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BACTERIAL CAUSES OF DECREASE IN PERFORMANCE OF BREEDER CHICKENS FLOCKS.

By

M. M. AMER and WAFAA A. ABD EL-GAHNY

Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University

SUMMARY

This study was carried out to investigate the possible bacterial causes affecting productivity in breeder hens. The hens under test proved to be positive for *Mycplasma gallisepticum (MG)* and *Mycplasma synoviae (MS)* and negative for *Salmonella gallinarum-pullorum* as tested by serum agglutination test. Ovaries with lesions were proved bacteriologically to have 12 bacterial isolates including 3 untyped *E. coli* , one O11, and one O78; 3 *Staph. aurous*; one *S.gallinarum pullorum (S. g-p)*; 2 *protus* and one *P.aeruginosa*. The antibiogram of the two identified *E. coli* strains, *S. g-p* as well as *P. aeruginosa* showed variable sensitivity. The tested organisms were totally sensitive to Colistin and Enrofloxacin. The selected *E. coli, S. g-p* and *P.aeruginosa* strains proved to be pathogenic to orally infected 3 day-old chicks, with induction of clinical signs, mortality, lesions and adverse effect on the body weight gain. On the other hand, E. coli O78 proved to be more pathogenic than O 11. The use of the above mentioned drugs for controlling of these infections in chicks was an effective as indicated with the results of the in vitro test. It is important to carry out bacteriological examination to breeder flocks to investigate the bacterial affections, with estimation of the changes in their sensitivity to the used antibiotics.

INTRODUCTION

As poultry rearing developed from backyard to be an organized industry, many bacterial pathogens had been incriminated as the cause of ovarian affection of laying hens. These affections including salmonellosis **(Elleman, 1960; Sokker et al., 1975; Netherlands, 1990; Al-Nakhal et al., 1999 and Shivaprasad, 2000)**, colibacillosis **(Gross and Siegel, 1959; Harry, 1964; Abd El-Nasser, 1976; Azzam,1983; Ibrahim and Sheha, 1985; Montogomery et al., 1999 and Srivasan et al., 2003)**, pseudomoniasis **(Sharma et al., 1980; Batra et al., 1982; Kheir El-Din et al., 1986; Shehata et al., 1988 and Sidu et al., 1989)**. These bacterial affections resulted in ovarian lesions including misshaped and discolored ovarian follicles, caseated and degenerated ova and egg peritonitis **(Saif et al., 2003)**. Drop in egg production varies according to the nature and type of such infection as *S.g-p* **(Shivaprasad, 2003)** and *E. coli* **(Bisgaard and Dam, 1981 and Gazdziniski and Barnes, 2002)** infections can cause 10-30%, while *P. aeruginosa* can cause 20-80% **(Kaul et al., 1992)**.

Pathogenicity to 1-3 day-old chicks was reported in *S. g-p* **(Sieburth and Johanson, 1957; Kosugi et al., 1985 and Gorham et al., 1994)**, *E. coli* **(Awaad, 1972; Khalid, 1990; Andreatti et al., 1993 and Johnson et al., 2001)** and *P. aeruginosa* **(Ray and Baujeri, 1969; Kheir El-Din et al., 1986 and Hamouda et al., 1987)**.
Antibiogram is recommended for detection of suitable drugs for controlling of such pathogens **(Saif et al., 2003)** as they acquired drug resistance by pervious, long, and hazard use of these antibacterial drugs.
Infection of chickens with *Mycoplasma gallisepticum* and *synoviae* increase susceptibility to pathogenic and potentially pathogenic organisms like *E. coli* **(Gross, 1990 and Nakamura et al., 1994)**.

This work was carried out to investigate the bacterial causes of ovarian lesions in broiler and layer breeder's flocks, the antiobiogram of the isolated bacterial strains, the pathogenicity of the strains to 3 day-old chicks and the treatment of experimentally infected birds as recommended with the results of the in vitro sensitivity test.

MATERAL AND METHODS

History:
Two broiler breeder flocks aged 35 and 56 weeks and one layer breeder flock aged 35 weeks showed lower egg p[production than the farm standard by 8%, 12% and 21% ; respectively. The hatchery parameters including fertility and hatchability were also reduced.

Samples:
1. Ovaries:
Thirty ovaries (10/flock) with misshapen, discolored and long stalk caseated cystic ovules were aseptically collected for bacteriological examination.

2. Blood samples:
From each flock, 100 blood samples were randomly collected from the wing vein and the sera were separated to be tested using slide agglutination test to confirm the results of the farm test.

