Study on the incidence of E. coli and salmonella in the enviro of some poultry farms in rural areas in Behera province.

Samaha, H.A.; Haggag, Y.N.; Noseir M. A. and Shita, H. M.
Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria Univer

Abstract

A total of 640 samples including air (160), water (160), feed (160), and litter (1 collected from 4 poultry farms (2 broiler and 2 layers) in rural areas in Behera in winter and summer seasons. The collected samples were subje bacteriological examination and revealed that the isolation rates of *Escherichia Salmonella species* from environmental samples of different farms were 19.5 %, respectively. The percentage of *E. coli* and *Salmonella spp.* was higher in of layers farms (22.2 and 18.6 %, respectively) than broiler farms (16.9 and respectively). Also, the highest percentage of *E. coli* was recorded in litter (26.9 %) followed by water (21.3 %), air (18.1 %) and lastly feed (11.9%). On hand, the highest percentage of *Salmonella spp.* was recorded in litter sample followed by water (15.6 %), feed (13.8 %) and lastly air (9.4 %). In addi percentage of *E. coli* was found to be higher in summer (22.8%) than in winter for all samples while the seasonal isolation of *Salmonella spp.* was nearly equa summer (15 %) and winter (14.4 %). Moreover, The serological identificat isolates of *Salmonella spp.* (3 isolates from feed and 3 isolates from litter) reve all the isolates were *S. enteritidis* which further confirmed by PCR. The results and the hygienic importance of *E. coli* and *Salmonella spp.* isolat different examined samples were discussed.

Introduction.

Poultry industry plays an important role in offering cheap source of animal pr human beings in Egypt where prices of red meat, milk products and other sc animal protein reached a very high levels that disable the ordinary consum those who called the income limited citizens from buying them in amounts tha all their needs regularly, so, the role of poultry industry arises markedly in o reasonable price source of animal protein.

Bacteria are of the major factors that influenced by the environmental c around poultry and at the same time affecting poultry health and production su *coli* and *Salmonella*. *E. coli* infection of chicken is considered as one of t serious problems affecting poultry industry including several forms of infection colibacillosis which is recognized as the primary cause of morbidity, mort condemnation of carcasses in the poultry industry worldwide (Goren, 1990 and et al., (2000). In addition, there are more than 2,000 species or serotypes of belonging to genus *Salmonella*; all are potential pathogens of poultry but serotypes are true poultry pathogens (*S. gallinarum and S. pullorum*). M *Salmonella* infections with other serotypes (*S. enteritidis, S. typhimurium, S* etc.) seldom cause disease in poultry, but they are of major concern to publi (Breytenbach, 2004).

The environment of poultry houses constitutes a dangerous vehicle for surv spreading of bacterial pathogen of both veterinary and public health import *Coliform* bacteria could be found in litter, faecal matter, water and dust o

houses and can persist for longer periods particularly in dry conditions. So, atter must be paid for the sources of infection inside poultry farms and hygienic construc of such building, disinfection of poultry houses, drinking water and using of sound also avoid contact with diseased birds and their contaminated environment (Draz e 1996).

Successful control of bacterial diseases entails isolating and identifying dise producing species, if present, and preventing multiplication and spread of the orgar within the bird's body or to other birds. So, this work is carried out to give further on the possible sources of *E. coli* and *Salmonella spp.* infections including air, w feed and litter in poultry farms in Behera Province, isolation and identification of *E.* and *Salmonella spp.* from different sources in poultry farms, Study the effect of s epidemiological factors including type of poultry farm and the season of the year or incidence of the isolated bacteria and discussing the problems caused by them available control measures.

Material and methods

1. Farms under investigation.

The present study was carried out in four poultry farms (2 broiler and 2 laye low capacity (5000 – 7000) in Behera Province in rural localities in both winter sea where the environmental temperature ranged between 9 and 24 °C with a rang relative humidity between 50 and 90 % and summer season where the environm temperature ranged between 29 and 38 °C with a range of relative humidity betv 40 and 70 %. All farms under investigation belong to the opened system with ma feeder and manual drinking system except one with automatic feeders and n drinkers.

2. Collection of samples:

A total of 640 environmental samples were collected from air (160), water (litter (160) and feed (160) in both winter and summer seasons. The collected sam were dispatched with minimum of delay to Laboratory of Animal Hygiene Zoonoses Department, Faculty of Veterinary Medicine, Alexandria University.

