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An application study for monitoring and evaluation of the hygienic status of poultry farms

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ABSTRACT

Proper application of hygienic measures is very essential to reduce the risk of infectious agents and increase the profitability of poultry farms. A cross-sectional study was conducted on twelve Egyptian poultry farms in Qalyubia Governorate with different types of housing systems and productions. A total number of 2160 samples included litter, feed, water, droppings and dust. Moreover, swabs from walls, birds' cloaca, workers' hands, and wheels of vehicles. The hygienic level of different poultry farms was evaluated according to the hygiene scoring system, the aerobic plate count (APC) and the percent of isolated *Salmonella*. The results showed a huge range in the hygienic level of poultry farms under study, the hygiene scores of different poultry farms ranged from (31.7%) to (90%). The APC of collected samples ranged from (log 3.78 CFU/g) to (log 12.31 CFU/g). In addition, the mean percentage of *Salmonella* in different poultry farms was (20%). Pen litter had the highest mean of APC (log 10.02 CFU/g) and *Salmonella* percentage (49.5%). A highly significant increase ($P > 0.05$) in the APC and *Salmonella* percentage was recorded in poultry farms with low hygienic levels. The poultry farms' hygiene is the strongest shield that protects the farm from the risks of bacterial contamination and many dangerous diseases that may be transmitted from the farm environment to the birds, thus preventing large economical loss and serious public health hazards.

1. INTRODUCTION

Poultry farming has seen remarkable growth globally because poultry provides an integral part of the global supply of animal protein; it remains trendy among consumers due to its lower cost compared to other animal protein sources (Elsayed *et al*., 2019). The application of basic hygienic measures such as the presence of a functional foot bath, disinfectant dip for vehicles' wheels, efficient cleaning and disinfection could reduce the introduction of bacterial contamination from the farm environment to the birds and from farm to others (Kouam *et al*., 2018).

Despite the great importance of farm hygiene, there are many common defects in the application of hygienic measures in poultry farms, the level of poultry farm hygiene must be continuously measured and evaluated by using hygienic scoring systems (Aengwanich *et al*., 2014). Moreover, using the aerobic plate count is an efficient method for the objective evaluation of the level of poultry farm hygiene, as well as some indicator organisms such as *Salmonella* (Capita *et al*., 2004). *Salmonella* infections act as a serious problem in the poultry industry because it has the ability to infect all ages and all types of poultry. It causes serious clinical symptoms and high mortality at young ages less than 6 weeks, older chicks may show stunting and uneven growth (Kim *et al*., 2007).

The hygienic scoring system is depending on the different aspects of hygienic measures such as infrastructure and the location of the farm, purchase of one-day-old chicks, off-farm movements of live animals, feed and water supply, removal of manure and dead birds, supply of materials and biological vectors (Gelaude *et al*., 2014).

The present study aimed to evaluate the hygienic level of different poultry farms and study the possible associations between poultry farm hygiene and bacterial contamination by counting the APC and isolation of *Salmonella*.

2. MATERIAL AND METHODS

2.1. Poultry farms

The current study was carried out on twelve poultry farms (three broiler chicken farms, three layer chicken farms, three breeder chicken farms and three breeder duck farms), all farms located in Qalyubia Governorate. The selection of the farms was based on the variation in the level of farm hygiene, housing system and the type of production. Each farm was visited three times at different ages. Broiler chicken farms were visited at the first, third and fifth weeks of age. Layer chicken, breeder chicken and duck farms were visited at fourth, sixth and eighth months of age. All farms used deep litter except layer chicken farm C used a battery system and all farms used open housing system except the three breeder chicken farms used a closed housing system. All farms used public chlorinated tap water as a water source.

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2.2. Hygiene evaluation and scoring system

The hygiene scoring system has been developed according to (Soliman and Abdallah, 2020) for quantifying and assessing the applied hygienic measures in each poultry farm. This system consists of different components related to farm hygiene including the external and internal farm environment such as farm buildings, workers, visitors, vehicles, litter, water, feed and birds. Each hygienic measure for each farm was evaluated and scored according to a score from zero to three, resulting in the highest total score of 60 for all measures. The final score of each farm was calculated according to (Table 1) and the following formula:

Farm hygienic score = (Total scores of applied hygienic measures / 60) x 100

2.3. Sampling

A total number of 2160 samples and swabs were collected from twelve poultry farms, 180 samples and swabs from each farm, in three visits at different ages, five samples were collected per visit from each type of samples and swabs. Samples were collected from stored feed, feeder, water source, drinkers, and source of litter, pen litter, dust and droppings (180 samples per each). Moreover, swabs were taken from birds' cloaca, farms' walls, workers' hands and wheels of vehicles (180 swabs per each). The collection of samples was approved with Institutional Approval Number (BUFVTM 04-07-22). The preparation of samples and swabs was carried out according to (Soliman and Abdallah, 2020).

2.4. Aerobic Plate Count (APC)

Tenfold serial dilutions were prepared and the APC was carried out according to (Chouhan, 2015).

2.5. Isolation and identification of Salmonella

Isolation of Salmonella was carried out according to (Hassan and Osama, 2021), Biochemical identification of Salmonella was carried out according to (FDA, 2012) and Serological identification of Salmonella was carried out according to Kauffman's white scheme (Kauffman, 1974).

2.6. Statistical Analysis

The statistical analyses were carried out by two-way ANOVA using SPSS, ver. 25 (IBM Corp. Released 2013) and the significance level was set at P< 0.05.

