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PASTEURELLA HAEMOLYTICA AND OTHER AVIAN PATHOGENS AS THE CAUSATIVE AGENTS OF SOME PROBLEMS IN BROILER AND LAYER CHICKEN FLOCKS IN ASSIUT GOVERNORATE

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الباستيرلا هيموليتيكا وبعض المسببات المرضية للطيور المسببة لبعض المشاكل في بدارى التسمين وقطعان إنتاج البيض بمحافظة أسيوط

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تم عزل ميكروب الباستيرلا هيموليتيكا من قطيعين من بدارى الدجاج والتي ظهرت به أعراض تنفسية ومن قطيع بياض ظهر به نقص في انتاج البيض مع انتاج عدد من بيض بدون قشرة. تم فحص عينات من قطعان البدارى والبياض بكتريولوجيا وسيرولوجيا. وكذلك تم عزل الميكروب القولوني من بدارى الطيور. تم اختبار ميكروب الباستيرلا المعزول ضد أنواع مختلفة من المضادات الحيوية وقد اتضح من الفحص السيرولوجى أن كل من قطعان البدارى والبياض ايجابى للاجسام المناعية ضد مرض الميكوبلازما جاليسيبتكم بواسطة اختبار التجمع الدموى السريع. وكان القطيع البياض ايجابيا للاجسام المناعية ضد ظاهرة انخفاض البيض باستخدام اختبار تلازن الدم الاحباطى.

SUMMARY

Pasteurella haemolytica was isolated from broiler flocks which showed respiratory signs and from layer flocks which showed drop in egg production and shelless eggs. Samples from broiler and laying flocks were examined bacteriologically and serologically. Also *E.coli* was isolated from broiler flocks. The isolated *Pasteurella haemolytica* were tested against different antimicrobial agents. The serological examination revealed that both of broiler and laying flocks were positive to agglutinating antibodies against *Mycoplasma gallisepticum* (MG) by

rapid serum plate agglutination test. The haemagglutinating inhibiting antibodies against EDS-76 were detected in sera of laying flocks.

Key words: *Avian pathogens, Broiler and Layer chicken*

INTRODUCTION

Pasteurella haemolytica (***P. haemolytica***) is a member of the normal flora of the respiratory tract of chickens but not of turkeys or ducks, under conditions of stress, however this bacterium may acquire the role of an opportunist, participating in pathological processes. Harbourne (1962) isolated *P. haemolytica* from livers of young chikens and turkeys. Harry (1962) and Mushin et al. (1980) isolated *P.haemolytica* from the upper respiratory tract. *P. haemolytica* has been associated with increased mortality and abnormalities in egg production in laying hens and increased mortality in pullet (Harbourne, 1962; Harry, 1962 ; Hacking and Pettit, 1974 and Rhoades and Rimler, 1984). Nicolet and Fey (1965) isolated *P. haemolytica* from young hens with salpingitis, the organism was often localized in the oviduct and was disseminated in other organs including lungs. Addo and Mohan (1985) isolated a typical strains of *P.haemolytica* that failed to ferment maltose were isolated from nodular necrosis in the liver and heart blood of domestic fowl. Shaw et al. (1990) and Suzuki et al. (1996) isolated *P.haemolytica* from the internal organs of laying hens and pullets. Lin et al. (1993) isolated *P.haemolytica* from the respiratory tract of sick birds suffering from a long-lasting respiratory syndrome or from the bone marrow. Birds at necropsy showed septicemia characterized by petechial haemorrhages of the heart, spleen and kidney and occasionaly of the muscles especially in younger birds. Kidneys were usually enlarged and showed focal areas of necrosis (Addo and Mohan, 1985 and Suzuki et al. 1997). The p.haemolytica infection was nearly always accompanied by nasal catarrh, helminth infection or leukosis or chronic respiratory disease or infectious bronchitis or colibacillosis or staphylococci (Nicolet and Fey, 1965; Hacking and Pettit, 1974) or *E. coli* infections and Gumboro disease (Lin, 1986). Egg drop syndrome (EDS-76) is a viral disease of economic importance consequences of the infection in laying hens can be costly mainly due to the drop in egg production, depressed hatchability in breeder flocks and laying poor shell quality (McFerran 1979).

Mycoplasma gallisepticum caused drop in egg production in laying hens and caused chronic respiratory disease (Yoder, 1991).

This work was planned:

1- To investigate the most avian pathogens causing respiratory disease in broiler flocks and cause drop in egg production and egg quality in laying hens.
2- Isolation and identification of the causative bacterial agents
3- Serological examination of the above flocks for antibodies against ***Mycoplasma gallisepticum*** and adenovirus infections.

