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Epidemiological Surveillance on Environmental Contaminants in Poultry Farms

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Abstract: A total of 416 environmental samples (litter, water, swabs and air) were collected from commercial poultry farms located in Ismailia and Zagazig Governorates during the period January through July of 2008. These samples were tested by conventional cultural methods and then were confirmed biochemically. The bacterial isolates that were identified included: *Citrobacter spp.*, *E. coli*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Pseudomonas aureuginosa*, *Salmonella sp.*, *Shigella sp.*, *Staphylococcus aureus*, *Streptococcus fecalis* and *Streptococcus pneumoniae*. The suspected colonies for *Salmonella spp.* were cultured onto a selective media (Selenite F broth and S-S agar) for further confirmation. Prevalence and frequencies of the microorganisms were calculated to detect the most predominant microorganisms. Swab samples showed higher prevalence of bacterial isolates (37.7%). Samples collected from closed house system had higher prevalence of bacterial isolates in swab samples (20.5%) as compared to samples from open house system (17.2%). *Citrobacter sp* (8.3%), *Proteus vulgaris* (8.3%) and *Pseudomonas aureuginosa* (16.7%) predominated in litter samples from closed house system. *E. coli* (35.7%) predominated in air samples of closed house system. *Klebsiella oxytoca* (10.0%) predominated in water of open house system. *Salmonella sp* (35%) predominated in swab samples of open house system. *Shigella sp* prevalence was similar between water samples of opened house system (6.0%) and swab samples of closed system (5.9%). *Staphylococcus aureus* (50.0%) predominated in air of closed house system. *Streptococcus pneumoniae* (17.8%) predominated in air samples of open house system. *Streptococcus fecalis* (5.3%) predominated in litter samples of open house system. A total of 266 environmental and non-environmental samples were collected during the period September of 2008 through January of 2009 by the Alabama State Veterinary Diagnostic Laboratory as part of the National poultry improvement plan. These samples were examined using highly selective media for *Salmonella sp*. The positive samples were confirmed biochemically and sero-grouped. The highest prevalence of *Salmonella spp.* was in environmental swabs (38.6%) with special reference to slat swabs (10.2%), fans (8.1%) and sills (6.9%). The highest predominant group of *Salmonella spp.* was C3 (50.4%) followed by group B (24.0%) and group C2 (13.9%).

Key words: *Salmonella*, surveillance, epidemiology, poultry

INTRODUCTION

In order to reduce human illness, epidemiological measures have been established because poultry and poultry products often serve as the vehicles for human salmonellosis (Bean and Griffin, 1990; Persson and Jendteg, 1992), the poultry industry and governmental agencies are focused on eradicating *Salmonella* in live birds and at the processing plant (Hargis *et al.*, 2001), *Salmonella Enterica serovar enteritidis* is the world leading cause of salmonellosis and is often implicated in over 60% of human Salmonellosis in Europe, in the United States it remains the second most common serotype of *Salmonellae* (Patrick *et al.*, 2004). The current world wide epidemic of *Salmonella enteritidis* started in the middle of 1980s (Ward *et al.*, 2000), the (USDA) and Food Safety Inspection Services (FSIS) implemented an "in plant" Hazard Analysis Critical Control Point (HACCP) program to reduce the prevalence of the food borne pathogen contamination in meat and egg. poultry are recognized as important

reservoir of *Salmonella* (Henson, 1997; Lynch *et al.*, 2006), the United States Department of Agriculture implemented to reduce the source(s) of infection. Because food animal are the main reservoir of *Salmonella enteritidis* is mainly poultry often carrying asymptomatic infection, which pass the human pathogen along the food production chain. Especially undercooked or raw eggs and frozen poultry meat represent a high risk for humans. This study was conducted for:

- Conduction of Epidemiological survey to understand the most frequently and persisting pathogenic microorganisms in the broiler's environment.
- Culture, isolation and identification of pathogens from the environmental samples collected from broiler's environment.
- Isolation, identification, sero-grouping of *Salmonella sp.*

MATERIALS AND METHODS

The samples for this study was collected on two sets:

First set

Sampling: A total of 416 environmental samples were collected from two different systems of bird houses (Natural-artificial). The samples were litter samples, (William *et al.*, 1975), Water samples, (Lawrence, 1988), Swabs (Collins *et al.*, 1991) that were collected from walls, floors, feed pads and drinkers, Air samples, (Cruickshank *et al.*, 1975).