3. RESULTS

Hygiene scoring system

The breeder chicken farms recorded the highest hygiene score (82.2%). In contrast, the duck farms recorded the lowest hygiene score (34.3%). The most applied hygienic measures in the tested poultry farms were good ventilation (100%), as shown in (Figures 1 and 2).

Aerobic plate count (APC):

There was a significant difference between the APC of the different poultry farms. The broiler chicken farm C (log 5.86 CFU/g) had the lowest APC, unlike the duck farm C (log 7.85 CFU/g) which had the highest APC, as shown in (Table 2).

Effect of the age on the APC

In broiler chicken farms, the lowest APC of collected samples and swabs at different ages (1st w, 2nd w and 3rd w) was shown at the young age (1st w). Moreover, in breeder chicken, layer chicken and duck farms, the lowest APC of collected samples and swabs at different ages (4 m, 6 m and 8 m) was at the young age (4 m), as shown in (Table 3).

Effect of the type of production the APC

The broiler chicken farms were the lowest APC (log 6.07 CFU/g), but the duck farms were the highest APC (log 7.54 CFU/g). Moreover, pen litter had the highest APC (log 10.02 CFU/g), unlike the water source had the lowest APC (log 4.85 CFU/g), as shown in (Table 4).

Salmonella percent

The mean percentage of isolated Salmonella from different types of poultry farms was 20%. The highest percentage of Salmonella was recovered from the duck farms (36.2%) and the lowest percentage was recovered from the breeder farms (10.2 %). Our result revealed that the pen litter was the highest Salmonella percentage among all collected samples and swabs (mean 49.45%), as shown in (Table 5).

Identification of Salmonella

Seven serotypes of Salmonella were isolated; the most isolated Salmonella serotype was S. Agona (39.76%) and the lowest one was S. Virchow (1.2%). as shown in (Table 6).

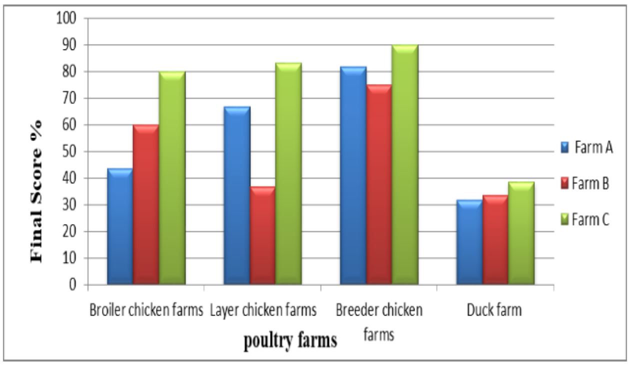

Figure (1): The hygienic score of the different production types of poultry farms.

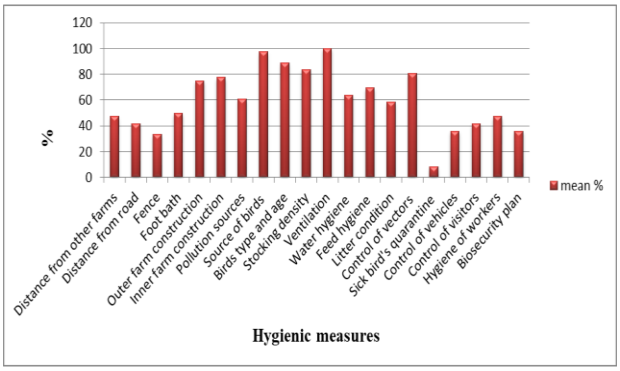

Figure (2): The score of applied hygienic measures in different poultry farms

Table 1 the hygiene scoring system for evaluation of poultry farms' hygiene.

