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EVALUATION OF THE EFFICACY OF CLEANING AND DISINFECTION IN BROILER FARMS BETWEEN REST PERIODS IN KHARTOUM STATE, SUDAN.

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ABSTRACT

This study was carried out during the period from November 2015 to November 2017 in Bahri locality, Sudan. The objectives of the study were to determine the efficacy of cleaning and disinfection of broiler farms between production cycles and evaluating the present biosecurity measures in poultry houses during rest period. A structured and standardized checklist containing the major aspects of Good Hygienic Practices (GHPs) and related factors was designed and conducted. In addition, a total of 60 samples were collected from 4 different broiler farms (each 15) which comprised 5 swab samples from suspended different ground surface areas after disinfection and before new litter were applied, 5 samples from the newly introduced litter and 5 water samples. Samples were collected before introducing new flock using systemic random sample technique. All samples were, serially diluted, spread plated into agar plates, incubated for 48 hr, at 37°C and enumerated. Total bacterial count (TBC) was investigated for each sample. Data was coded and analyzed using SPSS program. The study disclosed that all farms (100%) didn't apply appropriate cleaning and disinfection practices or water quality testing or saw dust (litter) fumigation. Whereas, all farms (100%) didn't perform bacteriological testing to verify the efficacy of cleaning and disinfection, 75% of them didn't apply longer period between two production cycles. The study also revealed a significant difference in the mean of bacterial count in different samples (water, litter And swab) with $p<0.05$ (0.007). Whereas, there was a significant difference in total bacterial count (TBC) between swab, water, swab and litter ($p=0.007$ and 0.045), respectively, the difference in total coliform count (TCC) between samples from different farms $p>0.05$(0.199) was insignificant. It could be concluded that all farms

(100%) didn't apply appropriate GHPs between production cycles and that the swab sample method was considered as main source of bacterial contamination in broiler farms between rest periods.

INDEX TERMS*:* Biosecurity**,** Broiler Farm, rest period, *cleaning and disinfection.*

I. INTRODUCTION

[1]emphasized the importance of improving biosecurity along the food chain to reduce the risk of disease spread and that biosecurity applies to public health measures that reduce contact between birds and man. Because of the incidence of food-borne diseases and birds health problems, many authors emphasized the importance of having developed standardized on-farm food safety programs in response to meet consumer demand.[2] As well designed biosecurity program can include traffic, segregation, cleaning and disinfection[3], it shall also include the "all-in–all-out" practice and a vacancy or rest period between production cycles as this will play an important role in breaking the cycle of disease in commercial flocks.[4] Poultry producers must make decisions on which elements of biosecurity to use and at what point.[5] The rest period between production cycles is an effective procedure to reduce the severity of infection. It is important to have an effective cleaning and disinfection program at the beginning and between production cycles as some microbes can survive for a long time in the environment even in the absence of poultry.[6]

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.[7] Wet cleaning consists of factors such as time, mechanical action, chemistry and temperature.[8] Soaking with detergent is preferably performed from the floor towards the ceiling, while washing is performed vice versa to avoid contaminating already cleaned surfaces.[9] After drying, surface disinfection, thermal fogging or fumigation can be carried out with a pressure cleaner.[10] Subsequently, a rest period shall be applied in order to dry the animal houses and further reduce the residual bacteria. The rest period is different from country to country i.e. Austria (14 days), Estonia (21 days), Luxemburg (21 days), Norway (30 days), Denmark (10-14 days), Spain (12 days).[11]

Monitoring of hygiene status after cleaning and disinfection is of paramount importance. This can be performed by agar contact plates (ACP),[12] swab samples, air samples, specific pathogen analyses.[13] Bacteriological monitoring often focuses on total aerobic bacteria.[14] It

is suggested that *Enterococcus* spp. to be an adequate hygiene-indicator organism for faecal contamination of surfaces.[15] To verify the efficacy of a disinfectant, tested reference bacteria must show a minimum 5 log reduction after exposure to this disinfectant.[16]

II. MATERIAL AND METHODS

Study area

This study was conducted in Bari locality, Sudan in 4 broiler farms.

Study design

Descriptive and experimental method was used.

