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تحضير لقاح فورمالينى متعدد ضد مرض الباستيرلا فى الأغنام

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تم تحضير لقاح فورمالينى متعدد من عترات أ ، ب ، هـ ، لميكروب الباستيرلا مالتوسيدا .
القوة الضاربة للماعز قد تم تقديرها بواسطة اختبار تلاقن الدم الغير مباشر
بعد اسبوعين وثلاثة وأربعة أسابيع من التحصين .

وقد أكدت الاختبارات أن هذه العترات لها صفة مناعية بالنسبة للأغنام .

PREPARATION OF A FORMALIZED POLYVALENT VACCINE FOR SHEEP PASTEURERLOSIS
(With 3 Tables)

By

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SUMMARY

A polyvalent formalized vaccine was prepared from *Pasteurella multocida* types A, D and E strains (virulent strains to sheep). The titre in vaccinated sheep were determined by the indirect haemoagglutination test after 2, 3 and 4 weeks following vaccination. The tests proved that *Pasteurella multocida* types A, D and E are immunogenic to sheep.

INTRODUCTION

Informations about protection of sheep against pasteurellosis were insufficient. In cattle, DHANDA (1959) described agglutination test to judge the immunity against haemorrhagic septicaemia. BAIN (1960) mentioned that serum from buffaloes and cattle immune to haemorrhagic septicaemia possessed both opsonins and bactericidal antibodies. CARTER (1963) concluded that type B and E strains can be easily distinguished immunologically by application of serum portection test in mice. In trials for detection and evaluation of antibodies to *Pasteurella multocida* in the serum, CARTER and RAPPY, (1963) applied the indirect haemoagglutination test.

The aim of this work was devoted for the preparation of polyvalent formalized vaccine from virulent strains to sheep and the evaluation of immunogenicity of such strains by in vitro tests.

MATERIALS AND METHODS

A- Materials:-

I- Pasteruella multocida organisms:

Lypholized ampules containing identified strains of *Pasteurella multocida* types A, D and E strains obtained from animal research health institute, Dokki, Cairo. (Strains A, D and E were standard and another E strain, a dog strain).

II- Experimental animals:

- a- Laboratory animals: 20 white mice of about 25 GMS weight, 4 weeks age.
- b- Sheep: 4 clinically healthy, 2-4 years old tested and proved to be free from internal and blood parasites.

III- Media:

- a- Sterile nutrient broth.
- b- 10% blood agar.

The media were prepared according to the methods described by (CRUICKSHANK, 1975).

- c- Petone media.

B- Methods:

Pasteurella strains were reconstituted by using sterile saline and then injected in mice. From the dead mice after inoculation, cultures were made on blood agar to isolate the orgainsm and to ensure its purity.

From the pure culture on the blood agar, organism was subcultured in nutrient broth for 6 hours then transferred to peptone water and incubated for 24 hours.

2- Safty and sterility tests to the vaccine were carried out as follows:

- a- Films stained by Gram's stain were made to detect any contamination.
- b- Subcultures from the vaccine were made on nutrient broth and blood agar after 24 hours, incubation at 37°C.
- c- Two white mice were subcutaneously injected with 0.2 ml of the vaccine to observe any deaths.

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3- Standardization of the vaccine was performed by matching using Macfer land nephelometer barium sulphate standard (ABDEL-MOTY, 1972).

IV- Evaluation of the vaccine used: For evaluation of the vaccine, 2 groups were used. Each group of sheep contains 4 animals. All sheep were injected with the prepared vaccine as follows:

Group No. 1:

Each animal was injected (S/C) with a dose of polyvalent formalized vaccine containing 1500 million organisms. This dose contained 500 million from both E standard and E dog strains and 1000 million organisms from A and D strains).

Group No. 2:

Each animal was injected (S/C) with a dose of bivalent vaccine containing 1000 million organisms from strains A and B. Sera were obtained from injected sheep 2, 3 and 4 weeks after injection. The titers were determined against a polyvalent antigen contained types A, D, E standard and E dog strain. Titers were determined also against each strain antigen using the indirect haemoagglutination test (CARTER, 1955).

RESULTS AND DISCUSSION

Titers after 2, 3 and 4 weeks were shown in Tables No: 1, 2 and 3.

The results of the indirect haemoagglutination tests after 2, 3 and 4 weeks indicated that *Pasteurella multocida* types A, D and E were immunogenic to sheep. This was confirmed by the detection of the specific immune antibodies in serum using the haemoagglutination test (BAIN, 1960, CARTER and PAPPY 1963).

However, the informations concerning sheep protection against sheep pasteurellosis were insufficient. The present work needs further investigations concerning the dose, route of application, duration of immunity and the suitable time for vaccination.

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Table (1): Results of indirect haemagglutination tests after 2 weeks.

Types of sera	Types of K antigen	Titre
<u>Group No. 1.</u>		
serum of the 1 st sheep	§ polyvalent K antigen	$\frac{1}{80}$
serum of the 2 nd sheep	Polyvalent K antigen	$\frac{1}{160}$
<u>Group No. 2.</u>		
serum of the 1 st sheep	palyvalent K antigen	$\frac{1}{20}$
serum of the 2 nd sheep	polyvalent K antigen	$\frac{1}{20}$
pooled sera from the 1 st group	K antigen of type A standard	$\frac{1}{10}$
	K antigen of type D standard	$\frac{1}{20}$
	K antigen of type E standard	$\frac{1}{20}$
	K antigen type E "dog strain"	$\frac{1}{40}$

§ contained K antigen of strains A,D,E (standard strains) and E "dog strain"

Table (2): Results of indirect haemagglutination tests after 3 weeks.

Types of sera	Types of K antigen	Titre
<u>Group No. 1.</u>		
serum of the 1 st sheep	polyvalent K antigen types	$\frac{1}{320}$
serum of the 2 nd sheep	A,D,E standard strains and type E (dog strain)	$\frac{1}{640}$
<u>Group No. 2.</u>		
serum of the 1 st sheep	polyvalent K antigen types	$\frac{1}{80}$
serum of the 2 nd sheep	A,D,E standard strains and type E (dog strain).	$\frac{1}{80}$
pooled sera from the 1 st group	K antigen of type A st.	$\frac{1}{80}$
	K antigen of type D st.	$\frac{1}{80}$
	K antigen of type E st.	$\frac{1}{20}$
	K antigen of type E E (dog strain).	$\frac{1}{20}$

Table (3): Results of indirect haemagglutination tests after 3 weeks.

Types of sera	Types of K antigen	Titre
<u>Group No. 1.</u>		
serum of the <u>1st</u> sheep	polyvalent K antigen types	$\frac{1}{320}$
serum of the <u>2nd</u> sheep	A,D,E standard strains and type E	$\frac{1}{640}$
<u>Group No. 2.</u>		
serum of the <u>1st</u> sheep	polyvalent K antigen types	$\frac{1}{80}$
serum of the <u>2nd</u> sheep	A,D,E standard strains and type E (dog strain).	$\frac{1}{80}$
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pooled sera from the <u>1st</u> group	K antigen of type A st.	$\frac{1}{80}$
	K antigen of type D st.	$\frac{1}{80}$
	K antigen of type E st.	$\frac{1}{20}$
	K antigen of type E (dog strain).	$\frac{1}{20}$