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## VACCINATION EXPERIMENTS ON PREGNANT MICE WITH E. COLI VACCINES PREPARED FROM SEPTICAEMIC AND ENTEROPATHOGENIC STRAINS OF E. COLI

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DAM, A.: *Vaccination experiments on pregnant mice with E. coli vaccines prepared from septicaemic and enteropathogenic strains of E. coli.* Acta vet. scand. 1973, 14, 691—699. — Experiments have been carried out with vaccination of pregnant mice against E. coli, followed by i.p. challenge of the offspring at one week of age.

With a septicaemic strain the results were highly significant, and the method is therefore recommendable for testing of vaccines against such strains of E. coli.

Results were less clear-cut with enteropathogenic strains of E. coli. However, with mortality rates of 40 to 45 % in baby mice born by non-vaccinated mothers and less than 15 % in baby mice born by vaccinated mothers, the difference in percentage mortality seems sufficient to warrant the use of the method also in the control of vaccines against enteropathogenic E. coli strains.

baby mice; E. coli vaccine; potency-test.

In the prophylaxis of colibacillosis in calves and pigs vaccination experiments with E. coli vaccines have been carried through in many countries, in Denmark on calves with vaccines prepared from septicaemic strains of E. coli (Dam 1968), and on piglets with vaccines prepared from enteropathogenic strains (Dam 1971). The present paper deals with attempts to work out a method for testing such vaccines by vaccination of pregnant mice and subsequent i.p. challenge of the baby mice at one week of age. The vaccines tested in this way had been found effective in challenge experiments on, respectively, calves and piglets (Dam 1968, 1971, 1972 and unpublished data), the commercial vaccine also in field trials (Smith 1972).

## MATERIALS AND METHODS

Vaccines employed: 1) A trivalent *E. coli* vaccine O15-78-115,  $5 \times 10^9$  organisms/ml, with Freund's incomplete adjuvant.

2) A monovalent *E. coli* vaccine O149 strain A<sub>1</sub>,  $5 \times 10^9$  organisms/ml, with the incomplete Freund's adjuvant.

3) A different batch of the monovalent *E. coli* vaccine O149, with the same density.

4) A commercial polyvalent *E. coli* vaccine, prepared from enteropathogenic strains of *E. coli* of piglet origin, including serogroup O149.

Vaccination of pregnant mice: 0.2 ml of vaccine was given s.c. from 3 to 22, in one case 48, days before delivery\*.

Challenge of baby mice: One week after birth, 0.1 ml of broth culture was given i.p., undiluted or diluted.

## RESULTS

The results of the experiments with a trivalent vaccine of septicaemic strains of *E. coli* belonging to serogroups O15, O78 and O115, are given in Table 1. The majority of baby mice from non-vaccinated mothers died when challenged with 0.1 ml of a 20 hrs. broth culture diluted 1/640, and 100 % when challenged with 0.1 ml of culture diluted 1/160, while no baby mice from vaccinated mothers died, when challenged with dilutions 1/640, 1/320, 1/160, 1/80, 1/40 or 1/20; about 50 % died when challenged with dilution 1/10 and 100 % when challenged with dilution 1/5.

The outcome of the experiments with pathogenic strains of *E. coli* is shown in Tables 2 and 3, which give the results of vaccination with two batches of a monovalent *E. coli* O149 vaccine, and in Table 4, which gives the results of vaccination with a batch of the commercial polyvalent *E. coli* vaccine. Finally, Table 5 gives the results obtained when the young of mothers vaccinated with a trivalent *E. coli* vaccine prepared from septicaemic strains of *E. coli* were challenged with an enteropathogenic strain of serogroup O149.

From the survey of results given in Table 6 it appears that, with a challenge dose of 0.1 ml of undiluted 20 hrs. broth culture

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given i.p., there was a mortality varying from 95 to 100 % in the non-vaccinated groups as well as in the group vaccinated with a heterologous vaccine and from 85 to 93 % in the vaccinated group. With a challenge dose of 0.1 ml of a 20 hrs. broth culture diluted 1/3, the mortality in the four non-vaccinated groups and the group vaccinated with a heterologous vaccine was, respectively, 79, 39, 30, 29 and 52 %, while in the groups vaccinated with a homologous vaccine it was, respectively, 18, 8 and 7 %.

Table 1. Intraperitoneal challenge experiments with strain K 667 (serotype O78:K80) on baby mice from, respectively, vaccinated and non-vaccinated mothers.