Data collection

- **Check list**

A structured and standardized checklist containing the major aspects of Good Hygienic Practices (GHPs) and related factors was designed and conducted e.g. appropriate cleaning and disinfection practices; bacteriological testing after cleaning and disinfection; period between two production cycles; saw dust fumigation; selection of cleaning agents; acceptable buffer zone between farms.

- **Swabs and samples**

A total of 60 samples were collected from 4 different broiler farms (15 each) before introducing new chicken using systemic random sample technique. Five swab samples were collected randomly from suspended different ground surface areas after disinfection and before new litter was applied, 5 samples from the newly introduced litter and 5 water samples.

Sample preparation

Swab technique was used for sample collection based on[17] as follow: sterile swab was removed from coded test tubes that contained 5 ml of sterile phosphate-buffered saline and the targeted areas were swabbed. Sampling was performed by swabbing the areas horizontally, vertically and diagonally using sterile templates. Swabs were placed back into the coded test tubes. Then samples were transported to the laboratory using an insulated ice box contained an ice pack at 4 C°. In the laboratory, the test tubes and the swabs were shaken vigorously for 10 sec. Next, the swabs were discarded and 0.5 ml of the sample was poured on selective media to conduct the bacteriological examinations. A litter sample weight 0.5g was then put in test tube and the litter was shaken vigorously for 10 sec. As regards to water sample, 0.5 ml was

transferred to next serial test tube contained 4.5 ml phosphate-buffered saline and was shaken vigorously for 10 sec before 0.5ml of the sample was poured on to selective media.

Examination of cultured media

- **Total bacterial count**

The pour plate count method to count total bacteria was carried out as described by.[18] The bacteria were cultured on plate count agar media.

- **Total Coliform count**

For coliform bacteria the same method of enumerating total bacteria was used. The bacteria were cultured on MacConkey agar media.

Data analysis

The collected 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 with the hypothesis that the compliance with biosecurity measures was uniform among all poultry farms.

III. RESULTS

The checklist results showed that all farms (100%) were located in heavily poultry populated areas but did not share any cleaning and disinfection equipments during cleaning and disinfection procedures. As for the most important biosecurity measures, all farms (100%) didn't apply appropriate cleaning and disinfection practices or water quality testing or saw dust (litter) fumigation. Also all farms (100%) didn't perform bacteriological testing to verify the efficacy of cleaning and disinfection. Whereas, 75% of the farms said that they applied longer period between two production cycles (rest period), only 50% applied all in – all out procedure (Table 1).

Table 1: Checklist of biosecurity good hygienic practices in broilers farms.

Parts	Farm 1	Farm 2	Farm 3	Farm 4	Overall %
All in – All out practice	NO	YES	NO	YES	50
Appropriate cleaning and disinfection practices	NO	NO	NO	NO	100
Bacteriological testing before cleaning and disinfection	NO	NO	NO	NO	100
Bacteriological testing after cleaning and disinfection	NO	NO	NO	NO	100

Long period between two production cycles applied	YES	YES	YES	NO	75
Short period between two production cycles applied	NO	NO	NO	YES	75
Saw dust fumigation	NO	NO	NO	NO	100
Expert consultation available during cleaning and disinfection	NO	NO	NO	NO	100
Selection of cleaning and disinfection agents	YES	NO	YES	YES	75
Applying water quality testing	NO	NO	NO	NO	100
House repairing	NO	NO	NO	YES	75
Acceptable buffer zone between farms	NO	NO	NO	NO	100
Sharing cleaning equipments between farms	NO	NO	NO	NO	100

High levels of bacterial contamination were evident (overall mean = 4.76 CFU) in all farms. The farms with high contamination level of bacteria were as follow: farm 3= 4.85, farm 1= 4.77, farm 2= 4.71 and farm 4 =4.69. Water samples scored the highest mean (4.96), litter (4.84) and swab (4.47), respectively (Table 2).

