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Salmonella occurrence in chicken eggs and environmental samples and their sero-prevalence in laying hens

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ABSTRACT

A study was carried out to know the status of *Salmonella* occurrence in chicken eggs and poultry environment and to find their sero-prevalence in live birds in a selected poultry farm. A total of 720 samples of egg, feed, water cloacal swab and faeces of layers collected from an organized poultry farm were screened for the presence of *Salmonella* spp, which were isolated from 3.61% of samples. Out of 180 chicken egg, 120 from poultry feed, 120, drinking water; 120, faecal and 180 cloacal swab samples screened, *Salmonella* spp. were isolated from 8 (4.4%), 3 (2.5), 4 (3.3%), 3 (2.5%) and 8 (4.4%), respectively. For detecting *Salmonella* infected birds, the micro agglutination test with antigen of *S*. Typhimurium and *S*. Gallinarum had a sensitivity of 83.3% and 75% respectively. The prevalence of *S.* Typhimurium in chicken eggs and environment and their sero-positivity in live birds indicated that a good correlation existed between the sero-positivity in layer birds and their eggs.

Key words: Chicken eggs, Layer farm, *Salmonella*, Sero-prevalence

Apart from zoonotic importance, *Salmonella* also causes infection in poultry birds. *S.* Gallinarum and *S.* Pullorum are the causative agents of fowl typhoid and pullorum disease in chickens, respectively, causing heavy economic loss to the poultry industry in terms of morbidity and mortality. In addition, feed contaminated with *Salmonella* is often a common source of animal infection and some serovars of *Salmonella* isolated from poultry feed ingredients, particularly from meat and bone meal are important from human health point of view (Maciorowski *et al.* 2004). The present study was designed since the information on occurrence of *Salmonella* in chicken eggs in India and its correlation with their sero-prevalence in live birds is scanty.

MATERIALS AND METHODS

Sample collection: In the present study, 720 samples (chicken egg, feed, drinking water, faeces and cloacal swab) were collected from commercial chicken laying farm. Chicken eggs and poultry feed samples were collected in sterile polythene film pouches, drinking water samples in sterile glass tubes and faecal samples in sterile glass vials. The cloacal samples were collected by sterile cotton swab. All these samples were transported to lab under aseptic conditions and processed immediately for the isolation of *Salmonella*. Apart from above, 60 blood samples were also collected in 15 ml centrifuge tube, transported to lab under aseptic conditions and serum were separated from blood samples by centrifugation and stored at –20°C till analyzed for antibody detection for *S.* Typhimurium and *S.* Gallinarum.

Isolation and identification of Salmonella*:* Isolation of *Salmonella* from chicken eggs (egg shell and contents) was carried as per Yadav *et al.* (2008). For isolation of *Salmonella* from poultry feed, 10 g of sample was inoculated in 90 ml of buffered peptone water (BPW) hygienically under Class II Biosafety cabinet while for isolation of *Salmonella* from drinking water and faecal samples, 1 ml of drinking water and 1g of faecal sample was inoculated with 9 ml of BPW separately. For isolation of *Salmonella* from cloacal swabs, the sterile cotton swab was first dipped in 10 ml of sterile BPW and was used for cloacal swabbing and then re-immersed in the same tube containing BPW. The incubated BPW was then incubated at 37°C for 18 h. One ml pre-enriched culture was added to 10 ml tetrathionate broth (TTB) and incubated at 43°C for 24 h. Similarly, 0.1 ml of the pre-enrichment was added to 10 ml Rappaport-Vassiliadis (RV) broth and incubated at 43°C for 24 h. Samples from TTB and RV enrichment broths were plated on hektoen enteric agar (HEA) and *Salmonella* differential agar (SDA) plates, respectively, and then the rest of the isolation and confirmation was done as per Yadav *et al.* (2008).