Number of young	Days from vacc. to birth	Challenge dose i.p.	Deaths
<i>Unvaccinated group</i>			
8		0.1 ml 20 hrs. broth culture dil. 1/20	8
10		" " " " " " " 1/40	9
4		" " " " " " " 1/80	4
18		" " " " " " " 1/160	18
8		" " " " " " " 1/320	7
12		" " " " " " " 1/640	10
<u>60</u>			<u>56</u>
<i>Vaccinated group</i>			
1	9	0.1 ml undiluted 20 hrs. broth culture	1
1	9	0.1 ml 20 hrs. broth culture dil. 1/3	1
3	9	" " " " " " " 1/5	3
4	9	" " " " " " " 1/10	4
3	10	" " " " " " " 1/10	0
4	5	" " " " " " " 1/20	0
3	10	" " " " " " " 1/20	0
4	5	" " " " " " " 1/40	0
4	16	" " " " " " " 1/40	0
4	5	" " " " " " " 1/80	0
4	16	" " " " " " " 1/80	0
4	5	" " " " " " " 1/160	0
4	16	" " " " " " " 1/160	0
4	5	" " " " " " " 1/320	0
4	16	" " " " " " " 1/320	0
4	5	" " " " " " " 1/640	0
<u>55</u>			<u>9</u>

Table 2. Intraperitoneal challenge experiments with strain A<sub>1</sub> (serotype O149:K91) on baby mice from vaccinated (vaccine O149<sub>1</sub>) and non-vaccinated mothers.

Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths	Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths
<i>Vaccinated group</i>									
14/9 1971	4	3	0.1 ml undil.	4	14/9 1971	3	5	0.1 ml dil. 1/3	0
14/9 1971	4	6	"	3	14/9 1971	4	6	"	0
15/9 1971	5	7	"	4	16/9 1971	4	8	"	1
16/9 1971	4	8	"	4	17/9 1971	3	6	"	0
17/9 1971	3	6	"	3	18/9 1971	5	9	"	1
18/9 1971	4	9	"	4	21/9 1971	4	10	"	3
21/9 1971	4	10	"	4	24/9 1971	4	15	"	3
2/11 1971	3	3	"	3	2/10 1971	4	22	"	0
4/11 1971	4	5	"	4	2/11 1971	4	3	"	0
4/11 1971	4	6	"	2	4/11 1971	4	5	"	0
8/11 1971	4	8	"	3	4/11 1971	4	6	"	1
22/11 1971	4	22	"	4	8/11 1971	4	8	"	0
	47			42	22/11 1971	4	22	"	0
						51			9
<i>Non-vaccinated group</i>									
9/9 1971	3		"	3	10/9 1971	4		"	4
8/11 1971	4		"	4	24/9 1971	4		"	4
10/11 1971	4		"	4	30/9 1971	4		"	1
12/11 1971	4		"	4	2/10 1971	4		"	3
	15			15	4/10 1971	3		"	2
					4/10 1971	4		"	4
					8/11 1971	4		"	3
					10/11 1971	4		"	4
					12/11 1971	3		"	2
						34			27

Table 3. Intraperitoneal challenge experiments with strain A<sub>1</sub> (serotype O149:K91) on baby mice from vaccinated (O149<sub>II</sub>) and non-vaccinated mothers.

Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths	Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths
<i>Vaccinated group</i>									
22/11 1971	4	4	0.1 ml undil.	4	22/11 1971	4	4	0.1 ml dil. 1/3	0
22/11 1971	4	5	"	4	22/11 1971	4	5	"	0
24/11 1971	4	6	"	3	24/11 1971	4	6	"	1
1/12 1971	2	5	"	0	1/12 1971	3	5	"	0
2/12 1971	4	6	"	4	2/12 1971	4	6	"	1
2/12 1971	3	6	"	2	2/12 1971	3	6	"	0
3/12 1971	3	7	"	1	3/12 1971	3	7	"	0
3/12 1971	4	7	"	2	3/12 1971	4	7	"	0
4/12 1971	4	8	"	4	4/12 1971	4	8	"	0
4/12 1971	4	8	"	4	4/12 1971	4	8	"	1
6/12 1971	4	9	"	4	6/12 1971	4	9	"	0
8/12 1971	4	21	"	4	8/12 1971	4	21	"	1
9/12 1971	4	22	"	4	9/12 1971	4	22	"	0
11/12 1971	4	15	"	4	11/12 1971	4	15	"	0
	52			44		53			4
<i>Non-vaccinated group</i>									
22/11 1971	4		"	4	22/11 1971	4		"	0
22/11 1971	4		"	4	22/11 1971	4		"	0
29/11 1971	3		"	3	29/11 1971	3		"	0
29/11 1971	4		"	4	29/11 1971	4		"	1
30/11 1971	4		"	4	30/11 1971	4		"	2
30/11 1971	4		"	4	30/11 1971	4		"	0
1/12 1971	4		"	4	1/12 1971	4		"	2
1/12 1971	3		"	3	1/12 1971	4		"	0
4/12 1971	4		"	4	4/12 1971	4		"	3
4/12 1971	3		"	3	4/12 1971	3		"	2
6/12 1971	4		"	4	6/12 1971	4		"	4
9/12 1971	4		"	4	9/12 1971	4		"	4
	45			45		46			18

