J. Vet. Malaysia (2000) 12(1): 41-43

SHORT COMMUNICATION

SELECTION OF SUITABLE SALMONELLA ISOLATE FOR VACCINE PRODUCTION IN BANGLADESH

M.M. Hoque[1], H.R. Biswas[1], K.M. Kamaruddin[2] and K.K. Pandit[2]

[1]Animal Health Research Division,
Bangladesh Livestock Research Institute,
Savar, Dhaka-1341, Bangladesh
[2]Bangaldesh Agricultural Research Councii,
Dhaka, Bangladesh

SUMMARY

The pathogenicity of two Bangladeshi isolates of salmonella; *Salmonella pullorum* (strain 9) and *S. gallinarum* (strain 10) was studied and compared to a reference *S. gallinarum* strain 9R SG in day old chicks. The incubation period of the disease ranged from 1-5 days. Following intraperitoneal injection, the mortality rate was 100% for strain 9 whereas strains 10 and 9R produced no mortality. Following oral administration, the mortality rate was 50% for strain 9 whereas no death was observed for the other two strains. All affected chicks died within 24-48 h. The clinical signs, gross pathology, bacteriological examinations and drug sensitivity tests were typical of infection caused by *S. pullorum* and *S. gallinarum*. *S. pullorum* was re-isolated from liver, heart blood and bone marrow. The strain 9 was found pathogenic for day-old chicks and was not considered to be a vaccine strain. Strain 10 was found to be as non-pathogenic as the reference strain 9R SG and was selected to be a possible candidate for vaccine production.

Keywords: *Salmonella pullorum, S. gallinarum,* vaccine, Bangladesh

INTRODUCTION

Salmonella pullorum and *S. gallinarum* are the causative bacteria of pullorum and fowl typhoid in chicken respectively. Fowl typhoid is an acute or chronic disease with high mortality rate whereas pullorum usually appears as an acute systemic form in chicks and poults but often as localised and chronic form in adults. Both diseases, however, produce carrier birds, which act as the principal sources of further propagation of the two diseases. Both diseases are transmitted through eggs (Pomeroy, 1994). Domestic poultry constitute the largest single reservoir of salmonella existing in the nature thus, salmonella is the most frequently reported contamination from poultry and poultry products (Barrow, 1993).

Amin et al. (1969), Rahman et al. (1976) and Sarkar (1976) have reported on salmonellosis in poultry in Bangladesh. In recent years, the disease has gained great economic importance in Bangladesh with the incidence rate of >10% and mortality rate to be 40-50% due to the mass poultry production in this country, particularly broilers. Moreover, the disease was found to reduce egg production for up to 20-30% and hatchability for up to 20-30% (Fehervari, 1994; Hoque *et al.* 1997). Heavy loses occur not only in broiler flocks but also in laying birds due to morbidity, mortality, reduced production and poor chick quality. The mortality rate from negligible to 10-30% or higher in severe outbreaks was also reported in laying birds (Kaura *et al.*, 1990; Williams *et al.*, 1990; Hoque *et al.*, 1997).

Incorporation of antibiotics in poultry feed can prevent mortality and reduce incidence of the disease (Pomeroy, 1984). However, in the breeding flocks this practice may lead to development of carriers. Thus, some government poultry farms in Bangladesh are using the live 9R commercial vaccine, which is imported, costly and giving insignificant protection level (Central Poultry Farm, Dhaka, 1995). Since many countries are now finding good results in controlling fowl typhoid through vaccination (Barrow, 1993), vaccination is considered to be the most appropriate method of controlling salmonellosis in breeding flock. The aim of this study was to select a local vaccine strain of salmonella by comparing the bacteriological, biochemical and serological characteristics of the local isolates with that of a vaccine strain contained in a commercial vaccine.

MATERIALS AND METHODS

The test organisms were isolated from either dead or infected birds with salmonellosis at the Central Disease Investigation Laboratories, Dhaka. A strain each of *S. pullorum* and *S. gallinarum* was selected as vaccine candidates on the basis of their bacteriological, biochemical and serological characteristics. *S. gallinarum* 9R SG strain, which contained in a live commercial vaccine (Intervet; The Netherland) was selected for comparison with the local isolates of salmonella. The test organisms were designated as strain 9 *(S. pullorum)* and strain 10 *(S. gallinarum)*.

