

牛における日本脳炎ウイルス感染の動向

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Status of Japanese Encephalitis Infection in Cattle: Survey of Antibodies in Various Geographical Locations in Japan

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ABSTRACT. Serum samples were collected from a total of 1339 cattle raised in the northern, central and southern regions of Japan during the years 1982–83, and their antibody titers against Japanese encephalitis virus were determined by the hemagglutination-inhibition test. Antibody prevalences were 59.7% (468/784) and 56.8% (179/315) in the central and southern regions, respectively, while only 2.1% (5/240) in the northern region. The distribution of positive reactors to the virus was limited to the southern regions in Spring but the positive cases appeared also in the northern regions in September.—**KEY WORDS:** antibody to JEV, Japanese cattle, Japanese encephalitis virus (JEV).

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Japanese encephalitis (JE), a viral zoonosis, occurs mainly in Eastern Asia (Japan, Eastern Siberia, China, Korea, Taiwan, Malaysia, Singapore and India). In Japan, severe epidemics occurred in humans (morbidity: 1.6–6.2/100,000) and horses (30.0–337.1) during the period 1948–51, followed by moderate epidemics in the human (1.7–5.0) and horses (3.0–46.0) in 1952–61 [9]. Since then the incidence of the disease has gradually decreased, although 20 to 80 persons were affected annually in the period of 1962–76. The disease in horses has also decreased markedly because of widespread reduction in total numbers of farm horses and the use of agriculture chemicals which have controlled the mosquito vector [4, 5]. In the past 10 years, no occurrence of the disease was recognized among Japanese horses with the exception of 5 cases in 1983 [8]. On the contrary, large-scale epidemics of JE virus (JEV) infection occurred recently in the Eastern Asia. For example, 5,459 persons contracted the disease in India [2]. It seems, therefore, that the main epidemic areas of the infection have moved to the Eastern Asian countries. The ecological nature of JEV in

the human and animal populations, however, has not yet been fully explained. In the present study, an attempt was made to elucidate the epidemiological significance of JEV infection in animals raised in Japan. The cattle were selected as the objects for the study because they have not been vaccinated against the disease.

MATERIALS AND METHODS

Serum: Serum samples were obtained from 240, 784 and 315 cows, ranging from one month to 14 years of age, raised in Iwate (northern region), Saitama (central region) or Kagoshima prefectures (southern region) during the period from July 1982 to Sept. 1983 (Fig. 1). They were Holstein Friesian breed except 47 in Kagoshima prefecture which were the Japanese Black breed. These sera were stored at -20°C before testing.

Serological test: The sera were treated with cold acetone as described by Clarke and Casals [1]. Some of the sera were pre-treated with 0.2 M 2-mercaptoethanol (2ME) (1 volume: 1 volume of serum) at 37°C for one hour before the acetone-treatment as indicated in

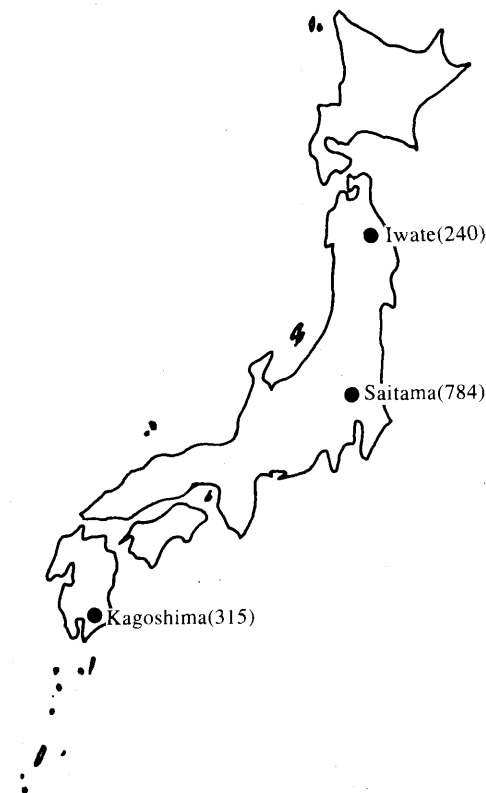


Fig. 1. Sampling sites for the survey of antibody titers against Japanese encephalitis virus. Numbers in parentheses indicate the sample sizes.

the text. For comparison, the swine positive reactions against JEV were obtained from the statistics published weekly in 1982 by the Division of Health Information, Bureau of Public Health, Ministry of Health and Welfare of Japan.