3. Preparation of the samples:

The collected samples from different sources were prepared for bacteriolo examination according to *Moubarak, 1989* and *Quinn et al., 1994.*

4. Isolation and identification of E. *coli* from different samples:

Collected samples were cultured as described by *Moubarak, 1989* and *Qui al., 1994* then subcultured on MacConkey's agar plates for 24 hours at 37 Suspected lactose fermented colonies were picked up and streaked on Ec methyline blue media for another 24 hours at 37 °C, suspected colonies (gree blackish color with metallic luster) were picked up and kept in slope aga identification that was carried out by morphological characteristics, motility biochemical tests *(Bailey and Scott 1990).*

5. Isolation of *Salmonella* from different samples:

Collected samples were cultured as technique described by *Moubarak, 198 Quinn et al., 1994* in Selenite F. broth and tetrathionate broth then subculture MacConkey's agar plates and brilliant green agar plates for 24 hours at 37 Suspected colonies were picked up and kept in slope agar for identification that carried out by morphological characteristics, biochemical tests and serolo identification.

6. Serological identification of the *Salmonella* isolates:

It was carried out in the Serology Unit in Animal Health Research Institute, I Giza. 6 isolates that were preliminary identified biochemically as *Salmonella* subjected to serological identification.

7. Detection of *S. enteritidis* by PCR:

Salmonella isolates were grown in selenite faecal broth for different tim incubation as mentioned before at 37 °C. 100 µl of broth culture were centrifuge the pellet was resuspended in distilled water. The genomic DNA was extract boiling of the suspension for 10 minutes in water bath to ensure lysis of cell complete denaturation of DNA and the supernatant was used as a templa polymerase chain reaction. The thermal cycler was programmed as follows: One for 5 minutes at 94°C to denaturate the DNA template followed by amplificatic cycles of denaturation, primer annealing and extention at 94°C for 30 seconds, for 90 seconds and 72 °C for 30 seconds, respectively. The 35 cycles were follow thermal cycle of extention at 72 °C for 10 minutes to ensure that the entire product in double stranded DNA. The annealing temperature (56 °C) was se according to Soumet et al. (1999) as it should be 1-5 °C lower than the lowest value of the primers. Finally, agarose gel was examined for the expected size amplified DNA fragment and visualized using Ultraviolet light on an UV transillumi

Results

The obtained results are presented in Tables (1, 2, 3, 4 and 5) and 1photo

Table (1): Incidence of *E. coli* in different samples in broiler and layers farms

Sample	Broiler (No. = 320)		Layers (No. = 320)		Total (No. = 640)	
	+ve	%	+ve	%	+ve	%
Air	10	12.5	19	23.8	29	18.
Water	20	25.0	14	17.5	34	21.
Feed	11	13.8	8	10.0	19	11.
Litter	13	16.3	30	37.5	43	26.
Total	54	16.9	71	22.2	125	19.

No = Number of examined samples

Table (2): Incidence of *E. coli* in different samples in summer and winter seasons

Samples	Winter (No. = 320)		Summer (No. = 320)	
	+ve	%	ve+	%
Air	11	13.8	18	22.5
Water	15	18.8	19	23.8
Feed	7	8.8	12	15.0
Litter	19	23.8	24	30.0
Total	52	16.3	73	22.8

Table (3): Incidence of *Salmonella spp.* in different samples in broiler and layer far

Sample	Broiler (No. = 320)		Layers (No. = 320)		Total (No. = 640)	
	+ve	%	+ve	%	+ve	%
Air	5	6.3	10	12.5	15	9.4
Water	6	7.5	19	23.8	25	15.(
Feed	11	13.8	11	13.8	22	13.8
Litter	12	15.0	20	25.0	32	20.(
Total	34	10.6	60	18.6	94	14.7

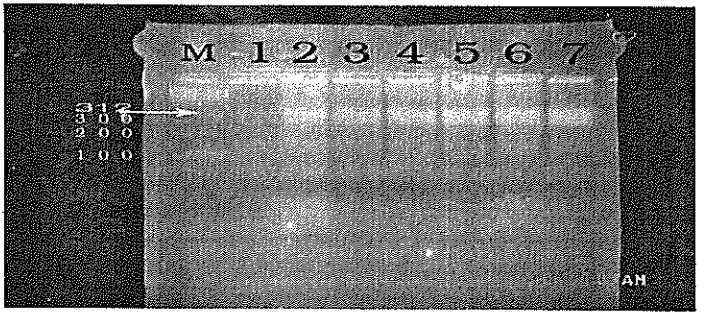
Table (4): Incidence of *Salmonella spp.* in different samples in summer and seasons

Samples	Winter (No. = 320)		Su (No. =
	+ve	%	ve+
Air	7	8.8	8
Water	14	17.5	11
Feed	10	12.5	12
Litter	15	18.8	17
Total	46	14.4	48

Table (5): Salmonella serotypes recovered from examined feed and litter sa collected from different farms.