Items		Score (0)	Score (1)	score (2)	Score (3)
Distance from other farms	Broilers	< 0.6 km	0.6 - < 0.8 km	0.8 km - 1 km	> 1km
	Layers	< 1km	1 - < 1.5 km	1.5 km - 2 km	> 2km
	Breeders	< 1 km	1 - < 3 km	3 km - 5 km	> 5 km
Distance from main road	Broilers	< 100 m	100 - < 200 m	200 -300 m	> 300 m
	Layers	< 200 m	200 - < 300 m	300 - 400 m	> 400 m
	Breeders	< 300 m	300 - < 400 m	400 -500 m	> 500 m
Fence		Absent	-	-	present
Foot bath		Absent	Present, but not regularly used	Disinfectant wasn't regularly changed	Functional foot bath
Outer farm construction		Very bad	Weak	Fair	Good
Inner farm construction		Very bad	Weak	Fair	Good
Pollution sources		High	Moderate	Low	Absent
Source of birds		Bad and not trustable source	Weak source	Fair source	Good and trustable source
Birds type and age		Different types or ages in same pen	Different ages in separated pens	Same, but from different hatcheries	Same and from same hatchery
Stocking density of broiler chicken at the end of production cycle	open system	> 25 kg/m^2	> 21 - 25 kg/m^2	18-21 kg/m^2	< 18 kg/m^2
	Closed system	> 40 kg/m^2	> 35 - 40 kg/m^2	30–35 kg/m^2	< 30 kg/m^2
Stocking density of broiler ducks at the end of production cycle	Open system	> 17 kg/m^2	> 15 -17 Kg/m^2	12-15 Kg/m^2	< 12 Kg/m^2
	Closed system	> 25 kg/m^2	> 20 - 25 kg /m^2	15- 20 kg/m^2	< 15 kg/m^2
Stocking density of breeder chicken, Open system	Rearing	> 15 bird / m^2	> 12 - 15 bird /m^2	10-12 bird/ m^2	< 10 bird / m^2
	Production	> 8 bird/m^2	> 6 - 8 bird/m^2	4-6 bird/m^2	< 4 bird/m2
Closed system	Rearing	> 20 bird/m^2	> 16 - 20 bird /m^2	13-16 bird /m^2	< 13 bird /m^2
	Production	> 10 bird / m^2	> 8 - 10 bird / m^2	6-8 bird / m^2	< 6 bird / m^2
Stocking density of breeder ducks, Open system	Rearing	> 9 bird / m^2	> 7 - 9 bird / m^2	6-7 bird / m^2	< 6 duck / m^2
	Production	> 5 duck / m^2	> 4 - 5 duck / m^2	3-4 duck / m^2	< 3 duck / m^2
Closed system	Rearing	> 11 duck /m^2	> 10-11 duck / m^2	9-10 ducks / m^2	< 9 duck / m^2
	Production	> 8 duck / m^2	> 6 - 8 duck / m^2	6-5 duck / m^2	< 5 ducks / m^2
Stocking density of layers in deep litter system, Open system	Rearing	> 15 bird / m^2	> 12 - 15 bird / m^2	10-12 bird / m^2	< 10 bird / m^2
	Production	> 8 bird / m^2	> 6 - 8 bird / m^2	6-5 bird / m^2	< 5 bird / m^2
Close system	Rearing	> 20 bird /m^2	> 17 - 20 bird / m^2	15-17 bird / m^2	< 15 bird / m^2
	Production	> 10 bird / m^2	8 - 10 bird / m^2<	7-8 bird / m^2	< 7 bird / m^2
Stocking density of layers in battery system, Open System	Rearing	> 15 bird / m^2	> 12 - 15 bird / m^2	10-12 bird / m^2	< 10 bird / m^2
	Production	> 25 bird / m^2	> 22 - 25 bird / m^2	20-22 bird / m^2	< 20 bird / m^2
Closed System	Rearing	> 20 bird / m^2	> 17 – 20 bird / m^2	15-17 bird / m^2	< 15 bird / m^2
	Production	> 30 bird / m^2	> 27 - 30 bird / m^2	25-27 bird / m^2	< 25 bird / m^2
Ventilation		Bad with strong ammonia odor	Little ammonia odor	Fair ventilation	Good
Hygiene of water		Bad	Weak	Fair	Good
Hygiene of feed		Bad	Weak	Fair	Good
Condition of litter		Caked litter	Damp litter	Fair condition	Good condition
Control of vectors		Bad	Weak	Fair	Good
Quarantine for sick birds		Absent	Isolated inside the pen	Isolated beside the pen	isolated far from the farm
Control of vehicles		Bad	Weak	Fair	Good
Control of visitors		Bad	Weak	Fair	Good
Hygiene of workers		Bad	Weak	Fair	Good
Biosecurity planning		Bad	Poor	Fair	Good

Table 2 Effect of the hygienic level on APC (log 10 CFU/g) of collected samples and swabs from different poultry farms (mean ± SE).

