Preliminary Studies on Fowl Cholera in Layers

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Abstract

Pasteurella multocida the etiological agent of fowl cholera was isolated from 16 layer flocks in and around Faisalabad. The flocks ranged in age from 18-24 weeks. Increased mortality and considerable drop in egg production were the main clinical signs in all affected flocks. Bacterial isolation and identification was conducted on the basis of cultural, morphological and biochemical characteristics. All the isolates were highly sensitive to enrofloxacin.

Key words: Fowl cholera, P. multocida, Enrofloxacin

Introduction

Fowl cholera (FC), caused by *Pasteurella multocida* is a severe septicaemic disease of domestic and wild fowl and remains an important havoc for the poultry industry (David *et al.*, 1991). It affects the birds of all ages, however, chickens less than 16 weeks of age are generally quite resistant. Death losses from FC in chickens usually occur in laying flocks (Rhoades and Rimler, 1991). High ambient temperature, poor management and malnutrition are among the plausible factors, which are incriminated to potentiate the incidence of the disease in Pakistan. High environmental temperatures were found to be influential in the development of fowl cholera in turkeys (Simensen and Olson, 2001).

Many poultry birds and most of the farm animals can be carriers of *P. multocida*, but not all of the isolates from these animals are capable of producing disease in poultry (Curtis *et al.*, 1980 and Snipes *et al.*, 1988). Once the disease introduced into a flock it spreads rapidly through the contamination of open watering systems and cannibalism of contaminated carcasses (Pabs-Garnon and Soltys, 1971).

Diagnosis depends on identification of the causative agent, *P. multocida*, following isolation from birds with signs and lesions consistent with this disease (Anonymous, 2000). Various antibiotics are used extensively with varying success for the treatment of FC.

Corresponding Author: M. Javed Arshed Department of Veterinary Microbiology, University of Agriculture, Faisalabad-Pakistan Sensitivity testing is often advantageous since strains of *P. multicida* vary in susceptibility to chemotherapeutic agens and resistance to antibiotics may develop especially during prolonged use of these agents (Rhoads and Rimler, 1991). The present study reports the isolation, biochemical characteristics and antibiotics sensitivity testing of *P. multocida* isolated from the field outbreaks of FC.

Materials and Methods

Layer flocks with suspected fowl cholera outbreaks in and around Faisalabad were surveyed. Hygienic and managemental conditions, clinical signs and postmortem lesions were recorded. Representative samples including liver, spleen and heart blood were collected aseptically and brought to the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan for bacterial isolation and identification.

Isolation of the organism(s) was carried out following the method described by Krieg and Hold (1984). Briefly the specimens were transferred to tryptic soy broth and incubated for 24 hours at 37°C under aerobic conditions. The cultures were examined for morphological studies. All those cultures showing the presence of gram negative coccobacilli were further shifted to dextrose starch agar with 5% avian serum, blood agar and MacConkey agar for the preliminary identification of the isolates.

The purified isolates were further differentiated on the basis of sugar fermentation and other specific biochemical tests (Krieg and Holt, 1984). All the isolates were subjected to antibacterial sensitivity testing by the agar plate method as described by Awan and Rahman (2002).

Results and Discussion

In the present study, drop in egg production (10-30%), foetid diarrhoea, mucus discharge from mouth, increased respiratory rate and variable mortality (6-12%) were recorded. In some cases the birds were getting down on their hocks before death. Postmortem findings included subserosal multiple petechial haemorrhages throughout the viscera, on the myocardium, in coronary and abdominal fat, at the base of the heart, congested and friable liver with multiple light yellow focal necrotic foci (corn meal liver), flaccid ovarian follicles. In some cases purulent hock joints, femoral osteomyelitis, swollen and cyanotic wattles were also observed. In 6 flocks purulent pleuropneumonia was also observed. Similar signs and symptoms have also been reported previously by Roades and Rimler (1991) and Anjum (1994).

At most of the farms with fowl cholera outbreaks, hygienic and managemental conditions were poor. Cats and rodents were also a problem on many of the premises. Malnutrition, parasitism and occurrence of other concomitant diseases were other stress factors. In the flocks where antibiotic therapy was administered, norfloxacin, enrofloxacin and tetracycline gave good response.

P. multocida was isolated from 12 out of 16 commercial layer flocks with suspected outbreaks of FC. All the isolates were examined for their cultural, morphological and biochemical characteristics. None of the isolates was able to grow on MacConkey agar. The colony characteristics were best studied on blood agar, where the growth was perfused with small grayish colonies without any evidence of haemolysis. Similar results have been reported by Divivedi and Sodhi (1989) and Collier *et al.* (1998).

All the isolates fermented glucose, fructose, mannose, mannitol, sucrose, sorbitol and xylose without the production of gas. However, the isolates were unable to ferment arabinose, inositol, lactose, maltose, salicin, dulcitol and raffinose. A positive reaction was also recorded for catalase, oxidase, indol production, nitrate reduction and H₂S production tests, while negative for methyl red, Voge's proskaur, urease activity and gelatin liquefication tests. These results are in line with the findings of Cruickshank (1975) and Rhoades and Rimler (1991).

