Age-Related Antibodies to Rotavirus in New Zealand Rabbits

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The prevalence of antibodies to rotavirus was determined in 199 New Zealand rabbits from a commercial rabbitry by an indirect immunofluorescent test. Virtually all rabbits less than 1 month old and greater than 2 months old had antibodies, whereas only 25% of rabbits 1 to 2 months old had antibodies.

Rotavirus has been recovered from rabbits with and without diarrheal disease (1, 2, 4). A serological survey of 91 young adult and adult rabbits from two commercial rabbitries in Canada revealed that 98% had antibodies to rotavirus (3). A survey in Japan of 39 adult rabbits revealed that 82% had antibodies (5). In an attempt to obtain rabbits seronegative for rotavirus, we conducted an age-based survey for antibodies to rotavirus at a commercial rabbitry. We found that 1-month-old rabbits, free of antibodies to rotavirus, remained free of rotavirus infection upon isolation.

Sera were obtained from 199 New Zealand white rabbits, 2 weeks to 2 years old, from a local commercial rabbitry. In addition, 12 does and two rabbits from each of their litters were bled. The rabbitry contained ca. 4,000 New Zealand white rabbits with ca. 240 breeding does. Litters were weaned at 5 to 8 weeks of age. Breeding rabbits were maintained in one building, and weaned rabbits were caged in another building by litters until 10 weeks of age and then individually until sold to laboratories.

For indirect immunofluorescence assays, monolayers of MA104 cells were grown in microtiter plates. When cells were confluent, Eagle minimal essential medium (without calf serum) containing ca. 100 fluorescent cell-forming units per well of canine rotavirus (K9 strain kindly provided by G. N. Woode, Iowa State University, Ames), was added. Plates were centrifuged at $1,400 \times g$ for 1 h, incubated overnight at 37°C, and fixed in methanol as previously described (6). Each rabbit serum tested was diluted 1:20, and doubling dilutions to 1:1,280 were made across the plate. Plates were washed after 1 h of incubation at 37°C, and fluorescein-conjugated goat anti-rabbit immunoglobulin (Nordic Immunologicals Ltd., El Toro, Calif.) was added. After a 1-h incubation, plates were washed, counterstained with Evans Blue, and read by using a vertical illuminator on a Zeiss microscope. The last well in which fluorescent cells could be clearly seen was the dilution endpoint.

Rotavirus antibodies occurred commonly in both young (<1-month-old) and older (>2-months-old) rabbits but infrequently in rabbits 1 to 2 months old (Table 1). All 14 2-week-old rabbits from five litters had antibodies to rotavirus. Antibody titers in rabbits less than 1 month old ranged from

1:20 to 1:160; about two-thirds of the rabbits had titers of $\leq 1:80$. In 149 rabbits greater than 2 months old, 95% had antibodies. Antibody titers in rabbits greater than 2 months old ranged from 1:20 to 1:1,280; about two-thirds of rabbits had titers of $\geq 1:320$. Only 25% of 36 rabbits 1 to 2 months old had antibodies to rotavirus. Within this age range antibodies were most commonly absent in rabbits 1 month old (Table 2). In litters aged 32 to 42 days old, only 1 of 10 rabbits had rotavirus antibodies, whereas 7 of 14 rabbits 44 to 57 days old had antibodies. Titers in the latter group ranged from 1:160 to 1:1,280, similar to rabbits greater than 2 months old. Litters of does 4564, 8472, and 8195, in which one littermate had undetectable antibodies and the other littermate had antibodies detectable in high titers, were retested for antibodies 3 weeks later. At this time littermates from all three litters had antibodies to rotavirus ranging from 1:320 to 1:1,280.

Antibodies to rotavirus, detected by the complement fixation test, have been reported in 98% of 91 young adult and adult rabbits (3) and 82% of 39 adult rabbits (5). Our study revealed that 95% of weanlings, young adults, and adults had antibodies to rotavirus as detected by an indirect immunofluorescent test. However, only 25% of preweanling rabbits had antibodies to rotavirus. Rabbits of ca. 1 month old were devoid of antibodies, whereas antibodies occurred more commonly as rabbits approached 2 months old. Since all rabbits less than 1 month old had antibodies, this suggests that maternal antibodies to rotavirus disappeared by 1 month of age. Rabbits soon succumbed to natural infection as virtually all weanlings (2 to 3 months old) had antibodies.

TABLE 1. Prevalence of antibodies to rotavirus in New Zealand white rabbits from a commercial rabbitry

Class	Sex	Age (mo)	No. tested	No. positive	% Positive
Neonates	M,F	<1	14	14	100.0
Preweanlings	M,F	1-2	36	9	25.0
Weanlings	M,F	2-3	18	17	94.4
Young adults	F	3-4	108	103	95.3
Breeding adults	F	>5	23	21	91.3

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TABLE 2. Antibodies to rotavirus in New Zealand white rabbit does and litters

Doe no.	Parity	Doe antibody titer ^a	Age of litter (days) ^b	Litter anti-body titers ^a
2117	3	80	32	<20, <20
5034	1	ND ^c	35	<20, 20
6249	6	640	40	<20, <20
1691	12	160	40	<20, <20
6598	11	160	42	<20, <20
4564	2	320	44	<20, 640
2750	7	640	44	1,280, 1,280
8472	10	160	45	<20, 1,280
8195	5	160	48	<20, 1,280
5635	8	320	50	<20, <20
5635	9	320	53	<20, <20
8195	4	<20	57	160, 640

^a Reciprocal of serum dilution.^b Two rabbits from each litter were tested.^c ND, Not done.

For experimental studies, rabbits seronegative for rotavirus have been obtained by weaning 1-month-old rabbits and transporting them in clean individual cages. At the University of Washington animal facility, rabbits were individually

maintained in a separate room. At 4 months after arrival, rabbits were still free of antibodies to rotavirus.

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