Bacteriological examination:
Aseptically collected ovaries were subjected to individual testing as 1 ml of the follicular content was aspired by a sterile syringe, inoculated into Selenite F broth and incubated at 42° C for 18 hours for salmonella sp isolation, another 1ml was inoculated in nutrient broth and incubated at 37° C for 24 hours. Each of the inoculated broth medium was subcultured onto selective agar medium (sheep blood, SS, MacConkey, nutrient and pseudomonas agar) plates, then incubated at 37° C for 48 hours. All the plates were examined for bacterial growth according to **Cruickshank et al. (1975)**.

Bacterial identification:

The obtained bacterial growths were purified, examined for colonial morphology, staining characters **(Cruickshank et al., 1975)** and subjected to biochemical identification **(Edwards and Ewing, 1972; MaCffaddin, 1980 and Quinn et al., 1994)**.

Serological typing:
The obtained *E. coli* and *salmonellae* sp. strains were serotyped using slide agglutination test against polyvalent and monovelant standard serum obtained from Behring Werk institute, Germany using methods of **Neville and Brgant, (1986)** and **Lee and Arp (1998)**.

Antibiogram:
All the isolated strains were tested for their in vitro sensitivity for the following chemotherapeutic discs: Neomycin 30 (µg), Oxytetracycline 30 (µg), Trimethoprim (25µg),, Streptomycin (10µg), Ampicillin (25µg), Nalidexic acid (30µg), Colistin (10µg) and Enrofloxacin (10µg) adpoting method of **Cruickshank et al., (1975)** and the results were interoperated according to **Bio-Merieux (1980)**.

Broth culture:
Twenty four hours broth cultures from *S.g-p*, *E.coli* O11 and O78 as well as *P.aeruginosa* were separately prepared and subjected to plate counting as described by **Collins and Lynn's (1989)**.

Slide agglutination antigen:
Slide agglutination colored antigen against both *MG* and *MS* as well as *S.g-p* were obtained from Intervet Co., Boxmeer, Holland.
Serum agglutination tests for *Mycoplasma* sp. and *S.g-p*. were carried out as stated in **NPIP**.

Experimental infection:
The used 3 day-old chicks were orally infected each with 0.2 ml containing $4X10^4$ CFU/ bird for *S. g-p*, $3X10^7$ CFU/bird for *E.coli* **(Awaad, 1972)** or $3X10^9$ CFU/bird **(Kheir El-Din, 1986)** for *P. aeruginosa.*

Antibiotics used for Treatment:
According to the results of the in vitro sensitivity test, Colistin 10% liquid in water (lot no. 1050020) was obtained from Ascor chimici, Forli, Italy and Enrofloxacin 10% (lot no. 604160) from Memphis Co. for Med. Ind., were used for the treatment of experimentally infected chicken groups in rate of 6 mg/kg for colistin and 10 mg/kg of Enrofloxacin in drinking water for 6 days each.

Experimental Chicks:
Three hundred and twenty two, day-old male LCL chicks were obtained from commercial hatchery. Ten chicks were bacteriologically examined for the freedom of bacterial pathogen. Rest of these chicks were floor reared and fed on commercial ration without antimicrobial feed additives and water was given *ad libitum*

Statistical analysis:
The obtained results were statistically analyzed using ANOVA test.

Experimental design:
At the 3rd day of life, the remaining 312 chicks were randomly divided into 13 equal groups (1-13); 24 chicks each. Chicks groups were treated as follows: groups 1-3, 4-6, 7-9 and 10-12 were orally infected with *S.g-p*, *E.coli* O11, *E.coli* O78 and *P.aeruginosa*, respectively; while chicks of group 13 were kept as non infected negative control. The infected chicks groups were observed daily for clinical signs and/or mortality.

At the first detection of clinical signs, birds of groups 1, 4, 7 and 10 were treated with Colistin, while those of groups 2, 5, 8 and 11 were treated with Enrofloxacin. The medicaments were given in the drinking water for 6 days. Birds of groups 3, 6, 9 and 12 were left as infected non treated controls.

All the groups were daily observed for 10 days with recording of clinical signs, mortality rate and lesions as well as bacterial reisolation from dead birds. At the 10th day post infections (3 days after stop medication), 10 randomly selected chicks /group were weighted, sacrificed and examined for lesions. Samples from liver, spleen and lung were collected from dead and sacrificed birds and subjected to bacterial reisolation.

The obtained results are shown in table (1-4) and figures (1-3).

RESULTS

Results of slide agglutination test revealed that the tested sera were positive to *M. gallisepticum, M. synoviae and S.g-p* in rates of 44.35%, 63% and 0 %; respectively.

