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دراسة أولية عن التحصين المزدوج في العجول البقري بلقاحي الطاعون البقري النسيجي والدرن

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في دراسة أولية عن التحصين المزدوج بلقاحي الطاعون البقري النسيجي والدرن في العجول البقري ـ اتضح أن التحصين المزدوج يحفز من سرعة تكون الأجسام المناعية المعادلة لفيروس الطاعون البقري في العجول المحقونة مسبقا بلقاح ب٠س٠ج ، وقد وجد أن بداية ظهور التأثير الباثولوجي لفيروس الطاعون البقري في خلايا الكلى البقري يتأخر عن المعتاد وذلك باختيار مصل العجول المحقونة بلقاح ب٠س٠ج فقط ، وقد ثبت أن استجابة العجول للحقن بمستحضر التيوبركلين متماثلة في الحيوانات المحقونة بلقاح ب٠س٠ج وتلك المحقونة بلقاحي ب٠س٠ج والطاعون البقري ٠

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A PRELIMINARY STUDY ON VACCINATION OF CALVES WITH RINDERPEST AND BCG VACCINES

(With 3 Tables)

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SUMMARY

A preliminary study on calves dually vaccinated with Tissue Culture Rinderpest (TCRPV) and BCG vaccines revealed that the development of serum-Rinderpest neutralizing antibodies was enhanced. The development of cytopathic effect (CPE) of Rinderpest virus on bovine kidney cells was retarted on carrying out serum neutralization test with serum of BCG-vaccinated calves. In calves dually vaccinated by (BCG & TCRPV) and by BCG, the skin reaction to tuberclin test was similar to mammalian and avian tuberclin and time of maximum reactivity.

INTRODUCTION

BCG vaccine, which is an attenuated strain of mycobacterium tuberculosis of bovine origin (HUMPHRY and WHITE, 1975), was reported to act as immunostimulant (FUDENBERG, et al. 1977). Apart from its specific use to control human and animal tuberculosis, it was reported to be non-specifically controlling some other diseases (BARAKAT, et al. 1981). TURK and PORTER (1969) and THOMAS (1970) reported that vaccination with BCG may result in untrue positive reaction to tuberculin test. BENTZON (1976) observed that human suffering from a viral infection or receiving viral vaccines show a decreased level of reaction to tuberculin inoculation.

The aim o this work was to speculate a probable immunostimulant effect of BCG to cattle plague and determine the reaction to tuberculin testing of calves vaccinated by (TCRPV & BCG) and BCG vaccines.

MATERIAL and METHODS

Virus:

Tissue culture adapted virulent Kabete O. virus at its 99th passage level in calf kidney cultures, kindly supplied by Dr. PLOWRIGHT and FERRIS (1962) was further passaged 1 to 3 times in primary calf kidney cells in Rinderpest Laboratory (SINGH, et al. 1967).

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Cell cultures:

Primary bovine kidney cultures were prepared by trypsinization. The dispersed cells were subsequently cultivated in growth medium consisting of Eagle's MEM with Hank's salts supplemented with 10% bovine serum. The medium contained 50 Ug of streptomycin and 100 units of penicillin per ml. Monolayers were prepared by adding 1 ml to 160-by-15 mm test tubes.

Vaccine titration:

Four ampoules of freeze-dried TCRPV were reconstituted with 8 ml of sterile Hank's balanced salt solution (HBSS). Tenfold dilutions were made with HBSS. For each dilution five calf kidney culture tubes were inoculated with 0.1 ml per tube of virus suspension. The tubes were incubated at 37°C and examined microscopically for CPE every other day. Medium was changed every 3 or 4 days. End points of 50% $TCID_{50}$ were culculated by the method of REED and MUENCH (1938).

Serum-neutralization test:

This was conducted qualitatively to determine the susceptibility of the test animals to Rinderpest. The quantitative test was done as follows:

Five-fold dilutions of sera collected from vaccinated calves at various intervals were prepared and each dilution was mixed with an equal quantity of TCRPV so that 0.1 ml of the virus dilution contains 100-200 $TCID_{50}$. After one hour of incubation at 25°C 0.2 ml of each serum virus mixture was added to each of the four culture tubes seeded 3 days previously with calf kidney cell suspension. The cultures were examined daily for 11 days for CPE. The medium was changed twice a week. Virus and normal bovine serum controls were included in all tests. The reciprocal of the final dilution of serum inhibiting the CPE of 100 to 200 $TCID_{50}$ was expressed as the serum titre (SINGH, et al. 1967).

