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عدوي باستيريلا ملتوسيدا وباستيريلا أناتيبيستفر فى البط
٢ - دراسات مناعية

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أمكن تحضير لقاح ضد ميكروبي باستيريلا ملتوسيدا وباستيريلا أناتيبيستفر لأول مرة فى مصر ، وبتقييم هذا اللقاح الزيتى أظهر كفاءة عالية عند حقنه مرتين أو ثلاثة فى حين أدى التحصين بجرعة واحدة الى نسبة منخفضة من المناعة عند قياسها بالعدوى المناعية .

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**PASTEURELLA MULTOCIDA AND PASTEURELLA ANATIPESTIFER
INFECTIONS IN DUCKS
II- IMMUNOLOGICAL STUDIES**

By

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SUMMARY

It is reported to the first time in Egypt on the preparation and evaluation of a bacterin against both *Pasteurella multocida* and *anatipestifer* infections. An oil-emulsion bacterin proved to be efficient when used twice or thrice, while single dose showed moderate dependable rate of protection as measured by challenge test.

INTRODUCTION

Various regimens have been used in recent years to immunize ducks against fowl cholera.

Vaccination have been attempted through the use of bacterins prepared from in vitro-propagated *Pasteurella multocida* containing various adjuvants (HEDDLESTON and REISINGER, 1960; MATSUMOTO and HELFER, 1977 and DAVIS *et al.*, 1970), or in Vivo and in Ovo-propagated *P. multocida* bacterins (HEDDLESTON and REBERS, 1972 and 1974).

In Egypt a trivalent formalized *P. multocida* oil-adjuvant bacterin is used for routine vaccination of ducks.

In the field, however, bacterins have been ineffective, probably because there are several serotypes which do not cross-protect (MATSUMOTO and HELFER, 1977). There are very few published reports on attempts to control *P. anatipestifer* infection through vaccination with monovalent or polyvalent bacterins (SANDHU, 1979).

This investigation was undertaken to develop and study the efficacy of a trivalent bacterin containing an inactivated locally isolated cell suspension of two *P. multocida* serotypes 5:A and 2:D and one serotype of *P. anatipestifer*.

MATERIALS and METHODS

Bacterin Preparation :

24 hours Tryptose broth cultures were prepared from isolated field strains of *P. multocida* serotypes 5:A and 2:D as well as *P. anatipestifer*.

Cultures were adjusted to 10 X McFarland No. 1 density, then broth cultures were mixed with each other, one liter of each.

Cells were inactivated by 0.25% Formaline at room temperature for three days with periodical shaking.

Water-in-oil emulsified bacterin was prepared from the bacterial suspension after HEDDLESTON(1962).

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Bacterin was tested for safety by bacterial isoaltion and mice inoculation.

Vaccination trials were performed under field condition in White Pekin Duck Farms of El-Wady El-Gadeed, at 2 week of age in fattening ducks and at 2,8 and 24 weeks in breeder ducks. 0.5 ml of bacterin was injected subcutaneously. Parallel non vaccinated controls were isolated till the end of exp.

Challenge :

Groups of 10 birds each were inoculated intramuscularly (IM) with 1 ml. of 24 hours Tryptose broth culture of *P. multocida* (5:A and 2:D) and *P. anatipestifer*.

Mortality was checked daily for two weeks, dead and servivor birds were necropsied and examined pathologically and subjected to bacterial reisolation.

RESULTS

Results of challenge of rardon samples from ducklings vaccinated at 2 weeks of age revealed variable rate of protection as shown in table (1).

Breeder ducks vaccinated at 2 and 8 weeks of age showed high rate of protection at 2,4,6 and 8 weeks post-vaccination, table (2).

Adult breeder ducks revaccinated at the onset of egg-production showed the maximal rate of protection table (3).

Challenge of the control non vaccinated ducklings and ducks resulted in 100% deaths and all birds were positive for reisolation of inoculated organisms.

Specificity of infection in dead challenged birds was assured by detection of specific lesions to Cholera and positive reisolation cultures, control vaccinated and non vaccinated non-challenged birds remained viable during the experiment.

DISCUSSION

Since the primary aim of this study was to develop a bacterin, a sever challenge method by intramuscular inoculation was employed to ascertain uniform infection. A better protection might result in birds when exposed by a milder method such as contact exposure or challenge via drinking water. It is often difficult to interpret laboratory results in terms of real protection, but when unvaccinated controls shows 100% infection, any protection of vaccinated birds indicates a relative immunity (MATSMUTO and HELFER, 19777).

Abacterin prepared from *P. multocida* and *P. anatipstifer* was prepared and evaluated to the first time in Egypt. It is clear from our results that ducklings vaccinated once at two weeks of age showed relatively moderate independable rate of protection ranging from 50-80%.

On the other hand birds vaccinated twice or three times showed high rate of protection which reached 80-100%.

It could be concluded that one or two booster doses of the bacterin are necessary to induce dependable rate of protection aganist possible field infection as described by SANDHU (1977).

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Table (1): Results of Challenge in fattening ducks vaccinated at 2 weeks of age.

Inoculated Organisms	Weeks Post-Vaccination							
	2 Weeks		4 Weeks		6 Weeks		8 Weeks	
	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%
<i>P. multocida</i>								
5:A serotype	4/10	60	5/10	50	4/10	60	5/10	50
2:D serotype	3/10	70	3/10	70	4/10	60	4/10	60
<i>P. anatipestifer</i>	2/10	80	3/10	70	3/10	70	4/10	60

Table (2): Results of challenge in breeder ducks vaccinated at 2 and 8 weeks of age.

Inoculated Organisms	Weeks Post-Vaccination							
	2 Weeks		4 Weeks		6 Weeks		8 Weeks	
	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%
<i>P. multocida</i>								
5:A serotype	2/10	80	1/10	90	1/10	90	0/10	100
2:D serotype	2/10	80	1/10	90	0/10	100	1/10	90
<i>P. anatipestifer</i>	0/10	100	1/10	90	1/10	90	2/10	80

Table (3): Results of challenge in breeder ducks vaccinated at 2, 8 and 24 weeks of age.

Inoculated Organisms	Weeks Post-Vaccination							
	2 Weeks		4 Weeks		6 Weeks		8 Weeks	
	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%	Dead/ Inoc.	Prot. Rate%
<i>P. multocida</i>								
5:A serotype	0/10	100	0/10	100	1/10	90	0/10	100
2:D serotype	0/10	100	0/10	100	0/10	100	1/10	90
<i>P. anatipestifer</i>	0/10	100	0/10	100	0/10	100	1/10	100

Challenge were done 14 days post-last vaccination.