

# The ERA Strain of Rabies Vaccine

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## SUMMARY

An antigenic extinction trial in cats showed that the ERA rabies vaccine had superior antigenic properties over Flury H.E.P. C.E.O. and killed tissue culture rabies vaccine.

Dogs and cats on a duration of immunity study of ERA rabies vaccine were challenged with fox salivary gland "street" rabies virus. The results of this challenge show a duration of immunity of five years in dogs and four years in cats.

Vaccination of dams in late pregnancy with ERA rabies vaccine resulted in transference of maternal antibody to the newborn, in both cattle and dogs. This maternally derived antibody interfered with the successful active immunization of the young calf. Calves free of antibodies for rabies could be successfully vaccinated as early as 17 days of age and were able to withstand a challenge with virulent "street" rabies virus two years later.

## RÉSUMÉ

Une expérience sur l'extinction antigénique effectuée chez des chats a révélé que le vaccin antirabique ERA possédait des propriétés antigéniques supérieures à celles du vaccin Flury H.E.P. C.E.O. et d'un vaccin tué, préparé sur culture tissulaire.

On infecta des chiens et des chats, au cours d'une expérience sur la durée de l'immunité conférée par le vaccin antirabique ERA, avec une souche de virus rabique "de rue" provenant des glandes salivaires de renards. Les résultats de cette expérience démontrent que l'immunité dure cinq ans chez le chien et quatre ans chez le chat.

La vaccination de femelles dont la gestation tirait à sa fin, avec le vaccin antirabique ERA,

provoqua le transfert d'anticorps maternels aux nouveaux-nés, tant chez les bovins que chez les chiens. Ces anticorps maternels firent échec à l'immunisation active efficace des jeunes veaux. On pouvait cependant vacciner avec succès des veaux dépourvus d'anticorps contre le virus rabique, dès l'âge de 17 jours. Ces sujets résistèrent à une infection expérimentale avec du virus rabique "de rue", deux ans plus tard.

## INTRODUCTION

The ERA strain of rabies vaccine propagated in primary porcine kidney tissues has been described in the literature and has been proved to be safe and antigenic in a variety of domesticated animals (1, 2). Trials in Argentina (4) and in Mexico (8) have shown the vaccine to be effective against rabies of bat origin. Field trials carried out in Bolivia (6) have shown the vaccine to be effective under field conditions.

The duration of immunity reported in the literature (3, 5) is four years in cattle, three years in dogs and two years in cats and horses. The purpose of this paper is to present results of the latest duration of immunity studies and other trials carried out on the ERA vaccine.

## MATERIALS AND METHODS

### ANIMALS

The dogs and cats used in these trials were obtained from farm sources as recently weaned litters. Following a quarantine period of about one month, they were assigned to specific groupings, vaccinated and kept under isolation until challenged. The cattle came from the closed breeding herd maintained by the Connaught Laboratories at Bolton, Ontario. White Swiss mice 10-12 grams and approximately four

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weeks of age were used in the serum neutralization test and virus titrations.

#### SAMPLES COLLECTED

Blood samples were collected, the sera removed and frozen at  $-20^{\circ}\text{C}$  until required, and inactivated at  $56^{\circ}\text{C}$  for 30 minutes prior to use.

Milk samples were obtained as soon after parturition as possible. To assist in the collection of milk from bitches, 0.2 ml of oxytocin<sup>1</sup> was administered intramuscularly. The samples were then stored at  $-20^{\circ}\text{C}$  until required, at which time they were thawed, 0.1% rennin solution added at the rate of 0.1 ml to 1.0 ml of milk, and placed in a water bath at  $40^{\circ}\text{C}$  for two hours. After this treatment, they were centrifuged and the clear fluid removed for rabies antibody determinations.

#### VIRUSES

The ERA rabies vaccine used in all the trials was produced in primary porcine kidney. The dosage was 2.0 ml and was administered intramuscularly in the gluteal region. The H.E.P. Flury vaccine was of chick embryo origin and was a regular commercial lot, whereas the tissue culture

vaccine was an inactivated and concentrated product for use in humans. All vaccines were produced by Connaught Laboratories under licensed methods.