| | Closed houses | Open houses | Total |
|--------|---------------|-------------|-------|
| Litter | 28 | 24 | 52 |
| Water | 56 | 48 | 104 |
| Swab | 84 | 72 | 156 |
| Air | 56 | 48 | 104 |

And the processing of the samples was as following

- All of litter, water, swabs and air samples were pre-enriched in 9 ml of Buffered Peptone Water and incubated at 37°C for 18-24 h.
- Inoculate non-selective triple plates of Blood Agar, Mac Conkey agar and Cystein Lactose Electrolyte Deficient Agar (CLED). Streak the plates for isolation using a sterile cotton swab. Incubate the inoculated plates at 37 degree C for 18-24 h.
- The growing colonies were first used for gram staining for identification. Gram Negative Bacilli; (Edward and Ewing, 1982), Gram Positive Cocci, (Cherry *et al.*, 1972; Cruickshank *et al.*, 1975).
- The colonies were then identified biochemically by inoculation onto TSI (Bialy and Scott, 1978; Edward and Ewing, 1982; Fingegold and Martin, 1982), SIM Citrate; (Cruickshank *et al.*, 1980), Indol test (Cruickshank *et al.*, 1980), Urease Utilization test (Cruickshank *et al.*, 1980), MR/VP test (Fingegold and Martin, 1982) Gelatin Liquefaction test (Cruickshank *et al.*, 1980), Potassium Cyanide (Cruickshank *et al.*, 1980) for the gram negative bacteria. Meanwhile the gram positive tested against Catalase test (Cruickshank, *et al.*, 1980), Coagulase test and Deoxyribo-nuclease test (Blair *et al.*, 1967).
- The colonies were suspected to be *Salmonella*. Three to five colonies were inoculated into 9 ml of Selenite F broth. The tubes then incubated at 37 degree C for 18-24 h. A loopful is stricked onto S-S agar and the plates were incubated at 37°C for 18-24 h. The positive result for *Salmonella* appeared as black colonies with hallow zone.

Second set

Sampling: A total of 266 environmental and non-environmental samples were collected by the Alabama State Veterinary Diagnostic Laboratory.

| Nature of Sample | No. |
|-------------------------|-----|
| Litter and Pinch | 44 |
| Environmental Swabs | 154 |
| Non-environmental Swabs | 24 |
| Organs Tissue | 44 |

The enrichment procedure used for *Salmonella* on environmental samples is a tetrathionate enrichment with Delayed Secondary Enrichment (DSE) and the procedures as following, National Poultry Improvement plan and Auxiliary Provisions (Manual for March, 2002). For chick box papers; drag swabs, swabs from chick boxes, fecal material, litter, dust or floor litter surface or nest box; drag swabs as the sample testes accordingly to the following steps:

- Add tetrathionate- iodine enrichment broth to the sample to give a 1:10 (sample to enrichment) ratio. Once it has already added iodine to the tetrathionate in the ratio of 1 ml of iodine to 50 ml of tetrathionate.
- Then incubate the samples at 37°C for 20-24 h.
- After the selective enrichment, inoculate selective plates as BGS and XLT4, streak the plates for isolated colonies. Incubate the plate at 37°C for 20-24 h. once they have incubated; the plates are examined for suspected colonies for *Salmonella*. Inoculate three to five suspect colonies from the plates onto TSI and LIA slants. Also inoculate onto SIM, Citrate, Urea and Malonate and perform the oxidase test. Then incubate the biochemical tubes at 37°C for 20-24 h. Screen colonies by serotyping with *Salmonella* poly O antisera and group specific antisera. The biochemical reactions are checked and if needed, an API strip is set up or a vitek card as a means of further identification and verification of identity as a *Salmonella* sp.
- If the initial selective enrichment is negative for *Salmonella*, the DSE or Delayed secondary enrichment is used.
- DSE involves leaving the original tetrathionate-enriched samples at room temperature for 5-7 days. Transfer 1 ml of the original TT culture into 9 ml of fresh tetrathionate-iodine enrichment broth and then incubated at 37°C for 20-24 h and plate to selective plates as BGS and XLT4. Then the plates are examined, once they have incubated for suspected colonies of *Salmonella*. Inoculate three to five suspected colonies from the plates onto TSI and LIA slants. Also inoculate onto SIM, Citrate, Urea and Malonate and perform the oxidase test. Incubates the biochemical tubes at 37°C for 20-24 h. Screen colonies by serotyping with *Salmonella* poly O antisera and group specific antisera. The biochemical reactions are checked and if needed an API strip is set up or a vitek card as a means of further identification and verification of identity as a *Salmonella* spp.

