قسم: أمراض الدواجــن٠ كلية الطب البيطري ـ جامعة أسيوط٠ رئيس القسم: د٠ مصطفى عبدالمطلب شحاته٠

حدوث اصابات السودموناس في الدجاج بصعيد مصر

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تم في هذا البحث عزل ٢٦ عترة بنسبة ٢٥,٥١٪ من ميكروب السودموناس من أجنة البيض الميت، هذا بالاضافة الى عزل نفس الميكروب من الكتاكيت وبدارى الدجساج وأيضا من الدجاج البياض والسليم ظاهريا بنسب عزل ١٨,٢٣٪، ١,٥٤٪، ٥٥,٥٪، ١,٥٤٪ على التواليي.

وقد صنفت السودموناس المعزولة (٦٨ عترة) بالتفاعلات البيوكيميائية الى الانسواع التالية: سودموناس أورجيتوزا بنسبة ٥,٥٨ سودموناس فيزكيولارز (٩٦,٤٪) سودموناس أورفيشنس (١,٠٤٪) سودموناس دايمنيوتا (١,٠٤٪) سودموناس فلافا (١,٠٣٪)٠

تم اجراء اختبار العدوى الصناعية في أجنة بيض الدجاج عمر ٧ أيام بالحقن في كيس المخ أو التغطيس في مستبت الشوربة الذي يحتوي على الأنواع المعزول وسودموناس أورجينوزا سودموناس فيزكيولار وكذلك أحد الأنواع المصنفة عالمي السودموناس أورجينوزا)، قد أثبت أنهذه الميكروبات ضارية جدا وقد تم الحصول على نفس النتائج تقريبا باستخدام نواتج نمو العترات السابقة عن طريق الحقن في كيسس المخ في حين أن استخدام نواتج نمو العترات السابقة عن طريق التغطيس قد وجد أنها أقبل ضراوة •

أما بالنسبة للعدوى الصناعية في الكتاكيت بنفس العترات السابقة الذكر أونواتج نموها فقد ثبت أنالحقن تحت الجلد كان أكثر الطرق تأثيرا لحدوث العدوى٠

باختبار مدى حساسية العترات المعزولة لمختلف المضادات البكتيرية وجد أنها عالية الحساسية لكل من الرميكتان ، النيوميسين ، والجاراميسين •

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OCCURRENCE OF PSEUDOMONAS INFECTIONS IN FOWL IN UPPER EGYPT

(With 4 Tables and 4 Figures)

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SUMMARY

26 (25.24%) isolates of Pseudomonas spp, were detected from 103 dead chicken-embryos. In addition 18.23%, 6.35%, 5.55% and 1.54 of Pseudomonas spp, were recovered from baby chicks, growing chickens, laying hens and apparently healthy birds respectively. The isolated Pseudomonas spp (68 strains), were bicohemically differentiated into 5 species including: Ps. aeruginosa (5.58%); Ps. vesicularis (4.96%); Ps. aureofaciens (1.24%); Ps. diminuta (1.24%) and Ps. flava (1.03%).

Pathogenicity tests in 7-day old chicken embryos by yolk-sac inoculation or dipping in broth culture of Ps. aeruginosa (Reference
strain), Ps. aeruginosa (clinical isolate) and Ps. vesicularis clinical
isolated proved that these organisms were highly pathogenic with
high mortality rate. Similar results were obtained by using the
filtrates of the previous organisms by yolk sac inoculation. On the
other hand results of less pathogenicity with low mortality were
recorded by using the filtrates of the previous organisms through
dipping.

The experimental infection in baby chicks by different routs with the previous cultures or their filtrates, revealed that the subcutaneous route of inoculation was highly effective.

The isolated Pseudomonas spp from dead embryos and baby chicks were sensitive in vitro to Rifampin, Neomycin and garamycin.

INTRODUCTION

Several microbial infections are factors in the losses of poultry industry, from an economicview point Pseudomonas aeruginosa infection is not only responsible for embryonic mortality but also for mortality in chicks and heavy losses of broilers (VALADAE, 1961; SAAD et al., 1981; ANDREEV et al., 1982 and BAPAT et al., 1985).

KRISTIANSEN (1983) studied different types of media for the rapid isoaltion of Pseudomonas species.

The biochemical reactions of strains of Ps. aeruginosa isolated from dead in shell embryos and fowls were reported by SADASIVAN et al.(1979) and KIM et al.(1982), while MEITERT et al. (1981) studied the phage types and serotypes of Ps. aeruginosa.

ALI (1980) proved that Ps. aeruginosa strains isolated from dead embryos were pathogenic to young chicks.

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Reports on the antimicrobial sensitivity of Pseudomonas spp isolated from animals and birds have been made by CRESTEA et al. (1969) and CHAKRABARTY (1980).

Pseudomonas infection in birds did not receive much care in our country, therefore the work reported in this paper was undertaken to give an idea about the following:

- The incidence of Pseudomonas spp in dead chicken embryos and chickens of different ages.
- Experimental infection using the isolated organisms and reference strain as well as their filtrates in chicken embryos and chicks.
 - The antimicrobial sensitivity of the isoalted Pseudomonas spp.

