Meteorological Factors Involved in Japanese Encephalitis Virus Infection in Cattle

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ABSTRACT. From 1982 to 1987 a total of 4,371 dairy cattle in Saitama Prefecture, were examined for levels of hemagglutination inhibition (HI) antibody to Japanese encephalitis virus (JEV) and the correlation with meteorological factors and an antibody positive rate was studied. Positivity rates of HI antibody from July to October each year (Y_i) ranged from 58.8 to 88.0% with a considerable annual variation. Simple regression analysis of Y_i with the comparative meteorological value (X_i) was determined from mean temperatures ($T_i, j-1$), rainfalls ($R_i, j-1$) for 10-day-periods each, and the number of days showing 25°C or above ($t_i, j-1$) from June to September, which yielded a correlation coefficient of 0.8147 ($p < 0.05$) and an equation for estimated HI antibody positivity rate: $\hat{Y}_i = -0.04X_i + 79.9$ ($p < 0.05$). Multiple regression analysis with the power values of three meteorological parameters as independent variables and Y_i as the dependent variable, showed a multiple correlation coefficient of 0.987 ($p < 0.05$) and a multiple regression equation: $\hat{Y}_i = -3991T^{1/13} - 0.0035R^{-12} + 1978t^{1/9} + 4187$ ($p < 0.05$), giving estimated positivity rates almost equal to the values from the observations. Therefore, a predictive equation was formulated reflecting positive and negative correlations of sero-positivity with the temperature and the precipitation, respectively.—**KEY WORDS:** Japanese encephalitis virus (JEV), JEV antibody, meteorological factor.

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Japanese encephalitis virus (JEV) is widely distributed in Southeast Asian countries including Japan, China and Korea as well as India. Considerable efforts have been made to prevent from Japanese encephalitis (JE) in Asian countries [5, 15]. In Japan, human cases started decreasing from 1967 and less than 40 cases have been recognized annually since 1978. However, the population of insecticide-resistant *Culex tritaeniorhynchus* (*Ct*) [15], the primary vector of JEV, have increased and there might be possibility of JE epidemic. In addition, the antibody positivity rate in fattened pigs, which play a major role in the infection cycle of JEV, has still been high [16]. Recently, JE has also been observed in pigs in a northern part of Japan, Hokkaido, suggesting possible recurrence of major JE epidemics in Japan

[22].

The role of cattle in the JEV infection cycle [14] would be of importance, since animals are longer-lived with no vaccination to JEV. We have reported sero-epidemiological evaluations of JEV infection in cattle [4, 17–19]. In this study, we examined cattle older than 6 years of age for changes in the positivity rate of HI antibody and the results were analyzed in relationship between meteorological factors and the positivity rates in the same geographic region.

MATERIALS AND METHODS

Serum: From 1982 to 1987 serum samples were monthly collected from a total of 4,371 daily cattle reared in farms in the eastern

Saitama Prefecture, Japan, and stored at -20°C . The serum samples were thawed, treated with acetone, the inhibitor was removed by adsorption on goose red blood cells, and the supernate was heated at 56°C for 30 minutes before use.

HI antibody assay: The antibody level was measured by the microtiter technique according to the method of Clarke & Casals [2], using the JaGAr#01 diagnostic dry antigen (Kaketsuken, Kumamoto). Sera showing HI antibody titers of 1:10 or above were regarded as positive, and monthly positivity rates for HI antibody, $Y_{i,j}$ were calculated each year. Of $Y_{i,j}$, the "i" represents the last number of years from 1982 to 1987, while the "j", the month from January to December, respectively, when the samples were collected. The relationships between the positivity rate and the meteorological factors were analyzed with the mean of the HI antibody positivity rates ($Y_{i,j}$) in 4 months from July to October, that is, $Y_{i,7}$ to $Y_{i,10}$. The mean HI antibody positivity rates in animals of different ages (Z_a) were also used for analysis. Of Z_a , "a" represents the age of the cattle.

Climatic parameters: Three meteorological parameters from June to September 1982–1987 were of the records of Kumagaya Regional Meteorological Observatory [8], including the mean temperature ($^{\circ}\text{C}$), rainfall (mm) for 10 day-periods, and the number of days showing 25°C or higher. In some studies, analysis were made with the meteorological parameters a month before the serum sampling; the total of the mean temperature for the 10 day-periods ($T_{i,j-1}$), the total of the mean rainfall ($R_{i,j-1}$) and the number of days showing 25°C or higher ($t_{i,j-1}$). "j-1" was attached to the "T", "R" and "t", because, in a given year (i), the changes in the antibody level in the serum samples collected in the month j were associated with the meteorological values of the previous month (j-1).