- All isolates are serogrouped that are identified as *salmonella* sp and are subsequently sent to NVSL for serotyping.

RESULTS AND DISCUSSION

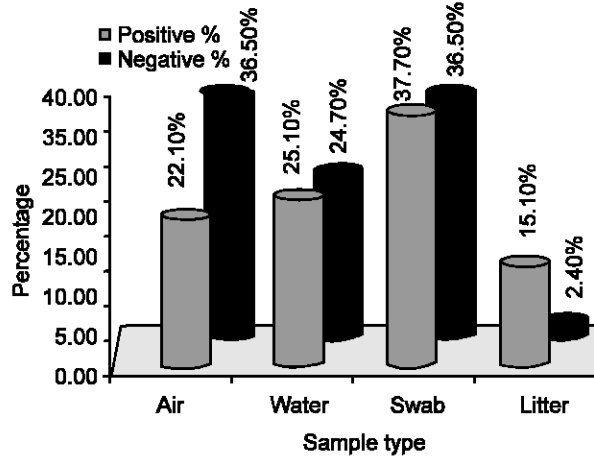


Fig. 1: Prevalence of positive and negative environmental samples collected at the period from January through July of 2008

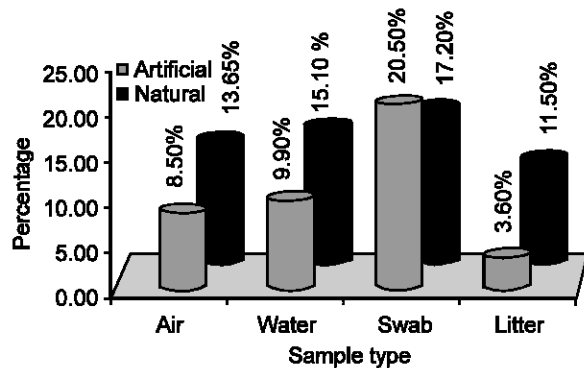


Fig. 2: Prevalence of positive environmental samples compared to the system of housing from which the samples were collected

The Fig. 1 showed that the higher prevalence of bacterial isolation rate in swab samples (125 positive samples out of 156 samples with percentage 37.7% of the total positive samples), this may be attributed to the higher exposure of the building walls and floor to the bird activity more than any other part. Water (83 positive samples out of 104 samples with percentage 25.1% of total positive samples), air (73 positive samples out of 104 samples with percentage 22.1% of total positive samples) and the last is litter, which actually gave the higher positivity rate of bacterial isolation, but the lower in relation to the total positive samples (50 positive samples out of 52 samples with percentage 15.1% of total positive samples).

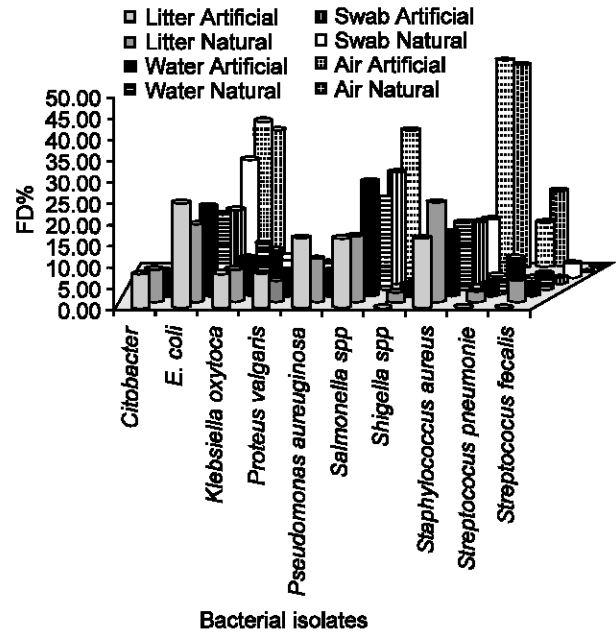


Fig. 3: Frequency distribution percentages of bacterial microorganisms isolated from litter, water, swabs and air of closed and open house system

