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Investigation of Japanese Encephalitis Virus Infections in Three- to Six-Year-Old Children in Rural Taiwan

Abstract

To understand the distribution of Japanese encephalitis antibodies among pre-school children in rural Taiwan, all three- to six-year-old children residing in Hsinchu, Taichung, Pingtung and Hualien Counties were selected for sero-epidemiological investigation. The plaque reduction neutralization test (PRNT) was used with a total of 2,281 children. The total positive rate of Japanese encephalitis neutralization antibodies found was 67% (1,520/2,281). After a series of three doses of Japanese encephalitis vaccine, the four-year-old children of all four counties showed the lowest neutralization antibody positive rate: these were 45% (56/125) in Hsinchu; 51% (57/111) in Taichung; 30% (39/128) in Pingtung and 66% (67/102) in Hualien. These figures are significantly lower than those for the other age group of the children immunized in each county. ($p=0.0001$, Chi-square test). Whether or not children had been immunized against Japanese encephalitis, the neutralization antibody positive rate went up after age five, reaching the highest rate (around 82 to 91%) at age six. This shows that in the areas of study, spontaneous infection with Japanese encephalitis generally existed. The antibody positive rate of the 47 non-immunized children also went up with age.

The neutralization antibody positive rate was 67% (1,122/1,665) for children who had had three doses of vaccine: 66% (64/97) for children with two doses; 33% (4/12) for children with only one dose and 40% (19/47) for children not immunized. The latter three groups had significantly lower rates than the ones who had had three doses of the vaccines ($p < 0.0001$, Chi-square test for trend). This shows that the spontaneous infection rate was relatively high. However, children with two or more doses of vaccine had higher protection. It appears that vaccines still play a very important role in the current prevention and control program against Japanese encephalitis.

Introduction

In the early days in Taiwan, there were outbreaks of so-called "summertime

encephalitis". In 1938, when Kobayashi succeeded in isolating the Japanese encephalitis virus from the brain tissue of patients[1], it was established that Japanese encephalitis and its virus did indeed exist in Taiwan. Later, the Japanese encephalitis virus was again isolated from patients' brains[2]. It was re-established that the virus was the major pathogenic source of Japanese encephalitis, that *Culex tritaeniorhynchus* and *Culex annulux* were the vectors[3] and that pigs were the most important reservoir[4] for these pathogens. The disease is more prevalent in the summertime months between May and September, affecting a large segment of the Island, particularly rural areas. Over the last 20 years, it has been noted that the peak month has moved ahead one month every 10 years[5]. This change in the epidemic season may have come about from changes in the weather pattern, or they may have resulted from changes in the genes of the virus itself. More studies are needed to clarify this.

Japanese encephalitis is a mosquito-borne viral infection with an incubation period of one to two weeks; the disease symptoms are high fever, headache and nausea. Inapparent infection is frequent[6]. However, once clinical symptoms appear, case fatality can be as high as 25%. The sequelae are severe. Half of all cases can develop permanent neurological symptoms or mental disorders, creating a heavy burden for families and society[7]. This is a serious health problem in Taiwan.

Japanese encephalitis was made reportable in Taiwan in 1955[8]. In 1965, T.C. Hsu, et al. conducted a study of three- to seven-year-old children in Hsinchu, Miaoli, Taipei and Taoyuan counties to assess the effectiveness of the vaccine. It was learned that protection against infection was low when children were given only one dose, but went up to around 80% for children with two doses[9]. Thus, from 1968 children under two were given two doses of the vaccines. In 1974, one booster was given a year later. The Government's immunization policy since 1983 has been to give a first dose at age 15 months, the second dose 2 weeks later, a single booster the next year and another booster upon entering primary school. Since this universal immunization was introduced, cases of Japanese encephalitis have sharply declined. More recently, however, each year 200-300 cases have been reported, of which about 10-30% have been confirmed[10].

It has been 27 years since universal immunization against Japanese encephalitis began. How high is the Japanese encephalitis antibody potency of the immunized children, and what is the distribution of antibody prevalence? Answers to both questions are important. With a view to better assessing its prevalence in children immunized against Japanese encephalitis, an investigation of the neutralization antibody of children immunized against the diseases was conducted. That study was done in cooperation with "The Survey of Prevalence of Hepatitis B and other Vaccine-Preventable Infectious Diseases in Children."