Items	Broiler chicken farms			Breeder chicken farms			Layer chicken farms			Duck farms		
	A	B	C	A	B	C	A	B	C	A	B	C
Wall swabs	6.64 ±0.02^{bcAB}	6.77 ±0.04^{bcA}	6.46 ±0.03^{cdB}	8.02 ±0.56^{bB}	9.19 ±0.37^{bA}	8.92 ±0.38^{bA}	8.59 ±0.41^{aC}	8.97 ±0.04^{bcB}	9.33 ±0.39^{aA}	7.35 ±0.21^{deC}	10.49 ±0.39^{bcA}	10.19 ±0.38^{bB}
Stored feed	4.65 ±0.03^{eAB}	4.73 ±0.07^{fA}	4.52 ±0.05^{ghB}	5.15 ±0.31^{eB}	5.54 ±0.04^{eA}	5.44 ±0.06^{eAB}	5.15 ±0.25^{efB}	5.68 ±0.02^{hA}	5.29 ±0.41^{fB}	4.39 ±0.05^{hC}	5.47 ±0.09^{fgA}	5.21 ±0.07^{gB}
Feeders	6.47 ±0.13^{cA}	6.62 ±0.23^{cA}	5.99 ±0.21^{eB}	7.90 ±0.31^{bcA}	7.90 ±0.34^{cdA}	8.03 ±0.26^{cA}	7.14 ±0.26^{cdB}	8.51 ±0.43^{cdA}	6.99 ±0.27^{dB}	8.02 ±0.63^{cC}	10.00 ±0.18^{cA}	9.70 ±0.18^{cB}
Water source	4.71 ±0.07^{eA}	4.65 ±0.06^{fA}	4.76 ±0.08^{gA}	4.73 ±0.04^{fA}	4.70 ±0.23^{fA}	4.74 ±0.12^{fA}	4.83 ±0.07^{fgB}	5.15 ±0.34^{iA}	5.15 ±0.31^{fA}	5.40 ±0.05^{gA}	4.19 ±0.41^{hC}	5.16 ±0.02^{gB}
Drinkers	6.97 ±0.21^{bA}	7.08 ±0.37^{bA}	6.37 ±0.23^{dB}	7.31 ±0.19^{cdB}	8.27 ±1.02^{cA}	8.21 ±0.81^{cA}	7.61 ±0.12^{bcC}	9.43 ±0.74^{bA}	8.48 ±0.43^{bB}	9.32 ±0.18^{bC}	10.77 ±0.20^{bA}	10.48 ±0.20^{bB}
Source of litter	5.77 ±0.26^{dB}	6.07 ±0.27^{dA}	3.78 ±0.25^{iC}	4.60 ±0.13^{fB}	5.37 ±0.34^{eA}	5.22 ±0.22^{efA}	4.96 ±0.36^{efgC}	6.94 ±0.03^{fA}	5.51 ±0.2^{fB}	5.25 ±0.55^{gC}	7.71 ±0.25^{dA}	7.42 ±0.24^{deB}
Pen litter	8.15 ±0.31^{aAB}	8.00 ±0.51^{aB}	8.19 ±0.22^{aA}	11.19 ±0.86^{aB}	11.65 ±0.58^{aA}	11.52 ±0.64^{aA}	8.29 ±0.40^{aB}	11.16 ±0.65^{aA}	7.66 ±0.17^{cC}	10.15 ±0.12^{aC}	12.31 ±0.37^{aA}	12.01 ±0.37^{aB}
Dust	5.49 ±0.38^{dA}	5.53 ±0.59^{eA}	5.14 ±0.10^{fB}	4.69 ±0.05^{fB}	5.68 ±0.14^{eA}	5.42 ±0.13^{eA}	4.63 ±0.07^{gC}	6.13 ±0.34^{ghA}	5.41 ±0.47^{fB}	6.78 ±0.04^{fA}	5.68 ±0.21^{fC}	6.51 ±0.05^{fB}
Cloacae swabs	6.97 ±0.01^{bA}	6.95 ±0.01^{bcA}	6.98 ±0.01^{bA}	7.25 ±0.01^{dA}	7.47 ±0.13^{dA}	7.38 ±0.08^{dA}	7.61 ±0.16^{bcA}	7.49 ±0.19^{eA}	7.72 ±0.10^{cA}	7.74 ±0.03^{cdA}	7.37 ±0.28^{dB}	7.63 ±0.05^{dA}
Droppings	6.72 ±0.01^{bcA}	6.69 ±0.00^{cA}	6.76 ±0.01^{bcA}	8.39 ±0.37^{bA}	7.89 ±0.05^{cdB}	8.28 ±0.23^{cA}	7.69 ±0.07^{bB}	8.21 ±0.29^{dA}	7.89 ±0.05^{cB}	6.78 ±0.00^{fB}	7.44 ±0.27^{dA}	7.25 ±0.21^{deA}
Hand swabs	4.46 ±0.30^{eA}	4.50 ±0.24^{fA}	4.38 ±0.39^{hA}	5.39 ±0.38^{eA}	4.95 ±0.41^{fB}	5.25 ±0.37^{efAB}	5.39 ±0.09^{eB}	6.19 ±0.27^{gA}	5.32 ±0.21^{fB}	5.30 ±0.36^{gAB}	5.20 ±0.30^{gB}	5.45 ±0.07^{gA}
Wheel swabs	6.92 ±0.10^{A}	6.90 ±0.13^{bcA}	6.93 ±0.07^{A}	4.61 ±0.14^{fB}	5.79 ±0.16^{eA}	5.53 ±0.13^{eA}	6.89 ±0.04^{dA}	7.02 ±0.21^{efA}	6.37 ±0.36^{eB}	7.25 ±0.35^{eA}	6.93 ±0.28^{eB}	7.15 ±0.29^{eAB}
Mean	6.16	6.21	5.86	6.60	7.03	7.00	6.57	7.57	6.76	6.98	7.80	7.85

a, b and c: There is no significant difference (P>0.05) between any two means for each farm separately, within the same column have the same superscript letter

Table 3 Effect of the age of birds on APC (log 10 CFU/g) of collected samples and swabs from different poultry farms (mean ± SE).

