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RESEARCH ARTICLE

The Prevalence of Salmonella Species as A Biosecurity Indicator in Poultry Farms in Sharkia Governorate, Egypt

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Abstract

Ward biosecurity, which means the protection of living agents, is a program aimed to keep birds safe from disease-causing microorganisms. The purpose of this study was to assess the biosecurity measures and the prevalence of *Salmonella* species in poultry farms in Sharkia governorate, Egypt. The sensitivity of recovered *Salmonella* Typhimurium isolates against various antibiotics and disinfectants was also assessed. A total of 84 samples (21 from each of four poultry farms) were obtained. Water, feedstuff, litter, cloacal swabs, wall, hand, and foot boot samples were taken in threes from each farm. According to the questionnaire, all of the farms surveyed received a "poor biosecurity" grade, with a biosecurity score (BS) of less than 50%. *Salmonella* species were found in 10.7% (9/84) of the all investigated samples. Cloacae had the most *Salmonella* species (41.7%), followed by litter (25%) and feed (8.3%). Biosecurity level and *Salmonella* species isolation have significant negative correlations. Based on serological examination, *Salmonella* Enteritidis and *Salmonella* Molade were the most frequent serotypes of *Salmonella* in litter, while *Salmonella* Larochelle and *Salmonella* Typhimurium were common in feed and cloacae. Ciprofloxacin and levofloxacin sensitivity was higher in the isolated *S*. Typhimurium, followed by amikacin, nitrofurantoin, ceftriaxone, and gentamycin. Virkon S (0.5%) was shown to be particularly efficient against *S*. Typhimurium, followed by Virocid (0.5%) and finally cid 2000 (2%). In conclusion, the results of this investigate showed the variance in biosecurity levels found across the farms studied in connection to *Salmonella* prevalence, with the lower percentage of biosecurity score indicating a greater degree of *Salmonella* prevalence. A variety of management and biosecurity practices, including extremely strict cleaning and disinfection procedures have been identified as protective factors in minimizing *Salmonella* species entry and persistence on poultry farms.

Keywords: Salmonella, Biosecurity, Antibiotics, Disinfectants.

Introduction

The concepts of disease prevention and control in poultry farming are based on flock management, vaccination, and biosecurity. The expression "biosecurity" refers to management strategies that reduce the possibility of infectious pathogens entering or spreading within a manufacturing unit. Indeed, biosecurity measures are beneficial in the poultry industry because their primary goals are to protect the facility and its surroundings from pathogen introduction or spread of pathogens to uninfected farms or other farms where the pathogen is already present [1, 2].

Salmonella species are non-spore-forming bacteria that are regarded as one of the most widespread disease-causing agents, particularly in low sanitation areas, and utilized as a food safety indication in the chicken industry. Birds are regarded to be the most significant natural reservoir of various *Salmonella* species serovars. Furthermore, *Salmonella* infection occurred through a polluted environment contaminated by germs from infected hosts' feces [3]. Avian salmonellosis is a worldwide infectious disease that affects many species of chicken and costs commercial poultry farms a lot of money. Interventions in the poultry sector to limit the propagation of these infections involve two fundamental strategies: pathogen prevention and pathogen eradication [4].

Antibiotics are used in the poultry industry for treatment, prevention, and growth enhancement. To lower the morbidity and mortality of salmonellosis in poultry, the current therapy relies on the use antibiotics such as amoxicillin, oxytetracycline, tetracycline, and others. On the other hand, because the majority of isolates are resistant to them, antibiotics are no longer an effective form of treatment [3, 5, 6].

The disinfectant programs in poultry facilities are aimed to kill or minimize the populations of disease-associated bacteria and prevent their spread between batches. Disinfectants are commonly used in poultry buildings to remove zoonotic infections such as *Salmonella* species. To efficiently decrease microbial populations in poultry houses, it was revealed that the disinfection process is affected by numerous disinfection processes including disinfection methods such as cleaning, disinfectant concentration, pH, temperature, and exposure time. In poultry farms, disinfectants such as halogens, oxidizing agents, chlorhexidine compounds, phenolics, and alcohols are utilized. The disinfection capability of hydrogen peroxide was demonstrated to be good against *Salmonella* [7, 8].

This study was to evaluate the biosecurity status and to detect the prevalence of S*almonella* species in different poultry farms at Sharkia governorates. The sensitivity of identified *S.* Typhimurium to the commonly used antibiotics and disinfectants was also assessed.

Material and Methods

Description of examined poultry farms

The study target four commercial broiler chicken farms distributed in Sharkia governorate, Egypt. The first farm is in El-Salheya, the second in El-khattara, the third in Kafr Saqr, and the last one in Awlad Saqr. All information were acquired from the records in each farm, included; location, farm area, stocking density of birds, kind of farmed poultry species, mortality rates, cycle duration, storage of poultry feed, type of floor, water sources, ventilation, and lighting system (Table 1).