BCG:

The strain used for human vaccination and produced by Jap. BCG Lab., Tokyo, Japan, was obtained through the Egy. Org. for Biol. and Vaccine production, Agouza, Cairo.

Tuberculin:

Both Mammalian and Avian tuberculins were obtained from the Serum and Vaccine Research Institute, Abbassia, Cairo.

Experimental Animals:

Sixteen calves of 6-10 months old were used. They were Rinderpest susceptible (as was checked by screening serum neutralization test), T.B. free (as proved by negative reaction to tuberculin test) and proved to be free from blood and internal parasites (as revealed by examination of peripheral blood samples stained with Giemsa stain and Faecal samples, (BODDIE, 1959).

Experimental design:

Calves were devided into four groups as follows:

Group (1): consisted of 6 calves dually vaccinated first with the BCG vaccine (in a dose of 10-20 million organisms) intradermally and the TCRPV (in a dose of 10^2 ID_{50}) subcutane-ously with six weeks apart.

Group (2): consisted of 4 calves vaccinated with TCRPV in the same dose as above.

Group (3): consisted of 3 calves vaccinated with BCG in the same dose mentioned before.

Group (4): Consisted of 3 non-vaccinated control calves.

Experimental calves were bled at indicated intervals and the serum samples were subjected to neutralization test for demonestration of the Rinderpest neutralizing antibodies.

Single intradermal comparative tuberculin test:

The method of HERBERT (1974) was applied in tuberculin testing of calves under experiment, before first vaccination in all groups, three months after BCG vaccination and thereafter three to four months later. Non-vaccinated control animals were also included in tests.

RESULTS

Serological response of BCG-sensitized and non-sensitized calves to TCRPV:

The results of serum neutralization test carried out on serum of calves vaccinated by (both BCG and TCRPV) and RCRPV vaccines, revealed that in group 1, Rinderpest neutralizing antibodies were not detected in BCG-vaccinated calves even after 40 days of vaccination. However, Rinderpest neutralizing antibodies could be detected 6 days posy TCRPV vaccination in 50% of these BCG-sensitized animals whereas in non-sensitized animals, Rinderpest neutralizing antibodies wer not demonstrable on the 6th day after vaccination. Thereafter, the antibody titre was approximately similar in both groups at 14, 21 and 60 days post vaccination, (Table 1).

Effect of BCG-sensitized calf serum on the onset of CPE of Rinderpest virus on bovine kidney cells:

It was found that rinderpest cytopathic effect in bovine kidney cells with serum samples of BCG-sensitized calves was first detected on the 4th, 5th and 6th day post inoculation of cells in each of 3 out of 9 (Table 2).

Reaction to tuberculin test in calves dually vaccinated by (BCG & TCRPV), BCG and TCRPV:

Negative rection to tuberculin was obtained in all calves before vaccinations (Table 3). It was also found that TCRPV - vaccinated group of calves negatively reacted to tuberculin test. On the other hand, dually vaccinated (BCG & TCRPV) and BCG-vaccinated calves reacted similarly to tuberclin test regarding reactivity which was more clear to mammalian than to avian tuberculin and maximum of reactivity which was at the 10th month post BCG-vaccination.

Effect of BCG - vaccinated calf serum on the onset of CPE of Rinderpest virus on bovine kidney cells:

Table 2, shows that the onset of CPE of Rinderpest virus on bovine kidney cells was delayed on carrying out serum neutralization with serum samples of BCG-vaccinated calves (Group I & III). It was first detected on the 4th day P.I. in 33.3%, on the 5th day P.I. in 33.3% and on the 6th day P.I. in 33.3% of the tested serum samples.

DISCUSSION

The live attenuated tissue culture Rinderpest vaccine proved to be safe and immunogenic for different types of Egyptian cattle and water buffaloes under a wide variety of field conditions (SINGH, et al. 1967 and OSMAN, et al. 1985). Serum Rinderpest neutralizing antibodies were reported to be detected on the 9th to the 11th day post vaccination (SINGH, et al. 1967). However, in the present study it could be detected on the 6th day post vaccination in 3 out of 6 BCG presensitized calves (Table 1). This can be interpreted in the light of the findings of CLARK, et al. (1970), who reported that BCG enhances the development of Rinderpest neutralizing antibodies. Neverthless, on the 21st day after TCRPV inoculation, antiboby titres were virtually identical in both BCG-sensitized and non-sensitized calves. Variations in antibody titres as revealed in table 1 could be attributed to individual immunological variations.