Seventy day-old white leghorn chicks, found to be free from salmonella infection was obtained from a flock with no history of salmonella. There was also no history of the use of antibiotics in the feed. The chicks were divided into 7 groups; two groups for each strain to be inoculated either intraperitoneally (i.p.) or per oral (p.o.). The last A group was the uninfected negative control group.

The organisms were grown on nutrient agar for 12-24 h before they were harvested, washed and suspended in sterile normal saline. For oral administration, each bird was inoculated with $5x10^7$ of live bacteria/ mL broth containing 300 mg preservative consisted of 40% chalk, 43% light kaolin and 17% Magnesium trisilicate (OIE Diagnostic Manual, 1992). For the intraperitoneal inoculation, $5x10^6$ live bacteria/mL sterile normal saline was used. The number of bacteria was determined using either the MacFarland scale no 2 or the plate counting method (Cappuccino and Sherman, 1987). The control birds were inoculated with sterile normal saline.

All birds were examined regularly for up to 14 days for any signs of the disease. Dead birds were subjected to routine post-mortem examination for gross pathological changes. The liver, heart blood and bone marrow samples were cultured in salmonella media. Isolation and identification were done according to Carter and Cole (1990) and OIE Diagnostic Manual (1992). Blood samples were collected from live chicks for serological investigation and full blood slide agglutination test. Samples found positive were submitted to a tube agglutination test to find out the end titre (OIE Diagnostic Manual, 1992). The rough and smooth strain of salmonella was determined according to the OIE Diagnostic Manual (1992.

The three strains were tested against various antibiotics and sulphur drugs including chloramphenicol 10μg, streptomycin 10μg, tetracycline 10μg, neomycin 10μg, ampicillin 10μg, furazolidane 15μg, compound sulphonamide 50μg and co-trimoxazole 25μg. The multidisks were supplied by Oxoid Ltd., England.

RESULTS

The incubation period for all strains was found to range between 1-5 days after inoculation. The mortality rate was 100% for test strain 9 when inoculated intraperitoneally but strains 10 and reference strain SG 9R produced no mortality. Following oral administration, strain 9 produced 50% mortality but no death with the other strains. The control group showed no mortality. The birds inoculated with strain 9 showed stunted growth, drowsiness, ruffled feather, crowding around the light with their head bent. There was white diarrhoea and the vent was found wet with faeces. The birds also showed the signs of dullness, depression and loss of appetite. Lesions typical to salmonellosis were recorded.

Bacteriological re-isolation following challenge with strain 9 either orally or i.p revealed 100% re-isolation that were confirmed as *S. pullorum*. In contrast, the bacteria failed to be re-isolated following oral or i.p challenge with strains 10 and 9R SG.

Following oral challenge with strain 9, a significant antibody titre was found in 100% chicks. Only 50% of the chicks administered either orally of i.p had antibody titre. The antibody titre was found in 50% and 40% of the chicks administered orally and i.p respectively with strain 9R SG.

Strain 9 was resistant to streptomycin, sulphonamide and co-trimoxazole but sensitive to chloramphenical, tetracyclin, ampicillin and furazolidone (Table 3). Strain 10 was sensitive to chloramphenical, streptomycin, tetracycline, neomycin, ampiciflin and furazolidone but resistance to sulphonamide and co-trimoxazole. The reference strain (9R SG) was sensitive to all drugs.

Strains 10 and 9R SG were characterised as rough strains while strain 9 as smooth strain.

DISCUSSION

To select a local vaccine strain of Bangladeshi isolates of salmonella, selected isolates were tested and compared for their pathogenicity, biochemical, serological and growth characteristics with a reference 9R SG vaccine strain. The reference strain 9R SG vaccine produced mild symptoms when applied per oral (p.o) with no mortality. When the same strain was applied intraperitoneally (i.p), no mortality was noted but antibodies against pullorum and fowl typhoid were produced. This is in agreement with Smith (1956) and Barrow (1992). The local strain 9, when administered either orally or intraperitoneally, was pathogenic for day old chicks when all the chicks died within 24 h. The antibody titre following challenge with strain 9 was very strong.