Items	Broiler chickens' age (week)			Breeder chickens' age (month)			Layer chickens' age (month)			Ducks' age (month)		
	1^{st} w	3^{rd}w	5^{th} w	4 m	6 m	8 m	4 m	6 m	8 m	4 m	6 m	8 M
Wall swabs	6.61 ±0.05^{cdA}	6.64 ±0.12^{bcA}	6.62 ±0.09^{A}	8.13 ±0.41^{bC}	8.42 ±0.42^{cB}	9.57 ±0.23^{bA}	8.58 ±021^{aB}	9.24 ±0.14^{aA}	9.07 ±0.52^{aA}	8.70 ±0.87^{cB}	9.70 ±1.03^{bA}	9.63 ±1.10^{bA}
Stored feed	4.60 ±0.00^{eA}	4.66 ±0.12^{gA}	4.64 ±0.06^{A}	5.45 ±0.01^{eA}	5.16 ±0.32^{efB}	5.53 ±0.02^{efA}	5.10 ±0.31^{efB}	5.45 ±0.19^{efAB}	5.56 ±0.35^{dA}	4.95 ±0.24^{hA}	5.05 ±0.38^{gA}	5.08 ±0.35^{ghA}
Feeders	6.24 ±0.03^{dA}	6.41 ±0.41^{cA}	6.44 ±0.19^{A}	7.50 ±0.02^{cB}	8.18 ±0.27^{cA}	8.15 ±0.25^{cA}	7.02 ±0.37^{cdB}	7.83 ±0.43^{bcA}	7.79 ±0.70^{bA}	8.58 ±0.91^{cB}	9.62 ±0.42^{bA}	9.52 ±0.52^{bA}
Water source	4.58 ±0.02^{eB}	4.82 ±0.03^{gA}	4.72 ±0.03^{AB}	4.75 ±0.03^{gA}	4.92 ±0.12^{efA}	4.50 ±0.11^{hB}	4.95 ±0.12^{fA}	5.04 ±0.39^{faA}	5.14 ±0.27^{dA}	5.17 ±0.09^{hA}	4.81 ±0.52^{fA}	4.77 ±0.50^{eA}
Drinkers	6.55 ±0.10^{cdB}	6.93 ±0.51^{bA}	6.94 ±0.25^{A}	6.69 ±0.17^{dC}	8.99 ±0.71^{bA}	8.12 ±0.37^{cB}	7.91 ±0.23^{abC}	8.38 ±0.52^{bB}	9.22 ±0.9^{aA}	9.81 ±0.42^{bB}	10.42 ±0.42^{bA}	10.35 ±0.49^{bA}
Source of litter	4.99 ±0.43^{eB}	5.30 ±1.00^{eA}	5.33 ±0.72^{A}	5.21 ±0.39^{efA}	5.20 ±0.36^{eA}	4.78 ±0.04^{ghB}	5.66 ±0.70^{efA}	5.95 ±0.51^{eA}	5.80 ±0.66^{dA}	6.69 ±0.38^{efB}	6.59 ±1.22^{deB}	7.09 ±0.73^{cdA}
Pen litter	7.44 ±0.23^{aC}	8.58± 0.04^{aA}	8.32 ±0.02^{B}	10.34 ±0.33^{aC}	11.31 ±0.05^{aB}	12.72 ±0.03^{aA}	8.39 ±0.78^{aB}	9.51 ±1.04^{aA}	9.21 ±1.44^{aA}	10.92 ±0.51^{aB}	11.80 ±0.74^{aA}	11.75 ±0.79^{aA}
Dust	4.69 ±0.17^{eC}	5.85 ±0.29^{dA}	5.61 ±0.24^{B}	5.47 ±0.35^{eA}	5.17 ±0.24^{efB}	5.15±0.29^{fgB}	5.00 ±0.41^{efB}	5.73 ±0.68^{efA}	5.45 ±0.37^{dA}	6.13 ±0.44^{fgA}	6.44 ±0.28^{eA}	6.40 ±0.27^{dA}
Cloacae swabs	6.98 ±0.02^{bcA}	6.96 ±0.00^{bA}	6.97 ±0.01^{A}	7.50 ±0.13^{cA}	7.33 ±0.06^{dAB}	7.27 ±0.01^{dB}	7.61 ±0.08^{bcA}	7.44 ±0.23^{cdA}	7.77 ±0.02^{bA}	7.37 ±0.30^{deB}	7.67 ±0.01^{cA}	7.70 ±0.03^{cA}
Droppings	6.71 ±0.01^{bcA}	6.73 ±0.03^{bcA}	6.72 ±0.02^{A}	7.81 ±0.08^{cB}	8.31 ±0.27^{cA}	8.44 ±0.26^{cA}	7.82 ±0.10^{abcB}	7.84 ±0'2.03^{bcB}	8.13 ±0.34^{bA}	6.84 ±0.03^{defB}	7.32 ±0.28^{cdA}	7.32 ±0.28^{cA}
Hand swabs	3.85 ±0.12^{fC}	4.88 ±0.01^{efgA}	4.62 ±0.00^{B}	4.91 ±0.03^{fgB}	4.74 ±0.23^{fB}	5.94 ±0.13^{eA}	5.75 ±0.47^{eA}	5.56 ±0.18^{efA}	5.58 ±0.31^{dA}	5.30 ±0.36^{ghA}	5.40 ±0.26^{fA}	5.24 ±0.18^{eA}
Wheel swabs	7.08 ±0.02^{abA}	6.73 ±0.05^{bcB}	6.94 ±0.00^{AB}	5.33 ±0.50^{efAB}	5.17 ±0.21^{efB}	5.42 ±0.37^{fA}	6.64 ±0.52^{dA}	6.87 ±0.10^{dA}	6.77 ±0.05^{cA}	7.61 ±0.07^{dA}	6.61 ±0.02^{deC}	7.11 ±0.24^{cdB}
Mean	5.86	6.21	6.16	6.59	6.91	7.13	6.70	7.07	7.12	7.34	7.62	7.70

a, b and c: There is no significant difference (P>0.05) between any two means for each farm separately, within the same column have the same superscript letter.

Table 4 Effect of the type of production on APC (log 10 CFU/g) of collected samples from different poultry farms (mean ± SE).

