6. SUMMARY

Fowl cholera is a contagious bacterial disease caused by *P. multocida* and affects domestic and wild birds causing devastating economic hazards of poultry industry.

The present study aimed to investigate the incidence of *P. multocida* in chickens layers and breeders flocks from 4 Egyptian governorates located in El-Delta by isolation and identification of *P. multocida*, then trial for preparation of locally inactivated vaccine from the predominant *P. multocida* isolated strains and determination of its efficacy. Moreover, detection of the *in-vitro* antibiogram of the predominant isolate of strains was done.

A total of 330 freshly dead birds (234 layers and 96 breeders) were collected from 55 farms belonging to El-Sharqia (22), El-Gharbia (14), El-Qalubia (11) and El-Minofia (8) governorates, Egypt. All the examined bird had history of septicaemia (congestion and cyanosis of comb and wattles), nasal and ocular discharge, conjunctivitis, greenish diarrhea, and increased mortality (5-10%), with lesions of severe septicaemia, spleen congestion, liver necrosis, fibrinous perihepatitis and fibrinous pericarditis. Liver, spleen and heart from each chicken were examined bacteriologically for isolation of *P. multocida.*

Out of 55 chicken flocks, 6 *P. multocida* isolates were detected with rate of incidence (10.9%), 5 isolates from layer (12.8%) and 1 isolate from breeder (6.2%) flocks. The incidence of isolation from different governorates was the highest in El-Sharqia followed by El-Minofia governorate, El-Qalubia and finally El-Gharbia.

The isolated *P. multocida* were identified based on morphological characteristics, biochemical reactions, serological and molecular identification.

Colonial morphology of *P. multocida* appeared as small mucoid and grayish dew drop like colonies.

Microscopical examination revealed small size Gram negative coccobacilli with Gram stain and bipolar organisms with Leishman's stain.

Biochemical reactions revealed that all the isolates were positive for oxidase, catalase, indole production, hydrogen sulphide production, nitrate reduction test, Voges-Proskauer test and sugar fermentation reactions of glucose, sucrose, fructose, mannitol and xylose, while negative for urease activity, sugar fermentation reactions of maltose, lactose, arabinose and dulcitol.

The isolates were pathogenic for mice as they died within 18-24 hr after artificial infection with lesions of congestion and septicaemia of the internal organs.

The isolated *P. multocida* were confirmed to be of serotype A:1 and A:3 by serological methods.

The isolated strains were confirmed to be related to *P. multocida* by kmt1 specific PCR (Universal primers) method as the isolates showed positive bands at 460 bp.

Molecular serotyping revealed that the isolates were within *P. multocida* type A.

Inactivated *P. multocida* vaccine was prepared from the isolated strains, and the vaccine was proved to be sterile and safe for chickens and mice.

Results of the mean ELISA antibody levels of the locally prepared inactivated *P. multocida* vaccine revealed that the highest level was at 5 weeks post vaccination.

The clinical signs, mortality rate and post-mortem lesion were mild in vaccinated and challenged birds while severe in non vaccinated and challenged birds.

The protection rate was (85%) when birds challenged with *P. multocida* serotype A:1, 80% after challenging with *P. multocida* serotype A:3, while the protection rate was (10%-20%) in non vaccinated challenged control groups.

The reisolation rates of *P. multocida* after challenge were the highest (95%) in non vaccinated-challenged birds with *P. multocida* serotype A:1, then (90%) in non vaccinated-challenged ones with *P. multocida* serotype A:3, (25%) in vaccinated-challenged groups with *P. multocida* serotype A:1, (15%) in vaccinated-challenged chickens with *P. multocida* serotype A:3 and (0%) in non vaccinated non challenged controls.

Histopathological examination of *P. multocida* vaccinated-challenged birds revealed mild congestion of the central vein of liver. The heart showed normal myocardium and pericardium or mild congestion of the myocardium. Spleen showed mild congestion of the red pulp as well as depletion of the lymphoid follicle.While in *P. multocida* challenged chickens, there were congestion of the portal vein of liver and the hepatocytes showed hydropic degeneration of cytoplasm with appearance of focal area of coagulative necrosis infiltrated with heterophils. The heart showed myocarditis with inflammatory cells infiltration, andprecardial and sub endocardial hemorrhages. Spleen showed congestion of the red pulp, focal area of necrosis and area of hemorrhage.

The antibiogram of *P. multocida* revealed that the strains were *in-vitro* sensitive to several antibiotics as chloramphenicol, tetracycline, trimethoprim /sulphamethoxazole, ofloxacin, penicillin G, norfloxacin, azithromycin, and erythromycin while they were resistant to ampicillin and clindamycin.