Table 1: Descriptive data of the examined poultry farms in four localities in Sharkia governorate, Egypt

	Categories	Farm I (El-Salheya)	Farm II (El Khattara)	Farm III (Kafr Saqr)	Farm IV (Awlad Saqr)
1.	**Location**	El-Salheya	El Khattara	Kafr Saqr	Awlad Saqr
2.	**Farm area / m^2**	650 m^2	700 m^2	750 m^2	780 m^2
3.	**Stocking density of bird / m^2**	8-10 birds	6-8 birds	8-10 birds	10-12 birds
4.	**Total capacity of the farm**	5500 birds	4500 birds	6000 birds	8000 birds
5.	**Reared Spp.**	Arbo Acres	Arbo Acres	Sasso	Ross
6.	**Cycle duration**	40 days	40days	50 days	60 days
7.	**Distance between farms**	20 m	50 m	150 m	200 m
8.	**Mortality rate/ cycle (%)**	5%	5-10%	10-15%	15%
9.	**Time interval between cycles**	1 month	Not fixed	Not fixed	Not fixed
10.	**Cleaning & disinfection between flock**	Chlorine & iodine	Phenol & chlorine	Formalin	Formalin
11.	**No. of windows / side**	7	8	10	12
12.	**Types of Floor**	Cement	Muddy	Muddy	Cement
13.	**Type of water source**	Private (Underground)	Private (Underground)	Public	Public

Assessment of the biosecurity score in the examined poultry farms

A biosecurity questionnaire was created to measure the degree of biosecurity in the chicken farms under investigation. Biosecurity levels are evaluated by a total of 23 questions, including; access to the farm, distance from nearest farm, distance from water source, disposal of dead birds, manure disposal and management, drinking water origin, rodent control, bird proofing, visitor restriction, vehicles conditions of chicks placing, birds density at one day, concrete floor, management of ill birds, water sanitation, types of drinkers, foot bath dip, contact of workers with other flock, cleaning and disinfection of farm between flocks, cleaning and disinfection of equipment and vehicles, cleaning and disinfection of footwear before and after visit, hand hygiene before and after poultry handling ,utilization of farm cloths and foot wear and disinfection of worker cloths.

The questionnaire responses earned a score of 0 (total lack of preventative measures) or 1 (complete existence of preventive measures) [9]. The biosecurity score (BS) percentages were then computed and compared to the conventional biosecurity grade "Good" if the farm's BS was greater than 50% and "Poor" if the farm's BS was less than 50% [10].

Samples and sampling procedures

A total of 84 samples were obtained randomly from four commercial broiler chicken farms (21 each) in Sharkia governorate, Egypt. Water, feedstuff, litter, cloaca, wall, hand, and foot boot samples were taken in threes from each farm and were obtained from November 2021 to July 2022. The upper layers (dry and wet) litter, 100 g of fully mixed feed, and 100 ml of water drinkers were aseptically taken from the poultry farms under investigation. Swab samples were taken from cloaca, wall, hand, and foot boot using sterile swabs that immersed into 9 mL pre-enrichment broth (Buffer Peptone Water) under aseptic conditions as previously adopted. Immediately after sampling with a minimum of delay, all samples were labeled and aseptically transferred to the laboratory for further investigation **[**11, 12].

Isolation and identification of Salmonella species

Pre-enrichment broth (Buffer Peptone Water) was mixed with 25 ml/g of each sample of analyzed water, homogenized feed material, and litter; swab samples were incubated in 9 mL Buffer Peptone Water [13]. All samples were incubated at 37˚C for 24 hours. Then 0.1 mL of pre-enriched tubes was inoculated into enriched broth Rappaport-Vassiliadis (RV) and then incubated at 41.5˚C**.** A loopful of the 24 hours-enriched cultured broth was streaked onto Xylose Lysine Deoxycholate agar (XLD) agar (Himedia, India) and then incubated at 37˚C for 24 hours [14]. Typical colony of suspected *Salmonella* has a black center and lightly transparent zone of reddish color was picked and identified using morphological characters and biochemical reactions (include; indole, methyl red, voges – proskouer, citrate utilization, urea hydrolysis, H_2S production, gelatin liquefaction and sugar Fermentation) [13, 15].

Serological identification of Salmonella species isolates

Four biochemically identified *Salmonella* isolates were selected from different sources for serotyping using monovalent O and H antisera according to

Alzwghaibi *et al.* [16] at The National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

Antibiotic Sensitivity Test

The antibiotic susceptibility testing for the recovered *S.* Typhimurium isolate was achieved using the disc diffusion method in accordance with the National Committee for Clinical Laboratory Standards' guideline [17]. *S.* Typhimurium colonies that had been identified and purified were added to the brain heart infusion broth, and it was then incubated at 35–37 ◦C for 24 hours. The surface of the nutritional agar on which the antibiotic discs were placed received 1 mL of turbid broth, which was then inoculated and incubated at 37 °C for 24 hours. Thirteen antibiotics were tested (BioMerieux F6980 Marcy Etoite France); ciprofloxacin (15µg), amoxicillin + clavulanic acid (30µg), gentamycin (10µg), sulbactam + ampicillin (10µg), nitrofurantoin (300 µg), fusidic acid (10µg), ceftazidime (30µg), amikacin (30µg), levofloxacin (5µg), penicillin (10µg), Cefotaxime (30µg), erythromycin (15µg) and ceftriaxone (30 µg). The sensitivity of *S.* Typhimurium isolates to different antibiotic discs were measured by the diameter of inhibitory zone and compared with antibiotic susceptibility testing sheet. Interpretation of the zones of growth inhibition's size in accordance with Aditi *et al.* [18].

