IV.

RESULTS

Present research work envisaged (1) Collection of data on prevalence of *Pasteurella multocida* infection in poultry based on PM reports from the Department of Pathology, Veterinary College, Hebbal, (2) Preparation, standardization of biofilm antigen of *Pasteurella multocida* organisms and experimental evaluation of immunity in Giriraja layer birds, (3) Comparison of immunity conferred by broth vaccine and commercial vaccine with biofilm vaccine and (4) An attempt was made to assess seroprevalence of Fowl Cholera in different poultry farm around Bangalore and Shimoga.

4.1 Prevalence of Fowl Cholera in different parts of Karnataka based on Postmortem Record between 1994-2004.

The data on 40,700 birds of various variety / breed in different localities, which died during the period 1994-2004, were included for analysis. The available information about variety / breed and location from postmortem (PM) findings was categorized (Table-1 & 2 and Fig.1).

The postmortem records available in Department of Pathology, Veterinary College, Bangalore were screened from 1994-2004. A total of 40,700 birds from different parts of the Karnataka were available. Apart from Pasteurella/Fowl Cholera other disease commonly recognized were Coccidiosis, Salmonellosis, Colibacillosis, IBD, Hepatitis, Arthritis and Fatty-liver-kidney syndrome.

The distribution of these disease conditions over the 11 years indicated that mortalities ranged from 32.56 per cent for Cocidiosis, 14.45 per cent for Pasteurellosis, 14.23 per cent Colibacillosis, 11.67 per cent for FLKS, 8.25 per cent for Salmonellosis,
6.83 per cent for miscellaneous, 6.61 per cent for IBD, 3.76 per cent for Hepatitis and 1.65 per cent for Arthritis (Table-1).

Out of 40,700 birds subjected to PM during 1994-2004, 7000 belonged to Kolar, 4000 to Mysore, 2000 to Tumkur, 8700 to Bangalore, 1000 to Hassan, 5000 to Bellary, 6100 to Shimoga, 3600 to Chitradurga, 2300 to Davanagere and 1000 to Mandya. Highest mortality percentage was observed in Bangalore district and lowest percentage in Hassan and Mandya (Table-1).

In Kolar district Arthritis was recorded in 80 birds (0.20%), Salmonellosis in 120 (0.29%), Colibacillosis in 200 (0.49%), hepatitis in 460 (1.13%), Pasteurellosis in 540 (1.33%), Coccidiosis in 600 (1.47%), miscellaneous in 2000 (4.91%) and FLKS in 3000 (7.37%) (Table-1).

In Tumkur district miscellaneous diseases were observed in 20 birds (0.05%), Salmonellosis in 50 (0.12%), IBD in 150 birds (0.37%). Colibacillosis in 220 (0.54%), FLKS in 300 (0.74%), Pasteurellosis in 360 (0.88%), hepatitis in 400 (0.98%), Coccidiosis in 500 (1.23%). Highest prevalence was Coccidiosis (1.23%) and there was no record of arthritis (Table-1).

In Bangalore district, arthritis condition was observed in 100 birds (0.25%), miscellaneous in 200 (0.49%), IBD in 500 (1.23%), Salmonellosis in 1000 (2.46%), Pasteurellosis in 1720 (4.23%), Colibacillosis in 2100 (5.16%) and Coccidiosis in 3080 (7.57%). Highest prevalence was in Coccidiosis (7.57%) and however there was no record of Hepatitis and FLKS.
In Hassan district, FLKS was seen in 50 birds (0.12%), Arthritis in 50 (0.12%), miscellaneous in 60 (0.15%), IBD in 90 (0.22%), Pasteurellosis in 100 (0.25%), Colibacillosis in 110 (0.27%), Salmonellosis in 220 (0.54%), and Coccidiosis in 320 (0.79%). Highest prevalence was in Coccidiosis (0.79%), however hepatitis was not recorded.

In Bellary district, Arthritis was seen in 100 (0.25%), hepatitis in 100 (0.25%), Colibacillosis in 150 (0.37%), FLKS in 200 (0.49%), Pasteurellosis in 300 (0.74%), Salmonellosis in 600 (1.47%), IBD in 750 (1.84%), and Coccidiosis in 2800 (6.88%). Highest prevalence was in Coccidiosis (6.88%) and lowest prevalence was in miscellaneous.

In Shimoga district, Arthritis and miscellaneous disease was recorded in 100 birds (0.25%), hepatitis in 120 (0.29%), FLKS in 350 (0.85%), IBD in 600 (1.47%), Pasteurellosis in 680 (1.67%), Salmonellosis in 850 (2.90%), Colibacillosis in 1200 (2.95%) and Coccidiosis in 2100 (5.16%). Highest prevalence was observed in Coccidiosis (5.16%) and lowest in Arthritis (0.25%).

