

Japanese encephalitis virus infection in cattle: comparison of antibody distribution in the central and southern regions of Japan

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日本中部와 南部의 牛에서 日本腦炎感染에 관한 疫學의 研究

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抄錄: 1982년부터 1984年度까지 3年間に 걸쳐, 日本內 中部 Saitama縣의 牛 1,306頭와 南部의 Kagoshima縣의 牛 536頭를 對象으로 하여 日本腦炎바이러스 (JEV)의 赤血球凝集抑制 (HI) 抗體 陽性率을 檢査한 바, Kagoshima縣에선 68.8% 그리고 Saitama 縣에서는 65.5%가 陽性이었다.

季節別로는 兩地域이 供히 夏節에 抗體陽性率이 높았고, 年齡別 陽性率은 Saitama 縣의 경우 64.0%부터 82.8%까지 分布하고, Kagoshima縣의 牛는 1歲群에서 29.4%, 2歲群에선 50.0%, 3歲群에선 47.4% 그리고 4歲群에선 74.5%의 陽性率을 나타내었다. 그리고 兩地域의 年齡과 抗體力價間에는 相關性이 없었고, Saitama縣 牛의 力價는 年齡에 따라 15.3~22.5, Kagoshima 縣牛는 20.0~32.3이었다.

Key words: Japanese encephalitis virus infection, cattle, Japan.

Introduction

Regarding the dissemination of Japanese encephalitis virus (JEV) in Japan, much importance has been attached to the basic transmission cycle in which mosquitoes play the role of vector and swine play the role of amplifier, and the latter role has already been elucidated in much detail.^{1,2} JEV, however, has also been isolated in domestic animals, wild animals, birds and reptiles, and the presence of JEV antibodies in their blood has been proved.^{3,4} It is probable, therefore, that these animals also are involved in the transmission cycle of JEV.^{5,6,7} The method by which JEV hibernates from late

autumn to spring, the non-epidemic season of Japanese encephalitis (JE), is little known to date. In order to further elucidate the nature of JEV in nature, we have been investigating the presence/absence of hemagglutination inhibitor (HI) antibody against JEV in cattle.⁸⁻¹¹ Cattle are known to be sensitive to JEV¹² and have a longer life-span than swine. Since cattle are not vaccinated against JE, the course of JEV infection can be accurately followed over a long period of time. In the present investigation, we conducted a comparative seroimmunological study on the distribution of HI antibody in cattle in Saitama prefecture (located in central Japan) and Kagoshima prefecture (located

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in southern Japan) during the period from early summer through early winter.

Materials and Methods

Bovine serum: Serum samples were collected from 1306 and 536 cattle, raised in Saitama and Kagoshima prefecture, respectively, during the period from 1982 to 1984 (Fig 1). Animals ranged from one to 18 years of age. Cattle in Saitama were of the Holstein-Friesian breed while those in Kagoshima were Japanese Black breed except for 33 Holstein-Friesians. All sera were stored at -20°C until use. At the time of determination of HI antibody titer, serum samples were thawed, treated with acetone, then adsorbed by goose erythrocytes. The resulting supernatant was incubated at 56°C for 30min before the testing.

Antibody determination: Serum antibodies against JEV virus were tested using HI test with a commercial antigen of the JEV virus strain JaGAR#01. The HI test was carried out according to the method of Clarke and Casal in a microtiter system.¹³ Titers were expressed as the reciprocal of the highest dilution of serum that completely inhibited hemaggluti-

nation by 16 units of the antigen. An HI antibody titer of 10 was recorded as positive. A serum known to be positive to JEV virus served as a control throughout the experiment.

Results

Monthly distribution of HI antibody: Over a three-year period, the antibody-positive rate in cattle was 65.5% in Saitama and 68.8% in Kagoshima. Statistically, there was no significant difference between the two groups. The antibody titers ranged from 1:10 to 1:320 in Saitama and from 1:10 to 1:640 in Kagoshima. The mean titer was 20.5 and 28.5 in Saitama and Kagoshima, respectively.