Items	Type of production				Mean
	Broiler chicken farms	Breeder chicken farms	Layer chicken farms	Duck farms	
Wall swabs	6.62±0.05^{d}D	8.71±0.29^{bC}	8.96±0.19^{aB}	9.34±0.53^{cA}	8.41
Stored feed	4.63±0.04^{ghC}	5.38±0.11^{fA}	5.37±0.16^{gA}	5.02±0.17^{iB}	5.1
Feeders	6.36±0.13^{e}D	7.94±0.15^{dB}	7.55±0.29^{dC}	9.24±0.37^{cA}	7.77
Water source	4.71±0.04^{gC}	4.72±0.08^{hC}	5.05±0.14^{hA}	4.92±0.22^{iB}	4.85
Drinkers	6.81±0.18^{bcd}D	7.93±0.41^{dC}	8.50±0.36^{bB}	10.19±0.24^{bA}	8.36
Source of litter	5.21±0.38^{C}	5.06±0.17^{g}D	5.80±0.32^{fB}	6.79±0.43^{fA}	5.72
Pen litter	8.11±0.19^{aC}	11.45±0.36^{aA}	9.03±0.58^{aB}	11.49±0.37^{aA}	10.02
Dust	5.38±0.21^{fBC}	5.26±0.16^{fgC}	5.39±0.27^{gB}	6.32±0.18^{gA}	5.59
Cloacae swabs	6.97±0.01^{bC}	7.37±0.05^{eB}	7.61±0.08^{dA}	7.58±0.1^{dA}	7.36
Droppings	6.72±0.01^{cd}D	8.19±0.15^{cA}	7.93±0.11^{cB}	7.16±0.14^{eC}	7.5
Hand swabs	4.45±0.16^{h}D	5.20±0.20^{fgC}	5.63±0.17^{fA}	5.31±0.14^{hB}	5.15
Wheel swabs	6.92±0.05^{bcB}	5.31±0.19^{fD}	6.76±0.16^{eC}	7.11±0.16^{eA}	6.53
Mean	6.07	6.88	6.97	7.54	6.86
LSD at 0.05	P			F	
	0.24			0.12	

a, b and c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B and C: There is no significant difference (P>0.05) between any two means for the same attribute, within the same row have the same superscript letter.

Table 5 The percent of *Salmonella* in the collected samples swabs and from different poultry farms (mean ± SE).

Items	Broiler chicken farms			Breeder chicken farms			Layer chicken farms			Duck farms			Total
	A	B	C	A	B	C	A	B	C	A	B	C	
Wall swabs	13.33 ±6.67^{cdA}	0±0^{dB}	6.67 ±6.67^{efAB}	0±0^{dA}	0±0^{eA}	0±0^{dA}	0±0^{cB}	20.00 ±20.00^{cdA}	0±0^{bB}	13.33 ±6.67^{eA}	20.00 ±11.55^{cdA}	20.00 ±0.00^{deA}	7.78
Stored feed	0±0^{dA}	0±0^{dA}	0±0^{fA}	0±0^{dA}	0±0^{eA}	0±0^{dA}	0±0^{cA}	0±0^{eA}	0±0^{bA}	0±0^{eB}	6.67 ±6.67^{deAB}	13.33 ±13.33^{defA}	1.67
Feeders	53.33 ±6.67^{abA}	6.67 ±6.67^{dC}	20.00 ±11.55^{cdeB}	6.67 ±6.67^{cdA}	6.67 ±6.67^{deA}	6.67 ±6.67^{cdA}	0±0^{cA}	0±0^{eA}	0±0^{bA}	80.00 ±11.55^{abcA}	53.33 ±17.64^{bB}	53.33 ±6.67^{abB}	23.89
Water source	0±0^{dA}	0±0^{dA}	0±0^{fA}	0±0^{dA}	0±0^{eA}	0±0^{dA}	0±0^{cA}	0±0^{eA}	0±0^{bA}	0±0^{eA}	0±0^{eA}	0±0^{fA}	0
Drinkers	40.00 ±11.55^{bA}	40.00 ±11.55^{bcA}	26.67 ±13.33^{bcdB}	0±0^{dB}	6.67 ±6.67^{deA}	6.67 ±6.67^{cdA}	0±0^{cB}	46.67 ±17.64^{bA}	0±0^{bB}	93.33 ±6.67^{aA}	66.67 ±6.67^{abB}	66.67 ±17.64^{aB}	33.34
Source of litter	13.33 ±13.33^{cdA}	0±0^{dB}	6.67 ±6.67^{efAB}	0±0^{dA}	0±0^{eA}	0±0^{dA}	0±0^{cB}	13.33 ±13.33^{dA}	0±0^{bB}	0±0^{eB}	20.00 ±11.55^{cdA}	26.67 ±6.67^{cdA}	6.67
Pen litter	53.33 ±17.64^{abA}	60.00 ±11.55^{aA}	40.00 ±23.09^{abB}	33.33 ±17.64^{aB}	40.00 ±11.55^{bA}	33.33 ±6.67^{aB}	60.00 ±0.00^{aB}	66.67 ±6.67^{aA}	6.67 ±6.67^{abC}	80.00 ±11.55^{abcA}	73.33 ±6.67^{aA}	46.67 ±6.67^{bB}	49.45
Dust	20.00 ±11.55^{cA}	6.67 ±6.67^{B}	13.33 ±13.33^{deAB}	13.33 ±6.67^{bcB}	26.67 ±6.67^{cA}	0±0^{dC}	0±0^{cB}	26.67 ±6.67^{cA}	0±0^{bB}	33.33 ±17.64^{dAB}	26.67 ±6.67^{cB}	40.00 ±0.00^{bcA}	17.22
Cloacae swabs	60.00 ±11.55^{aA}	53.33 ±6.67^{abcAB}	46.67 ±17.64^{aB}	20.00 ±11.55^{bB}	26.00 ±13.33^{cA}	20.00 ±11.55^{bcB}	40.00 ±0.00^{bA}	40.00 ±11.55^{bA}	6.67 ±6.67^{abB}	73.33 ±17.64^{bcA}	53.33 ±6.67^{bB}	66.67 ±17.64^{aA}	42.22
Droppings	53.33 ±6.67^{abA}	53.33 ±17.64^{abcA}	40.00 ±11.55^{abB}	13.33 ±6.67^{bcC}	60.00 ±11.55^{aA}	20.00 ±0.00^{bcB}	60.00 ±0.00^{aA}	60.00 ±11.55^{aA}	13.33 ±13.33^{aB}	86.67 ±13.33^{abA}	53.33 ±6.67^{bB}	40.00 ±11.55^{bcC}	46.11
Hand swabs	0±0^{dA}	0±0^{dA}	0±0^{fA}	6.67 ±6.67^{cA}	0±0^{eB}	0±0^{dB}	0±0^{cA}	s0±0^{eA}	0±0^{bA}	0±0^{eA}	6.67 ±6.67^{deA}	6.67 ±6.67^{efA}	1.67
Wheel swabs	0±0^{dB}	33.33 ±6.67^{cA}	0±0^{fB}	0±0^{dB}	20.00 ±11.55^{cdA}	0±0^{dB}	0±0^{cA}	0±0^{eA}	0±0^{bA}	66.67 ±13.33^{cA}	0±0^{eC}	13.33 ±6.67^{deB}	11.11
Total	22.22	21.11	16.66	7.78	16.11	6.67	13.33	22.78	2.22	43.89	32.22	32.78	7.78
Mean		21.1			10.2			12.8			36.2		20.07