Source	No.	*Salmonella* serovars
Litter	3	*S. enteritis*
Feed	3	*S. enteritis*

Photo (1): The ethidium bromide stained gel of the PCR products of isolates *enteritidis.*



Lane M: Marker, Lane (1): Negative control (empty), Lanes (2-7): Positive samples (1 band at 3 Positive samples show a single band in corresponding of 312 bp. indicating presence of *S. enteritidis*

Discussion

Today, poultry raising is big business so each step must be done by someone knows what should be done. Disease outbreaks cost poultry producers and rel industries millions of dollars a year in lost revenue and in order to minimize th losses, disease- prevention methods must be followed, including practices controlling disease-causing organisms (pathogens) and their vectors.

The data recorded in Table (1), firstly revealed that the total incidence of *E.* isolated from air samples of poultry farms was 18.1 %. This result was higher than obtained by Zaharan (1981) (14 %), Dainov (1985) (7.4 %), Hinz and Krause (1 (0.5 %), Sotohy (1989) (8.17 %), Rehab El-Zarka (2003) (17 %) and Moham (2005) (10.8 %) while it was lower than that recorded by Draz and Samaha (1 (21.4%), El-Kabany (1992) (23.3%), Draz et al. (1996) (26.5 %), Ola-Basha (1 (21.21 %), Abd El Haleem (2000) (58.3 %) and Ayoub (2007) (20 %). In addition percentage of *E. coli* isolated from air samples of layer farms (23.8 %) was higher that isolated from broiler farms (12.5 %). This recorded result disagreed with A (2007) who found that the incidence of *E. coli* in layer farms (16.25 %) was lower that of broiler farms (25 %). The higher incidence of *E. coli* in layer farms ma attributed to the longer periods that birds stay in layer houses than broilers where hygienic status of low capacity farms were not considered enough.

The data recoded in Table (1), secondly revealed that the total incidence of *E.* isolated from water samples of poultry farms was 21.3 %. This result was higher that obtained by Sotohy (1989) (9.19%), Ola-Basha (1997) (10.93%), Abd El-Hal (2000) (20.8 %), Mohammed (2005) (11.43%) and Ayoub (2007) (14.6 %) while it lower than that obtained by Abd El – Karim et al. (1993)(23 .30 %), Draz et al. (1 (36.7 %) and Rehab El-Zarka (2003) (25%). In addition the percentage of *E.* isolated from water samples of layer farms (17.5 %) was lower than broiler farms %). This recorded result agreed with Ayoub (2007) who found that the incidence *coli* in layer farms (5 %) was lower than broiler farms (22.5 %). This difference in *E* percentage in water samples that collected from both broiler farms and layer fa may be due to the variety of watering systems as layer farms use nipple wat system versus automatic and manual drinkers in broiler farms which are expose contamination from birds and litter particles. The results illustrated in Table (1), revealed that the total incidence of *E. coli* isolated from feed samples of poultry fa was 11.9 %. This result was lower than that obtained by Sambyal et al. (1981) (%), Zahran (1981) (15 %), Zakia (1984) (26 %), Mashhoor et al. (1987) (22. 67 Abd El –Haleem (2000) (22.2 %) and Ayoub (2007) (27.9 %). At the same time, it higher than that recorded by Ahmed et al. (1995) (7.69 %), Ola Basha (1997) (6.8 and Hoda El-ashker (2001) (7.8 %). The variation in the incidence of *E. coli* in samples collected from poultry farms may be attributed to the difference of sources, differences in storage conditions or different feeding systems. In addition obtained data presented in Table (1) illustrated that the incidence of *E. coli* in bro (13.8 %) was higher than in layers (10 %). This difference may be due to the us manual feeders in broiler farms which easily contaminated with bird's manure or it be due to the type of feed used in layer farms which is pelleted feed versus ordinary mashed feed in broiler farms as the hot pelting of feed resulted in reduction of *E. coli* and Gross (1990)). This result agreed with Ayoub (2007) who f that *E. coli* isolated from broiler and layer farms was 21.7 % and 12.5 %, respecti It was found that the total incidence of *E. coli* isolated from litter samples of po farms was 26.9 % (Table, 1). This result was higher than that obtained by Mashho

