J. Vet. Malaysia (2000) 12(1): 33-36

PREVALENCE AND ISOLATION RATES OF *SALMONELLA GALLINARUM* AND *SALMONELLA PULLORUM* IN CHICKEN IN BANGLADESH

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SUMMARY

A total of 33,204 birds at different age groups were investigated for salmonella infection. The morbidity and mortality rate due to salmonellosis among the birds was 4.90% and 1.83% respectively. The mortality rate was highest (68.53%) in the early age groups (0-3 months). It decreased with age and reduced to 0.25% in the twelve months and above age groups. The high breed were more susceptible compared to the exotic and local breed when mortality rate was found to be 24.00% in Starcross and Lohman brown breeds. The mortality rate due to salmonella infection was 63.43% during the monsoon (June to September), 21.25% in winter (October to January) and 15.32% in summer (February to May). *Salmonella pullorum* and *S. gallinarum* were isolated, identified and partially characterised on the basis of morphological, cultural, biochemical and serological tests. The pathogenicity of the isolated organisms was determined by infecting chicks before clinical sign and post-mortem findings were observed. The brilliant green agar was found to be most suitable compared to other media from where the highest number of organisms were isolated (50%). Liver and bone marrow (50%) were the most suitable samples compared to spleen and heart (40% and 48%).

Keywords: Salmonellosis, chicken, prevalence, isolation, Bangladesh

INTRODUCTION

Fowl typhoid or *Salmonella gallinarum* infection and pullorum disease or S. *pullorum* infection in chicken are the diseases of worldwide significance. The diseases were found to have adverse economic effect in south and central America, the Middle East, the Indian subcontinent and parts of Africa (Silva, 1985; Pomeroy *et al.*, 1991). Continuous increase in the incidence of fowl typhoid and pullorum over the last few years in Bangladesh is posing a great threat to the poultry industry (Amin, 1969; Fehervari, 1994; Hoque *et al.*, 1997).

The present study was under taken to determine the prevalence salmonellosis in Bangladesh, to isolate and identify the causative organisms and to test the pathogenicity of the isolates so that selection of suitable isolates for vaccine production can be made.

MATERIALS AND METHODS

Animals

A total of 33,204 dead and sick chicken of different age, breed and sex from different poultry farms were sent to the Central Disease Investigation Laboratory, Dhaka for routine examination. The birds that were diagnosed with either *Salmonella pullorum* or *Salmonella gallinarum* infection during the period of 1994 to 1995 were grouped into three groups. They were the high breed group, which included breeds such as Starcross, ISA-brown, Hi line, Hi-sex, Lohman, Babcock, Bv-300 and starBrow; the exotic group, which included breeds such as white leghorn and Fyomi and the cross and local group.

Isolation and identification

Suspected samples were inoculated into different non-selective and selective enriched media and broth consisted of nutrient broth, selenite broth, tetrathionate broth, MacConkey agar, thioglycolate broth, tryptose soy broth and semisolid media. The organisms were then sub-cultured onto blood agar, nutrient agar, desoxycholate citrate agar (DCA), brilliant green agar and Salmonella-shigella agar before being incubated at 37°C for 24 h (Cowan and Steel, 1977; Malik, 1988; Carter and Cole, 1990; OIE Diagnostic Manual, 1992). Isolates were further identified by cultural and morphological characteristics (OIE Diagnostic Manual, 1992), routine biochemical characteristics (Carter and Cole, 1990; OIE Diagnostic Manual, 1992) and by agglutination test.

For the agglutination test, a typical colony from nutrient agar and blood agar plate was transferred onto a slide before a drop of polyvalent salmonella O (A-G) antiserum (Welcome Laboratories, UK) was added. The slides were rocked for one min and observed for agglutination. Cultures, which were positive with polyvalent salmonella antiserum were further tested with a group specific sera for group D.

Experimental infection

A total of 20 salmonella free, 6-week old Lohman brown chickens were inoculated intramuscularly with fresh culture inoculum of either *S. pullorum* or *S. gallinarum*. Each bird received 1mL inoculum at the concentration of $5x10^6$ virulent organisms per mL. The birds were observed for clinical signs of salmonellosis for a period of 2 weeks. At the end of 2 weeks, all surviving birds were slaughtered. Post-mortem lesions and bacteriological investigations were carried out according to the methods described earlier (Hofstad, 1978; Carter and Cole, 1990; OIE Diagnostic Manual, 1992).

RESULTS

Prevalence of naturally occurring salmonellosis

Table 1 shows the prevalence of salmonellosis in Bangladesh. Out of 33,204 chickens examined, 1,628 (4.9%) were sick while 607 (1.8%) were dead. The mortality rate was highest (68.5%) at the younger age between 0-3 months old and was found to be lowest after one year of age. The high breed group was most susceptible with highest (24.4%) mortality in Starcross and Lohman brown breeds. Mortality was low in the White leghorn, Fyomi and other cross and local breeds. Fig. 1 reveals that highest mortality (63.5%) occurred during the monsoon (June to September), winter (October to January) recorded moderate mortality while summer (February to May) recorded the least mortality rate (15.3%).

