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التأثير الوبائى للتحصين بالكورايني أوفـــى على العدوى بجدرى الأغنام

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اتضح من هذه الدراسة أن حقن الأغنام بجرعة واحدة من الكورينى أوفز تحتوى على ٧٫٥ × $١٠^{٨}$ من البكتريا والمعامل بالكريستال فيوليت تركيز ١ / ٠٠٠٫٧٥٠ له تأثير مناعى مساعد ضد فيروس جدرى الاغنام الضارى . وكانت أعلى درجـــات المقاومة لفيروس الجدرى بعد ٢١ يوم من الحقن بالكورينى حيث هبطت بشدة بعد ذلك .

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THE PROTECTIVE EFFECT OF VACCINATION WITH CORYNE BACTERIUM OVIS ON SHEEP POX INFECTION

(With 2 tables)

By

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SUMMARY

Data presented in this study indicates that the injection of a single dose of 7.5×10^8 of c.ovis treated with 1 : 750000 crystal violet has a reasonable immuno-potentiating effect in sheep which enables them to show a measurable resistance to experimental inoculation with virulent sheep-pox virus. This c.ovis induced resistance which was highest at 21 days post inoculation and dropped sharply thereafter.

INTRODUCTION

The Egyptian farm animals are subjected to a wide range of stress factors (microbial, parasitic, nutritional, environmental) which in most cases show a side immunosuppressing effect. Thus a search for an immunopotentiating agent seems quite logic in our struggle against infectious diseases of animals.

BCG has already proved its value as an immunopotentiator as shown by international and local experiences (1,2,4).

However, for fear of side-hazzards and interference with T.B- eradication programm, another immuno-stimulant is urgentley needed.

More recent studies on crystal violet treated C.ovis have clearly demonstrated its value as a non-specific immunostimulant which can raise the resistance of sheep to artificial infection with potential pathogens. It has even an adjuvant effect when mixed with microbial or inert antigens (3).

The here presented study is a further step in that direction, aiming to through some light on the non-specific immuno-potentiating capascity of C.ovis as it can be measured in relation of such a dangrous viral disease as sheep pox.

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MATERIAL and METHODS

1) Virus:

a) Modified-live sheep pox virus vaccine "Romanian Strain, was grown in susceptible lambs, lyophilized and stored at -20°C until used. The field dose of the reconstituted vaccine was 0.5 ml containing 100 LD_{50} of infectious viral particles, inoculated intradermally (I/D).

b) Challenge virus: The Egyptian virulent sheep pox virus, lyophilized and stored at -20°C was used. The challeng dose of reconstituted virulent virus was 0.5 ml containing 1000 ID_{50} to be inoculated I/D.

2) C.ovis:

This is a local strain isolated from sheep (3). It was used in the form of a suspesion containing 7.5×10^8 bacterial cells/0.2 ml in a diluent containing variable concentration of crystal violet.

3) Sheep:

Susceptible Barky sheep were selected for this study. They ranged in age from 4-8 months. They had no previous history of sheep-pox or c.ovis infection or vaccination.

Determination of resistance of sheep by titration of the virulent sheep pox virus strain in sheep (challenge):

For the determination of the degree of resistance confered to sheep either due to their specific immunization by sheep pox vaccine or to non-specific protection by C.ovis a titration of the virulent virus was done in such sheep. Ten-fold dilutions of the virulent virus were inoculated at both sides of the sheep, each dilution was injected at 3 sites and each site receiving 0.5ml I/D.

EXPERIMENTAL and RESULTS

Experiment I:

Sixteen sheep were divided into 5 groups as follows:

1) Three sheep each inoculated I/D with 1 ml C.ovis vaccine suspended in 1 : 100 crystal violet.
2) Three sheep each inoculated I/D with 1 ml C.ovis suspended in 1 : 250.000 crystql violet.
3) Three sheep each inoculated I/D with 1 ml C.ovis suspended in 1 : 750.000 crystal violet.
4) Three sheep each inoculated I/D with one dose of sheep pox vaccine (0.5 ml) under the tail.
5) Two sheep were inoculated I/D with 1 ml C.ovis which was not treated with crystal violet.
6) Two sheep were kept as control without any inoculation. C.ovis injection was conducted on the right thigh and the animals were observed for any local reaction.

