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Original Research Article

Prevalence of Salmonella Spp in Some broiler farmsin different Egyptian Governorates

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ABSTRACT

This study was conducted to evaluatethe degree of biosecurity level with especial reference to Salmonella Spp as an example to explain the expected causes and risk factors that leads to spread them in poultry flocks in Egypt then studied farms evaluated for the sensitivity of Salmonella isolates to the most common disinfectants used in Egypt.About 300 samples (100 cloacal swabs, 100 liver and intestinal samples,100 litter samples) were collected from 10 broiler farms with different age (at 0 old day, one week,2,4 and 6 weeks of age)then the samples were investigated for Salmonella Sppand subsequently identified based on biochemical and serological tests.The obtained results showed that 35 Salmonella species were isolated from 10 broiler poultry houses (25%);(6%) and (4%) from cloacal swab;liver and litter, respectively. Average prevalence of Salmonella spp. was 11.33 % in open broiler houses whether raised Cobb, Ross or Sasso breeds. Salmonella Typhimurium, S. enteritidisand S. Kentuckywerethe most serovars out of the 35 detected isolates.There was great statistical significant difference in the sensitivity of Salmonella isolates to the most common disinfectants(P<0.05)asVerkon- S® achieved 3 log reduction' after 5-minute,Formalin and Phenique were achieved 3 log reductions against S. entriditisafter one-minute Aldekol Des- Gda® achieved one log reduction after one minute, TH4®,Biosentry® 904 and Iodophoreachieved 2 log reductions after 5 minutes.

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INTRODUCTION

The intensification of broiler production, associated with stressful environmental conditions and management practices, often does not include effective strategies for the control of environmental pathogens, being responsible for increasing health challenges (**Barrios, 2009**).Salmonella spp. is delicate, non-spore forming gram negative rod-shaped bacteria of the family Enterobacteriaceae, which are widespread in the environment. Intestinal tract of many animalsand humans is their usual place of habitat and propagation, hence they are released into the environment and can survive for a long time and grow in food, plant and animal waste, in general, wherever they have found organic matter and suitable conditions (**Mitov,2000**).

Salmonella are commonly found in the environment (**Murray, 1991**), and there are many instances throughout the grow-out phase in which birds can come into contact with Salmonella and other pathogens (**Reiber et al,1990**). These pathogens are able to survive for extended periods of time in the environment and can be commonly found in the litter on which the birds live. Salmonella infections are recognized worldwide as an important food borne human diseases. Approximately 13 million cases of paratyphoid infections occur worldwide annually (Murugkar et al., 2005). S. Enteritidis in poultry causes serious economic losses due to high rate of mortality (4-50%), loss of weight and decreased in egg production in addition to the public health impact due to infection with S. Enteritidis (**Haider et al., 2004**).

Poultry house sanitation plays a crucial role in the control and prevention of pathogenic infectious diseases. Agood sanitation program can benefit the grower by optimizing bird performance while lowering the incidence of contaminated flocks. In the same time the Improper, sanitation procedures can adversely affect disease prevention, and lowering birdperformance (**Davies, and Wray,1995; Corrier et al,1992**). For this reason, it is important to routinely re-evaluate the effectiveness of poultry house sanitation programs.Therefore, the aims of the study were:Investigation to the contamination of broiler farms with Salmonella species,and evaluation to the degree of biosecurity level of the farms with especial reference to Salmonella Spp. As well as tried to explain the expected causes and risk factors that leads to spread them in poultry flocks in Egypt. Finally, Determination the disinfectants sensitivity of Salmonella isolates to most common used disinfectant agents in Egypt.

MATERIALS AND METHODS

A total of 10 broiler houses were studied from November 2016to January 2018.The farms were visited at different ages (one day old, week one, week 2, week4 and week6 of age). The data collected form the visited farms were;description for their construction, bird species, stocking densities, traffic control, pest control, vaccination programm

disinfection protocol and other managemental criteria. The evaluation process was carried out through filling out a designed questionnaire and taking samples for the isolation of bacterial pathogens.