Disinfectants' efficacy against identified S. **Typhimurium**

The efficacy of Virocid (Glutaraldehyde, 0.5%), Virokin S (Potassium peroxymonosulfate and Sodium chloride 0.5 %) and Cid 2000 (Hydrogen peroxide and acetic acid, 2%) was assessed by applying each of them singly on *S.* Typhimurium isolates in the absence and presence of organic matter as previously described [19, 20]. To test the disinfectant's effectiveness in the presence of organic matter, 9.5 mL of brain heart infusion broth supplemented with 2% yeast extract was inoculated with 0.5 mL of *S.* Typhimurium strain. To prepare the microbial-disinfectant mixture without organic matter, a normal saline solution was used. The turbidity of the test suspension was compared with a 0.5 McFarland's turbidity (2 x 10^8 CFU/mL). Assessed disinfectants were added to tubes at the recommended level and subcultures for 1, 5, 10, 20, 30, 45, 60 minutes contact time and then 5µL of Tween-80 was added to halt the activity of disinfectants. The absence of microbial growth on plates of Xylose Lysine Deoxycholate agar (XLD) at 37 °C for 24-48 hrs was used as a measure of the disinfectant's effectiveness.

Statistical Analysis

Chi-square test and Pearson correlation were run to test differences and relations among four farms through biosecurity levels. $P < 0.05$ is considered statistically significant. All analyses were performed by SPSS version 24.0 (IBM. Corp., Armonk, NY) [21].

Results and Discussion

Evaluation of the biosecurity status in the investigated poultry farms

Table 2 revealed that the biosecurity was attained by 38.04% of the studied farms. There was no significant variance in biosecurity levels across the studied farms according to these data. All the investigated farms earned grade "poor" biosecurity score, where biosecurity levels were not more than 50%. Good education of broiler farmers and their staff was found to assist enhancing overall biosecurity on broiler farms in Europe [22]. These results were nearly similar to those previously mentioned [23, 24]. Material supply and disease management received the best marks, while manure and carcass removal received the lowest marks. These preliminary findings revealed that, despite the necessity of biosecurity, many biosecurity measures are poorly implemented, with opportunity for improvement. Location, ventilation, immunization status, and feeder and drinker cleaning are the most critical risk factors and biosecurity measures [25]. However, isolation, cleanliness, and movement restriction were recognized as the most important factors in limiting disease agent disseminating biosecurity measures, such as access control, vehicle disinfection and animal control, disinfection of house premises, demonstrated high biosecurity compliance, thereby representing an important phase in biosecurity implementation [26, 27].

***Salmonella* species prevalence in all studied farms**

The displayed results in Table 3revealed that *Salmonella* species were found in 9 out of 84 examined samples (10.7%). In El-Salheya, El-Khattara, Kafr Saqr, and Awlad Saqr farms, the isolation percentages of *Salmonella* species were 4.76 (1/21), 9.52 (2/21), 19.04 (4/21) and 9.52% (2/21), respectively. There was no statistically significant (p = 0.500 relationship between the occurrences of *Salmonella* in the studied farms. According to our findings, cloacal swabs exhibited the largest *Salmonella* species isolation percentages (41.7%), followed by litter (25%) and feed (8.3%). However, 75 of the samples obtained from water, feed, walls, workers' hands, and foot boots confirmed negative for *Salmonella* species. These findings were lower than those previously reported, in which cloacal swabs have been utilized to demonstrate *Salmonellae*'s chronic intestine colonization in individual birds. With a 55%, (44 out of 80) cloacal swab samples confirmed positive for *Salmonella* [28]. In another investigation, water samples showed the lowest *Salmonella* prevalence (15.1%) while, the greatest *Salmonella* prevalence were found in feces (23%) and feed (22.7%), followed by litter (20.3%) [29]. Our findings were supported by former research, who found *Salmonella* species in 35% of the samples, with the greatest frequency identified in cloacal swabs. *S.* Enterica serovar Typhimurium detection appeared to be significantly greater in cloacal samples (95.8%) than in litter and feed samples [30]. Furthermore, water and hand swab samples from a chicken farm were examined and found to be *Salmonella* free [31]. The greatest prevalence of *Salmonella* levels, on the other hand, was found in litter samples [32]. Moreover, cloacal swabs revealed the highest prevalence of *Salmonella* (2.2%) [33].Our findings contradict those who found 15.12% as a percentage of *Salmonella* [11]. The prevalence rates of *S.* Typhimurium in the poultry farms surveyed varied but were not statistically significant. The lowest level of prevalence was 10.64%, while the maximum level was 20.00%. These isolates were obtained from cloacal swabs, fresh feces, litter, and poultry drinking water samples, in that order: 11%, 18.7%, 40%, and 25%, respectively. A previously conducted study found that *Salmonella* species were found in cloacal swabs (14.8%) and hand swabs examined from farm attendants with a prevalence of 33.3%. The isolation rate of *Salmonella* was substantially greater in hand (33.3%) than in cloacal swabs (14.8%) [12]. In another investigation, the farm level point prevalence rate for *S.* Enterica was 55% (10 of 18 farms). Twenty-six (9%) of the total 288 farming environmental samples collected were positive. The rate of isolation varied based on the origin of the samples; water (27.5%); feces (10.6%); litter (8.6%); farm swabs (5%), and feed (1.8%) [34].