Table 4. Intraperitoneal challenge experiments with strain A<sub>1</sub> (serotype O149:K91) on baby mice from vaccinated (commercial vaccine) and non-vaccinated mothers.

Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths	Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths
<i>Vaccinated group</i>									
2/2 1972	2	6	0.1 ml undil.	2	2/2 1972	2	6	0.1 ml dil. 1/3	0
3/2 1972	4	7	"	4	3/2 1972	5	7	"	0
4/2 1972	4	8	"	4	4/2 1972	4	8	"	0
4/2 1972	4	8	"	4	4/2 1972	4	8	"	0
7/2 1972	4	10	"	4	7/2 1972	4	10	"	0
19/2 1972	4	9	"	3	19/2 1972	4	9	"	0
21/2 1972	4	11	"	4	21/2 1972	4	11	"	0
21/2 1972	4	11	"	3	21/2 1972	4	11	"	2
21/2 1972	2	11	"	2	21/2 1972	3	11	"	0
3/3 1972	4	7	"	4	3/3 1972	4	7	"	0
3/3 1972	4	7	"	4	3/3 1972	4	7	"	0
17/3 1972	4	48	"	4	17/3 1972	4	48	"	1
	44			42		46			3
<i>Non-vaccinated group</i>									
29/1 1972	4		"	4	29/1 1972	4		"	0
10/2 1972	4		"	4	10/2 1972	5		"	1
22/2 1972	4		"	4	22/2 1972	4		"	0
24/2 1972	4		"	4	24/2 1972	4		"	0
26/2 1972	4		"	4	26/2 1972	4		"	3
26/2 1972	4		"	4	26/2 1972	4		"	2
5/3 1972	4		"	4	5/3 1972	4		"	1
10/3 1972	4		"	3	10/3 1972	4		"	0
16/3 1972	4		"	3	16/3 1972	4		"	4
	36			34		37			11

Table 5. Intraperitoneal challenge experiments with strain A<sub>1</sub> (serotype O149:K91) on baby mice from vaccinated (vaccine O15-78-115) and non-vaccinated mothers.

Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths	Date	Number of young	Days from vaccin. to birth	Dose of 20 hrs. broth cult.	Deaths
<i>Vaccinated group</i>									
21/8 1972	3	4	0.1 ml undil.	3	21/8 1972	4	4	0.1 ml dil. 1/3	4
23/8 1972	5	7	"	5	23/8 1972	6	7	"	0
24/8 1972	3	8	"	3	24/8 1972	3	8	"	0
24/8 1972	4	8	"	4	24/8 1972	5	8	"	2
5/9 1972	4	19	"	4	5/9 1972	3	19	"	0
7/9 1972	3	22	"	2	7/9 1972	4	22	"	1
8/9 1972	6	22	"	6	8/9 1972	7	22	"	6
9/9 1972	5	24	"	5	9/9 1972	6	24	"	5
9/9 1972	5	24	"	5	9/9 1972	5	24	"	5
13/9 1972	5	28	"	5	13/9 1972	5	28	"	0
19/9 1972	4	34	"	4	19/9 1972	5	34	"	3
19/9 1972	4	34	"	3	19/9 1972	5	34	"	4
	51			49		58			30
<i>Non-vaccinated group</i>									
14/8 1972	4		"	4	14/8 1972	4		"	1
14/8 1972	4		"	4	14/8 1972	4		"	3
18/8 1972	5		"	5	18/8 1972	5		"	4
22/8 1972	4		"	4	22/8 1972	5		"	1
22/8 1972	4		"	4	22/8 1972	5		"	0
23/8 1972	4		"	4	23/8 1972	5		"	1
23/8 1972	4		"	4	23/8 1972	5		"	0
23/8 1972	4		"	4	23/8 1972	5		"	1
23/8 1972	4		"	4	23/8 1972	5		"	1
9/9 1972	4		"	3	9/9 1972	4		"	3
9/9 1972	4		"	4	9/9 1972	4		"	3
9/9 1972	4		"	4	9/9 1972	4		"	2
9/9 1972	4		"	4	9/9 1972	5		"	3
11/9 1972	4		"	4	11/9 1972	5		"	0
11/9 1972	4		"	4	11/9 1972	4		"	0
13/9 1972	4		"	4	13/9 1972	5		"	1
13/9 1972	4		"	4	13/9 1972	5		"	2
13/9 1972	4		"	4	13/9 1972	5		"	0
13/9 1972	4		"	4	13/9 1972	4		"	0
15/9 1972	1		"	1	15/9 1972	4		"	0
15/9 1972	1		"	1	15/9 1972	2		"	0
18/9 1972	4		"	4	18/9 1972	4		"	1
	82			78		94			27