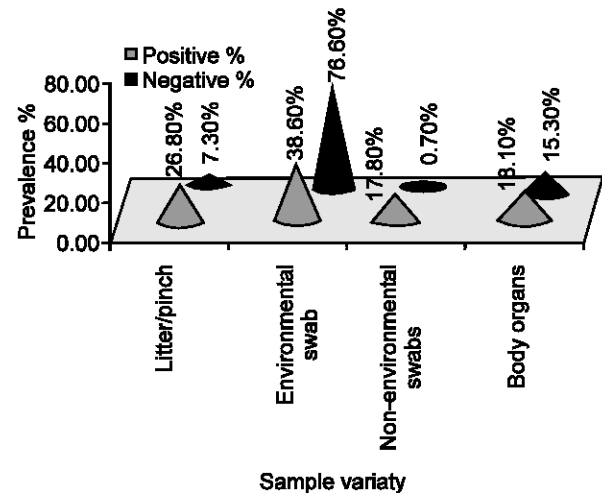


Fig. 4: Prevalence of positive and negative environmental and non-environmental samples collected at the period from September of 2008 through January of 2009

The Fig. 2 shows that open houses system had higher prevalence of bacterial isolates as compared with closed houses system. This was definite in water (15.10%), air (13.65%) and litter (11.50%). On the contrary swab samples of the closed houses system gave higher prevalence of bacterial isolates (20.5%) as compared to open houses system (17.2%).

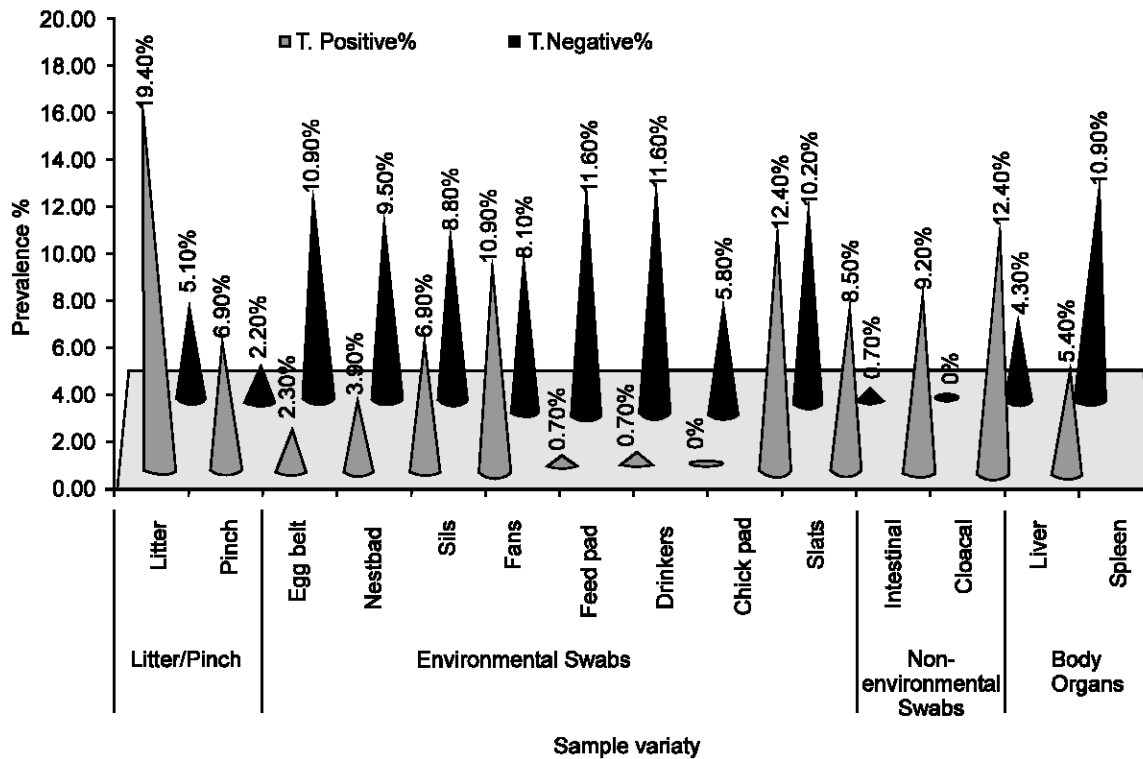


Fig. 5: Prevalence of positive and negative *Salmonella* sp samples collected at the period September, 2008 through January, 2009