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Micro-agglutination test (MAT): For detectopn of antibodies against 'O' antigen of *S.* Typhimurium and *S.* Gallinarum in serum samples, first the antigens for these serovars were prepared. For preparing antigen, *S.* Typhimurium and *S.* Gallinarum culture were inoculated in brain heart infusion (BHI) broth individually and incubated aerobically in shaking incubator for 24 h at 37°C. The culture was boiled for 2 h to destroy the 'H' antigen. After cooling, both the cultures were diluted with half of the amount of 0.5% formalin and kept at room temperature for 24 h. Thereafter the antigens were suspended to get OD 0.6 at 540 nm. The antigens prepared were tested for antigenicity with standard serum.

MAT for somatic 'O' antigen was performed as per Hudson and Hay (1991). Briefly, 2-fold serial dilution of test serum were made in normal saline solution in 96 well microtiter plate up to 11th well in a row, 12th well of the row were kept as negative control having 100 µl NSS only. After serum dilution, 100 µl of desired antigen was added into each well and incubated at 37°C for 24 h. Agglutination was observed by comparing the button formation in negative (NSS) control well and mat formation in positive wells. The titre was recorded equal to the highest dilution of serum yielding visible agglutination i.e. inhibiting the button formation.

RESULTS AND DISCUSSION

The occurrence of *Salmonella* in chicken eggs, poultry feed, drinking water, feaces and cloacal samples was 4.4, 2.5, 3.3, 2.5 and 4.4% respectively. Among the 8 chicken eggs found positive for *Salmonella* spp, 6 (75%) were contaminated on shell surface while 2 eggs (33.3%) were contaminated both in yolk as well as egg shell while no *Salmonella* could be detected in egg albumen. Isolates from egg shell belonged to *S.* Typhimurium (6), *S.* Senfteenberg (1) and *S. Kottbus* (1). The serovars from feed were *S.* Kottbus (1), *S.* Typhimurium (1) and *S.* II (1), from cloacal swab were *S.* Kottbus (1), *S.* Typhimurium (5) and *S.* II (1), from drinking water *Salmonella* serovars belonged to *S.* Kottbus (2), II (2), and faeces were *S.* Kottbus (2), *S.* II (1). Higher incidence of *Salmonella* on egg shell surface than egg contents were reported in earlier studies conducted in and around Chennai city (Krishnamoorthy *et al.* 2003) and in farm market eggs in north Indian states (Yadav *et al.* 2008). The survivability of *Salmonella* on egg shell surface was greatly influenced by the storage temperature (Radkowski 2002) and other contaminating factors which might lead to higher contamination on shell surface as compared to egg contents. Incidence of *Salmonella* in poultry feed, drinking water, faecal and cloacal samples was low, however, their possible role in contamination to chicken eggs cannot be ruled out with the fact that serovars of *Salmonella* isolated from environment samples were similar with the serovars isolated from the chicken eggs.

The detection of *S.* Typhimurium as a predominant serovar in this study indicated egg-borne salmonellosis in India which needs to be controlled for reducing *S.* Typhimurium incidence. Much more interesting findings of this study was that *S.* Enteritidis has not been detected as the main zoonotic serovar from poultry and poultry products. Murugkar *et al*. (2005) also observed pre-dominance of *S.* Typhimurium from cloacal swabs of poultry in north-eastern of India; however, S. Enteritidis was also detected in their study. The main reason for the prevalence of other *Salmonella* serovars instead of *S.* Enteritidis in poultry eggs and poultry environmental samples may be their more adaptability of environmental condition indicating the changing dynamics of *Salmonella* serovars occurrence in this region.

The results of sero-detection indicated that out of 60 serum samples screened, 50 samples were found positive for *S.* Typhimurium whereas 45 samples were found positive for *S.* Gallinarum. Humphrey *et al*. (1989) reported that eggs contaminated with *S.* Enteritidis have been traced back to flocks of laying hens that were culturally or serologically positive for the *Salmonella*. Their research had indicated that approximately 3% of the eggs from S. Enteritidis infected birds would be contaminated with *S.* Enteritidis. Due to the consistency and economy of serum and antigen required to perform MAT, this test is now in common use in most of the country for serotyping of *Salmonella* isolates (Wray and Wray 2000).

Our results indicated that pre-dominance of *S.* Typhimurium in chicken eggs and environment and their sero-positivity in live birds indicated a good correlation between the sero-positivity in live birds and eggs.

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