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بعض الدراسات عن كولوليرا البط في الوادي الجديد

٤- كفاءة اللقاحات المحضرة باستخدام الفورمالين

طلبه يونس ، بخيت سالم ، كمال الزناتي، مصطفى شحاته ، ابراهيم سكر

تم تحضير لقاح ذاتي من العترة (٢ د) من الباستيرلاملتوسيدا السابق عزلها عن الوادي الجديد ، وكذلك تحضير اللقاح الثنائي من العترات (٢ د + أ) وقد تم استنابت تلك العترات على التريتوز آجار •

كما تم تجريب اللقاح في البط الصغير السن بحقنه مرتين تحت الجلد في منطقة الرقبة عند عمر ثلاث سنوات وخمس أسابيع •

ولم تحدث أي عدوى في البط المحصن باللقاح (٢ د) أو (٢ د + أ) بعد حقنه بالعترة الضارية المماثلة على فترات بالمقارنة بالبط الغير محصن ، وقد استمرت المناعة ١٠٠% ستة شهور •

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**SOME STUDIES ON DUCK PASTEURELLOSIS IN THE NEW-VALLEY
IV. EFFICACY OF FORMALIZED MONOVALENT
AND BIVALENT BACTERINS**

By
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(Received at 9/5/1987)

SUMMARY

Autogenous bacterins were prepared from *Pasteurella multocida* (PM) type 2:D previously isolated from ducks in the New-Valley, as well as bivalent A and 2:D, which produced dense growth in tryptose agar. The bacterins were injected S/C into ducklings at 3 and 5 weeks of age, such ducklings experienced significant less mortality than unimmunized controls following challenge with live culture of the homologous strain at monthly intervals. Immunity lasted for (6) months with 100% protection.

INTRODUCTION

Vaccination is an ideal prevention of fowl cholera in ducks, and has been the subject of numerous studies, HILBERT and TAX (1938) and DOUGHERTY (1953).

Cholera is an economic problem in duck industry, autogenous bacterins prepared with formalin inactivated broth cultures has been reported to provide excellent control in the field.

At present in Egypt, an oil-emulsified bacterin is routinely used for protection of ducks whereas several outbreaks occurs, so this study was designed to develop a bacterin of higher immunogenicity than the most widely used types of bacterin currently available.

MATERIAL and METHODS

A) Materials:

1. Pasteurella multocida organisms:

The PM type 2:D were isolated from natural outbreaks in white pekin ducks at the New-Valley by ABDEL MOTELIB and SALEM (1986).

The PM type A (5, 8, 9) as lyophilized ampules were obtained from Serum and Vaccine Institute at Abbasia, Cairo.

2. Experimental birds and animals:

Sixty-one-day old balady ducklings were obtained from the duck farm Assiut Governorate and raised in isolated buildings. Drinking water and commercial duck feeds without antibiotics

T.Y. ABDEL MOTELIB, et al.

during challenge tests were given at libitum.

Thirty white mice of about 25 GMS weight and 4 weeks age were used.

3. Media:

a- Tryptose broth.

b- 10% blood agar: The media were prepared according to the methods described by CRUICKSHANK (1975).

c- Tryptose agar.

B) Methods:

Bacterin preparations:

Pasteurella strains were reconstituted by using sterile saline and then injected in mice; cultures were made on blood agar from dead mice to isolate the organism and to ensure its purity. From the pure culture, on the blood agar, the organism was subcultured in tryptose agar and after 24 hours cultures were washed with saline. Colony forming unit performed by matching using Macferland nephelometer barium sulphate standard, 10⁹ CFU per/ml.

Formalin was added in a concentration of 1% to inactivate the cultures for 48 hours, at room temperature. The adjuvants (mineral mixture and lanoline) were added in equal volume after autoclaving.

Two separate bacterins were prepared one from 2:D separately and the other from 2:D + A (5, 8, 9).

Safety and sterility tests for the bacterins were carried out as follows:

a- Films stained by Gram's stain were made to detect any contamination.

b- Subcultures from the bacterins were made on tryptose broth and blood agar after 24 hours, incubation at 37°C.

c- Two white mice were subcutaneously injected with 0.2 ml. of the bacterins to observe any death.

Evaluation of the bacterins:

For evaluation of the bacterin, 2 groups of ducklings, each group contained 24 ducks, the first group was immunized with bacterin of type 2:D and the other group with type 2:D + A using 0.5 ml. S/C in the neck twice at 3 and 5 weeks age, and were reared with 12 ducks as control.

They were challenged by intramuscular injection in the leg with 0.5 ml. of a 10⁻⁶ dilution of a 24 hours tryptose broth culture (150 CFU) of 2:D or A + D strains, 4 birds for each, at monthly interval, as well as two ducks as control birds.

Mortality was recorded for 14 days post-challenge, all ducklings that died were necropsied, blood films were taken as well as their organs were cultured for isolation of pasteurella by streaking on blood agar.

RESULTS

Monovalent and bivalent bacterins were effective in preventing deaths caused by homologous pasteurella multocida challenge at all intervals till (6) months, giving 100% survival rates.

DUCK PASTEURELLOSIS

Pasteurella multocida was isolated from the heart blood, liver, or spleen of all control ducks that died, while no isolations were recorded from immunized ducks killed at the end of the observation period.

None of the ducks killed at the end of the experiment yielded PM lesion scores.

DISCUSSION

The study demonstrated that the prepared bacterin was effective in inducing immunity in ducks.

When ducklings were immunized at 3 and 5 weeks of age with bacterin prepared from type 2:D and from A + 2:D, 100% protection at monthly challenge till (6) months that killed 100% of the controls.

Since the primary aim of this study was to develop a bacterin, with severe challenge method, i.e., intramuscular inoculation of 150 CFU, was employed to ascertain uniform infection, but when unvaccinated controls show 100% infection any protection of vaccinated birds indicates a relative immunity.

It is of interest that results of challenged vaccinated birds which were killed at the end of the observation period showed no lesions, except in one bird. These lesions are distinct from those observed in control birds dying acutely which, invariably showed lesions of septicemic nature and isolations of PM from all organs and tissues cultured, this findings are in agreement with MATSUMOTO and HELFER (1977).

The use of the agar-grown bacterin in immunizing ducklings produce 100% protection lasted for (6) months, more or less similar results were observed by HEDDLESTON and REISINGER (1959) who found that immunity persisted for 21 and 54 weeks in turkeys and chickens respectively.

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