Materials and Methods

1. The Samples

Three- to six-year old children in Taiwan's mountain areas were selected for study.

similarly-aged children from neighboring plain areas were used as controls. Communities were designated as one of four groups following the 1989 serological survey of hepatitis A prevalence conducted by the Department of Health, using Hsioulin Township of Hualien County as the "high infection rate group", Wufeng Township of Hsinchu County as the "second high infection group", Santimen Township of Pingtung County as the "moderate infection group" and Hoping Township of Taichung County as the "low infection group". Areas studied were: (1) in the northern area, Wufeng (N=179), Chienshih (N=114) and Hengshan (N=296) townships of Hsinchu County; (2) in the central area, Hoping (N=244) and Hsinshe (N=304) townships of Taichung County; (3) in the southern area, Santimen (N=132), Machia (N=168) and Kaoshu (N=308) townships of Pingtung County; and (4) in the eastern area, Hsioulin (N=294) and Hsinchen (N=242) townships of Hualien County. From each age group, either 75 children or the total child population, if the number were fewer than 75, were selected. The study population was, then, around 300 children in each township. A total of 2,281 serum specimens were collected between March and June, 1993. Of the townships named above, Hengshan, Hsinshe, Kaoshu and Hsinchen are the plain area townships; the rest are in mountain areas.

2. Laboratory Methods

The plaque reduction neutralization test (PRNT) was used to test for the JEV Nt Ab. The virus used for testing was the Nakayama-NIH strain. Specimens were diluted 10 times with 0.01M PBS + 5% FCS and placed in 56 degree C water for 30 minutes for inactivation treatment. Quantified viruses were diluted with a virus dilution containing minimum essential medium (MEM) 5%, fetal calf serum (FCS) and 3% guinea pig serum (GPS); the volume of the viruses was 600-700 PFU/ml. The result was placed together with an equal quantity (130 μ) of the inactivated specimens under 4 degrees C for 18-21 hours. This virus-serum mixture was inoculated into the kidney cells (BHK-21) of suckling mice. When the number of serum plaques was lower than 50% of those of the control (not containing serum) viruses, and the antibody potency of the positive control was between 40 and 80 with the negative control at 0, the finding was read as positive.

3. Immunization Records

Immunization is generally carried out in May of each year: (1) first dose at age 15 months, (2) second dose two weeks later, (3) third dose one year later, and (4) fourth dose upon entering primary school. In 1989, one or two schools in each county were selected for a pilot project in which children were given the third dose upon entering primary school, if they had failed to have it previously. This practice was extended to all schools throughout the Island in September 1992. Specimens for the present study were collected between March and June, 1993. The children who were three years old then, having been born in 1989, would have had all three doses by the previous year. Children born before 30 August 1986 (the six-year-olds) entered primary school in September 1992. Some of them would have had a fourth dose some

time between February and May 1993. Immunization records came from individual area Health Stations.