Designed questionnaire

Biosecurity parameters	Score (code)
1. Self proofing (bird and house)	(yes = 0.1, no = 0)
2. Rodent and wild bird proofing	(yes = 0.1, no = 0)
3. Ventilation area	(yes = 0.1, no = 0)
4. Adequate distance between farms and other poultry operations	(yes = 0.1, no = 0)
5. Hygienic disposable of carcass	(yes = 0.1, no = 0)
6. Self sufficient (farm equipment)	(yes = 0.1, no = 0)
7. Cleaning and disinfection	(yes = 0.1, no = 0)
8. Foot dips	(yes = 0.1, no = 0)
9. Traffic control	(yes = 0.1, no = 0)
10.Visitor restriction	(yes = 0.1, no = 0)

Sampling

1) Litter samples (10 gm each) were randomly collected from the commercial broiler farms. The total of 100 samples were collected from the broiler farms (Triple litter samples).
2) Cloacal swab samples were collected from the broiler farms (three Cloacal swabs).
3) Liver and intestine swab samples, the total of 100 samples were collected from the broiler farms (three samples).

Samples were collected aseptically and then brought to the laboratory in the Department of Veterinary Hygiene and Management, Faculty of Veterinary, Cairo University These samples were subjected to various bacteriological and biochemical examination in the laboratory.

Sampling procedures

Propagation of the bacterial isolates:

The bacterial isolates (Salmonella entriditis) were propagated using pour plate method, (Cruickshank et al., 1980). A loopful was transferred from all bacterial strains that was stored onto nutrient slopes into 10 ml nutrient broth and incubated at 37^{o}C for 20-24 h. (**Zelver et al., 1999; Herigstad et al., 2001**).

Preparation of source of organic matter:

5% stock solution of yeast suspension (5 g of dried yeast was added to 100 ml of sterile distilled water); the yeast suspension was dispensed into 5 ml tubes, sterilized by autoclaving for 20 min at 121^{o}C.

A- Salmonellae Growth media:

Nutrient Broth (NB) and Nutrient Agar (NA) were used to grow the organisms from the collected samples before performing biochemical test according to the procedure describe by **Cheesebrough (1984)**. Eosin Methylene Blue (EMB) agar medium was used for observing growth of E. coli (**Cheesebrough, 1984**). MC medium was used for culturing the organisms under the family Enterobacteriaceae (**Cheesebrough. 1984**).

SS agar medium was used as a selective medium for Salmonella organism which causes enhancement of the growth of Salmonella while inhibiting the growth of other contaminating organisms and shows typical colony characters (**Cheesebrough, 1984**). Brilliant Green Agar (BGA) medium was used as a selective medium for the isolation and identification of Salmonella organisms (**Cheesebrough, 1984**).

B-Identification of salmonellae

Suspected isolates of Salmonella organisms were identified according to **MacFaddin (2000)**.

C-Serological identification of Salmonellae

Serological identification of Salmonellae was carried out according to Kauffman – White scheme (**Kauffman, 1974**)for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan).

D-Tested Disinfectants

Disinfectants were chosen representing 7 different types:

i. Potassium proxy Monosulphat (Verkon- S® 1:120)
ii. Aldehyde / QUACS disinfectant (Aldekol des- Gda®)(0.4%).
iii. Quaternary ammonium compound disinfectant (biosentry® 904™) (0.4%).
iv. Quaternary ammonium compounds and glutaraldehyde (TH4® 1 ml of TH4® solution was added to 100 ml distilled water, pH 8.7).
v. Formalin (2.5%, pH 7.9).
vi. Iodophore1 % in water
vii. Phenique 3% in water.

Evaluation of the efficacy of chemical disinfectants

The laboratoryevaluation of the efficacy of the chemical disinfectantswas carried out using modified use-dilution test(**Robinson et al., 1988**). The test was repeated twice;once in the presence of organic matter and the secondtime in the absence of the organic matter.

Bacterial suspensionwas prepared and propagated. 10 ml of the testedchemical disinfectant were poured into a sterile testtubes, 0.1 ml of the bacterial suspension (1–2 x 10^8) wasadded and shaken thoroughly to give the chance for micro-organism to come in contact with the disinfectant.At time interval 1, 5, 10 and 30 min from original zero-time 1 ml of disinfectant-bacterial mixture were taken into tube containing 9 ml of in-activator (Tween 80 3%) in nutrient broth, mix thoroughly. One ml from in-activator tubes was used for the bacterial count using pour plate method (Cruickshank et al., 1980). The numbers of survival bacteria on each plate were counted. The calculation was carried out using the following formula: Log (average CFU/ drop vol.) (dilution factor) (Vol. scrapped into/ surface area) (**Zelver et al., 1999; Herigstad et al., 2001**).