In Chitradurga district, Arthritis and miscellaneous diseases were recorded in 100 birds (0.25%), hepatitis and IBD in 200 (0.49%), Salmonellosis and Colibacillosis in 250 (0.61%), FLKS in 600 (1.47%), Pasteurellosis in 900 (2.21%) and Coccidiosis in 1000 (2.46%). Highest prevalence was in Coccidiosis (2.46%) and lowest was in arthritis (0.25%).
In Davanagere district, Pasteurellosis was recorded in 80 birds (0.20%), Salmonellosis was in 170 (0.42%), miscellaneous in 300 (0.74%), Coccidiosis in 850 (2.09%) and Colibacillosis in 900 (2.21%). Highest prevalence was recorded in Colibacillosis (2.21%) and lowest was in Pasteurellosis (0.20%).

In Mandya district, 90 (0.22%) birds had Arthritis, 200 (0.49%) birds had IBD, and Salmonellosis, 110 (0.27%) had Colibacillosis, total 600 (1.47%) birds had Coccidiosis, hepatitis, FLKS. Highest prevalence was recorded in Coccidiosis, hepatitis and FLKS, lowest prevalence was recorded in Pasteurellosis.

4.2 Prevalence of Pasteurellosis in different Variety / Breed of Birds

Out of 40,700 birds subjected to PM, 5880 had Pasteurellosis (14.20%), which included Cobb, Hubex, BV and Giriraja variety birds (Table-2 and Fig. 3).

Of these 5880 cases of Pasteurellosis 2200 (37.41%) were Cobb, 1400 (23.80%) were BV, 1270 (21.59%) were Giriraja and 1010 (17.17%) were Hubex. Highest prevalence of Pasteurellosis was seen in Cobb (37.41%) and lowest prevalence was in Hubex (17.17%) variety (Fig. 3).

In Kolar district, out of 540 Pasteurella infected birds identified, 100 (7.49) each belonged to Hubex (Fig. 7 & 5) and BV, 140 (8.87) were Giriraja (Fig. 6) and 200 (10.62) were Cobb (Table-3 & Fig. 4).
In Mysore district, of 1200 Pasteurella infected birds identified, 200 (10.62) belonged to Giriraja (Fig. 6), 300 (13.05) each were Hubex and B.V. (Fig. 7) and 400 (15.11) to Cobb (Table-3 & Fig. 4).

In Tumkur district, out of 360 birds subjected to PM 45 (5.01) each belonged to BV and Giriraja (Fig. 5 & 6), 90 belonged to Cobb (Fig. 4) and 180 (10.98) Hubex (Table-3 & Fig. 7).

In Bangalore district, out of 1720 birds subjected to PM 40 (4.73) Hubex (Fig. 7), 150 (9.99) Giriraja (Fig. 6), 550 (17.80) BV (Fig. 5) and 980 (24.09) Cobb (Table-3 & Fig. 4).

In Hassan district, out of 100 birds subjected to PM, 100 (7.49) belonged to Giriraja (Table-3 & Fig. 6).

In Bellary district, out of 300 birds subjected to PM 20 (3.34) belonged to Cobb (Fig. 4), 80 (6.69) BV (Fig. 5), 90 (7.10) Hubex (Fig. 7) and 110 (7.86) Giriraja (Table-3 & Fig. 6).

In Shimoga district, out of 680 birds subjected to PM 100 (15.11) belonged to Giriraja (Fig. 6), 140 (8.87) to Hubex (Fig. 7), 200 (10.62) to BV (Fig. 5), and 240 (11.65) to Cobb (Table-3 Fig. 4).

In Chitradurga district, out of 900 birds subjected to PM, 100 (7.49) belonged to BV (Fig. 5), 130 (8.55) to Hubex (Fig. 7), 270 (12.37) to Cobb (Fig. 4) and 400 (43.21) to Giriraja (Table-3 & Fig. 6).

In Davanagere district, out of 80 birds subjected to PM 25 (3.77) belong to BV (Fig. 5), 25 (3.73) to Giriraja (Fig. 6) and 30 (4.09) to Hubex (Table-3 & Fig. 7).

In Mandya district, prevalence of Pasterullosis was not recorded (Table-3).
4.3 Experimental Evaluation of Immunity Engendered in Layer birds of Giriraja and Comparison of Immunity Conferred by Biofilm, Broth and Commercial Vaccines

Pre-immunization sera of all the birds had no PHA titers. The PHA titers of individual sera collected at different days of post vaccination are depicted in Tables-4, 5 and 6.