Salmonella isolation rates in different media

In this study, a total of 100 *S. pullorum* and *S. gallinarum* were isolated and identified with 70 isolates were identified as *S. pullorum* and 30 isolates were identified as *S. gallinarum*. The rate of salmonella isolation was highest from liver (50%) and bone marrow (50%) compared to spleen (40%) and heart (48%). Lowest isolation was from the ovary (Table 2).

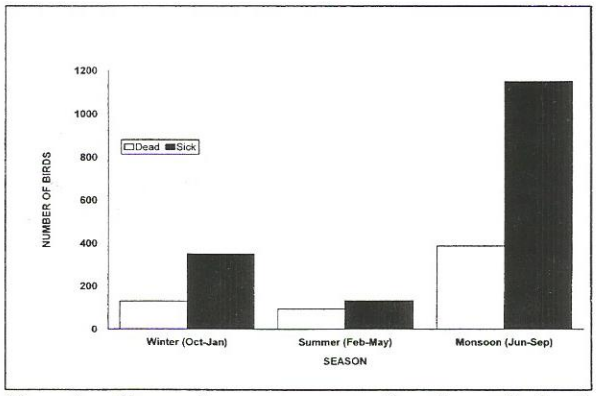

Fig. 1. Seasonal occurrence of salmonellosis in Bangladesh

Table 1. Incidence of salmonellosis in chicken of different age and breed

Breed	Total No.	0-3 mth A	0-3 mth B	4-7 mth A	4-7 mth B	8-11 mth A	8-11 mth B	12 above A	12 above B	Total A	Total B	Sick % of sick	Sick % of pop.	Dead % of sick	Dead % of pop
Star Cross	8835	100	94	72	25	16	7	63	22	251	148	15.4	0.8	24.2	0.5
ISA-Brown	5361	104	62	257	45	17	4	-	-	378	111	23.2	1.1	18.3	0.3
Hi-Line	4900	-	-	25	15	7	2	2	2	34	19	2.1	0.1	3.1	0.1
Hi-sex	5912	88	63	16	10	32	20	-	-	136	93	0.1	0.4	15.3	0.3
Lohman	2618	162	142	39	2	-	-	-	-	201	144	12.3	0.6	23.7	0.4
Bab Cock	350	-	-	-	-	2	1	-	-	2	1	0.1	6.0	0.2	3.0
Bv300	1050	-	-	500	36	-	-	-	-	500	36	30.7	1.5	5.9	0.1
Star Bro	1588	75	36	-	-	-	-	-	-	75	36	4.6	0.2	5.9	0.1
WLH	790	11	3	-	-	-	-	-	-	11	3	0.7	0.03	0.5	9.0
Fyomi	450	6	6	-	-	-	-	-	-	6	6	0.4	0.01	1.0	0.1
Cross	950	14	6	-	-	-	-	-	-	14	6	0.9	0.04	1.0	0.1
Local	400	20	4	-	-	-	-	-	-	20	4	1.2	0.1	0.7	0.1
TOTAL	33204	580	416	909	133	74	34	65	24	1628	607	4.9		1.83	
%		35.6	68.5	55.8	21.9	4.6	5.6	4.0	0.25						

A = sick bird; B =dead bird; pop = population

Table 2. Frequency of isolation of *Salmonella gallinarum* and *S. pullorum* from different birds using different media

Specimen	No of sample	No. of isolation				
		MacConkey agar	Blood agar	Brilliant green agar	Desoxycholate agar	Salmonella-shigella agar
Liver	200	75 (37.5%)	60 (30%)	100 (50%)	80 (40%)	75 (37.5%)
Spleen	200	60 (30%)	55 (27.5%)	80 (40%)	60 (30%)	55 (27.5%)
Ovary	80	15 (18.8%)	10 (12.5%)	25 (31.3%)	15 (18.8%)	12 (15%)
Heart	200	75 (37.5%)	60 (30%)	96 (48%)	76 (38%)	70 (35%)
Bone marrow	100	40 (40%)	30 (30%)	50 (50%)	40 (40%)	38 (38%)
Caecal junc.	200	50 (25%)	40 (20%)	74 (37%)	64 (32%)	60 (30%)
TOTAL	980	315 (32%)	255 (23%)	425 (43.4%)	335 (34.2%)	310 (31.6%)

Brilliant green agar media (43.4%) was the most reliable media to support the growth of salmonella followed by the desoxyeholate citrate agar (34.2%), MacConkey agar (32.1%) and Salmonella-shigella agar (31.6%).