MATERIAL and METHODS

Specimens and Bacteriological work:

Dead chicken embryos (103), dead baby chicks (181), growing and adults dead chickens (135) as well as 65 apparently healthy birds were obtained from governmental farms and balady hatcheries at Assiut and New-Valley provinces. Samples from embryonic-membranes, yolk sac, liver, embryonic fluids of chicken embryos and liver, spleen, kidney, heart blood, yolk sac of baby chicks and older birds were subjected to bacteriological examination. These samples were inoculated into Cetrimide agar and King's media, and incubated at 37°C for 48 hours. The suspected colonies were picked up and subjected to further identifications based on colonial and cellular morphology, pigment production, oxidase test, solubility of pigment in chloroform, sugar fermentation and other biochemical tests (BUCHANAN and GIBBONS, 1975 and WILSON and MILES, 1975).

Pathogenicity tests:

Embryonated chicken eggs and Baby chicks used in this study were considered healthy and free from Pseudomonas infection by bacteriological examination.

Reference strain: A reference Ps. aeruginosa, strain PAI was kindly obtained from J. BORST, National Institute of Public health, Bilthoven, the Netherland.

Filtrate preparation:

48 hours broth culture of the organism was taken, centrifuged and the supernatent was filtrated through seitz filter.

Experimental infection in chicken embryos :

Twelve groups (1-12), each consisting of 20 embryos of 7-day-old and another three groups (13-15), each of 10 embryos were used to study the pathogenicity test.

Groups 1,2 and 3, were inoculated via the yolk sac route by 0.1 ml. of 24 hours broth culture of Ps. aeruginosa (R), Ps. aeruginosa (C) and Ps. vesicularis (C) respectively, containal 14X10 viable cells per ml (SAAD et al., 1980).

- Groups 4,5 and 6, were dipped for 4 minutes in 24 hours broth culture of \underline{Ps} , $\underline{aeruginosa}$ (R), \underline{Ps} , $\underline{aeruginosa}$ (C) and \underline{Ps} , vesicularis (C) respectively at the same concentration used previously for inoculation.
- Groups 7,8 and 9 were inoculated via the yolk sac route by 0.1 ml of filtrate of the previous strains respectively.
 - Groups 10,11 and 12 were dipped in filtrate of the previous spp respectively.

Assiut Vet. Med. J. Vol. 20, No. 39, 1988.

- Groups 13 and 14 were inoculated and dipped in sterile broth respectively.
- Group 15 was left uninoculated as control.

N.B. (R) = reference strain (C) = clinical isolate.

All groups were separately reincubated and candled daily for 14 days. Embryos died during the incubation peroid and chicks that died after hatching were subjected to bacteriological examination and trials for reisolation of pseudomonas.

Experimental infection in baby chicks:

(285) 3-day-old chicks were classified into Nineteen equal groups each of 15 birds.

- Chicks of groups 1,2 and 3 were inoculated orally by 10⁸ organisms of Ps. aeruginosa
(R), Ps. aeruginosa (C) and Ps. vesicularis respectively.

- Chicks of groups 4,5 and 6 were inoculated intranasally by 10 of the previous organisms respectively.

Chicks of groups 7,8 and 9 were inoculated subcutaneously by 10⁸ of the previous organisms respectively.

Chicks of groups 10,11 and 12 were administered orally with filtrate of the previous spp. respectively.

Birds of groups 13,14 and 15 were inoculated I/N with filtrate of the same organisms respectively.

Birds of groups 16,17 and 18 were inoculated S/C with filtrate of these organisms respectively.

Birds of group 19 were Kept without treatment as control.

Inoculated and control birds were kept in isolated pens and observed for 4 weeks and specimens were collected for reisolation of Pseudomonas from dead bird.

Sensitivity test:

In vitro antibiotic sensitivity testing of identified Pseudomonas spp was performed by the disc plate technique described by BLAIR et al. (1970). Discs were prepared according to the method recorded by STAKES and WATER-WORTH (1972). The types of antibiotics and their concentration used included Rifampin (30 ug), neomycin (10 ug), gentamycin (10 ug), amikacin (30 ug), kanamycin (30 ug), streptomycin (10 ug), chloramphenicol (30 ug), tetracycline (30 ug), erythromycin (15 ug) and Penicillin (10 IJU).

RESULTS

Table (1) shows the frequency of different species of Pseudomonas isolated in this investigation. From table (1) it is shown that the incidence of Pseudomonas spp. was higher in dead embryos than that of other groups.

The results of pathogenicity tests in chicken embryes and baby chicks are given in Tables (2 and 3).

Table (4) illustrates the effect of different antibiotics on the isolated Pseudomonas strains.

DISCUSSION

Recently Pseudomonas infection is of considerable importance to the poultry industry as a cause of losses, especially in embryos and young chicks.

Assiut Vet. Med. J. Vol. 20, No. 39, 1988.

In this present study, out of 103 dead embryos during incubation, 12 isolates were identified to be Ps. aeruginosa. In similar studies the same organism have been isolated by the investigators, ZAGOEVSKI (1956) and ALI (1980). The percent of the isolated Ps. aeruginesa by the authors (11.65%) was nearly the same percentage reported by RANES and SZALY (1974) and NASHED (1981) from dead embryos and unhatched eggs.