Data analysis: Simple correlation and regression analysis were performed between the animal ages at the blood sampling (a) and the mean HI antibody titers. Then relationship between the meteorological values and the mean antibody positivity rate was examined by the following procedure. ($T_{i,j-1}$), ($R_{i,j-1}$), and ($t_{i,j-1}$) were raised to an arbitrary power, and between the values and the antibody positivity rate from July to October ($Y_{i,j}$) each year, correlation coefficients were determined. The power values showing the maximum coefficient were regarded as the optimal power for the corresponding meteorological parameter (m, n, and o). The meteorological values were raised to these optimal powers [$(T_{i,j-1})^m$, $(R_{i,j-1})^n$, and $(t_{i,j-1})^o$], and the multiplied meteorological value ($w_{i,j}$), mean multiplied meteorological value of the year i (W_i), total mean multiplied meteorological value ($\bar{W}$), and comparative meteorological value of the year i (X_i) were calculated by the following formulas.

$$w_{i,j} = T_{i,j-1}^m \cdot R_{i,j-1}^n \cdot t_{i,j-1}^o$$

$$W_i = \sum_{j=1}^{10} w_{i,j}/4$$

$$W = \sum_{i=1982}^{1987} W_i/6$$

$$X_i = (W_i/\bar{W}) \cdot 100$$

Simple correlation and regression between X_i and the antibody positivity rate (Y_i) were studied. Finally, multiple regression analysis was performed with ($Y_{i,j}$) as the dependent variable, and ($Y_{i,j-1}$), ($R_{i,j-1}$) and ($t_{i,j-1}$) or ($T_{i,j-1}$), ($R_{i,j-1}$) and ($t_{i,j-1}$) as independent variables.

RESULTS

Table 1 shows the antibody titers and the antibody positivity rates during the 6 years. The antibody titers were distributed from

1:10 or $\leq$ 1:320, and the mean positivity rate throughout the 6 year-period was 66.3%. The annual positivity rate varied from 50.3 to 74.1% with the maximum in 1985 and minimum in 1984. Positivity rates in 1985 were significantly higher ($p<0.05$) than those in 1983 and 1984. As presented in Fig. 1, sero-positivity rates during the 6 years were low in January to March, and increased slightly in April and May, considerably in June, reaching a peak in October.

The relationship between the animal age (a) and the mean antibody positivity rate (Z_a) are shown in Fig. 2, also indicating the correlation coefficient and the regression equation. The positivity rate was 45.5% in animals less than 1 year of age, but it increased with age reaching 79.4% in those

aged 9 years or over, with significant ($p<0.05$) correlation coefficient and regression.

Table 2 shows antibody titers and as well as means of monthly positivity rates (Y_i) from July to October when the positivity rate was notably increased. Throughout the 6 years the mean positivity rate from July to October was 74.9%. Y_i ranged from 58.8 to 88.0%, being highest (88.0%) in 1985 (Table 2) and lowest (58.8%) in 1982. In 1983, antibody levels of 1:10 or 1:20 were shown with many sera and there was no serum showing antibody titer of 1:160 or higher.

In Table 3, were shown the antibody positivity rates (Y_i), a mean meteorological value ($T_{i,j-1}$), ($R_{i,j-1}$) and ($t_{i,j-1}$) from

Table 1. Distribution of HI antibody titers to JEV in bovine sera

Year	HI antibody titer							No. positive/ No. tested (%)	
	<1:10	1:10	1:20	1:40	1:80	1:160	1:320		
1982	324 ^{a)}	130	153	114	56	7	3	463/ 787	(58.8)*
1983	187	109	75	40	6	0	0	230/ 417	(55.2)*
1984	255	123	102	27	5	1	0	258/ 513	(50.3)*
1985	377	294	372	282	111	18	1	1078/1455	(74.1)
1986	135	70	161	97	17	0	0	345/ 480	(71.9)
1987	194	166	230	104	25	0	0	525/ 719	(73.0)
Total	1472	892	1093	664	220	26	4	2899/4371	(66.3)

a) No. positive (1:10 or higher)/No. tested (%).

*Significantly (chi-square test) different from 1985 ($p<0.01$).

Fig. 1. Monthly positive rates of HI antibody to JEV in bovine sera (1982–1987).

Fig. 2. Age distribution of HI antibody titers to JEV in bovine sera (1982–1987).

June to September ($\bar{T}_i$, $\bar{R}_i$, and $\bar{t}_i$, respectively), a mean multiplied meteorological value (W_i) and comparative meteorological values (X_i) determined from $W_{i,j}$, antibody positivity rates estimated from the simple regression equation ($\hat{Y}_i$), and the total mean multiplied meteorological values ($\bar{W}$). Mean annual meteorological values were considerably fluctuated. By the optimal powers ($m=1/12$, $n=-20$, and $o=1/9$), ($W_{i,j}$), W_i , and X_i were calculated, resulting in that W_i and X_i were widely distributed from 0.7 to 6895.0 and from 0.1 to 500.1, respectively. The correlation and regression between X_i

and Y_i were both significant ($p<0.05$) with a correlation coefficient of $r=0.8147$ and a regression equation of $\hat{Y}_i = -0.04X_i + 79.9$. $\hat{Y}_i$ calculated by this equation was generally equal to Y_i . However, $\hat{Y}_i$ was not 80% or greater, because $\hat{Y}_i$ was 79.9% when X_i was 0.