The challenge virus was prepared from the salivary glands of rabies positive foxes supplied by the Canada Department of Agriculture. The challenge material was prepared from a 20% suspension by weight of finely ground salivary glands dispensed into ampoules which were then sealed and stored at  $-20^{\circ}\text{C}$ . The challenge pool contained approximately  $10^7$  mouse  $\text{LD}_{50}$  per 2.0 ml. The final challenge was diluted to contain approximately  $10^6$  mouse  $\text{LD}_{50}$  per 2.0 ml and was administered intramuscularly in the gluteal region. It is usual for deaths to occur up to 30 days. All animals are observed for six months post challenge before calculating the final results.

The CVS virus used in the serum neutralization test was initially received from the National Institute of Health, United States Public Health Service, and was a suspension of mouse brain. The test itself consisted of preparing five-fold serum dilutions to which rabies virus containing 50-100  $\text{LD}_{50}$  was added. After 90 minutes incubation at  $37^{\circ}\text{C}$ , this mixture was inoculated intracerebrally into mice at a dosage of 0.03 ml.

Virus titrations were carried out by inoculating ten-fold dilutions into 10-12 gram mice intracerebrally with 0.03 ml.

<sup>1</sup>Connaught Laboratories, Willowdale, Ontario.

TABLE I. Challenge and Serum Neutralization Results of Cats Vaccinated with Various Preparations

Vaccine	Virus Titre <sup>a</sup>	Dilution of Vaccine	Challenge Results <sup>b</sup> Surv/Chall.	Antibodies five Weeks Post-Vaccination $\text{PD}_{50}^c$					
				No. Samples	0	0-5	5-25	25-125	>125
ERA.....	4.31	Undil.	10/10	10	—	—	1	8	1
		1/10	9/9	9	—	—	3	6	—
		1/100	8/10	10	5	—	1	4	—
		1/1000	4/10	10	10	—	—	—	—
H.E.P.....	4.16	Undil.	5/10	10	9	—	1	—	—
		1/10	1/10	10	10	—	—	—	—
		1/100	0/10	10	10	—	—	—	—
		1/1000	5/10	10	10	—	—	—	—
Killed.....	—	Undil.	10/10	10	—	1	2	1	6
			10/10	10	—	1	4	4	1
Antigen.....	—	1/100	2/5	5	4	1	—	—	—
			3/11	—	11	—	—	—	—
Controls.....	—	—	3/11	—	11	—	—	—	—

<sup>a</sup> $\text{LD}_{50}$  per 0.03 ml intracerebral injection in 10-12 gram mice

<sup>b</sup>Cat Challenge dose =  $10^{6-9}$  mouse  $\text{LD}_{50}$  Interval between vaccination and challenge = five weeks

<sup>c</sup>Reciprocal serum dilution which protected 50% of mice. Reported as number of animals with this titre

The 50% end-points for titrations and serum neutralization tests were calculated according to the method of Reed and Muench (7).

## RESULTS

Table I shows the results obtained when cats vaccinated with various dilutions of either ERA, Flury H.E.P. C.E.O. or a killed rabies vaccine were challenged five weeks

later with virulent rabies virus. The ERA vaccine protected in all dilutions up to 1/100; the H.E.P. vaccine essentially failed to give a discernible antigenic response either by way of protection or antibodies, while the killed vaccine gave complete protection up to a 1/10 dilution of the stock vaccine.

Table II shows the results obtained when dogs vaccinated four years previously with ERA rabies vaccine, and unvaccinated dogs were challenged. Seventy per cent of the vaccinated dogs survived a challenge which

TABLE II. Results of Challenging Dogs Vaccinated Four Years Previously with ERA Vaccine

Dogs	Challenge Results*, Survived/Challenged	Survival %	Serum Neutralization Titres (PD <sub>50</sub> )					
			Pre Challenge			Seven Month Post Challenge		
			0	<5	5-25	0	<5	5-25
Vaccinated	7/10	70	0	5	5	0	0	7
Control	0/9	0	9	0	0	—	—	—