Table 2: The assumed score of biosecurity levels in the examined poultry farms

Biosecurity variables	El-Salheya Farm	El-Khattara Farm	Kafr Saqr Farm	Awlad Saqr Farm	Total score	
					No.	%*
1. Access to the farm	1	1	1	1	4	**100**
2. Distance from nearest farm	0	0	1	1	2	**50**
3. Distance from water source	1	1	0	0	2	**50**
4. Disposal of dead birds	0	0	0	0	0	**0**
5.Manure disposal & management	1	0	0	0	1	**25**
6. Drinking water origin	1	1	1	1	4	**100**
7. Rodent control	1	1	0	1	3	**75**
8. Bird proofing	0	0	0	0	0	**0**
9. Visitors restriction	0	0	0	0	0	**0**
10. Vehicles (allowed to enter farm)	0	0	0	0	0	**0**
11. Birds density at day 1 (chicks/m2)	0	0	1	1	2	**50**
12. Floor built with concrete	1	0	0	0	1	**25**
13. Management of ill birds	1	1	0	0	2	**50**
14. Water sanitation	0	0	0	0	0	**0**
15. Type of drinkers	1	1	1	1	4	**100**
16. Foot bath dip	0	0	0	0	0	**0**
17. Contact of workers with other flock	1	1	1	1	4	**100**
18. Cleaning & disinfection of farm between flocks	1	1	1	1	4	**100**
19.Cleaning & disinfection of equipment & vehicles	1	1	0	0	2	**50**
20.Cleaning &disinfection of footwear before & after visit	0	0	0	0	0	**0**
21.Hand hygiene before & after poultry handling	0	0	0	0	0	**0**
22.Utilization of farm cloths & foot wear	0	0	0	0	0	**0**
23. Disinfection of worker cloths	0	0	0	0	0	**0**
Total **No.**	11^a	9^a	7^a	8^a	35	
%*	47.8	39.1	30.4	34.8	38.04	

N.B. Total represent number of all variables for each farm that earned a score of 0 (total lack of preventative measures) or 1 (complete existence of preventive measures)

* The biosecurity level (%) = number of variables that farm earned / number of all variables – ^{abc} Means within the same row carrying different superscripts are significant.

- Chi square is no significance $\chi 2$ (3) = 1.614, p= 0.656

Table 3: Prevalence of *Salmonella* species recovered from the investigated poultry farms

SOURCES	No. of samples/ each farm	El- Salheya Farm		El-Khattara Farm		Kafr Saqr Farm		Awlad Saqr Farm		Total		
		Positive samples		Positive samples		Positive samples		Positive samples		Total No. of samples	Positive samples	
		No.	%	No.	%	No.	%	No.	%		No.	%
1. Water	3	0	0	0	0	0	0	0	0	12	0	0
2. Feed stuffs	3	0	0	0	0	0	0	1	33.3	12	1	8.3
3. Litters	3	0	0	1	33.3	2	66.6	0	0	12	3	25
4.Cloaca	3	1	33.3	1	33.3	2	66.6	1	33.3	12	5	41.7
5.Walls	3	0	0	0	0	0	0	0	0	12	0	0
6. Hands	3	0	0	0	0	0	0	0	0	12	0	0
7. Foot boots	3	0	0	0	0	0	0	0	0	12	0	0
Total	**21**	**1[a]**	**4.76**	**2[a]**	**9.52**	**4[a]**	**19.04**	**2[a]**	**9.52**	**84**	**9**	**10.7**

Chi square result revealed that there is no significance association between occurrence of *Salmonella* within the examined farms χ^2 (3) = 2.364, p = 0.500

In respect to hygiene, avian *Salmonella* infections are significant as a cause of clinical disease in poultry and a source of disease transmission to humans via food. The high prevalence of *Salmonella* isolation from feed may be due to inadequate sanitation, handling, and contamination across the chicken production chain, as well as cross contamination [35]. To limit the occurrence of *Salmonella*, it is essential that the equipment be cleaned and sanitized after each flock. Additionally, workers training in food safety and biosafety can help to minimize the spread of *Salmonella* in farm circumstances [29]. Effective biosecurity programs should be applied to avoid Salmonellosis [8].

Table 4 shows the Pearson correlation between biosecurity levels and *Salmonella* species identified from the studied poultry farms. There were significant negative correlations between the total biosecurity level and *Salmonella* species (p = or < 0.05). These findings were nearly identical to those of another study, which suggested that increased farm biosecurity might lead to a reduced prevalence of avian salmonellosis in poultry farms [36]. Standard biosecurity precautions are not widely used, and flocks frequently come into touch with wild birds, pets, and farm animals, making them more susceptible to infectious diseases. Furthermore, biosecurity measures are recognized to lower the probability of disease transmission [37].

Table 4: A Pearson Correlation between the biosecurity levels and *Salmonella* species recovered from the examined poultry farms

Parameter	Biosecurity	*Salmonella*
Biosecurity level	**1**	**-.893***
		.041

* Correlation is significant at the 0.05 level (2-tailed).

The findings in Table 5 declared that four *Salmonella* species were serotyped from litter (n=2), feed (n=1), and cloaca (n=1). *S.* Enteritidis and *S.* Molade were found to be the most frequent serotypes of *Salmonella* in litter (25% each). *S.* Larochelle and *S.* Typhimurium were more common in feed and cloaca (25% each). Previously, nearly identical results were obtained in Egypt, where *S.* Enteritidis and *S.* Typhimurium were common in poultry [38]. Furthermore, *S.* Typhimurium (16.7%) and *S.* Enteritidis (5.4%) were shown to be common. [39]. The frequency of *Salmonella* serotypes in chicken, on the other hand, was 3.35%. *S.* Enteritidis and *S.* Typhimurium were identified as the most common serotypes in the examined poultry and its product [40]. *S.* Typhimurium and *S.* Enteritidis were also found in high levels in chicken and feed.