MATERIAL and METHODS

Case history:

During the year of 1999, disease outbreaks were observed in 2 broiler flocks at 4 and 5 weeks of age and one Hyline laying flocks in Assiut Governorate. Mortalities in broiler flocks reached 2.5% in 4 days before treatment (250 chicks within 4 days out of 10000 birds and morbidity reached to 5%. The laying flock of about (20000 birds at 60 weeks of age, the most characterized signs were drop in egg production and shelless eggs.

Specimens:

Samples from trachea, lung, heart blood and liver were collected aseptically from 120 freshly dead and diseased broiler birds. Samples from uterine folds of oviduct, ovarian follicles, lungs, trachea, heart blood and liver were collected aseptically from 35 freshly dead and diseased laying hens.

Blood samples:

Blood samples were collected from broiler flocks and from laying flocks. Serum was separated and subjected to serological tests.

Bacterial isolation and identification:

Samples for bacterial isolation were inocualted on nutrient broth and tryptose soy broth, incubated for 24 hours at 37°C, then subcultured on McConkey agar, and blood agar (18-24 hours at 37°C), suspected colonies of *P.haemolytica* and *E. coli* were selected and purified, then pure colonies were picked and stained by Gram's stain.

Further identification of microorganisms were carried out according to Cruickshank et al. (1980) and Collins and Lyne (1991) including:

I- Cellular morphology.

II- Colonial morphology " colour, shape, size, odour and pigment production".

III- Biochemical reactions.

Sugar media for carbohydrate fermentation test (1% peptone water+ 1% of glucose, sucrose, lactose, maltose). Indole production test- Methyl red reduction test, Catalase test- Oxidase test- Urease test- Citrate utilization

Pathogenicity test on mice:

Suspected *P. haemolytica* colony was cultured in broth incubated for 24 hours at 37°C. Then white mice of 3-4 weeks obtained from laboratory Animal, Faculty of Medicine. Assiut University, was inoculated intrapritoneally with 0.1 ml of 10^6 CFU and kept under observation. Signs, lesions were recorded. Reisolation was carried out from mice. (Cruickshank et al. 1980).

Sensitivity of P.haemolytica isolates to antimicrobial agents in vitro:

Antimicrobial sensitivity patterns were carried out on ***P. haemolytica*** by disc diffusion method according to Cruickshank et al. (1980). Chemtherapeutic discs produced by Oxoid Basingstoke, England included Tetracycline (30 µg), Chloramphenicol (30 µg), Gentamycin (10 µg), Penicilin G (10 µg), Ampicillin (10µg), Oxytetracycline (30 µg), Neomycin (30 µg), Amoxycillin (10 µg), Flumequine (30 µg), Doxycycline hydrochloride (30 µg), Trimethoprim+ sulphamethoxazole (1.25+ 23.75 µg), Spectinomycin (10µg), Colistine (10µg) and Naladixic acid (30µg).

Serological tests:

1- Rapid serum plate agglutination (RSPA) test:

For detection of mycoplasma gallisepticum (MG) antibodies from broiler and layer flocks used stained antigens (Intervet, Box-meer, Holand) as described by Adler and Yamamoto (1956).

2-Haemagglutination inhibition (HI) test:

For detection HI antibodies against EDS-76 in laying hens. The micro HI test was carried out as described by Alexander et al. (1983). Four haemagglutinating units of antigen were used.

3-Agar gel precipitation (AGP) test:

For detection of precipitating antibodies against adenovirus (Phelps strain) in laying hens. (Yates et al. 1975).

Adenovirus ED76 haemagglutinating undiluted antigen and (phelps strain) were provided from Dr. S. Mousa, Poultry Dis. Dept., Faculty of Vet. Med. Assiut University.

RESULTS

Field outbreaks:

Broiler flocks showed respiratory signs (tracheal rales, nasal discharge and coughing) with morbidity and mortality rates of 5% and 2.5% respectively. The post mortem (P.M) examination showed septicemia as petechial haemorrhages of the heart, liver, spleen and kidneys. Severe tracheitis, congestion of trachea with bloody exudate in the trachea and lung congestion.

Layer flocks showed drop in egg production (about 10%) and shelless eggs (4%) and low morbidity and mortality. The P.M examination showed tracheitis with small amount of cloudy mucous, mild air saculitis, liver and kidneys were enlarged and showed areas of necrosis. Ovaries were regressing with blood clots present within the large, pale flacid follicles. Peritonitis with free yolk in the abdominal cavity was seen, these lesions revealed salpingitis.