Statistical Analysis

The data were analysed by the student t test and One-Way analysis of variance (ANOVA) according to **Shott (1990).**

RESULTS AND DISCUSSION

1- Prevalence of Salmonellae

Types of samples	No. of samples	Salmonella spp.
Cloacal swab	100	25%
Liver \Intestine	100	6%
Litter	100	4%
Total %	**300**	**11.33 %**

Table (1) showing average incidence of Salmonellae in broiler poultryfarms.

The obtained results showed that 35 Salmonella species were isolated from 10 broiler poultry houses (25%);(6%) and (4%) fromcloacal swab;liver and litter, respectively. Average prevalence of Salmonella spp. was 11.33 % in open broiler houses whether raised Cobb, Ross or Sasso breeds.

Serotypes	Number of isolates	Percentage
S. Typhimurium	9	25.71
S. entenidis	7	20
S. Kentucky	7	20
S Molade	5	14.29
S. Tamale	3	8.57
S. Papuana	2	5.71
S. Inganda	1	2.85
S. Larochelle	1	2.85
Total	**35**	

Table (2) showing Prevalence of Salmonella serotypes in all farms.

Prevalence of Salmonellae serovars

The most prevalent Salmonella serovars in broilers were: Salmonella Typhimurium (25.71%); S. entenidis (20 %) Salmonella Kentucky(20 %), SMolade (14.29 %), S. Tamale (8.57 %), Salmonella Papuana(5.71 %), Salmonella Inganda (2.85%) and S. Larochelle(2.85%) had been isolated from poultry

The most prevalent Salmonella serovars in broilers isolated by some authors were S. entenidis (25%) S.typhimuiurium (15 %), S., S.infantis (5%) and S. kenlucky (5%). Salmonellae had been isolated from poultry litter by many authers (**Pieskus et al., 2008; Dhanarani et al., 2009; Andreatti-Filho et al., 2009**).

A European baseline survey on the prevalence of Salmonella in commercial broiler flocks of Gallus gallusin 2005 and 2006 showed that in the European Union (EU), 23.7% of the broiler flocks were Salmonella positive (EFSA,2007). However, the Salmonella prevalence and serovars distribution varied widely among the EU member states. The five most frequently isolated Salmonella enterica serovars in Europe were those classically observed, like serovar Enteritidis (33.8%), serovar Infantis (22.0%), serovar Mbandaka (8.1%), serovar Hadar (3.7%), and serovar Typhimurium (3.0%). In Germany, the flock prevalence of Salmonella was 15.0% among the 377 broiler flocks investigated. In contrast to the well-known serovars described above, the predominating serovar was monophasic serovar 4,12: d: with a prevalence of 23.6%. This serovar was also isolated in Denmark and the United Kingdom, with prevalence of 15.2% and 2.8%, respectively.

In Egypt, Salmonellae were isolated from 5.3% of litter samples collected from Kafr El-Sheikh province, the only identified one of the collected samples was S. enteritidis (**Mohammed et al., 1999). Trawinska et al. (2008**) isolated S. typhimurium from geese, broiler chickens and reproductive laying hens, S. enteriditis proved the most commonly reported serovar in poultry isolated by **Trawinska et al., (2008**).

Bio-sec	Mort. %	Salmonella spp.
0.6	12	12.5%
0.4	15	43.8%
0.8	8	25%
0.5	15	75%
0.4	20	18.8%
0.8	6	0%
0.4	20	75%
0.5	15	62.5%
0.7	12	18.7%
0.7	12	18.7%

Table (3) showing biosecurity score, mortalityrate andSalmonella in farms.