Pathological changes following experimental infection

Lesions typical to salmonellosis included enlarged, bronze-coloured liver, some with white spot necrotic foci. The heart and spleen were enlarged and haemorrhagic while the kidneys were enlarged with white band of uric acid deposition, forming a thread beneath the kidneys. Other pathological changes included inflammation in the intestines and haemorrhagic cloaca filled with white faeces.

DISCUSSION

The clinical signs, post-mortem lesions and cultural biochemical characteristics of salmonella and salmonellosis observed in this study were similar to the earlier findings (Hofstad, 1978; Carter and Cole, 1990; Kaura *et al.*, 1990; OIE Diagnostic Manual 1992). The haemorrhage with white necrotic foci in the liver was commonly observed in this study whereas Kaura *et al.* (1990) reported the bronze-coloured liver was a common and important observation.

This study revealed that the most suitable samples for isolation of salmonella were liver and bone marrow while brilliant green agar was the most suitable agar. The results are in agreement with the findings of Ghosh and Panda (1988), who also isolated highest number of salmonella from liver samples inoculated into brilliant green agar. However, contrary to our result, they failed to grow any salmonella on the MacConkey agar. Generally, the rate of salmonella isolated from the liver samples in this study was less (50%) compared to the 100% rate reported by Ghosh and Panda (1988). Waltman *et al.* (1991) successfully isolated 43% salmonella using the brilliant green agar as compared to 50% isolation rate in the brilliant green agar in this study. Smith (1956) reported that salmonella were mostly present in the intestinal organs while bone morrow was also identified as a reliable source for the isolation of the organisms.

The birds, which were sent to the laboratory were sometimes decomposed or in latent stage of infection. Thus, salmonella isolation was not possible even with antibiotic treatment. However, salmonella was successfully isolated in most cases when the birds were in stressed condition due to heavy rain, high temperature or draught. Similar observation on the effect of drought and stressful condition on the rate of salmonella isolation was reported by Smith(1956). Kaura *et al.* (1990) reported that stress due to extremely hot or cold weather or handling of birds could aggravate the onset of clinical disease and mortality in flock, as observed in our study.

It was noted that the high breed was more susceptible than exotic and local. Aire and Ojo (1974) reported that local Nigerian cockerels were more resistant to infection by *S. gallinarum* than White Leghord cockerels. This might be due to genetic or climatic factors. Moreover, local chickens have been observed to survive better than exotic under semi-intensive or free range system because they more adapted to harsh temperature, high humidity, poor nutrition and housing common in Bangladesh. It was also noted that highest number of death occurred during 0-3 months of age compared to older age because chicks are more susceptible during that age.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Nazir Ahmed, Director General, Department of Livestock Services and Dr. Afzal Hossian Miah of CDIL and Dr. Fazlur Rahman, CSO of AHRD and Mr. Amal Kumar Shah, senior technician of AHRD for their sincere co-operation throughout the working period. We also gratefully acknowledge the financial support from IFAD and DANIDA. Thanks are also due to Mr. Hans and Mr. Dolburgh for their co-operation and to Tamas Fehervari, the avian pathologist and FAO consultant for the technical assistance.

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RINGKASAN

KADAR PREVALENS DAN PENGASINGAN SALMONELLA GALLINARUM *DAN* SALMONELLA PULLORUM *PADA AYAM DI BANGLADESH*

Siasatan mengenai jangkitan salmonella telah dijalankan terhadap 33,024 ekor ayam dari pelbagi peringkat umur. Kadar kemorbidan dan kemortalan akibat salmonelosis pada ayam adalah masing-masing 4.90% dan 1.83%. Kadar kemortalan adalah tinggi pada kumpulan berumur diantara 0-3 bulan (66.53%). Ini berkurangan mengikut peningkatan umur dan menjadi 0.25% pada ayam yang berumur melebihi 12 bulan. Kerentanan adalah lebih tinggi pada ayam baka tinggi berbanding dengan baka yang lain. Kadar kemortalan akibat jangkitan salmonella adalah 63.43% semasa monsun (Jun-September), 21.25% semasa musim sejuk (Oktober-Januari) dan 15.32% ketika musim panas (Februari-Mei). Salmonella pullorum *dan* S. gallinarum *diasingkan, dikenalpasti dan dicirikan secara separa berdasarkan ujian morfologi, kultur, biokimia dan serologi. Kepatogenan organisme yang diasingkan ditentukan dengan mengjangkiti sebelum petanda klinikal dan post mortem kelihatan. Agar hijau geliga di dapati sebagai yang paling sesuai berbanding media lain dimana jumlah organisme yang terbanyak diasingkan (50%). Hepar dan sum-sum tulang merupakan sampel terbaik berbanding dengan limpa dan jantung (40% dan 48%).*