Findings

1. Sero-positivity rates of JEV Nt Ab, by age

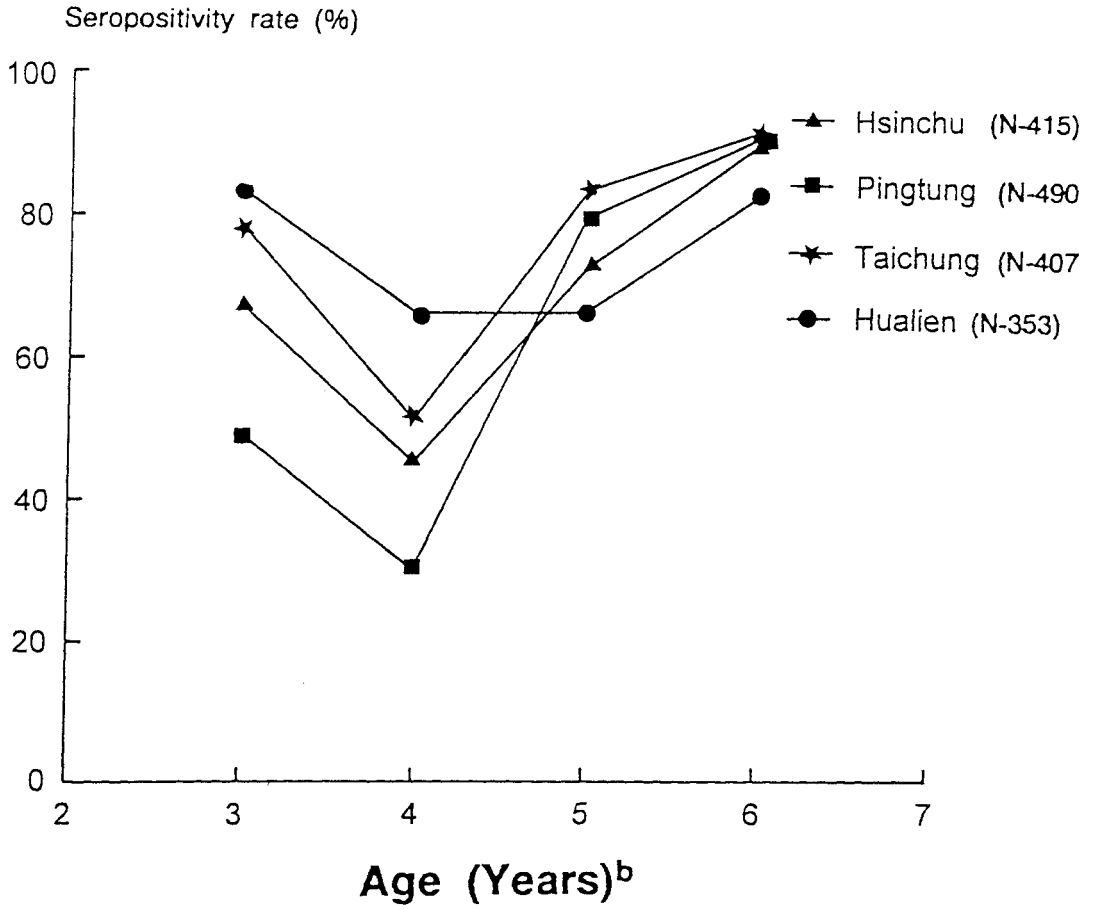
By using the Nakayama-NIH strain as the antigen in laboratory testing, of the 2,281 of the three- to six-year-old children, the total JEV Nt Ab positive rate was found to be 67% (1,520/2,281). For children who had had three doses of vaccines, the Nt Ab positive rates were: 68% (325/481) for the three-year-olds; 47% (219/466) for the four--year-olds; 76% (336/445) for the five-year-olds and 87% (242/273) for the six-year-olds (see Table 1). Of all age groups, the four-year-olds had the lowest positive rate: 45% (56/125) in Hsinchu; 51% (57/111) in Taichung; 30% (39/128) in Pingtung; and 66% (67/102) in Hualien, significantly lower than that of children in other age groups ($p<0.0001$, chi-square test). The Nt Ab positive rate went up after age five, and reached the highest point at age six, or around 82-91%. (see Table 1, and Figure 1)

Table 1. Seropositivity Rates of JEV Nt Ab, 1993[a]

Item	Hsinchu		Taichung		Pingtung		Hualien		Total	
	No.	Rate (%)	No.	Rate (%)	No.	Rate (%)	No.	Rate (%)	No.	Rate (%)
Age (Years)[b]										
3	108	67	111	78	150	49*	112	83	481	68
4	125	45*	111	51*	128	30*	102	66*	466	47*
5	109	72	107	83	134	79	95	66*	445	76
6	73	89	78	91	78	90	44	82	273	87
Immunized										
3 doses	415	65	407	75	490	59**	353	73	1,665	67
2 doses	32	72	7	43	21	62	37	68	97	66
1 dose	2	50	3	33	4	25	3	33	12	33
none	8	50	9	56	13	39	17	24	47	40***

a. Nakayama-NIH strain used in the plaque reduction neutralization test
b. includes only children completed with three doses
* comparing with children of different ages in the same county, $p<0.05$, Chi-square test
** comparing Pingtung with other counties, $p<0.05$, Chi-square test
*** comparing the unimmunized with children with one, two or three doses of vaccines, $p<0.05$, Chi-square for trend test