Antibody determinations: Antibodies to JEV was tested by the hemagglutination-inhibition method using a commercially prepared antigen of JEV strain JaGAR#01 (Takeda Chemical Industries, Ltd., Osaka). The HI test carried out by the Clarke and Casals' method in microtiter system [1]. The titer was expressed as the reciprocal of the highest dilution of serum which completely inhibited the hemagglutination by 16 units of the antigen. An HI antibody titer of ≥ 10 was recorded as positive. A known positive serum to

JEV served as the control throughout the experiments.

RESULTS

Regional distribution of HI antibody to JEV: Distribution of HI antibody to JEV was investigated among the cattle raised in three geographical locations (Table 1). In Iwate prefecture, although the test sera were collected in Sept. 1983, immediately after the summer season in the region, the positive rate was as low as 2.1% with low titers. In Saitama and Kagoshima prefectures, more than one half of the sera tested in July 1982 through Sept. 1983 showed positive titers ranging from 10 to 640. The high HI titers of 160 or over were in 10.6% (19/179) of the positive sera in Kagoshima, but only in 2.1% (10/468) of those in Saitama. There was no significant difference in the positive rates between the Holstein Friesian (23/30, 73.3%) and the Japanese Black (35/47, 74.5%) breed raised in the same area of Kagoshima prefecture.

Fig. 2 shows the antibody prevalence in the sera of cattle and swine in the three prefectures according to the week and month of sample collection. In Iwate prefecture, all the positive sera of cattle collected even in 1983 (5/240, 2.1%) were distributed only in the first week of Sept., while in the swine sera, positive rate was 5% (1/20), two and three weeks later in 1982. In Saitama and Kagoshima prefectures, the positive rates in cattle gradually increased from 21.3% (19/89) to 82.7% (149/180) in the former, and from 10.5% (2/19) to 66.7% (10/15) in the latter, from July to Sept. 1982. In the swine sera, parallel increase was observed from 10% (2/20) to 100% (20/20) in Saitama, and from 3.3% (1/30) to 96.7% (29/30) in Kagoshima with one to two weeks delay the comparison with the cattle. A significant reduction of HI titers (8- to 128-fold) was observed after the treatment with 2ME in 12 (92.3%) of

Table 1. Distribution of HI Antibodies against JEV in Bovine Sera Collected in Three Prefectures (1982-83)

Prefecture	No. of sera	No. of sera with antibody at indicated HI titers								≥10 (%)
		<10	10	20	40	80	160	320	640	
Iwate	240	235	4	1	0	0	0	0	0	5(2.1) ^{a)}
Saitama	784	316	132	154	114	58	7	3	0	468(59.7)
Kagoshima	315	136	48	59	39	14	9	6	4	179(56.8)
Total	1339	687	184	214	153	72	16	9	4	652(48.7)

a) Significantly different from Kagoshima by chi-square test at $p < 0.001$.

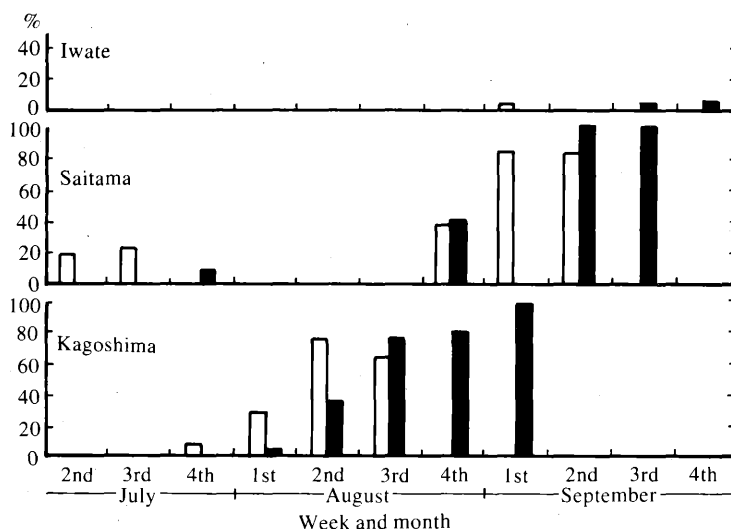


Fig. 2. Weekly positive rates of HI antibody to JEV in bovine (□) and swine (■) sera in three prefectures (1982). The result on bovine sera in Iwate prefecture was obtained from the sera collected in 1983.

the 13 bovine sera obtained in Aug. 1982 and 7 (77.8%) of the 9 bovine sera collected in Apr. 1983. In contrast, none of the positive sera collected in Dec. 1982 (five cases) showed any significant reduction of the titers after 2ME-treatment.