The bacteriological examination of ovaries with lesions showed isolation of 12 bacterial isolates. The obtained isolates were morphologically, staining and biochemically identified to be (5) *E. coli*, (3) *staphylococci*, (2) *Protus*, (1) *Pseudomonas* and (1) non motile *Salmonella*. These isolates were further identified to be (3) untyped *E. coli*, (1) O11 and the other was O78; the (3) Staphylococcal isolates were *Staph. aurous*, (2) *Protus*. Salmonella isolate was *S.gallinarum pullorum* and the last one was *P. aeruginosa*.

Identified strains of *E. coli*, *S.g-p* and *P. aeruginosa* were isolated from the more severe ovarian lesions. They showed variable sensitivity to the available antibiotics when tested in vitro. The tested organisms were totally sensitive to Colistin and Enrofloxacin; resistant to Oxytetracyclin and Trimethoprim; and variable to the others.

The infected chicks showed signs of illness at the 2nd day post oral infection with rates of 1/24 (4.16%) for all groups except for *E. coli* O78 infected group that showed 2/24 (8.32%) (Table 1). The most predominant signs were off food and ruffled feather. One chick was found died in *S.g-p* group at the 2nd day.

At the 3rd day of infection, the signs progressed to be 4, 5, 2, and 2 (16.64%, 20.83%, 8.32% and 8.42 %) for *E. coli* O11, *E. coli* O78, *S.g-p* and *P.aeruginosa* infected groups; respectively, while the mortality was 1, 2 , 2 and 3 (4.16%, 8.32%, 8.32% and 12.49 %) for *E. coli* O11, *E .coli* O78, *S.g-p* and *P. aeruginosa* ; respectively.(Table 1).

The detected signs were diarrhea, pasty vent, off food and huddling together for *E. coli* O11and *S.g-p* infected groups, while the signs were more severe in *E. coli* O78 group. Group (5) infected with *P. aeruginosa* showed ataxia, incoordination, off food and diarrhea.
All dead birds revealed lesions of septicemia and enteritis except for *E. coli* O78 dead chicks showed slight airsaculitis.

At the end of the 10^{th} day post infection, the total morbidity rates were 62.5%, 95.83%, 91.60% and 79.16% and the mortality rates reached 20.83%, 45.83%, 58.50% and 50% ; in groups infected with *E. coli* O11, *E. coli* O78, *S.g-p* and *P. aeruginosa* ; respectively. The control non infected group (1) showed no signs or mortality. Dead birds proved positive results for bacterial reisolation.

Results of body weight at the end of observation showed that the infected recovered birds were stunted than the non infected group (Table 3). The recorded mean weights
were the highest 71.32 gm for control, followed by 61.41, 58.16, 56.36 and 51.83 gm in descending manner for groups 5, 3, 2 and 4; respectively.
Results of body weight in infected non treated group (table 3) proved significant difference between group (1) and infected groups 2, 3, and 4 at $P>0.05$. While, the treated groups (table 4) proved significant difference only between group 2 and groups 4 and 5 at $P > 0.05$.

Groups received Colistin or Enrofloxacin in drinking water at the 3^{rd} day post infection showed the following results (Table 2, Fig. 3):
Clinical signs began to subside at the 5^{th} day post treatment in all treated groups except those of group 2 and 9, where signs were stopped at the 6^{th} day. Total morbidity was 25.00 in treated group 2, 6 and 20.83 in both 1 and 7, while other groups showed lower rates (table 2). Groups 5 and 9 those treated with Enrofloxacin showed mortalities lower than those treated with Colistin (4 and 8), this result was reversed in groups infected with *E.coli* O11 and *P.aeruginosa.*

The infected groups showed in table 1 and fig 1 showed higher morbidity and mortalities than those in treated groups in table 2.

DISCUSSION

Many bacterial infections have economical importance in laying breeder flocks including *E.coli* **(Bisgaard and Dam, 1981 and Gazdziniski and Barnes, 2002)**, *Salmonella gallinarum pullorum* **(Shivaprasad, 2000)** and *P. aeruginosa*; through there effect on ovary inducing pathological lesions, and lowered fertility, hatchability and egg production **(Saif et al., 2003)**.
The positive results of serum plate agglutination test to *Mycoplasma* sp. indicated that the flocks under test are infected. It was found that *Mycoplasma gillisepticum* and *synoviae* increase the susceptibility to infection with *E. coli* as stated by **(Gross, 1990; Nakamura et al., 1994; MacOwan et al., 1982 and Van de Zande et al., 2001)**.