The antibody-positive rate in Saitama was 38.2% in July, 50.5% in August, 83.1% in September and 81.5% in October. As demonstrated by the results, a significant increase in this value was observed during the summer season. The antibody-positive rate in Kagoshima was 10.5% in July, then markedly increased to 70.8% in August. In September, the rate was 75.9%, almost the same as in August. The rate remained high even in December (70.5%) (Fig 2).

In both prefectures, increases in the monthly mean antibody titer paralleled those of the antibody-positive rate, and decreased from September. In Kagoshima, however, the monthly mean antibody titer was still high (26.6) even in December as was the case with the antibody-positive rate; the value was equal to that observed in Saitama in September (26.6).

Distribution of HI antibody as a function of age: In Saitama, the antibody-positive rate based on age ranged from 64.0% to 82.2%, with no correlation between the positive rate and age. In Kagoshima, on the other hand, the antibody-positive rate was low in the one-year-old cattle group (29.4%), but much higher in the two-year-old group (50.0%), the three-year-old group (47.7%) and the four-year-old group (74.5%) with the positive rate significantly increasing with age. In cattle older than four years, a slight increase in the positive rate was observed with age, but the results were not statistically significant (Fig 3).

In Saitama, the mean antibody titers in each age

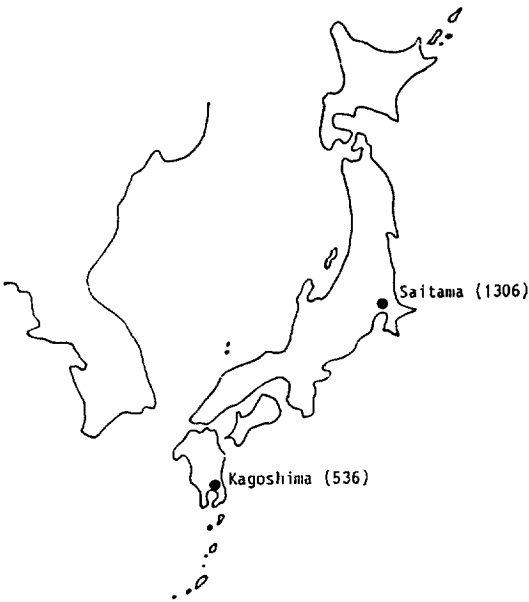


Fig 1. Sampling sites for the survey of antibody titers against Japanese encephalitis virus. Numbers in parentheses indicate the sample sizes.