\*Dog Challenge Dose — 10<sup>6.9</sup> mouse LD<sub>50</sub>

TABLE III. Results of Challenging Dogs Vaccinated Five Years and Cats Vaccinated Four Years Previously with ERA Vaccine

Animals	Results* Survived/Challenged	Survival %	Serum Neutralization Test (PD <sub>50</sub> )								
			Pre Chall.				Ten Wk. Post Chall.				
			0 <sup>b</sup>	<5	5-25	>25	0	<5	5-25	>25	
Dogs	Vaccinated	13/14	92.8	7	5	1	1	0	3	6	4
	Unvaccinated Controls	5/14	35.7	14	0	0	0	2	1	0	2
Cats	Vaccinated	8/8	100	1	4	2	1	ND <sup>c</sup>	ND	ND	ND
	Unvaccinated Controls	1/10	10	10	0	0	0	ND	ND	ND	ND

\*Challenge Dose — 10<sup>5.4</sup> mouse LD<sub>50</sub>

<sup>b</sup>Sera with apparent titres of 1 or less were considered as negative

<sup>c</sup>ND = Not Done

TABLE IV. Results of Challenging Calves Vaccinated with ERA Vaccine at 17-67 Days

Group	Results* Survived/Challenged	Survival %	Serum Neutralization Titres (Average)				
			Pre Vaccination	Post Vaccination			
				Four Weeks	Ten Weeks	14 Weeks	102 Weeks
1 dose	8/8	100	0	10.7	11.0	8.9	3.2
2 doses <sup>b</sup>	8/8	100	0	19.6	6.9	>78.4	1.7
Controls	0/5	0	ND	ND	ND	ND	0

\*Challenge dose — 10<sup>6</sup> mouse LD<sub>50</sub>. Interval between vaccination and challenge two years

<sup>b</sup>Second dose ten weeks after first injection

\*ND = Not Done

killed nine out of the nine control dogs.

Dogs and cats which had been previously vaccinated five and four years respectively with ERA rabies vaccine were challenged with virulent rabies virus. Table III shows that 13 of 14 vaccinated dogs and all of eight vaccinated cats survived the challenge which killed nine of 14 control dogs and nine of the ten control cats.

A trial was carried out in which calves from non-vaccinated cows were inoculated with ERA rabies vaccine at 17-67 days of age. Half of the vaccinated calves were re-vaccinated 70 days later. All the vaccinated calves and five unvaccinated animals were challenged with virulent virus 102 weeks after the first vaccination. Table IV shows the results of the challenge. All the

vaccinated calves survived a challenge which killed the five control animals. It should be noted that the revaccination had a transient booster effect.

Calves from cows which had been vaccinated in late pregnancy with ERA rabies vaccine were vaccinated at one week, one month or four months after birth. A similar number of animals were left unvaccinated. All the calves were subsequently challenged four months after the last vaccination had been carried out.

Table V indicates the number of calves with maternal antibodies at various times after birth. The results are arbitrarily grouped according to the titre of their dams at calving. It can be seen that maternal antibody in some of the calves persisted

TABLE V. Persistence of Maternal Antibodies in Calves Born to Vaccinated Dams

Mother's Titre <sup>a</sup> at Calving	Number of Animals with Maternal Antibodies <sup>b</sup>							
	Months							
	0	1	2	3	4	5	6	7
0 <sup>c</sup>	0/2	—	—	—	—	—	—	—
0-5	5/5	5/5	4/5	1/5	1/5	1/5	0/5	0/5
5-25	3/3	3/3	3/3	3/3	2/3	1/3	0/3	0/3
>25	8/8	7/8	7/8	7/8	6/8	3/8	2/8	0/8

<sup>a</sup>Protective Dose 50

<sup>b</sup>Number with antibodies/number tested in that group

<sup>c</sup>Serum Neutralization Titres < 1 considered as 0

TABLE VI. Challenge and Serum Neutralization Results of Parentally Immune Calves Vaccinated at Various Times after Birth with ERA Vaccine