al. (1987) (25.33 %), Draz et al. (1996) (17.8 %) El- kabany (1997) (7.8 %), Ola-E (1997) (13.8 %) and Lamiaa Mohammed (2004) (6.25 %) while, it was lower tha recorded by Sambyal et al. (1981) (90 %), Rehab El-Zarka (1998) (44 %), Ab Haleem (2000) (72.2 %), Hanson et al. (2002) (36.8 %) and Ayoub (2007) (27. The difference of *E. coli* incidence in litter samples obtained in this study and obtained by the different authors may be due to different litter type used, syste breeding, different states of hygiene inside farms, ventilation systems, age of bird seasons of the year. In addition, the incidence of *E. coli* isolated from litter samp broiler farms (16.3 %) was lower than layer farms (37.5 %). This result disagree Ayoub (2007) who recorded that the incidence of *E. coli* isolated from litter samp broiler farms (39.2 %) was higher than that recorded in layers farms (12.5 %).

The results presented in Table (2) illustrated the effect of season on the isolation of *E. coli* from different environmental samples inside poultry farms. It revealed th incidence of *E. coli* isolated from air samples in winter season (13.8 %) was lowe its incidence in summer (22.5 %). These results agreed with those reported by fakar (1994) who found that the incidence of *E. coli* in winter (25 %) was lower t summer (54.5 %). On the other hand, these results disagreed with the results ob by El-Kabbany (1997) who found that the incidence of *E. coli* in summer (4.2 % lower than in winter (6.2 %) and Ayoub (2007) who found that the incidence of *E.* winter (24.17 %) was higher than in summer season (15.8 %). The higher incide summer season may be attributed to that the coliform bacteria can persist for periods in dry conditions (Draz et al. 1996). In addition, it revealed that the incide *E. coli* isolated from water samples in summer season (23.8 %) was highe isolated in winter season (18.8 %) (Table, 2). These results disagreed with (1994) who found that the incidence of *E. coli* in winter (20.6 %) was higher t summer (5.66 %) and Ayoub (2007) who recorded an incidence of 18.3 % in season and 10.8 % in summer season. Table (2) revealed that the incidence of isolated from feed samples in winter season (8.8 %) was lower than its incide summer (15 %) which agreed with El–Kabbany (1997) who found that the incide *E. coli* in winter (2.1 %) was lower than in summer (3 %) with little seasonal while, they were different from those obtained by Sahar (1994) who record incidence of 15.4 % in winter and 3.8 % in summer season and Ayoub (2007 found that winter incidence (16.7 %) was higher than summer incidence (13.3 %) the results in Table (2) revealed that the rate of *E. coli* isolation from litter samp winter (23.8 %) was lower than in summer (30 %). These results disagreed with (2007) who found that winter incidence (30.8 %) was higher than the summer (25 The data illustrated in Table (3) shows that the total incidence of *Salmonell* isolated from air samples of poultry farms was 9.4% which was higher than obtained by Zahran (1981) (2 %), Sotohy (1989) (0.9 %), Ola Basha (1997) (4. and Rehab El-zarka (2003) (1 %).On contrary, was lower than the results recor Mohammed (2005) (13.8 %) and Orji et al. (2005) (12.5 %). The obtaine presented in Table (3) showed that the percentage of *Salmonella spp.* isolated fr samples of layer farms (12.5 %) was higher than broiler farms (6.3 %) which m due to the hygienic status of the examined farms and these data were lower than obtained by Poppe et al. (1991) (21.6 %), Limawongpranee (1999) (14.3 %) an et al.(2001) (39.3%) while were higher than those obtained by Chambers et al. (4.3 %). Secondly, table (3) shows that the total incidence of *Salmonella spp.* is from water samples of poultry farms was 15.6 % which was higher than those ob by Sotohy (1989) (0.74 %), Poppe et al. (1991) (12.3 %), El-Kabbany (1997) (4