The Fig. 3 shows that each bacterial isolate has higher frequency in certain sample type in relation to certain system of housing. *Citrobacter* (8.3%), *Proteus vulgaris* (8.3%) and *Pseudomonas aureuginosa* (16.7%) predominated in litter samples of closed houses system. *E. coli* (35.7%) predominated in air samples of closed houses system. *Klebsiella oxytoca* (10%) predominated in water samples of open houses system. *Salmonella* (35%) predominated in swab samples of open houses system. *Shigella* (6%) (5.9%) was similar in frequency between water samples of open houses system and swab samples of closed house system. *Staphylococcus aureus* (50%) predominated in air samples of closed house system. Finally *Streptococcus pneumoniae* (17.8%) predominated in air samples of open houses system and *Streptococcus fecalis* (5.3%) predominated in litter samples of open houses system.

The Fig. 4 showed that the higher prevalence of *Salmonella* spp. was isolated from Environmental swabs (38.6%), Litter samples (26.8%), organs as liver and spleen (18.1%) and non-environmental swabs (17.8%).

The Fig. 5 showed that despite of being the environmental swabs was the highest in *Salmonella* isolation. It varied in its nature. The highest prevalence

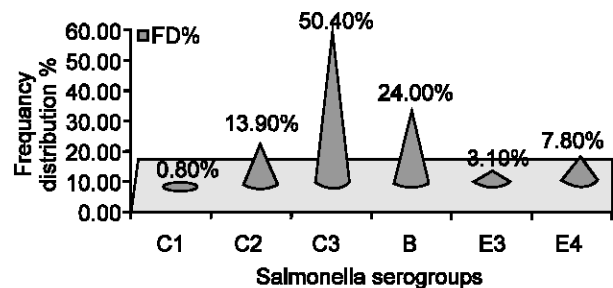


Fig. 6: Frequency distribution of *Salmonella* serogroups isolated from positive samples, were collected at the period September, 2008 to January, 2009

among environmental swabs was detected in slat swabs (10.2%), Fans (8.1%) and Sils (6.9%). Meaning while among the non-environmental swabs, the highest positive prevalence was among cloacal swabs (9.2%) then intestinal swabs (8.5%). Litter was much higher in positive prevalence (19.4%) when compared to that of pinch samples (6.9%). And last but not the least; the body organs where liver was also having higher positive prevalence (12.4%) when compared to that of the spleen (5.4%). Also with calculating the frequencies of *Salmonella* group and represent it as percentage from the total obtained isolates. The highest

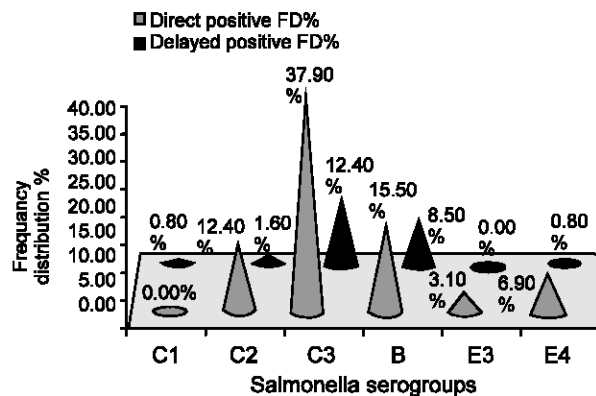


Fig. 7: Frequency distribution of direct and delayed *Salmonella* serogroups isolated from positive samples were collected at the period September, 2008 to January, 2009

predominant group was C3 with percentage of 50.4%; but this percentage was divided into (37.9%) as direct positive and (12.4%) as delayed positive samples, group B with percentage 24.0%; that include (15.5%) as direct positive and (8.5%) as delayed positive, Group C2 with percentage 13.9% including (12.4%) as direct positive and (1.6%) as delayed positive and Group E4 with percentage 7.8%; (6.9%) as direct positive and (0.8%) as delayed positive; as revealed in (Fig. 6, 7).

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