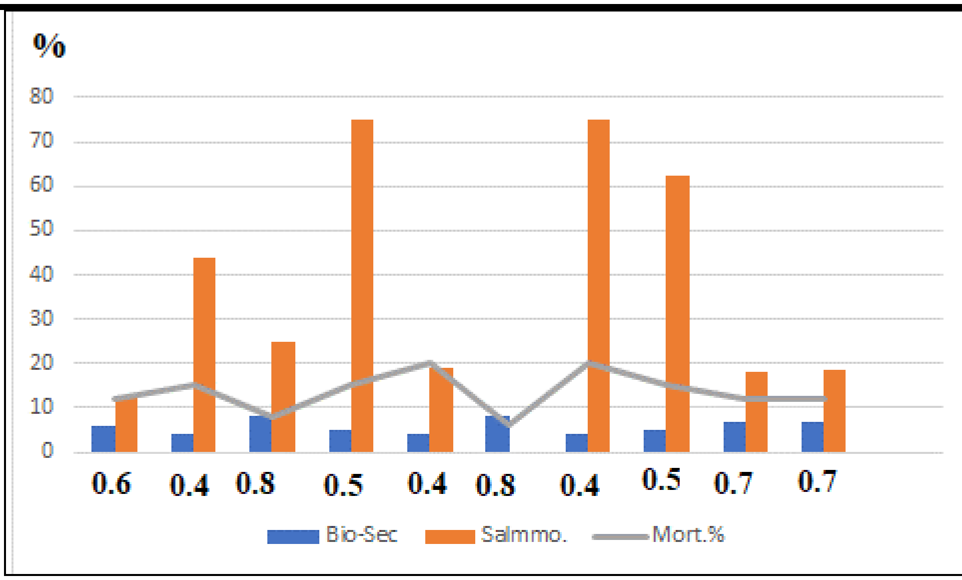

Figure: (1) showing biosecurity score, mortality rate and Salmonella in farms

The relationship between biosecurity level and Mortality rate: Form Table3,we found that there was strong association concerning level of applied biosecurity in broiler farms and mortality rate **(significant negative** P<0.05**)** **(**R= 0.8545), its means that the mortality rate ' will be reduced significantly (P<0.05) if the satisfactory biosecurity applied to such farms.

The relationship between biosecurity level and Salmonellaspread: Form Table 3**,** we found that there was a very strong association concerning level of applied biosecurity in broiler farms and Salmonellaspread**(significant negative** P<0.05) The value of R is NaN. This is a strong negative correlation, which means that high X variable scores go with low Y variable scores

Disinfectant/ contact time	Initial count	1 min	5 min	10 min	30 min
Verkon-	**1.5×10^8**	log6	log5	log5	log5
Aldekol	**1.6×10^8**	log6	log6	log6	log5
TH4®	**3.6×10^8**	log7	log6	log6	log6
Biosentry	**1.6×10^8**	log7	log6	log6	log6
Iodop™	**1.5×10^8**	log7	log6	log6	log6
Form	**1.2×10^8**	log^5	log^5	log^5	log^5
Pheni	**1.5×10^8**	log^5	log^5	log^5	log^5

Table (4) The Mean viable colony count (cfu/ml) of the tested disinfectants in In the absence of organic matter

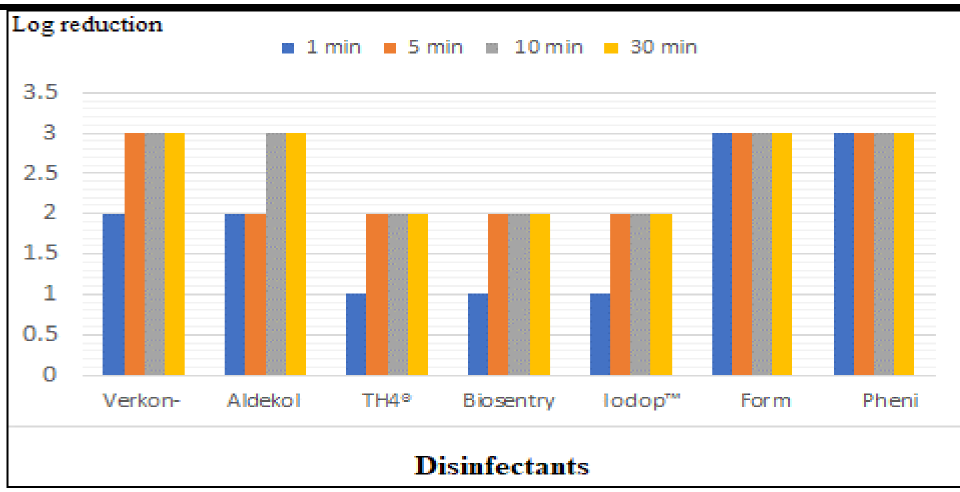

Figure (2): The Mean log reduction (Log 10) of S. entriditis after contact time with the tested disinfectants in the absence of organic matter.

