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EXPERIMENTAL INFECTION OF HEDGEHOGS, HAMSTERS, RABBITS AND FERRETS WITH THE VIRUS LEDNICE

This paper is a sequel to our previous communication concerning our investigations on possible hosts of the virus Lednice (Málková D. et al., Folia parasit. (Praha) 25: 113—114, 1978). We directed our attention to other mammal species occurring in littoral zones of fishponds, the biotope of *Culex modestus*. For the time being it is the only known mosquito species from which the virus has been isolated in this country (Málková D. et al., Acta virol. 16: 93, 1972; Folia parasit. (Praha) 21: 363—372, 1974).

MATERIAL AND METHODS

Animals. Exclusively the young were used in the experiment. Due to difficulties in obtaining a sufficient number of wild animals we used (except hedgehogs) related mammal species available from breeding farms. The young of hedgehogs (*Erinaceus europaeus*), each weighing 120—160 g, were captured in the Prague area. Their age was supposed to be 6—8 weeks. The young of golden hamsters (*Mesocricetus auratus*) were 8 days old and were supplied by the farm Velaz. The 2-3-day old suckling rabbits (*Oryctolagus cuniculus*) were made available to us from the laboratory colonies of the Institute of Physiology, Czechoslovak Academy of Sciences. The 4-8-day old young of ferrets (*Putorius furo*) were obtained from private breeders.

Virus and infection. Strain 6118 of the virus Lednice (antigenically closely related to the virus initially designated Yaba 1 from the group Turlock, later registered in the Catalogue of Arboviruses as the virus M'Poko) was passaged on suckling mice. All tests were based on stock lyophilized 20 % brain suspension. After rehydration in redistilled water the virus was titrated in PBS with 10 % calf serum and antibiotics. The animals were inoculated scut with 0.1 ml virus suspension diluted 10^{-2} or 10^{-4} per animal, namely in the occipital part of the head.
Collection of blood and regional lymphatic nodes. Blood was collected for isolation tests within the first ten days p.i. from vena tibialis into heparin; for serological test blood was drawn from the heart 3—6 weeks p.i. Regional lymphatic nodes were taken from hamsters and ferrets and isolation tests were conducted simultaneously with blood testing on 1st, 3rd, 5th, 7th and 10th day p.i.
Detection of virus. Blood for isolation tests was titrated immediately after collection and each dilution was inoculated (icer) into 2-day-old suckling SPF mice, in a dose of 0.01 ml per mouse. The animals were kept under observation for 14 days. Suspension from regional lymphatic nodes was tested in a similar way.
Detection of antibodies. Inactivated plasma, diluted with PBS 1 : 2 and titrated antigen (log 10), was used for neutralization test. After

incubation of the antibody-antigen mixture at 37 °C/60 min. It was inoculated ícer into 2-day-old suckling mice. One mouse litter numbering 10 young was used for each dilution. The mice were under observation for 14 days. Titres were calculated after Reed and Muench.

RESULTS

The results are summarized in Table 1. They indicate that viremia was not detected in any animal tested. Likewise, examination of regional lymphatic nodes in hamsters and ferrets did not reveal the virus. As for the antibody response it was negative in hedgehogs and hamsters, and very low in rabbits, in which it was demonstrated as late as 6 weeks p.i. and only in some individuals. The most important antibody response was noted in ferrets in which antibodies were detected as early as 3rd week p.i.

Table 1. Experimental infection of hedgehogs, hamsters, rabbits and ferrets with the virus Lednice

Animal		Inoculum log/animal	Virus		Antibodies (NJ in log)	
Order	Species		Blood	RLN	3rd week p.i.	6th week[2]) p.i.
Insectivora	Hedgehog	3.9	0	ND	0.31; 0.22	0.04; 0.27; 0.64
Rodentia	Hamster	3.59	0	0	0.18; 0.68	0.0; 0.17; 0.21; 0.34
		1.59	0	0	0.08; 0.17	ND
Lagomorpha	Rabbit	4.0	0	ND	0.35; 0.36	0.52; 0.70; 1.12; 1.43
		2.0	0		0.19; 0.32	0.08; 0.22; 0.91; 1.05
Carnivora	Ferret	3.5	0	0	$\geqq 1.77$[3])	$\geqq 2.58$[3])

[1]) Serum mixtures from 2—3 animals; [2]) Individual examination; [3]) In both groups (3rd week) or in all 4 animals (6th week)

DISCUSSION

The results clearly showed that no viremia formed in any animal tested. The antibody response, however, was not so clearcut. It proved to depend on the amount of antigen administered and on the different reactivity of animals. Neither hedgehogs nor hamsters formed antibodies to the virus; though the reactivity in rabbits was low, it was well demonstrable and in ferrets the antibody response was distinct despite the fact that a lesser amount of antigen was administered to them per 1 kg of weight (50—100 units/g) than to hamsters (230 to 280 units/g).

The results consequently confirmed our previous experience that mammals are not current hosts of the virus Lednice (Málková D. et al., Acta virol. 17: 74—78, 1973; Acta virol. 20: 226—231, 1976; Folia parasit. (Praha) 25: 113—114, 1978; Kolman J. M. et al., Folia parasit. (Praha), in press).

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