Table 5: Serotypes of *Salmonella* species recovered from the examined poultry farms

Serotypes	Source of isolates n=4	Total	
		NO.	%
S. Enteritidis	**Litter**	**1**	25.0
S. Molade	**Litter**	**1**	25.0
S. Larochelle	**Feed**	**1**	25.0
S. Typhimurium	**Cloaca**	**1**	25.0
Total		4	100.0

***Antibiotic sensitivity testing of S.* Typhimurium**

The findings of the antibiogram of the isolated *S.* Typhimurium against thirteen antimicrobial agents were shown in Figure 1. Ciprofloxacin and levofloxacin sensitivity was higher in the *S.* Typhimurium isolate (++), whereas amikacin, nitrofurantoin, ceftriaxone, and gentamycin sensitivity were intermediate (+). Fusidic acid and amoxicillin & clavulanic acid had the highest rates of resistance, followed by ceftazidime, sulbactam & ampicillin, cefotaxime, penicillin, and erythromycin. These findings confirm previous research that found *Salmonella* species resistant to erythromycin in both healthy and sick chickens [41]. *Salmonella* species displayed resistance towards the following antimicrobials: erythromycin (100%), chloramphenicol (76.2%), tetracycline (62%), ampicillin (47.7%), sulfamethoxazole/trimethoprim (42.9%), ciprofloxacin (4.8%), nalidixic acid (9.6%), streptomycin (19%) and kanamycin (28.6%), while cephalothin and, and gentamicin showed no resistance [42]. Previous research discovered that *Salmonella* species isolates were highly sensitive to amikacin (100%) and other remaining antibiotics; ceftriaxone, gentamicin, levofloxacin, ciprofloxacin, colistin, and tetracycline were found to be resistant [43]. However, *Salmonella* was more resistant to ciprofloxacin (77%), sulfisoxazole (73%), and ampicillin (55%) [44].

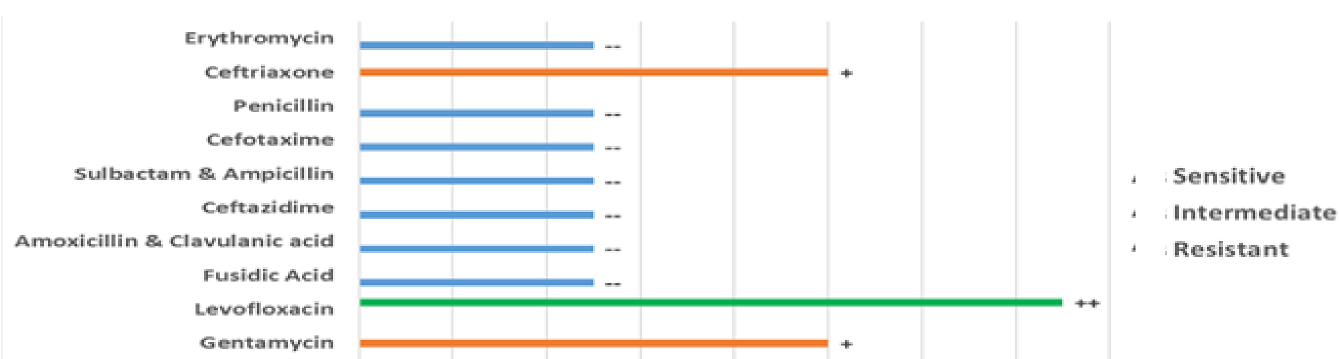

Figure 1: Patterns of antibiotic susceptibility for *Salmonella* Typhimurium isolated from the examined poultry farms

Assessment of the efficacy of certain disinfectants on the identified S. Typhimurium

The efficiency of certain disinfectants against isolated *S.* Typhimurium from the examined chicken farms after 1, 5, 10, 20, 30, 45 and 60 minutes of contact time in the presence or absence of organic matter was revealed in Figure 2. Virkon S (0.5%) was very effective against *S.* Typhimurium in the absence or presence of organic matter, with a kill time of less than one minute, followed by Virocid (0.5%), with contact times of 5 and 20 minutes in the absence and presence of organic matter, respectively. Cid 2000 (2%) had longer contact duration (45 and 60 minutes) in the absence and presence of organic matter. In surface contamination evaluation, glutaraldehyde-based treatments were much more successful than the other products. Although there were different perspectives within products within a chemical group, chlorocresol-based products were determined to be the most effective for usage in boot dips and aldehyde-based products for surface disinfection [45]. In poultry farms, disinfectants such as halogens, oxidizing agents, chlorhexidine compounds, phenolics, and alcohols are utilized. The disinfection capability of hydrogen peroxide was demonstrated to be good against *Salmonella* [2, 7, 8]. To minimize resistance, disinfectants should be used appropriately to reduce avian salmonellosis in poultry farms. Long-term disinfectant usage can have the opposite effect, such as the development of cross-resistances or an increase in biofilm formation [46].

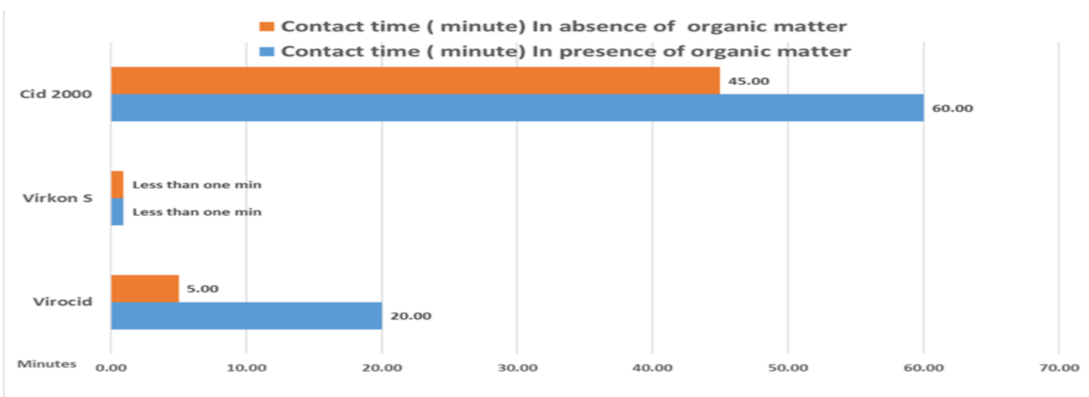

Figure 2: Efficiency of disinfectants against *Salmonella typhimurium* in the absence and presence of organic matter