Disinfectant/ contact time	Initial count	1 min	5 min	10 min	30 min
Verkon-	**1.5×10^8**	log6	log6	log6	log6
Aldekol	**3.6×10^8**	log6	log6	log6	log6
TH4®	**1.6×10^8**	log7	log7	log7	log7
Biosentry	**1.6×10^8**	log7	log7	log7	log7
Iodop™	**1.5×10^8**	log7	log7	log7	log7
Form	**1.2×10^8**	log5	log5	log5	log5
Pheni	**1.5×10^8**	log7	log7	log7	log7

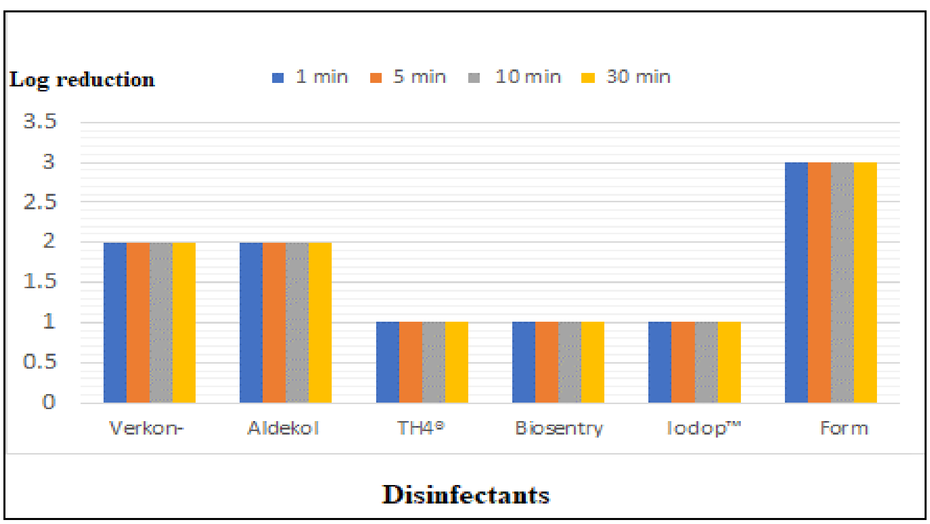

Figure (3): The Mean log reduction (Log 10) of S. entriditis after contact time withthe tested disinfectants in the presence of organic matter.

Verkon- S®achieved 3 log reduction' after 5 min, Aldekol Des- Gda® achieved one log reduction after on min,TH4® achieved 2 log reductions after 5 min, Biosentry® 904 and Iodophore achieved 2 log reductions after 5 min, Iodophore™, while Formalin and Phenique were achieved 3 log reductions against S. entriditisafter one min ($p<0.05$).In the presence of organic matter,Verkon- S® and Aldekol achieved S. entriditisreduction 2 log after one min ($P<0.05$) without any log reduction after words.TH4®, Biosentry® 904™ and Iodophore™achieved one log reduction after one min without any log reduction after words.In case of Formalin, it achieved 3 log reduction' after one minwhile Phenique achieved one log reduction' after one min without any log reduction after words. The result matched with **Williams ,1980,** who stated thatFormalin is widely use at 5% strength as a general disinfectant, but it needs contact time to be effective, the best reduction in total bacterial count could be obtained with 10% formalin solution followed by creolin 3% while lower efficiency was recorded with iodophors **Williams (1980); Ka-oud (1986); Sainsbury (2000) and Mandel et al, (2005)** recommended using the following disinfectants, formalin, iodophors, and phenique for disinfection of poultry houses and the most common disinfectant is formalin, due to it is cheap and available in market.

From the findings we can concluded that the most prevalent Salmonella serovars in broilers were: Salmonella Typhimurium, S. entenidis,Salmonella Kentucky and S Molade.There was a strong association concerning level of applied biosecurity in broiler farms and mortality rate, its means that the mortality rate will be reduced significantly if the satisfactory biosecurity applied to such farms. Also, the variables, such as application rate, disinfectant type, time of exposure, and the presence or absence of organic matter, are important considerations when including a chemical disinfectant application into a sanitation program. The potassium peroxymonosulfate, nascent oxygen, formalin and phenol products provided the best Salmonella reductions in the laboratory trials.

REFERENCES

Andreatti-Filho RL, Lima ET, Menconi A, Rocha TS, Goncalves GA. (2009). Research in Salmonella spp. In drag swabs originating from poultry houses. Veterinaria Zootecnnia.16:1,190-194.

Barrios PR.(2009). Detection of chicken infectious anemia virus. Belo Horizonte: Universidade Federal de Minas Gerais.

Cheesbrough, M. (1984). Culture Media. In: Cheesbrough, M., Ed., Medical Laboratory Manual for Tropical Countries, Vol. 3, Tropical Health Technology and Butterworth-Heineman, Cambridge, 60-69, 407-428.