Fig. 3 illustrates the relationship between the comparative meteorological value (X_i) and antibody positivity rate (Y_i). If $X_i \leq 100$ was regarded as the high risk zone for a major epidemic of the disease, the risk of the disease was considered to be high in all years including 1984 ($Y_i=72.4\%$), except

Table 2. Distribution of HI antibody titers to JEV in bovine sera collected from July to October

Year	HI antibody titer							No. positive/ No. tested (%) (Y_i)	
	<1:10	1:10	1:20	1:40	1:80	1:160	1:320		
1982	324 ^{a)}	130	153	114	56	7	3	463/ 787	(58.8)*
1983	39	68	63	34	6	0	0	171/ 210	(81.4)
1984	93	110	102	27	5	1	0	245/ 338	(72.4)
1985	91	85	231	231	98	18	1	655/ 756	(88.0)
1986	113	58	156	96	17	0	0	327/ 440	(74.3)
1987	98	105	183	81	18	0	0	387/ 485	(79.7)
Total	758	556	888	584	200	26	4	2258/3016	(74.9)

a) No. of positive sera (1:10 or higher).

* Significantly (chi-square test) different from 1985 ($p<0.01$).

Table 3. Estimated positivity rates of HI antibody to JEV in bovine sera collected from July to October by simple regressive analysis and multiple regressive analysis

Year	Positive rate	Meteorological value ^{a)}			Multiplied meteorological value	Comparative meteorological value	Estimated positive rate	
							Simple analysis	Multiple analysis
(i)	(Yi)	$\overline{T}(^{\circ}\text{C})^{\text{b)}$	$\overline{R}(\text{mm})^{\text{c)}$	$\bar{t}^{\text{d)}$	(Wi) ^{e)}	(Xi) ^{f)}	($\hat{Y}$ i) ^{g)}	($\hat{Y}$ i) ^{h)}
1982	58.3	274.1	93.3	1.279	6895.0	500.1	59.3	58.9
1983	81.4	277.3	62.6	1.353	57.3	4.2	79.7	80.9
1984	72.4	290.7	43.5	1.451	0.7	0.1	79.9	74.7
1985	88.0	280.8	75.6	1.443	556.6	40.4	78.2	88.6
1986	74.3	276.2	76.8	1.312	669.7	48.6	77.9	74.5
1987	79.7	289.9	65.1	1.459	92.9	6.7	79.6	77.1
Total	454.6	1689.0	416.9	8.297	8272.2	600.1	454.6	454.7
Mean	75.7	281.5	69.5	1.383	1378.7	100.0	75.8	75.8

a) June-September. b) Total mean temperature in 10-day periods. c) Total mean rainfall in 10-day periods. d) No. of days showing 25°C or higher. e) $W_i = \bar{T}^{1/12} \times \bar{R}^{-20} \times \bar{t}^{1/9}$. f) $X_i = W_i/\bar{W} \times 100$. g) $\hat{Y}_i = -0.04X_i + 79.9$ ($p<0.05$). h) $\hat{Y}_i = -3991\bar{T}^{1/13} - 0.0035\bar{R}^{-12} + 1978\bar{t}^{1/9} + 4187$ ($p<0.05$).

Fig. 3. Relationship between the positivity rate of HI antibody to JEV in bovine sera and comparative meteorological values.

1982 ($Y_i = 58.3\%$).

Antibody positivity rates estimated from the regression equation ($\hat{Y}_i$) are shown in Table 3. By multiple regression analysis with the dependent variables if the mean antibody positivity rates (Y_i) and the independent variables of three power values, the following results were obtained: a variance ratio of 25.39 ($p < 0.05$), a multiple correlation coefficient of 0.987 ($p < 0.05$), and a multiple regression equation of $\hat{Y}_i = -3991T^{1/13} - 0.0035R^{-12} + 1978t^{1/9} + 4187$. The estimated values on the extreme right in Table 3 were determined by the above equation, ranged from 58.9 to 88.6%, being nearly equal to the Y_i values.

DISCUSSION

Pigs have been examined for antibody positivity rates to JEV in Japan since 1965 to estimate the risk of human infection as a national project for prevention of JEV epidemics. The pig-mosquito cycle has been evidenced in nature and viremia has been observed in cattle, mules, sheep, goats, wild boars, dogs, and rabbits as well as in pigs and horses [1, 3, 7, 10, 13]. Therefore, epizootiological informations in these animals would be importance for clarifying the ecological background of JEV.

