION

The study concluded that poorly sanitized surfaces in meat processing operations lead to high microbial load which may pose food safety hazards. The overall evaluation of the PRPs and Sanitation Program Operational Procedures in Lean slaughterhouse were satisfactory**.**

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