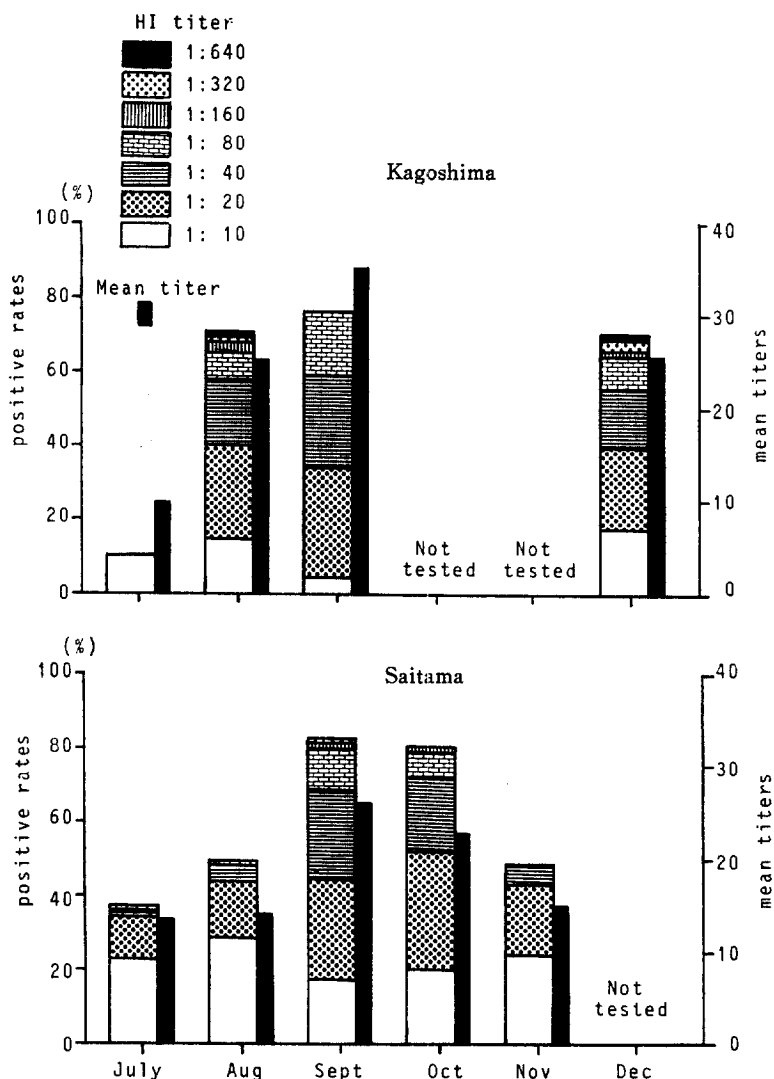


Fig 2. Seasonal distribution of HI antibodies against JEV in bovine sera collected in Saitama and Kagoshima prefectures.

group ranged from 15.3 to 22.5, with no significant correlation between this value and age being observed. The mean antibody titer in Kagoshima was 26.9 in the one-year-old group, 25.8 in the two-year-old group and 25.0 in the three-year-old group with the titer being almost the same in these three age groups. This parameter was only slightly higher in the eight-year-old group (30.5) and the nine-year-old or older group (32.3). Thus, the mean antibody titer showed no remarkable increases with age (Fig 4).

Discussion

Seroimmunological studies on JEV infection in cattle have been reported in Japan, Southeast Asian countries^{14,15} and India.^{16,17} The number of such reports, however, is small, and no detailed study has ever been made. In the present report, we investigated the positive rate for HI antibody against JEV in cattle as well as seasonal fluctuations in this parameter and its relationship to the age of the carrier. This study was performed using serum samples



Fig 3. Age distribution of positive reactors in HI tests with JEV in bovine sera collected in Saitama (o-o, N=1306) and Kagoshima (•-•, N=536) prefectures.

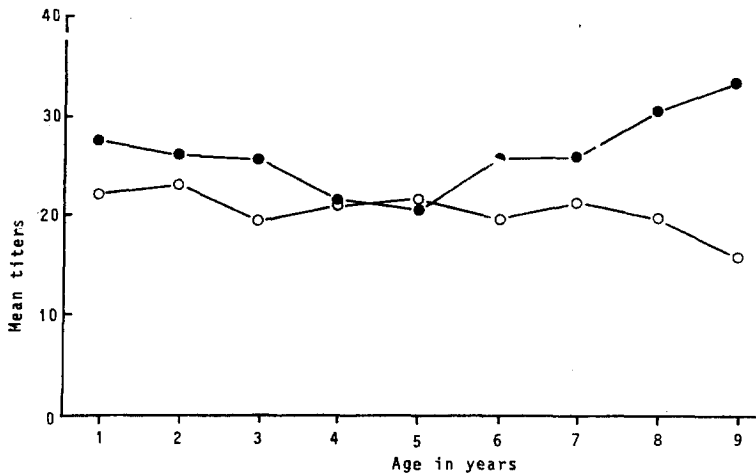


Fig 4. Age distribution of mean HI antibody titers in bovine sera collected in Saitama (o-o) and Kagoshima (•-•) prefectures

collected from 1306 cattle in Saitama prefecture and 536 in Kagoshima prefecture during the three-year-period from 1982 through 1984. The results from each prefecture were compared.