Groups	Calf No.	Serum Neut. Pre Vacc.	Titre (PD <sub>50</sub> ) Pre Chall.	Challenge Results <sup>a</sup>
Vaccinated one week after birth	14	0	16.7	Survived
	27	5	0	Died
	18	>25	0	"
	51	>25	0	"
	44	>25	0	"
Vaccinated one month after birth	13	0	5.5	Survived
	38	>25	0	"
	21	>25	0	Died
	23	9.7	0	"
Vaccinated four months after birth	15	0	0	Died
	50	5	0	"
	16	8.1	0	"
	31	11.3	0	"
Unvaccinated Controls	20	ND <sup>b</sup>	0	Died
	34	"	0	"
	39	"	0	"
	22	"	0	"

<sup>a</sup>Challenge Dose — 10<sup>6.5</sup> mouse LD<sub>50</sub>

<sup>b</sup>ND = Not Done

to six months of age if the dam possessed high levels herself.

Table VI demonstrates the results obtained when these same calves were subsequently challenged. Only three vaccinated animals survived. Two of these animals, numbers 13 and 14, were born 24 and 48 hours respectively after the mothers had been vaccinated and as a consequence, had no maternal antibodies to interfere with vaccination. The other calf, number 38, had no antibodies prior to and post challenge but died of rabies when rechallenged some time later. It is possible that this animal did not receive a proper challenge dose of virus on the first challenge.

In order to study maternal antibodies to rabies in puppies, fully susceptible pregnant bitches were vaccinated with ERA rabies vaccine 15-29 days prior to whelping. Table VII contains the results of serum neutralization tests carried out on these samples. In the first case, no antibodies were transferred via the colostrum to the puppies, even though the mother had a substantial titre of circulating antibody. In the remaining litters, all puppies showed the presence of maternal antibodies in their sera. By ten weeks of age, all trace of their antibodies had disappeared.

## DISCUSSION

In the vaccination extinction trial in cats, the serum neutralization results closely paralleled the actual challenge results. The antigenic superiority of ERA over H.E.P. Flury C.E.O. in cats could not be attributed to virus content because both vaccines appeared to have approximately the same virus titre.

In the duration of immunity trials in dogs, half of the animals still had circulating antibodies even after a five year period. In cats antibodies were detectable in seven out of eight at four years post vaccination.

Calves with no maternal antibodies can be successfully vaccinated as early as 17 days of age. Revaccination of these animals had a temporary booster effect, but this did not seem to have any observable advantage since at challenge, the antibody level of the revaccinated calves had returned to the same level as those animals which had received only one injection.

Maternal antibodies in calves interfered with successful vaccination, and if animals are vaccinated early in life, it would seem

TABLE VII. Maternal Antibodies to Rabies in Puppies from Immune Dams

Trial No.	Dam No.	Serum Neutralization Titre									
		Dam				Pups					
		Pre Vacc.	Post Vacc. (Days)	Colostrum	No.	2 <sup>a</sup>	4	6	8	10	
1	20 U	0	252	(56)	NL <sup>b</sup>	201	ND	ND	0	ND	ND
						202	"	"	0	"	"
						203	"	"	0	"	"
						204	"	"	0	"	"
						205	"	"	0	"	"
						206	"	"	0	"	"
29	0	238	(18)	328	2	160	56	9	11	0	
					4	125	70	11	7	0	
					6	280	48	48	13	0	
30	0	>625	(23)	>625	8	419	67	0	0	0	
					10	485	107	0	0	0	
					12	20	17	48	0	0	
2	E79	0	237	(29)	>625	21	47	12	5	0	0
						22	52	37	2	5	0
						23	9	8	1	2	0
V22	0	625	(25)	280	16	485	13	9	0	0	
					18	20	47	3	2	0	
					20	625	11	2	0	0	

<sup>a</sup>Age of pups in weeks

<sup>b</sup>ND = Not done

that a second vaccination at six to seven months of age when most maternal antibodies have disappeared would be indicated. It appeared that the level of antibody in the serum of the mother at calving time influenced the persistence of maternal antibodies in the calf.

Maternal antibodies in most of the puppies tested persisted until ten weeks of age. If these antibodies interfere with successful vaccination as they did in calves, then dogs should not be vaccinated until after ten weeks of age.

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