All sheep were challenged with sheep pox 21 days post vaccination. Results of this experiment are shown in table I.

It is clear from table I that, vaccination of sheep with C.ovis had a fair protective effect against challenge (infection) by virulent sheep pox virus. A dose of coryne ovis suspended in 1/750.000 crystal violet showed more protection against sheep pox than the same dose of C.ovis suspended in 1/100 or 1/250.000 crystal violet. The $\log_{10}$ reduction in the titre of challenge

virus in case of sheep vaccinated with C.ovis suspended in 1/750.000 crystal violet was 4.2 ID_{50} /0.5 ml, while in case of sheep receiving the conventional sheep pox vaccine, this reduction reached $\log_{10}$ 6.5 ID_{50}/0.5 ml, with a protection $\log_{10}$ diference of 2.3 ID_{50}/0.5 ml.

Experiment II:

From the results obtained by experiment I, it was found that the best concentration of crystal violet leading to the highst level of resistance against sheep pox infection, is 1/750.000. The bacteria were concentrated 5 times in the treated culture by centrifugation at 3000 r.p.m. for 20 minutes. In this experiment 36 sheep were also used, divided into five groups as follows:

1) Eight sheep, each was inoculated I/D with 0.2 ml C.ovis suspended in 1/750.000 crystal violet (one dose).
2) Eight sheep each inoculated I/D with 0.4 ml C.ovis suspended in 1/750.000 crystal violet (2 doses).
3) Four sheep each inoculated I/D with 0.8 ml C.ovis suspended in 1/750.000 crystal violet (4 doses).
4) Eight sheep each inoculated I/D with one dose of sheep pox vaccine (0.5 ml) under the tail.
5) Eight sheep were kept control without any inoculation. Sheep were kept under close observation for any local reaction. Two sheep from each group were challenged with virulent sheep pox virus 21 days-vaccination; Another 2 sheep from each group were challenged with virulent sheep-pox virus 3,6 and 9 months post-vaccination. Decimal dilutions of the virus were inoculated at both sides of the sheep, where each dilution was injected at 3 sites, each receiving 0.5 ml intradermally.

The degree of protection against challenge produced by the differents chedules of vaccination was determined both qualitatively and quantitatively. Thus, beside measuring the quantitative reduction in the titre of the challenge virus in vaccinated animals as compared with unvaccinated controls; it was determined also qualitatively the degree of protection by evaluating the decrease in size and duration of the local skin reaction to the intradermally inoculated challenge virus in vaccinated animals as compared with controls. The degree of qualitative protection against challenge with sheep pox virus was categorized as follows:

1) Complete Protection:

Where no fever or dermal reaction occured.

2) Good protection:

When the diamete of the plaque-like skin and dermal reaction was not greater than one third of that for the control sheep.

3) Fair protection:

In which the dermal reaction was reduced (less than half the diameter of those in the controls).

4) No protection:

When the type and duration of skin reaction were similar to those of the control challenged sheep.

Results of this experiment are shown in table II.

N.B.:

All of the lambs inoculated with 0.8 ml of concentrated C.ovis suspended in 1/750.000 crystal violet (4 doses) died on the 4th day post vaccination and the post-mortem examination revealed toxaemia and jaundice.

Table II indicated that a great difference could be detected between the reaction of group (1) of sheep which were vaccinated by the sheep pox vaccine and those of other C.ovis vaccinated groups. Two doses of C.ovis (0.4 ml) showed more protection against sheep pox infection (4.4 log reduction 21 day post-vaccination) than the 0.2 ml dose (4.2 log reduction 21 day P.V.). By studing the duration of immunity confered by different inocula, it is found that it was nearly lost after 3 months in case of C.ovis inoculated lambs, while it persisted in the group vaccinated by sheep pox vaccine for more than 9 months.