a, b and c: There is no significant difference (P>0.05) between any two means for each farm separately, within the same column have the same superscript letter.

Table 6 Different *Salmonella* serotypes were isolated from different poultry farms.

Salmonella strains	Broiler chicken farms	Breeder chicken farms	Layer chicken farms	Duck farms	Percentage of total serotypes (%)
S. Agona	+Ve	+Ve	+Ve	+Ve	39.60
S. Kentucky	+Ve	+Ve	-Ve	+Ve	23.80
S. Derby	+Ve	+Ve	-Ve	+Ve	17.45
S. Typhimurium	+Ve	+Ve	+Ve	+Ve	7.76
S. Enteritidis	+Ve	-Ve	+Ve	+Ve	7.20
S. Molade	-Ve	-Ve	-Ve	+Ve	2.22
S. Virchow	-Ve	-Ve	-Ve	+Ve	1.66

(+Ve) positive, (-Ve) negative.

4. DISCUSSION

Poultry farms' hygiene is a critical point in the poultry industry because insufficient application of hygienic measures in poultry farms or poor biosecurity leads to increasing bacterial contamination, high mortality and serious losses in poultry farms. Routine monitoring of hygiene and bacterial levels inside poultry farms is very important for the prevention of serious diseases caused by pathogenic bacteria and for increasing the quality of the production (Gibbens *et al*., 2001; Conan *et al*., 2012). Therefore, the hygiene scoring system was used in the current study for monitoring the hygienic level of the tested poultry farms.

The results showed that the final hygienic scores of the tested poultry farms revealed that, the breeder farms had the highest score (82.2%). Moreover, the farms which had the highest individual hygiene score (broiler chicken farm C, breeder chicken farm C, layer chicken farm C and duck farm C) were the farms that applied basic hygienic measures, such as the presence of a fence, traffic control, hygienic dead bird disposal, usage of protective clothes, control of wild birds and rodents (Aiyedun *et al*., 2018).

The results of this study showed that the most applied measures in the tested farms were good ventilation followed by the trustable source of birds, same types and ages of the birds, good stocking density and control of the vectors. On the other hand, the lowest applied measures were sick birds' quarantine and the presence of a fence. The absence of sick birds' quarantine leads to the spreading of infections from sick to healthy birds. In addition, the absence of fencing around the farms promotes the free movement of rodents, wild birds and pets which are potential sources of contamination of poultry farms by various pathogenic microbes that could allow the spread of pathogenic microorganisms from one farm to another (Dosso, 2014). In a previous study in Sudan, 68.9% of the farms applied the sick birds isolated, 26.7% kept different species of birds other than poultry, 17.8% had good vector control and the majority of the farms didn't apply most of the biosecurity measures (Ali *et al*., 2014). In recent study in Côte d'Ivoire, 45% of poultry farms had no fence around the farms (Goualie *et al*., 2020).

There was a wide range of APC in the different poultry farms. The broiler chicken farm C had the lowest APC (log 5.86 CFU/g), unlike the duck farm C which had the highest APC (log 7.85 CFU/g) among the twelve poultry farms. This significant difference between the different poultry farms in the APC was due to the difference in the hygienic level, the used housing system, the type of production and the age of birds (Ashry and El Baghy, 2019).

The results revealed that the age of birds had a significant effect on the bacterial contamination of poultry farms as the APC at the young age (1st week in broiler and 4th month in layer and breeder) was lower than the older age. Moreover, the broiler chicken farms, which are the youngest, had lower APC than breeder chicken, layer chicken and duck farms. This indicated that the bacterial contamination of poultry farms increased by increasing the age of bird (Awad *et al*., 2016).

There was a highly significant difference between the APC of the examined samples and swabs from different poultry farms. Pen litter had the highest APC (log 10.02 CFU/g). Also, the APC of all litter samples from pens were higher than the APC of litter source, the poultry litter may be contaminated by birds' excreta, feathers and dust. Moreover, the increased moisture content of pen litter caused by the droppings and the drinking water could encourage the growth of bacteria, so special attention should be given to litter management for decreasing its moisture (Dumas *et al*., 2011).