Bacteriological examinations of dead birds (chicks) revealed that the organism was isolated in a high percent (18-23). The results agreed to some extent with those of RAY and BANERJI (1969), MAZZETTI (1972), MARKARYAN (1975) and AWAAD, et al. (1980) who detected the organism from dead chicks associated with mortalities from 4-5 to 90%.

The present work recorded the isolation of Pseudomonas spp from chickens other than those of young ages with a low percentage.

It is worthy to mention that the isolation of Ps. vesicularis, Ps. aureofaciens, Ps. diminuta and Ps. flava from embryos and chickens were reported for the first time in this work.

Experimental infections of 7-days-old chick embryos either by inoculation via yolk sec or dipping on broth culturs of Pseudomonas spp indicated that the isolates were highly virulent leading to deaths of 75-100% of the infected embryos. Similar findings were reported by SAAD et al. (1980). The pathogenic effect of Ps. aeruginosa in chicken-embryos was studied also by SRINIVASAN (1977) and NASHED (1981) who concluded that the organism was responsible for a high percent of unhatched eggs and embryonic deaths.

The experimental infections in baby chicks by different routs with cultures of Pseudomonas spp revealed that the subcutaneous route of inoculation was highly effective with high mortality rate varied from 86.67%-100 %. The pathogenic effect of Pseudomonas spp in chicks was reported also by VALADAE (1961), RAY and BANERJI (1969), MARKARYAN (1975) and AWAAD et al. (1981). Reisolation of the organism from dead and sacrified birds were conducted, this result disagreed with the work of EL-NASAAN et al. (1975).

In this present study, the experimental infections in chicken embryos through yolk sac inoculation by using the fitrates of Ps. aeruginosa (R), Ps. aeruginosa (C) and Ps. vesicularis (C). proved that the filtrates were highly pathogenic and the mortality rate ranged from 65-100%. While results of less pathogenicity with low mortality (25-35%) were recorded by using the filtrates of the previous organisms through dipping. On the other hand the experimental infections in chicks with filtrates of the previous species indicated that the subcutaneous route was highly effective with moderate mortality rate.

The antibiotic sensitivity of Pseudomonas strains isolated from dead chicken embryos and baby chicks in this study in a descending order was as such: rimectane, neomycin, garamycin. Amikacian, Kanamycin, streptomycin, chloramphenicol and tetracycline. While, erythromycin and penicillin had no effect at all. Our results are in agreement with those published by CRESTEA et al. (1969) and SHIMITZA and SHIBITA (1969). Our results agreed but to some extent with the findings of AWAAD et al. (1981), CHAKRABARTY et al. (1980) and BAPAT et al. (1985).

In conclusion, it is worthy to say that, recently Pseudomonas infections appeared to be of high significance among the poultry flocks.

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Table (1): Shows the frequency of different species of Pseudomonas

Laying hen old chick 4-week-Above chicks Baby Dead healthy birds Apparently embryo Specimens Total 484 65 103 63 181 72 No. of samples Ps. aerug-27 No inosa 12 12 0.00 0.00 11.65 4.76 6.63 96 Ps.vesicularis No 14 24 6 4.96 0.00 5.55 0.00 4.97 5.83 96 Ps.aureofaciens Ps.diminuta No 6 1.24 0.00 96 0.00 0.00 1.66 2.91 No. 6 1.24 0.00 0.00 0.00 1.66 2.91 200 Ps.flava 1 No 1.03 1.54 0.00 1.59 0.55 1.94 96 33 8 26 No Total 14.05 18.23 1.54 5.55 6.35 25.24 96

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Days Infection Table (3): Showing the results of pathogenicity in chicks 46.67 oral Ps.ae ruginosa 33.33 1/N S/C 100 (C) 40.02 Inoculation with broth culture oral Ps. 26.66 aeruginosa (R) Z S/C 86.67 20.01 Oral Ps.vesicularis (C) 1/N S/C 13.33 93.33 20.01 20.01 66.67 Ps-aeruginosa (C) oral I/N 5/C Inoculation with filtrate 13.33 oral Ps.aeruginosa (R) 6.67 N S/c 53.33 20.04 oral I/N Ps.vesicularis 13.33 S/C 60 Coatrol

Penicillin Erythromycin Tetracycline Chloramphonical Streptomycin Kanamycin Amikacin Garamycin Neomycin Rimectane Antibiotics NO 22 26 Dead chicken embryos (26 strains) 69.23 84.62 84.62 92.31 50.00 96 No 50.00 30.77 15,38 15.38 53.85 100 200 No Baby-chicks (33 strains) 69.69 84.85 78.79 87.88 51.57 84.85 42.42 36 10 No R 48.48 57.58 30.30 21.21 15.15 15.15 12.12 96 50 No Total S 44.07 50.85 69.49 81.36 84.75 88.14 93.22

Table (4): Antibiotic sensitivity of Pseudomonas spp isolated from dead chicken embryos and Baby chicks.