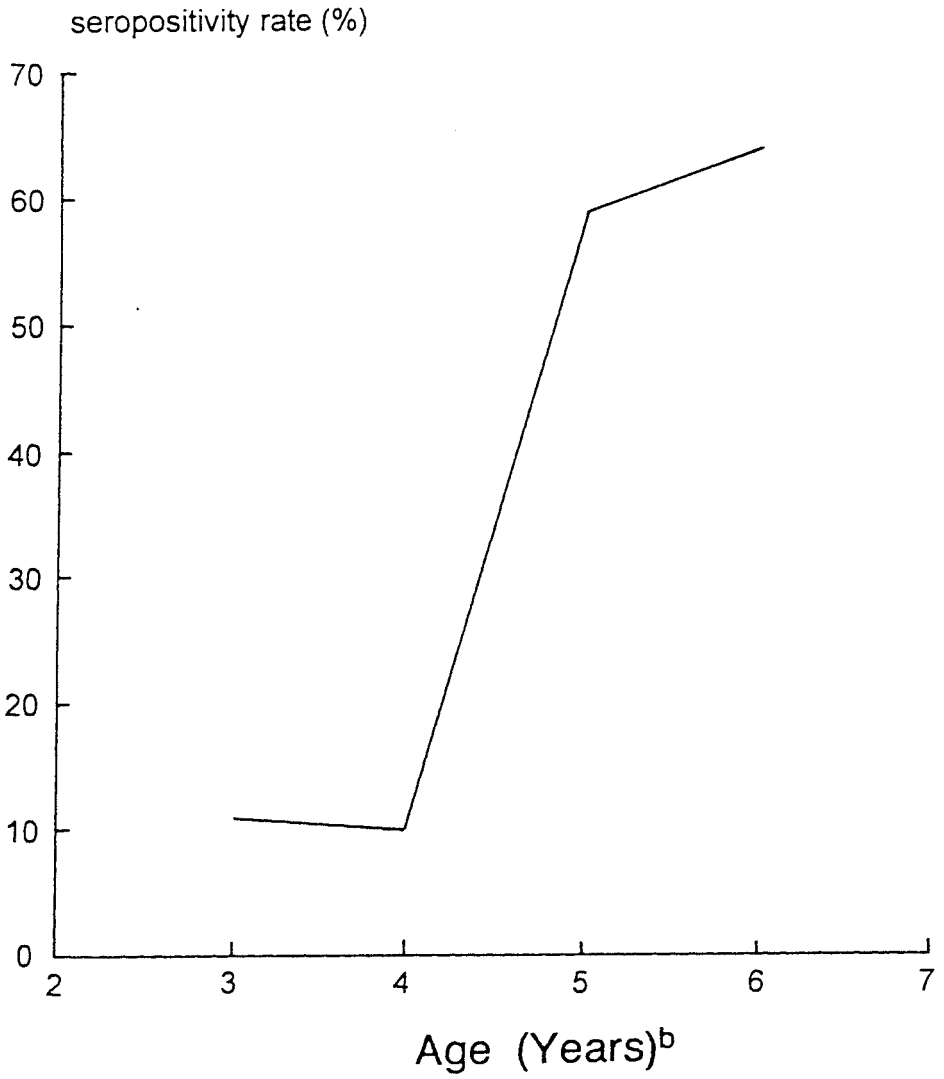
Figure 1. Seropositivity Rates of JEV Nt Ab Against Nakayama-NIH Strain Among 3-6 Year-Old Children in Taiwan, 1993[a]



a. Nakayama-NIH strain used in the plaque reduction neutralization test
b. includes only children with doses of vaccines

For non-immunized children, the Nt Ab positive rates showed a similar trend: 11% (1/9) for the three-year-olds; 10% (1/10) for the four-year-olds; 59% (10/17) for the five-year-olds; and 64% (7/11) for the six-year-olds (Figure 2). By area, the four-year-old children of the mountain areas in Hsinchu (33%, 18/55), Taichung (32%, 13/38) and Pingtung (31%, 23/74) had the lowest JEV Nt Ab positive rates.