Table 2: Means of total bacterial counts

	Farms	Mean	Std. Deviation	N
Water	Farm.1	4.87	.39	5
	Farm.2	5.38	.68	5
	Farm.3	4.82	.40	5
	Farm.4	4.78	.56	5
	Total	4.96	.54	20
Litter	Farm.1	4.98	.39	5
	Farm.2	4.61	.52	5
	Farm.3	4.99	.39	5
	Farm.4	4.75	.60	5
	Total	4.83	.47	20
Swab	Farm.1	4.44	.42	5
	Farm.2	4.14	.20	5
	Farm.3	4.74	.42	5
	Farm.4	4.55	.62	5
	Total	4.47	.46	20
Total	Farm.1	4.77	.44	15
	Farm.2	4.71	.71	15
	Farm.3	4.85	.39	15
	Farm.4	4.69	.57	15
	Total	**4.76**	.53	60

High mean of total coliform counts (log10 CFU) for the different farms were shown in (Table 3). High level of coliform contamination (5.44 CFU) was evident in all farms. The farms with high contamination level of coliform count were as follow: farm 3 (5.54), farm 2 (5.46), farm 1

(5.42) and farm 4 (5.29), respectively. Litter samples scored the highest mean (5.55), water (5.49) and swab (5.31), respectively.

Table 3: Means of total coliform counts.

Type of Samples	Farms	Mean	Std. Deviation	N
Water	Farm.1	5.12	.32	5
	Farm.2	5.55	.41	5
	Farm.3	5.52	.42	5
	Farm.4	5.75	.19	5
	Total	5.49	.39	20
Litter	Farm.1	5.67	.44	5
	Farm.2	5.69	.19	5
	Farm.3	5.74	.19	5
	Farm.4	5.10	.54	5
	Total	5.55	.43	20
Swab	Farm.1	5.45	.51	5
	Farm.2	5.40	.53	5
	Farm.3	5.34	.51	5
	Farm.4	5.02	.43	5
	Total	5.31	.49	20
Total	Farm.1	5.41	.46	15
	Farm.2	5.56	.39	15
	Farm.3	5.53	.41	15
	Farm.4	5.29	.51	15
	Total	5.44	.45	60

Table (4) shows significant differences in the mean of bacterial count in different samples (water, saw dust and swab) with $p<0.05$ (0.007). The significant difference in TBC between swabs, water, swab and saw dust was P =0.007 and 0.045, respectively (i.e**.** swabs was the main source of bacteria).

Table 4: Significance in the means of total bacterial counts.

(I) Type of Samples	-(J) Type of Samples	Mean Difference (I-J)	Sig.
Water	Litter (saw dust)	.13	.693
	Swab	.49	.007
Saw dust	Water	.12	.693
	Swab	.36	.045
Swab	Water	-.49	.007
	Saw dust	-.36	.045

There were also significant differences between group's samples in the mean of total bacterial counts as shown in Table (5).

Table 5: Significance in the mean of total bacterial count.

	Sum of Squares	df	Sig.
Between Groups	2.669	2	.007
Within Groups	13.979	57	
Total	16.648	59	

There was insignificant difference in the mean of total bacterial and total coliform counts between different farms (total bacterial with p>0.05(0.86) and coliform with p>0.05 (0.36).

IV. DISCUSSION

Biosecurity practices designed to minimize the transmission of infectious diseases between and within farms are an important component of modern flock health programs.[19] It was stated that poultry density in the vicinity of the farm is an important factor for those pathogens for which transmission is density dependent.[20] In addition to farm location, borrowing equipments or brought into the farm by contractors may introduce pathogenic agents into the farm.[21]

In the present study the checklist results showed that all farms (100%) were located in heavily poultry populated areas but did not share any cleaning and disinfection facilities during cleaning and disinfection of premises. This may render biosecurity status in these farms at greater risk.

One day old chicks shall be prevented to come in contact with litter, dust, feathers, and other debris from the previous flock as some pathogens can survive for a long time in the environment without the presence of poultry.[6]

As for the most important biosecurity measures in the present study, all farms (100%) didn't apply appropriate cleaning and disinfection practices or saw dust (litter) fumigation. This practice may predispose new broiler flocks to contamination by all kinds of pathogens.[7]

This study concluded that 100% of the farms didn't perform bacteriological testing to verify the efficacy of cleaning and disinfection. The lack of testing drinking water for pathogenic microorganisms is a serious biosecurity malpractice.[7]

Prolonged vacancy period of the animal houses are an essential part of the hygiene management.[22] Whereas, 75% of the studied farms said that they applied longer period between production cycles (rest period) only 50% applied all in – all out procedure. This may

explain the relatively high bacterial contamination in broiler houses after cleaning and disinfection.