The negative results of serum plate agglutination test to *S.g-p* can be attributed to that reported by **Gast and Beard. (1990)** where hens infected with antigenically intermediate or variant strains of *S.pullorum* were detected as seropositive less often than hens infected with antigenically standard strains. Therefore, ovaries with lesion were recommended by **Shivaprasad (2000)** to be involved in sampling for isolation.

Results of bacteriological isolation and identification proved the isolation of *E. coli* as reported by **Abd El-Nasser (1976)**, **Osklokov and Saltykov (1976)**, **Zahdeh (1982)** and **Azzam (1983)**. Serologically, these isolates were typed to be O11 as stated by **Abd El-Nasser (1976)** and **Andrawis (1980)**; while O78 was reported also by **Karmy et al., (1987) Singab (1987)** and **Riad (1994)**.
S.g-p was identified as an ovarian isolate of laying chickens; this result was stated by **Saif et al. (2003)**.
On the other hand *P. aeruginosa* was detected from ovaries with lesions **(Kheir El-Din et al., 1986; Batra et al., 1982 and Sharma et al., 1980)**.
The in vitro sensitivity test of such strains proved variable results to the used drug discs. This point was reported by **Azzam (1983)** and **Raid (1994)**. The most effective antibacterial drugs were Colistin sulphate and Enrofloxacin; as detected previously by **Raid (1994)**, while **Abd El-Ghafar (1979)** proved resistance of all tested isolates to Colistin. **Abd El-Wahab (1977)**, **Welsh et al. (1997)** and **Salmon and Watts (2000)**, reported the sensitivity of the isolates to Enrofloxacin.

For studying the pathogencity of the identified organisms to 3 day-old chicks, oral rout was used for the experimental infection with 24 hours broth cultures. *E. coli* infected birds showed clinical signs and lesions similar to those reported by **Abd El-Wahab (1977)**, **Bassiouni et al. (1979)** and **Azzam (1983)**.

Mortality in groups infected with *E. coli* ranged 20.83 in group 2 (O11) to 45.83 in group3 (O78), these results are higher than those of **Davis (1938)** as *E. coli* induced losses 15-40% in chicks less than 10 days of age. The detected lesions are similar to those previously reported by **Azzam (1983)** and **Singab (1987)** who reported gross lesions including pericarditis and perihepatitis 3-7 days post infection; while airsaculitis was also reported by **Butra et al. (1973)**.

Results of infection with *E. coli* O11 and O78 and the control non infected group (Table 1 Fig. 1). Also, results of body weight supported that *E.coli* affect significantly body weight as compared with non infected control. Results in group 3 that received O78 are lower than those of group received O11. It is clear that O 78 was reported to be commonly pathogenic to chickens **(Harry, 1964 and Sojka, 1965)**. Moreover, **Bassiouni et al. (1979)** and **Burkhanova (1980)** reported that *E. coli* O78 was highly pathogenic to 3 day-old chicks and it was more pathogenic than O11 to the inoculated chicks. Both **Butra et al. (1973)** and **Alian (1978)** recorded 50% mortality in day-old *E. coli* O78 orally infected chicks.

Group 4 infected with *S.g-p* showed signs, lesions, morbidity rate (91.60 %) and mortality rate (58.50 %). **El-Kady (1986)** reported similar signs and lesions in 2 day-old chicks but only 8.54% mortality rate.

Our recorded signs and lesion in chicks of group 4 infected with *P.aeruginosa* are similar to those recorded by **Hamouda et al. (1987)**. The recorded mortality (50.0 %) was lower than those of Hamouda et al. (1987) which was 56%; while, **Lin et al. (1996)** sated that *P.aerugienosa* can cause 50-100% mortality in experimentally inoculated 4 week-old chickens.

Body weight in group 4 is lower than that of group 1. Similar result as found by **Kheir El-Din et al. (1986)** who reported lowered growth rate in inoculated 7 day-ld chicks.

Results of the effect of Colistin and Enrofloxacin in the treatment as recommended by the results of the in vitro test pointed out that both drugs were effective in reducing morbidity, mortality and restoring body weight in the treated groups ; regardless the organism; as compared with negative and non treated control.

This study pointed out that *E. coli*, *Salmonella gallinarum-pullorum* as well as *P.aeruginosa* can cause reduction in the breeder flocks performance, especially when they are serologically positive to *Mycoplasma* sp. The antibiogram is the must for controlling of such infections. Using of low price old antibiotics can give results similar to the new generation of them when ideally used.

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