Rinderepst cytopathic effect on bovine kidney cells is known to be first detected on the 3th day after inoculation. This effect was shown to be delayed when testing serum samples of BCG-sensitized calves. It could be detected, hence, first on the 4th, 5th and 6th day post inoculation of cells in each of 3 out of 9 samples (Table 2). This retarted CPE may be attributable to a BCG stimulating effect on the reticuloendothelial system (SELL, 1972), or to nonspecific interferon production that may inhibit virus replication (RAFFAEL, 1971 and SALVIN, et al. 1973); or it could be attributed to both factors.

It was evident that vaccination of calves with TCRPV did not influence the immune response to BCG vaccination as revealed by reaction to tuberculin (Table 3). This was not in agreement with the findings of BENTZON (1976) who reported a decreased reaction to tuberclin in calves vaccinated with some viral vaccines. The controversy might be due to differences in viral vaccines.

CONCLUSION

It could be concluded that BCG-sensitized calves respond immunologically earlier to tissue culture Rinderpest vaccination. This might be of value in controling emergency cases of Rinderpest.

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Table (1)

Neutralizing antibody response of calves, BCG-sensitized and non-sensitized to TCRPV

Group of calves	Serial No. of calves	Serum Rinderpest neutralizing antibody * Titres (day P.I.) 40 days after BCG Vacc.	6	14	21	60
	1	0	0	16	100	100
	2	0	0	16	100	100
I	3	0	5	16	80	80
(BCG-sensitized)	4	0	0	10	80	80
	5	0	5	10	90	90
	6	0	5	16	80	80
	7		0	16	90	90
II	8		0	20	100	100
(non-sensitized)	9		0	10	80	80
	10		0	16	100	100
	14		0	0	0	0
IV	15		0	0	0	0
Control	16		0	0	0	0

* = Reciprocal fo sera dilutions.

Table (2)

Effect of BCG-snsitized calf serum on the onset of CPE of Rinderpest virus on bovine kidney cells

Animal group	Serial No. of calves	Time of sampling	onset of CPE	Time of sampling	onset of CPE
I (BCG-sensitized)	1	40 days after BCG sensitization	4*	21 days after RCRPV vaccination	
	2		5		
	3		5		
	4		6		
	5		4		
	6		6		
II	7	Non-sensitized (40 days)	3	21 days after TCRPV vacc.	
	8		3		
	9		3		
	10		3		
III (BCG-sensitized)	11	40 days after BCG sensitization	6	Non RCRPV-vaccinated	
	12		4		
	13		5		
IV Control	14	Non sensitized	3	Non TCRPV-vaccinated	
	15		3		
	16		3		

* = days post inoculation.

Table (3)

Reaction to tuberclin test in calves vaccinated by (BCG & TCRPV), BCG-vaccinated and TCRPV vaccines

Animal group	No. of calves	Vaccines used	Time of tuberculin test	Skin reaction m.m after application of tuberculin mamalian before ‰	mamalian after ‰	mamalian increase %	Avian after ‰	Avian incr. %
I	6	BCG & TCRPV	before vacc.	7.6	7.9	0.2	7.8	0.1
			3 month after vacc.	7.7	9.8	27.3	9.2	19.5
			6 after vacc.	7.6	12.0	57.9	10.8	42.1
			10 after vacc.	7.5	14.9	98.7	12.9	72.0
			14 after vacc.	7.6	14.4	89.5	12.4	63.2
II	4	TCRPV	before vacc.	5.8	6.0	0.3	5.9	0.17
			3 months after vacc.	5.8	6.0	0.3	5.9	0.17
			6 after vacc.	5.7	6.1	0.7	6.0	0.5
			10 after vacc.	5.7	6.0	0.5	6.3	1.1
			14 after vacc.	5.6	6.2	1.0	6.4	1.7
III	3	BCG	before vacc.	6.5	6.7	0.3	6.6	0.2
			3 months after vacc.	6.6	8.3	25.8	7.8	18.2
			6 after vacc.	6.5	10.1	55.4	9.1	40.0
			10 after vacc.	6.4	12.6	96.4	10.9	70.3
			14 after vacc.	5.5	12.1	86.2	10.6	63.1
IV	3	Non vacc. controls	At start of exper.	6.1	6.6	0.8	6.5	0.7
			At 3 months	6.1	6.4	0.5	6.3	0.3
			At 6 months	6.2	6.7	0.8	6.6	0.6
			At 10 months	6.2	6.8	1.0	6.7	0.8
			At 14 months	6.3	7.7	1.3	6.9	1.0