

ASSESSMENT OF CARRIER STATUS OF *SALMONELLA* PULLORUM AND GALLINARUM INFECTION IN HEALTHY FLOCKS

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Poultry are commonly infected with a wide variety of salmonella serovars and infection is generally subclinical (Wray *et al.*, 1996). Poultry farming is often hampered by reduction in productivity due to infections with large number of bacteria, salmonellosis being the most important causing great economic losses due to mortality and reduction in egg production (Khan *et al.*, 1998). Under the new naming system the *Salmonella* serovar Gallinarum is divided into biovar Gallinarum and Pullorum, which are identified to cause Fowl typhoid and Pullorum disease respectively. While fowl typhoid is a disease of mature birds, pullorum causes mortality of embryos and chicks. Infection with these pathogens are responsible for considerable economic losses in poultry production. The aim of this study was to determine the serological prevalence of these two biovars in apparently healthy domestic chickens and thereby assess the carrier status.

A total of 150 sera samples were collected from apparently healthy chickens from northern parts of Tamil Nadu and stored at -20°C until use, for detection of antibodies by Rapid Serum agglutination test (RST). A total of 222 cloacal swabs were collected aseptically in Ames transport medium for isolation and characterisation.

Standard *Salmonella pullorum* coloured antigen obtained from Institute of Veterinary Preventive Medicine-Ranipet, Tamil Nadu was used in Rapid serum agglutination test (RST) for detection of *Salmonella pullorum* antibodies in the sera samples. The RST was performed as described in OIE Manual (2008). Cloacal swabs of birds were subjected for isolation and identification of *Salmonella Pullorum* and *Salmonella Gallinarum*. The cloacal swabs were inoculated in selenite broth and incubated at 42°C for 24 hours. A loopful of Selenite broth inoculum was streaked onto Mac Conkey agar medium and Brilliant Green agar and incubated at 37°C for 24 hours and the colonies were identified as described by OIE (2008). The isolates were confirmed by bio-chemical tests as described by Quinn *et al.*, (1994).

Further the isolated colonies were also identified by inoculating on to Triple Sugar Iron (TSI) agar slant by streaking and incubation at 37°C for 24 hours.

Antibiogram of the *Salmonella* isolates was carried out by agar disc diffusion method as per Bauer *et al.*, (1966). The antibiotics used were Amikacin (10mcg/disc), Amoxicillin (30mcg/disc), Ciprofloxacin (5mcg/disc), Enrofloxacin (5mcg/disc), Tetracycline (30mcg/disc) and Sulphadiazine (300mcg/disc).

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Of the 150 sera samples screened for the antibodies against *Salmonella* Pullorum by RST, 24 sera samples (16 per cent) had antibodies but serological assessment for *Salmonella* Gallinarum was not conducted as *Salmonella* Pullorum was the only biovar isolated. The seroprevalence observed in this study was in agreement with the finding of Sikder *et al.*, (2005) who also reported 15-20 per cent seroprevalence. To overcome the false positive reactions the test sera were inactivated by heating at 56°C for 30 minutes in a water bath according to OIE Manual (2008).

Out of 222 cloacal swabs, *Salmonella* Pullorum was the only biovar isolated from 6 birds (2.7 per cent) and identification based on biochemical characters. The biovars *Salmonella* Gallinarum and Pullorum were differentiated based on TSI agar slant inoculation and different sugar fermentation tests. The biovar, Gallinarum can ferment galactose and dulcitol but in our study none of the biovars fermented galactose and dulcitol and this indicated the isolates were not biovar Gallinarum. All the isolates fermented glucose and were confirmed as Pullorum. This biovar differentiation concurs with the report of Proux *et al.*, (2002) who differentiated biovar by the use of sugars such as maltose, dulcitol and glucose.

Several authors reported that unlike other *Salmonella* serotypes, Pullorum and Gallinarum are not excreted extensively in faeces (Berchieri *et al.*, 2001 and Proux *et al.*, 2002). Proux *et al.*, (2002) observed that in the absence of clinical signs bacteriological assay of faecal swabs for Pullorum or Gallinarum will not be easy.

The most important observation in this study was the excretion of *Salmonella* Pullorum in healthy flocks which needs to be considered

when flocks are stressed due to other factors as such excretions can be a source of infection in multiage farms. *Salmonella* enterica serovar Pullorum is worldwide a poultry pathogen of considerable economic importance, particularly a developing poultry industry. In addition to the characteristic high mortality rates amongst young chicks, one of the features of *Salmonella* serovar Pullorum infection is that it persists for long periods in convalescent chicks in the absence of clinical disease. This can lead to colonization of the reproductive tract of chickens and at sexual maturity can result in infected progeny through transovarian transmission to eggs. *Salmonella* serovar Pullorum colonized both the ovary and the oviduct of hens and led to 6 per cent of laid eggs being infected by *Salmonella* serovar Pullorum. The colonization of several different sites of the reproductive tract suggests that *Salmonella* serovar Pullorum may employ more than one mechanism of egg infection (Wigley *et al.*, 2001).

Threfall (2002) stated that in developed countries, antimicrobial resistance of zoonotic salmonellosis has been attributed to the injudicious use of antimicrobials in food producing animals including poultry. Under this contention the isolates from this study were subjected to an antibiogram employing the most commonly used antibiotics in poultry flocks which included Amikacin, Amoxycillin, Ciprofloxacin, Enrofloxacin, Tetracycline and Sulphadiazine. All six isolates showed sensitivity to Amikacin (96 per cent) followed by Ciprofloxacin (91 per cent) and Enrofloxacin (88 per cent) but resistant to commonly used antibiotics like Tetracycline, Amoxycillin and Sulphadiazine.

Capita *et al.* (2008) reported that *Salmonella* Pullorum isolates were Multi-Drug Resistant (MDR) to Amoxycillin, Fluoroquinolones, Sulphadiazine and Tetracycline. The observation in this study was almost similar except for the fluoroquinolones which

was found to sensitive to the isolates as their use is possibly is still restricted to poultry practice.

The present study therefore recommends a wide spread screening of chicken flocks to assess the carrier status which should enable to institute effective control measures. The rapid serum agglutination test and culture protocol followed in this study can be adequate measures for assessing the carrier status. In view of the resistance observed in this study, it is to be cautioned that antibiotics in poultry production should be judiciously and strategically chosen and employed, to avoid large scale resistance development.

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