Seasonal distribution of HI antibodies to JEV: In Saitama prefecture, a total of 784 sera were examined on three occasions for the presence of HI antibody to JEV (Table 2). In July and Aug. 1982, 22.6% and 38.8% of the test sera showed positive titers ranging from 10 to 80, and only few cases had titers of 40 to 80. On the contrary, in Sept. 1982, the

percentage of positive sera elevated to 82.6%. The titers ranged from 10 to 320, and about one half of the positive sera (173/347, 49.9%) showed high titers of 40 or over. Likewise in Kagoshima prefecture (Table 3), 315 sera were examined on three occasions. In July-Aug. 1982, 50.7% of the test sera showed positive titers, and the positive rate in Dec. 1982 increased to as high as 71.3%. In Apr. 1983, however, the positive rate decreased again to near the initial value (52.4%). The HI titers ranged from 10 to 320 in July-Aug. and Dec. 1982, and from 10 to 320 in Apr. 1983, although there was no

Table 2. Seasonal Distribution of HI Antibodies against JEV in Bovine Sera Collected in Saitama Prefecture

Date of serum collection	No. of sera	No. of sera with antibody at indicated HI titers							≥10 (%)
		<10	10	20	40	80	160	320	
July 1982	124	96	20	6	0	2	0	0	28(22.6) ^{a)}
Aug. 1982	240	147	58	28	4	3	0	0	98(38.8) ^{a)}
Spet. 1982	420	73	54	120	110	53	7	3	347(82.6)
Total	784	316	132	154	114	58	7	3	468(59.7)

a) Significantly different from Sept. 1982 by chi-square test at $p < 0.001$.

Table 3. Seasonal Distribution of HI Antibodies against JEV in Bovine Sera Collected in Kagoshima Prefecture

Date of serum collection	No. of sera	No. of sera with antibody at indicated HI titers								≥10 (%)
		<10	10	20	40	80	160	320	640	
July-Aug. 1982	146	72	19	26	16	4	4	3	2	74(50.7)
Dec. 1982	87	25	19	22	13	3	1	2	2	62(71.3)
Apr. 1983	82	39	10	11	10	7	4	1	0	43(52.4)
Total	315	136	48	59	39	14	9	6	4	179(56.8)

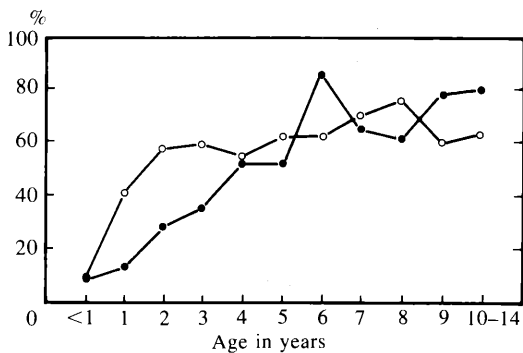


Fig. 3. Age distribution of positive reactors in HI tests with JEV in bovine sera collected in Saitama (○—○, N=744) and Kagoshima (●—●, N=295) prefectures.

marked difference in the distribution of them among the three observation times.

Fig. 3 represents the age distribution of positive reactors of cattle to JEV in Saitama and Kagoshima prefectures. The positive ratio in each age was calculated from the 40 to 130 cattle in Saitama and the 20 to 30 cattle in Kagoshima. In Saitama prefecture, an

incidence of positive reactors to JEV in the cattle aged one to 2 years was as high as 40.8 to 57.5%, as compared to 9.4% in the animals 3 to 11 months old. The positive rates remained almost at the same levels ranging from 59.2% (3 years old) to 76.0% (8 years old). On the contrary, in Kagoshima prefecture, the positive reactors to JEV increased gradually from 8.3% (1 to 11 months old) to 85.7% (6 years old), with the advance in age. Thereafter, the positive rates ranged from 65.0% (7 years old) to 80.2% (10 years or older), with a difference of about 20%.