Figure 2. Seropositivity Rate of JEV Nt Ab Among 47 unvaccinated 3-6 Year-Old Children from Four Areas in Taiwan, 1993[a]



a. Nakayama-NIH strain used in the plaque reduction neutralization test
b. includes only children not immunized at all

2. Sero-positivity rates of JEV Nt Ab by area

The positive rate (71%) of the plain townships in the four counties was significantly ($p=0.0001$, Chi-square test) higher than that of the mountain townships (63%). In particular, the positive rates of the plain townships in Hsinchu and Taichung counties were significantly ($p=0.007$, Chi-square test) higher than those of the mountain

townships. This was not the case in the other two counties (see Table 2). Of the plain townships, Pingtung County had the lowest rate (60%, 157/260); in the mountain townships, Hsinchu County had the lowest rate (55%, 99/179). In both plain and mountain townships, Hualien had the highest positive rates (75%, 107/142; and 72%, 152/211, respectively).

Table 2. Seropositivity Rates of JEV Nt Ab by age, Township, 1993[a]

Item	Hsinchu		Taichung		Pingtung		Hualien		Total	
	Plain	Mountain	Plain	Mountain	Plain	Mountain	Plain	Mountain	Plain	Mountain
	Rates (No.)	Rates (No.)	Rates (No.)	Rates (No.)	Rates (No.)	Rates (No.)	Rates (No.)	Rates (No.)	Rates (No.)	Rates (No.)
Age (Year)[b]										
3	63% (59)	71% (49)	80% (71)	73% (40)	42% (81)	58% (69)	82% (56)	84% (56)	65% (267)	71% (214)
4	54% (70)*	33% (55)	62% (73)*	32% (38)	30% (54)	31% (74)	63% (41)	67% (61)	53% (238)	41% (228)
5	89% (61)*	50% (48)	82% (72)	86% (35)	79% (72)	79% (62)	72% (29)	64% (66)	82% (234)	69% (211)
6	94% (46)	81% (27)	92% (62)	88% (16)	91% (55)	87% (23)	88% (16)	79% (28)	92% (179)	83% (94)
Total	73% (236)*	55% (179)	78% (278)**	66% (129)	59% (262)	58% (228)	75% (142)	72% (211)	71% (918)***	63% (747)

a. Nakayama-NIH strain used in the plaque reduction neutralization test
b. includes only children completed with three doses
* comparing children of the same age group in the same county by either plain or mountain townships, $p<0.05$, Chi-square test
** comparing plain and mountain townships in the same county, $p<0.05$, Chi-square test
*** comparing plain and mountain townships, $p<0.05$, Chi-square test

By age, in both plain and mountain townships, Pingtung County had the lowest rates: 42% (34/81) and 58% (40/69), respectively, for the three-year-olds; and 30% (16/54) and 31% (23/74) for the four-year-olds, respectively. Hualien County, on the other hand, had the highest rates: 82% (46/56) and 84% (47/56) for the three-year-olds, respectively from plains and mountains; and 63% (26/41) and 67% (41/61) from plains and

mountains, respectively, for the four-year-olds. The positive rates in the plain townships of both Hsinchu and Taichung counties were significantly higher ($p<0.0001$, Chi-square test) than those of the mountain townships (54%, 38/70 and 33%, 18/55, in Hsinchu County; and 62%, 45/73) and 32%, 12/38 in Taichung County).

3. Sero-positivity rates by doses of vaccines

In all four counties, the children who had had three doses of vaccine had a JEV Nt Ab positive rate of 67% (1,122/1,665). For children who had had two doses, the positive rate was also as high as 66% (64/97). Among children who had had only one dose, the positive rate was 33% (4/12), a figure close to the positive rate for non-immunized children whose positive rate was 40% or 19/47. The positive rate of those non-immunized was significantly lower ($p<0.0001$, Chi-square test) than that of children with two or more doses. This shows that the number of doses was related to the distribution of positive rates (Table 1). Children who had had two doses had the lowest rate in Taichung County (43%, 3/7); of those who had had three doses, the positive rate was the lowest in Pingtung County (59%, 289/490), suggesting that quality control of the JE vaccines seems to differ from place-to-place.

Discussion

The availability of vaccines has successfully reduced the incidence of Japanese encephalitis and brought it under effective control. As assessed by T.C. Hsu et al. in 1961 in four counties of northern Taiwan, the effectiveness of the vaccine used was assessed to be 80% after two doses of immunization[9]. In 1985 in northern Thailand, the effectiveness of the vaccine after two doses was assessed to be 91%[10]. Though these two large-scale community surveys indicated that the vaccines were quite useful, 21 (13%) of the 162 cases confirmed during the 1986-1991 period in Taiwan had complete immunization records[12]. Therefore the distribution of the JEV Nt antibodies by age groups, and the power of the protection they afford in a mass immunization program, ought to be further studied.