Table 6. Survey of results giving percentage mortality among young of vaccinated and non-vaccinated mothers.

<i>Vaccine O149<sub>I</sub></i>	Vaccinated			Non-vaccinated		
	total	deaths	% deaths	total	deaths	% deaths
0.1 ml undil.	47	45	92	15	15	100
0.1 ml dil. 1/3	51	9	18	34	27	79
<i>Vaccine O149<sub>II</sub></i>						
0.1 ml undil.	52	44	85	45	45	100
0.1 ml dil. 1/3	53	4	8	46	18	39
<i>Commercial vaccine</i>						
0.1 ml undil.	44	42	93	36	34	95
0.1 ml dil. 1/3	46	3	7	37	11	30
<i>Heterologous vaccine</i>						
0.1ml undil.	51	49	96	82	78	95
0.1 ml dil. 1/3	58	30	52	94	27	29

## DISCUSSION

When baby mice were challenged i.p. with 0.1 ml of undiluted 20 hrs. culture of *E. coli* O149, there was no significant difference between litters born by, respectively, vaccinated and non-vaccinated mothers, the mortality varying from, respectively, 85 to 93 % and 95 to 100 %. When challenge was made with 0.1 ml of a 20 hrs. broth culture diluted 1/3, the average mortality was from 40 to 45 % among the young of non-vaccinated or heterologously vaccinated mothers, and less than 15 % among the young of homologously vaccinated mothers. This difference would seem to warrant the use of the method in routine testing of *E. coli* vaccines, provided that the evaluation is based on a sufficient number of litters of baby mice. In a so sensitive test, chance variations may lead to false conclusions if just a few litters are compared. This is apparent from the experiments with vaccine O149<sub>I</sub> (Table 2), in which three mice out of four died in each of the litters 21/9 and 24/9.

With the septicaemic strains of *E. coli* the mouse test gave highly significant results and thereby proved to be a valuable tool in the efficacy testing of vaccines against *E. coli* septicaemia.

Whether vaccination takes place in early or late pregnancy seems to be of no influence on the results.

While the present work was being carried out, results of vaccination of mice followed by challenge of the baby mice were



published from Belgium (Huygelen *et al.* 1971) as part of a more comprehensive work on immunization of mice against *E. coli*. However, in the experiments on transfer of passive immunity from vaccinated mice to their offspring, only *E. coli* strains causing systemic disease in calves were included. In these experiments a highly significant protection was obtained, a result which was confirmed in the present experiments. Huygelen's experiments included but one enteropathogenic strain, belonging to serogroup 9, and with this strain just a low degree of protection was obtained.

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## SAMMENDRAG

*Vaccinationsforsøg på drægtige mus med E. coli vacciner fremstillet ud fra septikæmiske og enteropatogene stammer af E. coli.*

Der er gennemført forsøg med vaccination af drægtige mus med *E. coli* vacciner og påfølgende i.p. challenge af afkommet i 7 dages alderen.

Ved forsøg med septikæmiske stammer fik man udtalt signifikante resultater, og metoden kan derfor anbefales til kontrol med vacciner mod septikæmiske stammer af *E. coli*.

Resultaterne var mindre udtalte ved forsøg med enteropatogene stammer af *E. coli*. Med en mortalitet på 40—45 % blandt museunger efter uvaccinerede mødre og under 15 % blandt museunger efter vaccinerede mødre synes forskellen i mortalitet tilstrækkelig til at betragte metoden anvendelig også i kontrollen med vacciner mod enteropatogene stammer af *E. coli*.

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