In most infectious diseases including JE epidemics used to be affected by a number

of environmental factors. The present authors previously published a series of sero-epidemiological studies of JEV in cattle demonstrating an infection with the virus [4, 17–19]. Recently, positivity cases were increased in number in the northern Japan with the appearance of the vector insects, showing seasonal fluctuations peaking in summer [4, 17]. This was confirmed in the present study. The distribution of JEV was shown to be greatly affected by meteorological factors, influencing the growth of vector mosquitoes [6, 9, 12, 20, 23]. In the present study, we analyzed the relationship between the meteorological factors and the antibody positivity rates.

To produce mosquitoes with transmission capability, a mean temperature of 23°C was required [9]. At 20°C JEV grows in the body of the mosquitoes but viral transmission efficiency was markedly reduced [21]. The growth of the vector was shown to be dependent on the mean temperature ($T_{i,j-1}$) and rainfall ($R_{i,j-1}$) of 10 day-periods. The ability of vectors to transmit the disease was associated with the number of days showing 25°C or above ($t_{i,j-1}$) [12, 23]. The climate from June to September would be a determinant factor for the antibody positivity rate from July to October (Y_i) when the positivity rate notably increased reaching a peak. The examination of the relationship between the comparative meteorological values (X_i) determined from the three meteorological parameters of ($T_{i,j-1}$), ($R_{i,j-1}$), and ($t_{i,j-1}$) and the antibody positivity rates (Y_i) revealed significant correlation and regression ($p < 0.05$). Antibody positivity rate was estimated by a formula: $\hat{Y}_i = -0.04X_i + 79.9$. It was nearly equal to the positivity rate based on the observations (Y_i). The sero-positivity rate (Y_i) was correlated with the mean temperature ($T_{i,j-1}$) and the number of days showing 25°C or higher ($t_{i,j-1}$). Inversely it was negatively correlated with the mean rainfall ($R_{i,j-1}$).

These observations were compatible with Morita *et al.* [12]. In 1982, when the values of (Ti) and (ti) were the lowest, and that of (Ri) was the largest, the sero-positivity was the lowest reflecting these meteorological data. The epidemic size of human Japanese encephalitis was negative correlated with total summer precipitation [11].

Multiple regression analysis with the power values of the three meteorological parameters (independent variables) and the antibody positivity rate (Yi) (the dependent variable) gave a correlation coefficient of 0.987 ($p < 0.05$). The regression equation was: $\hat{Y}_i = -3991T^{1/13} - 0.0035R^{-12} + 1978t^{1/9} + 4187$. The positivity rate estimated from this equation ($\hat{Y}$) was nearly equal to the positivity rate observed (Y_i). Thus, meteorological factors were shown to influence on the antibody positivity rate by the multiple regression analysis. The growth of the vector mosquitoes might be greatly affected also by the hours of sunshine or wind as well as other environmental factors such as agricultural pesticides and factor of rice paddies, while these factors were not examined. On the other hand, precipitation was reported not to influence the appearance of mosquitoes [6]. The total rainfall should be studied further in the multiple meteorological equation.

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要 約

牛における日本脳炎 HI 抗体陽性率と気象要因の相関と回帰：酒井健夫・高橋 薫・久末修司・堀本政夫・滝沢隆安¹⁾（日本大学獣医衛生学研究室，¹⁾農林水産省家畜衛生試験場）——1982～1987年に、埼玉県東部地域の延べ4,371頭の乳牛について、日本脳炎ウイルスに対する HI 抗体陽性率とベクターの活動性に影響を与える気象要因との相関と回帰を調べた。各年次の7月～10月の抗体陽性率(Y_i)は58.8～88.0%で、各年次によって差がみられた。6月～9月の旬平均気温総和($T_i, j-1$)、旬平均降雨量総和($R_i, j-1$)および気温25°C以上の日数値($t_i, j-1$)から求めた気象比較値(X_i)と Y_i を単回帰分析すると、相関係数は0.8147 ($p < 0.05$)、HI 抗体陽性率推定式は $\hat{Y} = -0.04X + 79.9$ ($p < 0.05$)であった。一方、3種類の気象ベキ乗値を説明変数に、 Y_i を目的変数とする重回帰分析すると、重相関係数は0.987 ($p < 0.05$)、重回帰式は $\hat{Y}_i = -3991T^{1/13} - 0.0035R^{-12} + 1978t^{1/9} + 4187$ ($p < 0.05$)であった。これらの推定式から求めた推定陽性率は、実測陽性率にほぼ一致し、陽性率に正に相関する気温および負に相関する降雨量が反映した予測式を作ることが可能であった。