PHA Antibody Titers due to:

a) Biofilm Vaccine

Geometric mean titers (GMT) at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 days post vaccination (PV) were 7, 17, 42, 79, 79, 128, 194, 256, 338, 446, 549, 426, respectively. GMT ranged from 7 to 549, lowest GMT was observed on 15 days PV and highest GMT was observed on 165 days. There was gradual trend of increase in PV, GMT (Table-4 and Fig. 8).

b) Broth Vaccine

GMT at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 days pv were 4, 6, 21, 42, 42, 79, 56, 52, 45, 30, 16 and 11. GMT was ranging from 4 to 79, lowest GMT was observed on 15 days PV and highest was on 90 days PV (Table-5 & Fig. 9).

In broth vaccine GMT initially showed a rise upto 90 days and later there was a gradual decline in GMT upto end of the experiment.

c) Commercial Vaccine

GMT at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 days PV were 4, 7, 69, 34, 45, 52, 56, 52, 45, 32, 32 and 13. GMT was ranging from 4 to 56. Lowest GMT was observed on 15 days PV and highest was on 105 days PV. In commercial vaccine, GMT showed aberrant rise upto 105 days and later there was a sudden decline in GMT upto end of experiment (i.e., 180 days) (Table-6 & Fig. 10).

d) Unvaccinated

Control Group: There was no difference in titre at all.

The above data was subjected to statistical analysis, which indicated that there was a significant difference in GMT between biofilm, broth and commercial vaccine sera titers. However, there was no significant difference between broth and commercial vaccines (Fig. 11).
4.4 PHA Antibody Titers in Commercial Farms: In each farm about 25 sera were collected and subjected to PHA tests and results are as follows:

In farm-I, PHA titers were ranged from 1 : 2 to 1 : 128 (Table-7).

In farm-II, PHA titers were ranged from 1 : 2 to 1 : 64 (Table-8).

In farm-III, PHA titers were ranged from 1 : 2 to 1 : 64 (Table-9)

DNCB Test Result: The skin thickness (mm) measured during DNCB test revealed no changes in skin thickness in all the groups of birds at zero hour, at 24 hours post-inoculation and 48 hrs post-inoculation and 7 days later.

4.5 Challenge Studies

Calculation of LD50 Values in Mice: Pasteurella multocida organisms were given at the dilution of $10^4$, $10^5$, $10^6$, $10^7$, $10^8$ and $10^9$ to the mice intraperitonally and results were recorded (Table-10).

The mortality rate in mice, which received $10^4$ and $10^5$, were nil, whereas, mortality was 4 out of 6, at $10^6$ and $10^7$ dilution, mortality was 5 and at $10^8$ dilution, mortality was 6 out of 6 mice at $10^9$ dilution at 12 hour interval. Mortality was ranging from 0 to 100 per cent. Highest mortality percentage was observed at highest concentration ($10^9$) and lowest mortality was observed ($10^4$ and $10^5$).

Challenge Study Results

The birds, which had received $10^9$ organisms in control groups, showed mortality at 24 hrs (1 bird), 30 hours (1 bird), 36 hours (4 birds), 42 hours (3 birds) and 48 hours (1 bird). Mortality was highest between 36 and 42 hours post-inoculation.

Birds, which received biofilm vaccine, showed no mortality. Whereas, birds which received broth vaccine showed mortality at 36 hours post-inoculation (2 birds). Birds, which had received commercial vaccines showed mortality at 42 hours (3 birds) (Table-11).

In the present challenge studies, mortality rate ranged between 0 to 100 per cent. Highest mortality was in control (100%) group and lowest was in biofilm vaccinated group (0%).

4.6 Post-vaccinal Systemic Manifestation in Experimental Birds

Birds, which were immunized against fowl cholera using biofilm antigen showed swelling at the site of injection on first (1st) and second day (2nd), swelling gradually reduced by day third (3rd) and fourth (4th) and subsided on later days on both primary and booster vaccination. Erythema was present on second and third day and by fourth day intensity was reduced and later on it was absent. Pain at the site of injection was observed
on second and third day and later it was absent, pain was recognized by limping of birds whereas body temperature was normal throughout the observation time (Table-12).

Birds which had broth vaccine exhibited swelling at the site on first, second and third day, erythema was noticed on second, third and fourth day, pain on third and fourth day whereas hyperthermia was recorded on second day (Table-13).

In the group, which received commercial vaccine, birds manifested swelling on first and second day by third and fourth day, swelling was reduced and later on it was not evident. Erythema was seen on second and third day, pain was observed on second, third, fourth, fifth, sixth and seventh days after the injection. Body temperature was high in second and third day only (Table-14).

In control group there were no observable and recordable events.