Conclusion

In conclusion, all examined broiler farms earned grade "poor biosecurity farms. Many biosecurity measures are poorly implemented, with opportunities for improvement. Location, ventilation, feeder and drinker cleaning are the most critical risk factors and biosecurity measures. several management and biosecurity measures, including strict cleaning and disinfection methods have been found as protective factors in reducing the introduction and persistence of *Salmonella* species on poultry farms.

Education of farmers about the importance of biosecurity in poultry farming, role of various disinfectants need to be discussed in details with them and also organizing interactive session with workers and exposure them to practical aspects of broiler industry.

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Conflict of interest

The Authors declare that they don't have any conflict of interest.

References

[1] Georgiades, E.; Fraser, R. and Jones, B. (2016): Options to strengthen on-farm biosecurity management for commercial and non-commercial aquaculture. Aquaculture Unit, Technical Paper: 24-25.

[2] Kaoud, H.; Khalil, M. and Abdelhamed, M. (2022): Effect of cold fog disinfection on Escherichia coli affecting commercial egg layer flocks. Advanced Research and Reviews, 10 (1): 133-144.

[3] Malik, Y. S.; Milton, A.A.P.; Ghatak, S. and Ghosh, S. (2021): Avian salmonellosis. Role of Birds in Transmitting Zoonotic Pathogens, ed Y. S. Malik (Singapore: Springer), 79–91. doi: 10.1007/978-981-16-4554-9_6

[4] Ricke, S. C. (2021): Strategies to improve poultry food safety, a landscape review. Annual review of animal biosciences, 9: 379-400.

[5] Andrew, S. L; Mohammed H. Z.; Manyelo, T. G. and Mabelebele, M. (2020): The current status of the alternative use to antibiotics in poultry production: An African perspective. Antibiotics, 9(9): 594.

[6] Kamil, M. A. I. M.; Abu-Bakar, L.; Reduan, M. F. H.; Kamaruzaman, I. N. A.; Mahamud, S. N. A.; Azmi, A. F. M. and Shean, S. C. S. (2023): The Use of Medicinal Plants in Avian Colibacillosis Management: A Review. Veterinary Integrative Sciences, 21(2): 507-522.

[7] Aksoy, A.; El Kahlout, K. E. and Yardimci, H. (2020):Comparative evaluation of the effects of binzalkonium chloride, iodine, glutaraldehyde and hydrogen peroxide disinfectants against avian Salmonellae focusing on genotypic resistance pattern of the Salmonellae serotypes toward benzalkonium chloride. Brazilian Journal of Poultry Science, 22 (01):1-12

[8] Nabil, N. M. and Yonis, A. E. (2019): Isolation of Salmonella Characterized By Biofilm Formation and Disinfectant Resistance From Broiler Chickens. Alexandria Journal for Veterinary Sciences, 62 (2): 1-11.

[9] Maduka, C.; Igbokwe, I. and Atsanda, N. (2016): Appraisal of chicken production with associated biosecurity practices in commercial poultry farms located in Jos, Nigeria. Scientifica, 1914692

[10] Ismael, A; Abdella, A. Shimelis, S. Tesfaye, A. Muktar, Y. (2021):Assessment of Biosecurity Status in Commercial Chicken Farms Found in Bishoftu Town, Oromia Regional State, Ethiopia. Vet Med Int., 5591932.

[11] Abunna, F.; Bedasa, M.; Beyene, T.; Ayana, D.; Mamo, B. and Duguma, R. (2016): Salmonella isolation and antimicrobial susceptibility tests on isolates collected from poultry farms in and around Modjo, Central Oromia, and Ethiopia. J Anim Poult Sci, 5(2):21-35.

[12] Abdi, R. D.; Mengstie, F.; Beyi, A. F.; Beyene, T.; Waktole, H.; Mammo, B. and Abunna, F. (2017): Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the

poultry industry in Southern Ethiopia. BMC Infect Dis ,17(1): 1-12.

[13] Cruichshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, H.A. (1975): Medical microbiology. The practice of medical microbiology .12Ed. Vol. 11 Churchill, Edin burgh. 587-587.

[14] Humphrey, T.J.; Baskerville, A.; Mawer, S.; Rowe, B. and Hopper, S. (1989): Salmonella enteritidis phage type 4 from the contents of intact eggs: a study involving naturally infected hens. Epidemiol & Infec, 103(3): 415-423.

[15] Koneman, E.; Steven, D.; Dowell, V.; William, M. and Washington, C. (1997): Diagnostic Microbiology, J.B Lippineatt Co. Philadelphia, USA, p253-320.

[16] Alzwghaibi, A.B. ;Yahyaraeyat, R. Fasaei, B. N.; Langeroudi, A. G. and Salehi, T. Z. (2018): Rapid molecular identification and differentiation of common Salmonella serovars isolated from poultry, domestic animals and foodstuff using multiplex PCR assay. Archives of Microbiology, 200: 1009-1016.

[17] NCCLS (National Committee for Clinical Laboratory Standards (2002): Performance Standard for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. 2nd Ed., Approved Standard M31-A2, Wayne, PA.