Strain 10, when compared with the other two isolates (9 and 9R), produced mild symptoms with no mortality. Antibody titres were found in 40-50% of the chichs, which varied from 1:16 to 1:64. It was also found that the strain 10 was rough, indicating a mild strain with respect to ability to cause disease.

From the above findings, it was revealed that strain 10 is rough and non-pathogenic and may be considered as a candidate of vaccine against fowl typhoid or pullorum disease. Since strain 9 is smooth and very pathogenic, it might be a candidate for production of a killed vaccine

REFERENCES

Amin.M.M., Chowdhury, T.I.F. and Choudhury, M.U.A. (1969). Isolation of Salmonella from wild birds, rodents and insects. *Pakistan J. Vet. Sc.* **3**:41-47.

Barrow, A.P. (1993). Salmonella control: past, present and future. Xth World Poultry Association Congress, Sydney. pp127-137.

Cappuccino, G.J. and Sherman, N. (1987). *In:* Microbiology: A Laboratory Manual, 2nd ed. The Bengamin Publishing Co.

Carter, C. and Cole, J.R. (1990). *In:* Diagnostic Procedures in Veterinary Bacteriology and Mycology. 5th ed. Academic Press Inc., San Diego, California.

Central Poultry Farm (1995). Personal communication

Fehervari, T. (1994). Small-holder Livestock Development Project, Bangladesh, Animal Health Component, Second Mission Report, Dhaka, Bangladesh.

Hoque, M.M., Biswas, H.R. and Rahman, L.M. (1997). Isolation, identification and production of *Salmonella pullorum* coloured antigen in Bangladesh for the rapid whole blood test. *Asian Australasian J. Anim. Sc.* **10**: 141-146

Kaura, Y.K., Singh, J, Kaushil, R.K., Kulsherestha, R.C. and Chaturvedi, G.C. (1990). Salmonellosis in India. *Indian J. Anim. Sci.* **60**:127-130.

Office International Des Epizootic (1992). Manual of Standards for Diagnostics Tests and Vaccines. 2nd ed. Paris. France.

Pomeroy, B.S. (1994). Fowl typhoid. *In:* Diseases in Poultry. 8th ed., Hofsted M.S., Barnes, H.J., Calnek, B.W., Reid, W.M. and Yoder, H.W. (eds). Iowa State University Press. Ames. Iowa. pp.79-91.

Rahman, M.M., Chowdhury, T.I.M.F., Rahman, M.M. and Hossain W. I. M.A. (1979). Surveillance of salmonella and escherichia organisms in poultry feed. *Bangladesh Vet. J* **13**: 59-62.

William, J.E., Mallinson, E.T. and Snoeyenbos, G.H. (1990). *In:* Salmonellosis and Arizonosis: Isolation and Identification of Avian Pathogens. 2nd ed. The American Association of Avian Pathologists. Creative Printing Co. Inc., New York.

RINGKASAN

PEMILIHAN ISOLAT SALMONELLA SESUAI UNTUK PENGHASILAN VAKSIN DI BANGLADESH

Kajian kepatogenan dua isolat salmonella Bangladesh: Salmonella pullorum *(strain 9) dan* S. gallinarum *(strain 16) dan dibandingkan dengan rujukan strain 9R SG pada anak ayam berumur satu hari. Tempoh eraman penyakit berjulat di antara 1-5 hari. Selepas suntikan intraperitoneum dan pemberian oral, kadar kemortalan adalah masing-masing 100% dan 50% bagi strain 9 manakala tiada kematian berlaku pada dua strain yang lain. Kesemua ayam terjangkit mati dalam tempoh 24-48 jam. Petanda klinikal, patologi matakasar, ujian bakteriologi dan kepekaan drug adalah tipikal dengan jangkitan oleh* S. pullorum *dan* S. gallinarum. Salmonella pullorum *diasingkan semula daripada hati, darah jantung dan sum-sum tulang. Strain 9 didapati sebagai berpatogen bagi anak ayam berumur sehari dan tidak boleh digunakan sebagai strain vaksin. Strain 10 didapati sebagai bukan berpatogen sepertimana strain rujukan 9R Sg dan dipilih sebagai calon penghasilan vaksin.*