Corrier, D. E., A. Hinton, Jr., B. Hargis, and J. R. DeLoach. (1992). Effect of used litter from floor pens of adult broilers on Salmonella colonization of broiler chicks. Avian Dis. 36:897–902.

Cruickshank, R., J.P. Duguid, B.P. Marimion and R.H. Swain, (1980). Medical microbiology. E.L.B.S. 12th Ed., vol. 11, reprinted Churchill Livingstone and Robert Stevenso. Edinburgh, EHI, 3AF.

Davies, R. H., and C. Wray. (1995). Observations on disinfection regimens used on Salmonella Enteritidis infected poultry units. Poult. Sci. 74:638–647.

Dhanarani TS, Shanker C, Park J, Dexilin M, Kumar R, Thamaraiselvi K. (2009). Study on acquisition of bacterial antibiotic resistance determinants in poultry litter. Poultry Science .88:1381- 1387.

European Food Safety Authority. (2007). Report of the Task Force on Zoonoses Data Collection on the analysis of the baseline survey on the prevalence of Salmonella in broiler flocks of Gallus gallus, in the EU, 2005-2006. EFSA J. 98:1-85.

Haider, M. G.; Hossain, M. G.; Hossain, M. S.; Chowdhury, E. H.; Das, P.M. and Hossain, M. M. (2004). Isolation and characterization of Enterobacteria Associated with Health and Disease in Sonali Chickens. Banganladesh Journal of Veterinary Medicine 2 (1):15-21.

Herigstad, B., M. Hamilton and J. Heersink, (2001). How to optimize the drop plate method for enumerating bacteria. J. Microbiol. Meth., 44: 121-129.

Ka-oud HA. (1986). Hygienic Studies on E.coli in Poultry Farms in Egypt. J. Egypt .Vet Med .Ass 46: No 3 :243-249.

Kauffman, G. 1974. Kauffmann white scheme. J. Acta. Path. Microbiol. Sci., 61:385.

MacFaddin, J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. Baltimore, MD: Lippincott Williams & Wilkins

Mandel AB, Yadav AS, Johri TS, Pathok NN. (2005). Prevention of Infectious Diseases of Poultry in (Nutrition and Disease Management of Poultry). First Ed. pp 325-341. International Book Distributing Co. Printed at Army Printing Press.

Mitov I. (2000). Salmonellas. In: Microbiology, Mitov G, editor. Arso, Sofia, (in Bulgarian). 2000.

Mohammed LN, Samaha H A, Draz A A. Haggag Y N. (1999). Salmonellae among birds and human beings.Alex.J.Vet.Sc.,15 (1) :147-154. Glob. J. Sci. Res., 1 (1): 8-13, 2013

Murray, C. J. (1991). Salmonellae in the environment. Revue Scientifique et Technique de l'Office Internationale des Epizooties 10: 765–785.

Pieskus L, Kazeniauskas E, Butrimaite-Ambrozeviciene C, Stanevicius Z and Mauricas M. (2008). Salmonella incidence in broiler and laying hens with the different housing systems. Journal of Poultry Science.,45(3) :227-231.

Reiber , M. A. , R. E. Hierholzer, M. H. Adams, M. Colberg, and A. L. Izat. (1990). Effect of litter condition on microbiological quality of freshly killed and processed broilers. Poult. Sci. 69:2128–2133.

Robinson, R.A., H.L. Bodily, D.F. Robinson and R.P. Christensen, (1988). A suspension method to determine reuse life of chemical disinfectants during clinical use. Appl. Environ. Microbiol., 54:158-164

Shott, S. (1990): Statistical for health professionals. W.B. Saunders Co. Philadelphia, 313–336.

Sainsbury D. (2000). Poultry Health and Management. 4th Edition, Blackwell Science, Printed by Hartnolls ITD, Bodmin, and Cornwall.

Trawinska B, Saba BL, Wdowiaka l, Ondasovicova O, Nowakowicdebeck B. (2008). Evaluation of Salmonella rod incidence in poultry in the Lublin Province over the years 2001-2005.Annals of Agriculture and Environmental Medicine.,15(1) :131-134.

Williams, JE. (1980). Formalin Destruction of Salmonella in Poultry Litter. Poultry Science, 59: 2717-2724.

Zelver, N., M. Hamilton, B. Pitts, D. Goeres, D. Walker, P. Sturman and J. Heersink, (1999). Measuring antimicrobial effects on biofilm bacteria: in RJ Doyle et al. (Eds), biofilm: methods in enzymology, Academic Press, San Diego, CA, pp: 608-628.