For the total of sixty samples examined in this study, water samples scored the highest mean of total bacteria counts, followed by saw dust and finally swabs. In contrast to bacteria, coliform in saw dust scored the highest mean of counts, followed by water and eventually swabs. The contamination with these bacteria in this study may be due to differences in cleaning and disinfection application between broilers farms or because extra biosecurity measures such as specific pathogen control programs and pest control during the vacancy period are not applied.[22]

The findings of this study with regard to total bacterial count were within the acceptable range. This finding was in compliance with[23] who stated that satisfactory range is ≤ 5.0 log10 and[22] who found that the mean total aerobic bacteria determined by swab samples decreased from 7.7 + 1.4 to 5.7 + 1.2 log colony forming units (CFU)/625 cm² after cleaning and to 4.2 + 1.6 log CFU/625 cm² after disinfection. Lower bacterial counts were reported by[24] in North Carolina (5.05 cfu) and[25] in Belgium who reported 7.3 log10 cfu.

The findings of this study disagreed with[23] for total coliform count who reported that the satisfactory range is <4.0 log 10 and with[22] who reported 4.0 log10cfu for coliform count, but agreed with[25] in Belgium who reported similar findings (5.8 log10 cfu). The relatively high coliform count in this study may be attributed to lack of effectiveness of cleaning measures practices or to low quality cleaning and disinfectant agents.

The present study indicated that the swabs sample represented the main sources of bacterial contamination. The significance in bacterial counts of mean log, may be attributed to location, different cleaning steps, awareness of personal hygiene and the commitment in the implementation of the biosecurity measure in broiler production farms.

The results of this study showed insignificant difference in the mean of total bacterial and coliform counts (TCC) between different farms. This means that there was similarity in hygienic measures practiced in all broilers farms.[2] in Canada reported similar results in that the means of total bacteria counts were not significantly different (P=0.7892), but in contrast to our study they reported that the mean log coliform bacterial counts were significantly different (P= 0.0029). Different results were obtained by[26] in Canada who studied 3 broiler houses after

cleaning regimens found out that the means of total bacterial counts of different farms were significantly different ($P \leq 0.05$).

V. CONCLUSION AND RECOMMENDATIONS

It could be concluded that all farms (100%) didn't apply appropriate biosecurity GHPs between production cycles and that the swab sample method was considered as main source of bacterial contamination in broiler farms between rest periods.

As little research has been published on the effectiveness of cleaning methods in poultry houses in Sudan, an on-farm evaluation and comparison of different cleaning protocols could help farmers in selecting the most appropriate cleaning method and in reducing bacterial contamination.

VI. REFERENCES

1. World Health Organization (WHO) Public Health Interventions for Prevention and Control of Avian Influenza. A Manual for Improving Biosecurity in the Food Supply Chain: Focusing on Live Animal Markets., 2006.
2. Ward, P.J., Fasenko, G.M., Gibson, S., McMullen, L.M. A microbiological assessment of on-farm food safety cleaning methods in broiler barns. J. Appl. Poult. Res. 2006; 15: 326–332.
3. Vaillancourt, J.p., How do you determine the cost-benefit of a biosecurity system? Zootecnica- International, 2001; 24: 20-27.
4. Doyle, M. P., and M. C. Erickson. Reducing the carriage of foodborne pathogens in livestock and poultry. Poult. Sci. 2006; 85: 960–973.
5. Segal, Y. Farm biosecurity for better perfomance and higher profit. Chick Program Online, 2011; 35.
6. Butcher G.D., Miles R.D. Disease prevention in commercial poultry (CR1079). University of Florida IFAS Extension, 2012. http://edis.ifas.ufl.edu/pdffiles/VM/VM01100.pdf
7. Lister, S. A. Biosecurity in poultry management. M. Patisson, P. F. McMullin, J. M. Bradburry, and D. J. Alexander, ed. in Poultry Diseases. 6th ed. Saunders Elsevier, Beijing, China. 2008; 48-65.
8. Friis, A., Jensen, B.B.B. Improving the hygiene design of closed equipment in Handbook of hygiene control in the food industry. Woodhead Publishing Limited, Cambridge, Engelenad., 2005.