Ola-Basha (1997) (2.73 %), Rehab El-Zarka (2003) (3 %), Lamiaa Mohammed (2 (2.5 %), Mohammed (2005) (3.6 %) and Jafari et al. (2006) (12.5 %). On the hand, this result was lower than that obtained by Sasipreeyajan et al. (1996) (36 The obtained data presented in Table (3) showed that the percentage of *Salmo spp.* isolated from water samples of layer farms (23.8 %) was higher than broiler fa (7.5 %). This recorded result disagreed with Lamiaa Mohammed (2004) who found the incidence of *Salmonella spp.* in layer farms (1 %) was lower than broiler fa (3.75 %). The data illustrated in Table (3) shows that the total incidence of *Salmo spp.* isolated from feed samples of poultry farms was 13.8 % which was lower than obtained by Sasipreeyajan et al. (1996) (28 %) while it was higher than that repo by Barbour et al. (1983) (4.13 %), El–Kabbany (1997) (6.2 %) and Al-Zenki e (2007) (0.7 %). The obtained data recorded in Table (3) showed that the percen of *Salmonella spp.* isolated from feed samples of layer farms (13.8 %) was simil that of broiler farms (13.5 %). This result was higher than those obtained by La Mohammed (2004) who recorded a higher incidence of *Salmonella spp.* isolati boiler farms (3.8 %) than layer farms (1 %).

This variation may be due to the difference between the numbers of exam samples in both studies. The data illustrated in Table (3) shows that the total incid of *Salmonella spp.* isolated from litter samples of poultry farms was 20 %. This r nearly resembled Barbour et al. (1983) (19.15%) and agreed with Hanson et al. (2 who found that *Salmonella* spp. isolated from litter samples ranged between 2 % 25 %. At the same time, it was lower than that recorded by Sasipreeyajan et al. (1 (42 %) and Payne et al. (2006) (50 %) while it was higher than that recorded b kabany (1997) (7.3 %), Lamiaa Mohammed (2004) (5 %) and Al-Zenki et al. (2 (1.5 %).The obtained data presented in Table (3) showed that the percentag *Salmonella spp.* isolated from litter samples of layer farms (25 %) was higher broiler farms (15 %).

The data presented in Table (4) illustrated the effect of season on the isolation rat *Salmonella spp.* from different environmental samples inside poultry farms. Firs revealed that the incidence of *Salmonella spp.* isolated from air samples in w season (8.8 %) was lower than its incidence in summer (10 %). Secondly, it sh that the incidence of *Salmonella spp.* isolated from water samples in winter se (17.5 %) was higher than isolated in summer season (13.8 %). These results ag with El-Kabbany (1997) who found that the incidence of *Salmonella spp.* in winter %) was higher than in summer (1.04 %) while disagreed with Lamiaa Moham (2004) who found that the incidence of *Salmonella spp.* in winter (2.3 %) was l than in summer (3.33 %). Salmonella recovered from water may be attributed to fa contamination (Seligman and Reither 1966).

In addition, table (4) records the incidence of *Salmonella spp.* isolated from samples in winter season (12.5 %) was lower than isolated in summer season (1 These results agreed with El-Kabbany (1997) who found that the incidenc *Salmonella spp.* isolated from feed samples in winter (1.04 %) was lower tha summer (2.1 %). Moreover, Table (4) revealed that the incidence of *Salmonella* isolated from litter samples in winter season (18.8 %) was lower than its inciden summer (21.3 %). This result disagreed with El–Kabbany (1997) who found tha summer incidence (2.1 %) was lower than winter incidence (3.1 %) of *Salmonella s* The results illustrated in Table (5) clarified that the serological identification Salmonella serotypes obtained from litter and feed revealed that all the serotypes *S. enteritidis*. This result agreed with Poppe (1994), Limawongpranee (1999), Ca

al. (2001), Kinde et al. (2004), Al-Zenki et al. (2007), Jafari et al. (2007) and Lee (2007). On the other hand, this result disagreed with Chambers et al (1998 Lamiaa Mohammed (2004). *S. enteritidis* caused an invasive infection in poultr leads to septicemia and subsequent chronic infection of various organs and whe ovary is infected, transmission of the organisms to the contents of the egg could In addition, transmission of *S. enteritidis* through direct contact with infected bird indirect contact with contaminated environmental surfaces are known to be imp factors in the dissemination of *S. enteritidis* in poultry flocks (Gast et al. 1998).