Over the 3-year period, there were no striking differences of the positive rates for HI antibody against JEV in Saitama and those observed in Kagoshima. In Saitama, both the mean monthly antibody-positive rate and antibody titer from July through December increased in summer and decreased thereafter demonstrating seasonal changes with a peak occurring in summer. For cattle in Kagoshima as well, both the mean antibody-positive rate and

the mean antibody titer increased in summer, and both parameters remained high levels even in December. The distribution of the antibody-positive rate is greatly influenced by meteorological conditions, which affect the generation of mosquitoes, a known JEV vector.^{8,18} Hence, it is postulated that differences in the meteorological conditions of the two prefectures were in part responsible for the difference observed in the two antibody-positive rates.

On the other hand, Takahashi¹⁹ reported that differences found in the *Culex tritaeniorhynchus* line were related to differences in the mosquito's ability to intermediate JEV. Thus, it is possible that this

factor also played a role in the difference in antibody-positive rate between the two regions. In Saitama, the antibody titer increased in autumn while a low level of antibody could be detected in winter. The antibody-positive rate decreased in winter, with the cattle detected positive for HI antibody again following summer as a result of JEV re-infection. Thus, the antibody-positive rate also increased again in summer. It is surmised that, due to the repetition of such a transmission cycle, all cattle in Saitama showed a similar pattern of changes in the antibody-positive rate and antibody titer independent of age. In Kagoshima, on the other hand, the high antibody-positive rate observed in summer was also noted in winter. Since the antibody-positive period in cattle was prolonged, the antibody-positive rate did not decrease even in the following year. The following summer, cattle which had previously tested negative for the antibody were newly infected with JEV. Consequently the number of antibody-positive cattle, the antibody-positive rate, increased due to this accumulation in numbers. As a result of this phenomenon, there was no increase in the mean antibody titer with age while there was an increase in the antibody-positive rate. In an earlier study on 29 cattle in Saitama prefecture,⁸ only 6.3%(2/32) of the cattle detected positive for the antibody during one-year period. This supports the hypothesis presented above. An increase in the antibody-positive rate with age has also been reported in humans and swine.²⁰⁻²² In most humans, except for those living in special regions such as isolated islands,²³ the antibody-positive rate increases with age. In the present study, although the same phenomenon was observed in cattle in Kagoshima, no remarkable increase was detected in Saitama. We believe think that the relationship between age and the antibody-positive rate in cattle differed in the two regions. This theory is based on the following facts: (1) no striking increase in the antibody titer was observed after JEV infection in cattle, unlike in humans and swine, (2) no vaccination for Japanese encephalitis has been performed in cattle and (3) the line of mosquitoes may be different in the two regions and possibly differ in their respective abilities to inter-

mediate JEV.

Summary

The frequency of appearance of HI antibody against Japanese encephalitis virus was investigated over the three-year period 1982 through 1984. The sample population consisted of 1306 cattle in Saitama prefecture (located in central Japan), and 536 cattle in Kagoshima prefecture (located in southern Japan). During the study period, there was no significant difference in the average antibody-positive rate between cattle in Kagoshima (68.8%) and those in Saitama (65.5%). Variations in the monthly antibody-positive rate and mean monthly antibody titer in the Saitama cattle followed a mountain-like pattern with the peak observed during summer. In the Kagoshima cattle, both parameters also increased in summer and these increases persisted even in winter. The distribution of antibody-positive rate as a function of age ranged from 64.0% to 82.8% in Saitama, though no correlation was observed between these two factors. In the Kagoshima cattle, however, the antibody-positive rate increased with age as follows; the values averaged 29.4% in the one-year-old cattle group, 50.0% in the two-year-old group, 47.4% in the three-year-old group and 74.5% in the four-year-old group. There was no significant correlation between the mean antibody titer and age in either Saitama cattle (15.3~22.5) or Kagoshima cattle (20.0~32.3).

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