The water source had the lowest APC (log 4.85 CFU/g), This result can be explained by the fact that all tested poultry farms used the public tap water, which is usually treated with chlorine, as this treatment kills the bacteria, resulting in reducing the bacterial contamination of the water (Ellis-Iversen *et al*., 2009). All drinking water samples were higher APC than those of the water source, this result can be explained by fecal contamination of drinkers. In addition, defects in drinkers washing and disinfection (Folorunso *et al*., 2014). Moreover, there was a significant difference in APC of all feed samples from the feeders and those collected from stored feed at the same time, this contamination may be caused by fecal contamination of feeders by the birds (Metawea, 2000).

Salmonella infections are a serious threat facing poultry industries and public health, improper hygienic practices lead to increasing *Salmonella* contamination in poultry farms (Majowicz *et al*., 2010). The results of the current study demonstrated that the mean percent of isolated *Salmonella* from different types of poultry farms in Qalyubia Governorate was 20% (Omara *et al*., 2017). *Salmonella* was recovered from all tested poultry farms, but at different rates. Broiler chicken farm A, breeder chicken farm B, layer chicken farm B and duck farm A had a higher *Salmonella* percentage than the other two farms of the same type of production. This high *Salmonella* rate is due to the poor hygienic level of these farms as they didn't apply many hygienic and biosecurity measures. The level of hygiene has a strong effect on *Salmonella* contamination in different poultry farms (Ashry and El Baghy, 2019).

The used housing system had an effective relationship with the level of *Salmonella* contamination in poultry farms; the breeder chicken farms with a closed system were the least contaminated. This revealed that the farms with a closed system had a higher level of hygiene and biosecurity measures compared to other poultry farms with an open system (Soliman and Abdallah, 2020). On the other hand, the least contaminated individual farm with *Salmonella* among all tested poultry farms was layer farm C with a cage rearing system, this is due to the higher hygienic level of the cage rearing system than the deep litter system because the birds avoid the direct contact with their droppings, the absence of wet litter and the ease of cleaning and disinfection (Heitmann *et al*., 2020).

Environmental sampling of poultry farms is an effective method for monitoring the presence of *Salmonella* and detecting their important risk factors (Fagbamila *et al*., 2017). The contamination of *Salmonella* was the highest in pen litter (49.5%), so the most important risk factor of *Salmonella* contamination in poultry farms was the contaminated litter (Blaak *et al*., 2015). *Salmonella* was isolated from birds' droppings and cloaca with high rates

(46% and 42% respectively). This high percent of *Salmonella* in bird samples because poultry can be contaminated horizontally during the rearing period from litter, feed, drinking water, dust, and contaminated equipment, then the bacteria transmitted from infected poultry to the environment to infect the healthy birds and so on (Djeffal *et al*., 2018).

Our findings suggest that the water and poultry feed were considered potential risk factors for *Salmonella* contamination in poultry farms, as *Salmonella* was isolated from drinkers and feeders by (33.3% and 24% respectively) (Fagbamila *et al*., 2017; Ashry and El Baghy, 2019). Water can be contaminated by the farm environment and birds as a result of poor hygiene, since every time the birds drink this contaminated water, they are exposed to the risk of infection, so disinfection of the water and daily washing of drinkers reduced the bacterial contamination of water (Folorunso *et al*., 2014). Many factors could affect *Salmonella* contamination of poultry feed as fecal contamination by birds, the type of feed, processing treatments, storage conditions and moisture content. In addition to communication with wildlife and rodents could contaminate the feed by pathogenic organisms (Maciorowski *et al*., 2007; Davies and Wales, 2010).

The farm walls and dust may be a reservoir of *Salmonella* contamination in poultry farms, as their rate of *Salmonella* contamination is alarming. Once the environment has been contaminated with *Salmonella*, the pathogen remains within the following poultry flocks, so positive dust samples may be more indicative of previous infections with defects in cleaning and disinfection, as *Salmonella* has been reported to survive in dust for up to 53 weeks (Fagbamila *et al*., 2017). Moreover, the walls might be contaminated by workers' or visitors' hands, dust and poultry droppings (Namata *et al*., 2009). On the other hand, the transport vehicles which were allowed to enter the poultry farms were considered an important source of *Salmonella* contamination (Lister, 2008).

No *Salmonella* was isolated from the water source of all farms because all tested poultry farms used chlorinated public tap water, which may kill *Salmonella* (Ellis-Iversen *et al*., 2009).

Identification and serotyping of isolated *Salmonella* are essential for their epidemiological surveillance and investigations. In this study, the most predominant *Salmonella* serotype was *S. agona* (39.8%) followed by *S*. Kentucky (24 %). On the other hand, the lowest isolated *Salmonella* serotypes were *S. molade* and *S. virchow* (2.4%, 1.2%) respectively (Elsayed *et al*., 2019).

5. CONCLUSION

Many defects occur in the application of hygienic measures in poultry farms as a result of the lack of knowledge of farm staff about how to apply proper hygiene, which increases the risks of the introduction and transmission of dangerous pathogens. Therefore, all poultry farms must constantly monitor and improve their hygiene. The applied hygienic measures, the used housing system and the type of production have a significant effect on the level of bacterial contamination in poultry farms. On the other hand, appropriate management, proper hygiene and biosecurity measures are very essential to control *Salmonella* and other bacterial contamination.

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