The present investigation found that the JEV Nt Ab of children of Hsinchu, Taichung, Pingtung and Hualien counties increased after age five, indicating that spontaneous infection was still present. Although the present study was cross-sectional and was not able to identify the true infection rate, estimating from the antibody positive rate of the 47 non-immunized children, those four and five years old were estimated to have an annual infection rate of around 50%. With the exception of the five-year-olds of Hualien County, in the other three counties the antibody positive rates increased with age for those children who had received three doses of vaccine.

Inapparent infection by Japanese encephalitis is frequent. Statistics of the Republic of China's National Institute of Preventive Medicine show that, between 1987 and 1992, there were, (including confirmed cases and deaths) 1, 13, 16 and 12 cases in Hsinchu, Taichung, Pingtung and Hualien Counties, respectively. This again indicates that

spontaneous infection did in fact exist in those areas. An accurate infection rate, however, can only be detected through sero-epidemiological cohort studies. Surveillance of pig blood shows that the sero-positive rates of antibodies in pigs reach 100% soon after mid-May[13], which indicates that the viruses are still prevalent in the natural ecology. The sharp decline in the number of reported cases, however, proves once again the effectiveness of the vaccine.

The mountain townships and their neighboring plain townships selected for study in the present investigation are rather remote, hilly, sparsely populated, with few paddy-fields for the breeding of *Culex tritaeniorhynchus*. Through what mechanism(s) do the viruses and the vectors survive? With the decline in land area available for agricultural purposes, and the modernization of pig farming, the ecological environment has changed. That a relatively high spontaneous infection rate of Japanese encephalitis has been noted in the present survey deserves further investigation.

The Nt Ab positive rate of the three-year-old children (one year after the three doses of vaccine) in the four counties was higher than that of the four-year-old children (two years after the three doses). This can perhaps be explained by a reduction in antibody potency of the vaccines, since that potency is known to decline gradually. Oya of Japan therefore recommends one booster every three to four years to maintain immunity against Japanese encephalitis[4]. The need for such a booster should be assessed.

Finding the number of doses of vaccine to be in direct proportion to the distribution of the Nt Ab positive rates, and further finding that the total antibody positive rate (38%) of the 47 non-immunized children was lower than that of children with two or three doses has indicated that children who have had three doses should be better protected than those with only two, a single dose, or no immunization at all. In 1970, Kanamitsu et al. also established that three doses of the vaccine would not improve antibody potency, but also extend the continuity of antibodies[15]. Studies of adults by the US Centers for Disease Prevention and Control (CDC) showed similar findings[16]. This suggests that, though the spontaneous infection rate is high in certain areas, children with the basic three doses of vaccines are more highly protected and that, in the current control program against Japanese encephalitis, vaccines still play a very important role.

Study has demonstrated that the antibody distribution in children with three doses was lowest (59%) in Pingtung County, and that the total positive rate (71%) for the plain townships was significantly higher than the rate (63%) for the mountain townships that the antibody positive rates of the plain townships in Hsinchu and Taichung counties were higher than those for the mountain townships, but this was not the case for Pingtung and Hualien Counties. All these data suggest the involvement of such factors as the different geographic conditions in different areas, different spontaneous infection rates in different areas, different approaches taken by workers in different areas, different temperatures used to store vaccines in different areas or even cause question whether the virus strains used for immunity in different areas might be different. Further investigation into these variables is needed.

The present investigation also showed that the JEV Nt antibody of those four-year-

old children studied was declining. If children of this age group can be received follow-up, whether the antibody is declining and what the level of spontaneous infection is in these four counties can be clarified. Since the areas studied are rural, spontaneous infection rates tends to be higher. Study of an urban location should be helpful in noting the difference in spontaneous infection rates in rural and urban places. The effects of natural ecological environments on the JEV can also be observed. Those environments have changed with the decline in numbers of rice paddies, the modernization of pig farming and even changes in the weather. None of these factors is in favor of the survival and transmission of JEV, yet the JEV infection rate is still relatively high in rural areas. That observation deserves further study.

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