[18] Aditi, F. Y.; Rahman, S. S. and Hossain, M. M. (2017): A study on the microbiological status of mineral drinking water. The open Microbiol, 11:31-44

[19] Pilotto, F.; Rodrigues, L.; Santos, L.; Klein, W.; Colussi, F. and Nascimento, V. P. d. (2007): Antibacterial efficacy of commercial disinfectants on dirt floor used in poultry breeder houses. Brazilian Journal of Poultry Science, 9:127-131.

[20] Aidaros, H.; Hafez, E. and El Bahgy, H. (2022): In vitro efficacy testing of some commercial disinfectants against pathogenic bacteria isolated from different poultry farms. Adv Anim Vet Sci, 10-18

[21] McHugh, M. L. (2013): The chi-square test of independence. Biochemia medica (Zagreb). 23(2):143-9.

[22] Van Limbergen, T.; Dewulf, J.; Klinkenberg, M.; Ducatelle, R.; Gelaude, P., Méndez, J. and Maes, D. (2018): Scoring biosecurity in European conventional broiler production. Poultry Science, 97(1): 74-83.

[23] Morishita, T. Y. and Greenacre, C.B. (2021): Biosecurity. Backyard Poultry Medicine and Surgery: A Guide for Veterinary Practitioners. 107-116.

[24] Schweitzer, P. M.; Susta, L.; Varga, C.; Brash, M. L. and Guerin, M. T. (2021): Demographic, Husbandry, and Biosecurity Factors Associated with the Presence of Campylobacter spp. in Small Poultry Flocks in Ontario, Canada. Pathogens, 10 (11): 1471.

[25] Cuc, N. T. K.; Dinh, N. C.; Quyen, N. T. L., and Tuan, H. M. (2020): Biosecurity level practices in pig and poultry production in Vietnam. Adv Anim Vet Sci, 8 (10): 1068-1074.

[26] Tilli, G.; Laconi, A.; Galuppo, F.; Mughini-Gras, L. and Piccirillo, A. (2022): Assessing biosecurity compliance in poultry farms: a survey in a densely populated poultry area in north east Italy. Animals, 12(11): 1409: 1-9.

[27] Delpont, M.; Guinat, C.; Guérin, J. L.; Vaillancourt, J. P. and Paul, M. C. (2021): Biosecurity measures in French poultry farms are associated with farm type and location. Preventive Veterinary Medicine, 195. 105466: 1-7.

[28] Islam, M. J.; Mahbub-E-Elahi, A. T. M.; Ahmed, T. and Hasan, M. K. (2016): Isolation and identification of Salmonella spp. from broiler and their antibiogram study in Sylhet, Bangladesh.

Applied Biology and Biotechnology. 4(3): 046-051.

[29] Fagbamila, I. O.; Barco, L.; Mancin, M.; Kwaga, J.; Ngulukun, S. S.; Zavagnin, P. and Muhammad, M. (2017): Salmonella serovars and their distribution in Nigerian commercial chicken layer farms. PLoS One, 12(3): e0173097.

[30] Alam, S. B; Mahmud, M.; Akter, R.; Hasan, M.; Sobur, A.; Nazir, K. N. H.; and Rahman, M. (2020): Molecular detection of multidrug resistant Salmonella species isolated from broiler farm in Bangladesh. Pathogens, 9(3): 1-12.

[31] Dagnew, B.; Alemayehu, H.; Medhin, G. and Eguale, T. (2020): Prevalence and antimicrobial susceptibility of Salmonella in poultry farms and in contact humans in Adama and Modjo towns. Ethiopia. Microbiology, 9 (8): e1067.

[32] Gutierrez, A.; De, J. and Schneider, K. R. (2020): Prevalence, concentration, and antimicrobial resistance profiles of Salmonella isolated from Florida poultry litter. Journal of Food Protection, 83(12): 2179-2186.

[33] Khan, A. S.; Georges, K.; Rahaman, S.; Abebe, W. and Adesiyun, A. A. (2022): Occurrence, risk factors, serotypes, and antimicrobial resistance of Salmonella strains isolated from imported fertile hatching eggs, hatcheries, and broiler farms in trinidad and tobago. Journal of Food Protection. 85(2): 266-277.

[34] Sharma, S.; Fowler, P. D.; Pant, D. K.; Singh, S. and Wilkins, M. J. (2021): Prevalence of non-typhoidal Salmonella and risk factors on poultry farms in Chitwan, Nepal. Veterinary World, 4 (2): 426–436.

[35] Waltman, W. D. and Richard K. G. (2016): Salmonella enterica. In Isolation and Identification of Avian Pathogens, 6th edition, Williams, S. M., L.; Dufour-Zavala, M. W.; Jackwood, M. D.; Lee, B.; Lupiani, W. M.; Reed, E. ; Spackman, and P. R. Woolcock, eds. American Association of Avian Pathologists, Athens, GA. pp. 103-112.

[36] Fraser, R. W.; Williams, N. T.; Powell, L. F. and Cook, A. J. C. (2010): Reducing Campylobacter and salmonella infection: two studies of the economic cost and attitude to adoption of on farm biosecurity measures. Zoonoses and Public Health, 57(7-8): e109-e115.

[37] Goualie, G. B.; Bakayoko, S. and Coulibaly, K. J. (2020): Practices of biosecurity measures and their consequences on poultry farms in Abidjan district. Food and Environment Safety Journal, 19(1): 84-91.

[38] Barbour, E. K.; Ayyash, D. B.; Alturkistni, W.; Alyahiby, A.; Yaghmoor, S.; Iyer, A. and Harakeh, S. (2015): Impact of sporadic reporting of poultry Salmonella serovars from selected developing countries. Journal of Infection in developing countries, 9(01): 001-007.