DISCUSSION

It is well-known that most of the cattle in Japan have positive antibodies to JEV as do horses or swine [3, 9, 11, 12]. The virus also is widely disseminated among cattle in Indonesia [7]. Successful isolation of JEV from the blood sample of one out of the five sentinel cattle was achieved by Otsuka *et al.*

[10] in July 1967 in Japan. However, little is known about a role of cattle in the ecology of JEV infection in human and animal populations. In the present study, the serological evidence of JEV infection among the Japanese cattle has been confirmed. In the regional distribution of cattle, the very high incidences of 57% and 60% in bovine positive reactors to JEV were observed in Kagoshima (N 31°30', E 130°30') and Saitama (N 35°50', E 139°45') prefectures, as compared to 2% in Iwate (N 39°40', E 141°15') prefecture. In addition, high HI titers were recognized in more samples in Kagoshima (11%) than in Saitama (2%). On the other hand, Goto *et al.* [6] described that no response to JEV in HI test was observed in all of the 76 cattle raised in the eastern district (N 42°50', E 143°55') of Hokkaido, the northernmost island of Japan. Yamada *et al.* [12] also reported that in the northern Hokkaido (N 43°55', E 141°40') none of the 70 cattle showed positive antibodies to JEV in serum neutralization test, although 9% of the 534 cattle had low antibody titers of 5 to 40 in the southern districts (N42–43°, E 140–141°). These findings, including the present results, apparently indicate that the bovine positive reactors to JEV have distributed from the southern to the northern regions of Japan, except the northeastern Hokkaido.

In Kagoshima prefecture, the southernmost region in this study, one half of the cattle tested had positive antibodies to JEV and 7 of the 9 positive sera tested showed the 2ME-sensitive antibodies, in Apr. of 1983. Positive rates of the serum samples tested in July-Aug. and Dec. 1982 were 51% and 71%, respectively. Following the Kagoshima prefecture, the percentages of positive reactors in Saitama prefecture (the central region) gradually elevated from 23 in July to 83 in Sept. 1982. In Iwate prefecture (the northern region) only 5 (2%) of the 240 cattle had positive antibodies to JEV even in Sept. 1983. This trend also was very similar to

regional or seasonal distribution of swine sera showing HI antibodies positive to JEV, although the swine positive reactors appeared one to two weeks later than those of cattle (Fig. 2). The same finding was demonstrated in the swine and bovine sera collected during the period from June to Sept. 1967 in the central region of Japan [11]. Thus, it is likely that the bovine positive reactors to JEV have gradually distributed from the southern to the northern regions of Japan over a period from Apr. through Sept.

The positive rates of bovine sera in Kagoshima prefecture (Fig. 3) gradually increased according to the advance in age. On the contrary, in Saitama prefecture the cattle one to 2 years old showed relatively high percentage of positive reactors. This finding suggested that in this area a large-scale dissemination of JEV occurred among the cattle in 1981–82.

The present result, revealed the fact that bovine antibodies against JEV appeared high rates on some occasions or earlier than the swine positive reactors. As to the significance of the cattle in JE epidemic, it will need more frequent isolation of JEV from the bovine blood in a field trial, although the evidence of viremia in cattle was demonstrated only in one case as described above [10]. Such studies are in progress for the cattle raised in Saitama prefecture.

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要 約

牛における日本脳炎ウイルス感染の動向：わが国の3地域における抗体保有状況：酒井健夫・堀本政夫・後藤仁¹⁾（日本大学獣医衛生学研究室，¹⁾帯広畜産大学家畜微生物学教室）——1982～1983年にわたって、岩手県、埼玉県および鹿児島県で採取された牛血清1,339検体について、日本脳炎ウイルスに対する赤血球凝集抑制(HI)抗体価を測定した。抗体陽性率は、埼玉県では59.7% (784頭中468頭)、鹿児島県では56.8% (315頭中179頭)であったが、岩手県では2.1% (240頭中5頭)であった。抗体陽性牛の分布は、4月～9月にかけて漸次北上する傾向がみられた。