DISCUSSION

Previous studies have clearly shown the non specific protective potentials of BCG against bovine and ovine diseases of different aetiology (1, 2, 4).

However, in order to avoid any possible hazzards for BCG application among the livestock, we tried here to draw the attention to the possible use of C.ovis as another candidate in the new-rapidly growing list of immunopotentiating agents.

The here presented study shows clearly that a dose of C.ovis containing 7.5×10^8 organisms treated with 1/750.000 crystal violet inoculated 21 days before exposure to a titrative challenge with virulent sheep pox strain resulted in a 4.2 log reduction in the titre of that virus as compared to unprotected susceptible controls. This figure although it denotes that treated C.ovis was able to induce some sort of a nonspecific resistance in sheep against infection with the virulent strain of sheep pox, yet this resistance is not as high in its protective effect as the specific immunity conferes by the attenuated sheep pox vaccine (a reduction of 6-5 logs in the titre of the virulent virus).

This level of non-specific protection compares well with the results obtained with BCG as immunopotentiation in face of sheep pox (2).

In the second experiment we tried to determine the duration of the non-specific protection produced by C.ovis vaccine treated with 1/750.000 crystal violet when it is given in one and double doses. As shown in table II, it is clear that this non-specific protection was highest 21 days post inoculation with C.ovis, while it steadily decreased and completely vanished by the 9th month post inoculation.

Meanwhile, the specific immuncresponse elicited by the sheep pox vaccine was still clearly demonstrated at the 9th month post vaccination. A slight but non significant difference could be detected between the effect of one or two doses of the C.ovis vaccine. However, it has to be stressed here that 4 doses of that C.ovis-crystal violet vaccine was rapidly fatal to 100% of inoculated sheep, a matter which should draw the attention to the danger of over dosage.

From the above data it could be concluded that the C.ovis crystal violet vaccine beside its protective specific immune response directed against C.ovis infection in sheep, it also produces

a certain level of non-specific protection of the inoculated sheep against one of the most dangerous virus diseases of sheep,namely sheep pox. The result without doubt would raise the demand for more comprehensive studies directed to analyse this non specific protective mechanism on the cellular level and throw some more light on the immuno-potentiating effect of C.ovis.

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Table (I)

The degree of protection confered by C.ovis vaccine with different concentration of crystal violet against challenge with sheep pox virus

Inoculum	$\log_{10}$/virus titre (ID_{50})	Protection as $\log_{10}$ reduction in ID_{50}	C.ovis protection in relation to sheep pox virus protection log differences	Qualitative protection
Sheep pox vacc.	1.8	6.5	0	Complete
C.ovis + C.v 1/100	4.7	3.6	2.9	Fair
C.ovis + C.v 1/120000	4.8	3.5	3.0	Fair
C.ovis + C.v 1/750000	4.1	4.2	2.3	Fair
Control (virulent sheep pox)	8.3	0	6.5	No

N.B. The two lambs which were inoculated with C.ovis died on the 4th day.

Table (II)

Duration of C.ovis protection against sheep pox virus in relation to sheep pox vaccine

Inoculum	$\log_{10}$/virus titre (ID_{50}) at different time				Protection as $\log_{10}$ reduction in ID_{50}/ at different times				C.ovis protection in relation to SPV protection (log difference)			
	day 21	3M	6M	9M	day 21	3M	6M	9M	day 21	3M	6M	9M
Sheep pox vacc.	1.8	2.1	2.5	3.1	6.5	6.2	5.8	5.2	0.0	0.0	0.0	0.0
One dose of C.ovis (0.2 ml)	4.1	5.4	6.8	8.0	4.2	2.9	1.5	0.3	2.3	3.3	4.3	4.9
Two doses of C.ovis (0.4 ml)	3.9	5.0	6.5	8.0	4.4	3.3	1.8	0.3	2.1	2.9	4.0	4.9
Control	8.3	8.3	8.3	8.3	0.0	0.0	0.0	0.0	6.5	6.2	5.8	5.0

SPV = Sheep pox vaccine.
M = month.