Bacterial findings:

Out of 120 samples from broiler flocks, 72 samples (60%) were positive for bacterial isolation. 30 samples (25%), 25 samples (20.1%), 17 samples (10.4%) were positive for *P.haemolytica, E. coli* and mixed infection of *P.haemolytica* and *E. coli* infections respectively.

Out of 35 samples from laying flocks, 18 samples (50.1%) were positive for bacterial isolation, 7 samples (20%) were positive for *P.haemolytica.*

Identification of *P. haemolytica*:

P. haemolytica appeared as gram negative, aerobic, non motile short rods occuring single or in pairs. In tissues, and recently isolated culture the organism stained bipolar. It was hemolytic on blood agar (Beta-hemolysis) and showed small red colonies on McConkey agr. All isolates gave indole -ve , urease -ve, M.R. -ve, citrate -ve, catalase +ve, oxidase+ve, glucose +ve, sucrose +ve, Maltose -ve, lactose +ve

The isolated *E.coli* stains gram were negative bacilli, smooth, glossy and translucent rose-pink in colour on McConkey's media.

Pathogenicity test on mice:

No clinical signs or death were noticed in mice within 7 days after inoculation.

Results of sensitivity test:

P.haemolytica which isolated from both broiler and layer flocks gave the similar results with antimicrobial therapy. They were sensitive to Oxytetracycline, Trimethoprim+ Sulphamethoxazole and Gentamycin, less sensitive to tetracycline, Chloramphenicol, Neomycin, Flumequine, Doxycycline hydrochloride, Spectinomycine, Colistine and Naladixic acid, and resistant to Penicillin G, Ampicillin and Amoxycilline.

Serological tests:

Both broiler flocks and the layer flocks were positively for antibodies against MG. The layer flock possessed antibodies to EDS virus with a titer of 1/64 and negative to adenovirus (Phelps strain) antibodies.

DISCUSSUIN

Many reports discussed the role of *P.haemolytica* as a cause of disease in poultry. It is postulated that *P.haemolytica* is a member of the normal flora of the respiratory tract of chickens, under stress conditions this bacterium acquires pathogenic role. (Mushin et al., 1980; Mirle et al., 1991; Rimler and Glisson 1997). Broiler flocks showed respiratory signs (nasal discharge, tracheal rales and coughing. These signs of *P.haemolytica* were also reported by Harbourne (1962) and Lin et al. (1993). The post mortem (P.M.) examination of broilers revealed the evidence of septicemia in the form of petechial haemorrhages of the heart, spleen and kidney, tracheitis with catarrhal or body exudate. Similar findings of *P.haemolytica* were reported by Addo and Mohan (1985) and Lin (1986).

The most characteristic signs in affected laying flocks were drop in egg production and shell-less egg similar signs have been reported about the abnormalities in egg production in laying hens by Harvy (1962). Salpingitis was the most P.M. lesions shown in laying hens affected with *P.haemolytica* as reported by Nicolet and Fey (1965); Hacking and Pettit (1974) and Suzuki et al. (1997).

The isolation of P. heamolytica from trachea, lung, liver and heart blood from broilers and from uterine folds of oviduct, ovarian follicles, lungs, trachea, heart blood and liver from laying hens indicates that these microorganisms with other microorganisms acquire pathogenic role.

P.haemolytica was isolated from laying flock at rate of 20% nearly of this result recorded by Mirle et al. (1991), they isolated *P.haemolytica* at a rate of 27.4%.

Concerning the *P.haemolytica* which isolated from broiler and laying flocks were sensitive to Oxyteracycline, Gentamycin, Trimethoprim+ Sulphamethoxazole, resistant to Penicillin G, Ampicillin and Amoxycillin, the other antibiotics were very low in effectiveness. These results nearly agreed with the response of some antibiotics against *P.haemolytica* used by Shaw et al. (1990).

The condition in broiler flocks associated with E. col infection and positive to MG antibodies and the layer flocks associated with positive HI antibodies to EDS virus. Similar results were recorded by Harbourne (1962); Nicolet and Fey (1965); Lin (1986) and Mirle et al. (1991), they isolated *P.haemolytica* with *E. coli* and M.G. Similar results for egg quality and quantity and salpingitis in naturally infected laying hens with EDS virus were reported by McFerran (1979) and Yamaguchi et al. (1981).

It is concluded that *P.haemolytica*, *E. coli* and mycoplasma infections were the cause of the respiratory signs in broiler flocks while the *P.haemolytica*, mycoplasma and adeno virus (egg drop syndrome) were the cause of egg drop problems in layer flocks.

The experimental infection of *P. haemolytica* and the other avian pathogens which were isolated will planned in another research.

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