[39] Li, Y.; Yang, Q.; Cao, C.; Cui, S.; Wu, Y.; Yang, H. and Yang, B. (2020): Prevalence and characteristics of Salmonella isolates recovered from retail raw chickens in Shaanxi Province, China. Poultry science, 99(11): 6031-6044.

[40] Shivaning, K. N.; Benakabhat, M. C.; Agalagandi, G. S.; Hiremath, J.; Shivanagowda, P. G. and Barbuddhe, S. (2020): Prevalence of Salmonella serotypes S. Enteritidis and S. Typhimurium in poultry and poultry products. Journal of Food Safety. 40(6): e12952.

[41] Tawyabur, M.; Islam, M. S.; Sobur, M. A.; Hossain, M. J.; Mahmud, M. M.; Paul, S. and Rahman, M. T. (2020): Isolation and characterization of multidrug-resistant Escherichia coli and

Salmonella spp. from healthy and diseased turkeys. Antibiotics, 9(11): 770: 1-14.

[42] Ibrahim, S.; Wei Hoong, L.; Lai Siong, Y.; Mustapha, Z.; CW Zalati, C. S.; Aklilu, E. and Kamaruzzaman, N. F. (2021): Prevalence of antimicrobial resistance (AMR) Salmonella spp. and Escherichia coli isolated from broilers in the East Coast of Peninsular Malaysia. Antibiotics, 10(5): 579.

[43] Khanal, S.; Kandel, M.; and Shah, M. P. (2019): Antibiogram pattern of Escherichia coli, Salmonella spp. and Staphylococcus spp. isolates from broiler chicken. Nepalese Veterinary Journal. 36: 105-110.

[44] Xu, Y.; Zhou, X.; Jiang, Z.; Qi, Y.; Ed-Dra, A. and Yue, M. (2020): Epidemiological investigation and antimicrobial resistance profiles of Salmonella isolated from breeder chicken hatcheries in Henan, China. Frontiers in Cellular and Infection Microbiology. 10: 497.

[45] Gosling, R. J.; Breslin, M.; Fenner, J.; Vaughan, K.; West, E.; Mawhinney, I. and Davies, R. H. (2016): An in-vitro investigation into the efficacy of disinfectants used in the duck industry against Salmonella. Avian Pathology, 45 (5):576-581.

[46] Becker, E.; Projahn, M.; Burow, E. and Käsbohrer, A. (2021): Are there effective intervention measures in broiler production against the ESBL/AmpC producer Escherichia coli. Pathogens, 10(5): 608.

الملخص العربي

مدى انتشار أنواع السالمونيلا كمؤشر للأمن الحيوي في مزارع الدواجن بمحافظة الشرقية، مصر

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الأمن الحيوي يعني حماية الكائنات الحية، هو برنامج يهدف إلى الحفاظ على سلامة الطيور من الكائنات الحية الدقيقة المسببة للأمراض. كان الغرض من هذه الدراسة هو تقييم تدابير الأمن الحيوي وانتشار أنواع السالمونيلا في مزارع الدواجن في محافظة الشرقية، مصر. كما تم تقييم حساسية المضادات الحيوية والمطهرات لبكتيريا السالمونيلا تيفيموريوم المستردة. تم الحصول على إجمالي 84 عينة (21 من كل مزرعة من مزارع الدواجن الأربعة). تم أخذ عينات من المياه والأعلاف والقمامة والمذرق والجدران واليد والقدم على ثلاث دفعات من كل مزرعة. وفقاً للاستبيان، حصلت جميع المزارع التي شملتها الدراسة على درجة "ضعيفة في مجال الأمن الحيوي"، حيث كانت درجة الأمن الحيوي أقل من 50%. تم العثور على أنواع السالمونيلا في 10.7% (84/9) من العزلات. كان للمذرق أكبر عدد من أنواع السالمونيلا (41.7%)، يليه الفضلات (25%) والأعلاف (8.3%). مستوى الأمن الحيوي وأنواع السالمونيلا المعزولة لها ارتباطات سلبية كبيرة. بناءً على الفحص السيرولوجي، كانت السالمونيلا المعوية والسالمونيلا مولاد هي الأنماط المصلية الأكثر شيوعًا للسالمونيلا في الفرشة، في حين كانت السالمونيلا لاروشيل والسالمونيلا تيفيموريوم شائعة في الأعلاف والمذرق. كانت حساسية السيبروفلوكسين والليفوفلوكساسين أعلى في جميع سلالات السالمونيلا التيفيموريوم المعزولة، يليها الأميكاسين، والنيتروفورانتوين، والسيفترياكسون، والجنتاميسين. وقد تبين أن (0.5Virkon S %) فعال بشكل خاص ضد السالمونيلا تيفيموريوم ، يليه (0.5Virocid %) وأخيرًا (2000 cid -2%). ختاما فقد أظهرت نتائج هذا البحث التباين في مستويات الأمن الحيوي الموجودة في المزارع التي تمت دراستها فيما يتعلق بانتشار السالمونيلا، حيث تشير النسبة المئوية المنخفضة لدرجة الأمن الحيوي إلى درجة أكبر من انتشار السالمونيلا. تم تحديد مجموعة متنوعة من ممارسات الإدارة والأمن الحيوي، بما في ذلك إجراءات التنظيف والتطهير الصارمة للغاية، كعوامل وقائية لتقليل دخول أنواع السالمونيلا واستمرارها في مزارع الدواجن.