Enrofloxacin proved most effective antibiotic to which all the isolates of *P. multocida* were highly sensitive. The rest of the antibiotics in descending order included norfloxacin. amoxicillin, gentamicin, ampicillin, trimethoprim + sulphamethoxazole, chloramphenicol, doxycycline, oxytetracycline, kanamycin and erythromycin. The same was also reported by Gergis et al. (1986) and Sander and Glisson (1989). Abeynayake et al. (1993) confirmed the high sensitivity of P. multocida to enrofloxacin. Majority of P. multocida isolated from commercial turkeys was found susceptible to amikacin, ampicillin, ceftiofur, cephalothin, enrofloxacin, florfenicol, gentamicin, neomvcin. novobiocin, oxacillin, sarafloxacin, tilmicosin and trimethoprim with sulphadiazine (Ave et al. 2001). The efficacy of doxycycline against P. multocida infection in broiler chickens was investigated by Semjen et al. (1998) and reported that doxycycline is highly effective for the treatment of experimental pasteurellosis in chickens.

Majority of the isolates (87%) were found resistant to sulphadizine, clindamicin and tylosin in the present study. These results are in line with the findings of Donahue and Olson (1972) Abeynayake *et al.* (1993) and Aye *et al.* (2001).

The results of the present study showed that *P*. *multocida* isolated from field outbreaks of FC varied in susceptibility to chemotherapeutic agents so the only choice to prevent or control the disease and avoid economic losses is good management and vaccination of the birds. At present, a number of imported inactivated vaccines are being used in Pakistan with variable results. There is a dire need to develop vaccines against fowl cholera from local strains of *P*. *multocida*.

References

- Abeynayake, P., Wijewardana, T.G. and Thalagoda, S.A. Antimicrobial susceptibility of *P. multocida* isolates. In: Pasteurellosis in production animals: Proceedings of an International Workshop, Bali Indonesia, 1993. pp: 193-198.
- Anjum, A.D. Avian pasteurellosis. In: Poultry Diseases, 2nd Ed. F.Y. Corporation publisher, pp: 271-276, 1994.
- Anonymous, Principals of veterinary vaccine production. In: Mannual of standards for diagnostic tests and vaccine. World Org. Anim. Hlth., Paris, 2000.
- Awan, J.A. and Rahman, S.U. Microbiology Manual. Umitech Communications Faisalabad, pp: 99. 2002.
- Aye, P.P., Angrick, E.J., Morishita T.Y. and Harr, B.S. Prevalence and characteristics of *P. multocida* in commercial turkeys. Avian Dis., 2001, 45:182-90.
- Collier, L., Balows, Sussman, A.M. Microbiology and microbial infections. 9th Ed. Topley and Wilsons, Oxford Univ. Press, New York. pp: 1203-1207. 1998.
- Cruickshank, R. Medical Microbiology. Vol.II.12th Ed. Churchill Livingstone, Edinburgh, London, New York, 1975.
- Curtis, P.E., Ollerhead, G.E. and Ellis, C.E. *Pasteurella multocida* infection of poultry farm rats. Vet. Rec., 1980, 107: 326-327.
- David, W.H., Carpenter, T.E., Snipes, K.P., Hirsh D.C. and McCapes, Control study of fowl cholera outbreaks in meat turkeys in California from August 1985 through July 1986. Am. J. Vet. Res., 1991, 52: 212-216.
- Divivedi, P.N. and Sodhi, S.S. Morphological cultural and biochemical characterization of *P. multocida* isolated from poultry. Poultry Adviser, 1989, 22: 69-71.

- Donahue, J.M. and Olson, L.D. The *In vitro* sensitivity of *P. multocida* of turkey origin to various chemotherapeutic agents. Avian Dis., 1972, 16: 506-511.
- Gergis, S.M., Ghani, M.A., Safwat, E.E.A. Soliman, A.K. and Saad, M.K. *In vitro* sensitivity of *P. multocida* from fowl cholera epornithics to antibiotics. Agri. Res. Rev., 1986, 64:1985-1991.
- Krieg, N.R. and Holt, J.G. Bergeys Manual of Systemic Bacteriology. Vol.1. Williams and Wilkins Baltimore, London. 1984.
- Pabs-Garnon, L.F. and Soltys, M.A. Methods of transmission of fowl cholera in turkeys. Am. J. Vet. Res., 1971, 32: 1119-1120.
- Rhoades, K.R. and Rimler, R.B. Avian pasteurellosis. In: Diseases of poultry, 9th Ed. 1991.

- Calnek, B.W., Barnes, H.J., Beared, C.W., Ried, W.M. and Yoder, H.W. Iowa State University Press, Ames, Iowa. pp:145-162.
- Sander, J.E. and Glisson, J.R. Fowl cholera in broilers. Avian Dis., 1989, 33: 816-819.
- Semjen, G., Magyar, T. and Laczay, P. Therapeutic efficacy of doxycycline against experimental *P. multocida* infection in broiler chickens. Acta Vet. Hungarica., 1998, 46: 85-93.
- Simensen, E. and Olson, D. Relationship of increased fowl cholera outbreaks in turkeys with high environmental temperatures. Avian Dis., 2001, 45: 461-466.
- Snipes, K.P., Carpenter, T.E., Corn, J.L., Kasten, R.W., Hirsh, D.C., Hird, D.W. and McCapes, R.H. *Pasteurella multocida* in wild mammals and birds in California: prevelence and virulence for turkeys. Avian Dis., 1988, 32: 9-15.