PCR assay was used for detection of *S. enteritidis* in the 6 serologically ide *Salmonella* isolates. PCR detected that all of the examined samples were *S. ente* (100 %). Several epidemiological studies have shown that the incidence *enteritidis* serotype is worldwide and it appears that this serovar has replac *typhimurium* as the commonly foodborne serotype (Dawson, 1988). Photo shov agarose gel analysis stained with ethidium bromide to detect the DNA band of 3 as an indication for the presence of *S. enteritidis* in isolates of litter and feed sa collected from poultry farms. Lane 1 represented an empty lane to serve a negative control; the lanes from 2 to 7 represented the positive samples where at 312 bp were detected. This result agreed with the finding of Eyigor and Carli (and **Allgayer** et al. (2008) who found that 312 bp notifies the presence of *S. ente* DNA.

From the above mentioned results obtained, it can be concluded that there measurable difference in the isolation rate of *E. coli* and *Salmonella* between sa collected from broiler farms and those collected from layer farms which was hig layers than broilers. This difference may be due to the longer periods that birds s layer houses. Also, the results showed a variable rate of isolation of *E. co Salmonella* from air, water, feed and litter samples in poultry farms under investig So the following hygienic measures should be undertaken to avoid spreadi bacterial pathogens in poultry farms as strict hygienic measures should be appl improve the water quality; sanitary control measures should be applied to litter by periodically sound fresh litter, improvement of air quality inside poultry farms may be achieved by hygienic construction of the farms, good ventilation syster avoid overcrowding of birds, the choice of feed should be undertaken with stric also hot pelted feed is preferred to be used than fine mashed feed, and als hygienic conditions of feeders should be considered to avoid contamination o from litter to bird droppings and periodical examination of environmental sa inside the farm to determine the degree of contamination and determine the sou infection with different bacterial pathogens.

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العربـــــى

ة عن مدى تواجد الميكروب القولونى والسالمونيلا فى بيئة بعض مزارع الدواجن فى المناطق الريفية ظة البحيرة

احة، د. ياســـــر نصر حجاج، د. محمد نصير، هانى شتا

ران والأمراض المشتركة – كلية الطب البيطرى – جامعة الاسكندرية

تم تجميع عدد ٤٦٠ عينة من البيئة الخاصة ببعض مزارع الدواجن سواء للتسمين أو البيـاض مـن المناطق الريفية بمحافظة البحيرة وشملت العينات كل من الهواء (١٦٠)، الماء (١٦٠)، العلف (١٦٠) ، ة (١٦٠) في فصلى الشتاء و الصيف على حد سـواء وتـم فحصـها بكتريولوجيـا. أسـفر الفحـص يولوجى عن عزل الميكروب القولوني منْ العيناتِ البيئيةِ منْ المزارعِ المختلفة بنسبةْ ١٩,٥% و كانـت لعزلَ في عيناتِ مزارعِ التسمين (١٦,٩%) أقل من مزارع البياض (٢٢,٢%). وقد سُجّلتْ أعلى نسـبة للميكروب القولوني في عيناتِ الفرشة (٢٦,٩%) تليها عينات المياه (٢١,٣%) ثم الهـواء (١٨,١%) و العلف (١١,٩ %). وُجدَ أيضا أن نسبة عَزلَ الميكروب القولوني كانتَ أعلى في فصل الصـيف (٢٢,٨ نْ الشتاءِ (١٦,٣%). على الجانب الآخر كانت نسبة عزل السالمونيلا منْ العيناتِ البيئيةِ مـنْ المـزارعِ ية هى١٤,٧% و كانت نسبة العَزلَ في عيناتِ مزارعِ التسمين (١٠,٦%) أقل مـن مـزارع البيـاض %). وقد سُجّلتْ أعلى نسبة عَزلَ للسالمونيلا في عيناتِ الفرشة (٢٠%) تليها عينات المياه (١٥,٦%) ف (١٣,٨%) وأخيرا الهواء (٩,٤%). وُجدَ أيضا أن نسبة عزلَ السالمونيلا كانتَ متساوية تقريبا فـى صلين. هذا وقد تم إجراء التصنيف السيرولوجى لعدد ٦ معزولات من السالمونيلا ووجد أن المعزولات من نوع سالمونيلا انتيريتيدس وتم تأكيد نتيجة الفحص باستخدام اختبار تفاعل البلمرة المتسلسل هذا وقد شة النتائج والأهمية الصحية للميكروبات المعزولة من العينات المختلفة.