9. Cox, M., Van Meirhaeghe, H. Hier is hygiëne troef - hygiënemanagement op het pluimveebedrijf. (An English brief)., 2009.
10. Gradel, K.O. Disinfection of empty animal houses- scientific evidence for applied procedures in Environmental microbiology research trends. Nova Science Publishers, Inc., New York, USA., 2007.
11. EFSA Scientific opinion on a quantitative estimation of the public health impact of setting a new target for the reduction of *Salmonella* in broilers. EFSA J. 2011; 9: 1–93.
12. Vangroenweghe, F., Heylen, P., Arijs, D., Castryck, F. Hygienograms for evaluation of cleaning and disinfection protocols in pig facilities. 8th Int. Symp. Epidemiol. Control foodborne Pathog. pork- Safepork. 30 Sept. - 2 Oct. 2009, Quebec, Canada 2009; 220–23.
13. Merialdi, G., Galletti, E., Guazzetti, S., Rosignoli, C., Alborali, G., Battisti, A., Franco, A., Bonilauri, P., Rugna, G., Martelli, P. Environmental methicillin-resistant *Staphylococcus aureus* contamination in pig herds in relation to the productive phase and application of cleaning and disinfection. Res. Vet. Sci. 2013; 94: 425–7. doi:10.1016/j.rvsc.2012.10.020
14. Hancox, L.R., Le Bon, M., Dodd, C.E.R., Mellits, K.H. Inclusion of detergent in a cleaning regime and effect on microbial load in livestock housing. Vet. Rec. 2013; 173: 167–170. doi:10.1136/vr.101392
15. Gradel, K.O., Sayers, A.R., Davies, R.H. Surface disinfection tests with *Salmonella* and a putative indicator bacterium, mimicking worst-case scenarios in poultry houses. Poult. Sci. 2004b; 83: 1636–1643.
16. European Commitee for Standardization European standard EN 1656. Chemical disinfectants and antiseptics - Quantitative suspenstion test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field - Test method and requirements (phase2/step1)., 2000.
17. ISO 18593:2004(en). Microbiology of food and animal feeding stuffs — Horizontal methods for sampling techniques from surfaces using contact plates and swabs. Prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.
18. Harrigan, W.F. and MacCance, M.E. Laboratory Methods in food and Diary Microbiology.Academic Press New York., 1976.
19. Dorea, F. C., Berghaus, R., Hofacre, C. and Cole, D. J. (2010). Survey of biosecurity protocols and practices adopted by growers on commercial poultry farms in Georgia, U. S. A. Avian Dis. 2010 Sep; 54(3): 1007-15.
20. Truscott, J., T. Garske, I. Chis-ster, J. Guitain, D. Pfeiffer, L. Snow, J. Wilesmith, N. M. Ferguson, and C. Ghani. Control of highly pathogenic H5N1 avian influenza outbreak in

the GB poultry flock. Proceedings of the Royal Society: Biological Science 2007; 274: 2287–2295.

21. Vieira, A. R., C. L. Hofacre, J. A. Smith, and D. Cole. Human contacts and potential pathways of disease introduction on Georgia poultry farms. Avian Dis. 2009; 53: 55–62.
22. K. Luyckx, S. Millet, S. Van Weyenberg, L. Herman1, M. Heyndrickx, J. Dewulf3 and K. De Reu (2016). A 10-day vacancy period after cleaning and disinfection has no effect on the bacterial load in pig nursery units. BMC Veterinary Research, 2016; 12: 236.
23. Ashtown Teagasc (2005). STANDARD OPERATING PROCEDURE FOR MICROBIOLOGICAL EXAMINATION OF POULTRY. Food Research Centre. https://www.teagasc.ie/media/website/publications/2008/Poultry_SOP.pdf
24. Payne JB, Kroger EC, Watkins SE. Evaluation of disinfectant efficacy when applied to the floor of poultry grow-out facilities. The Journal of Applied Poultry Research 2005; 14(1): 322-329.
25. Luyckx, K.,VanWeyenberg, S., Dewulf, J., Herman, L., Zoons, J., Vervaet, E., Heyndrickx, M., De Reu, K. (2015b). On-farm comparisons of different cleaning protocols in broiler houses.Poult. Sci. 2015b; 94: 1986–1993.
26. Rathgeber et al Microbiological evaluation of poultry house wall materials and industrial cleaning agents, 2009.