都市近郊酪農地域の乳牛における牛ロタウイルス抗体保有状況

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A Serological Survey of Bovine Rotavirus Infection in Dairy Cattle in a Suburban Farming Area

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ABSTRACT. Holstein dairy cattle reared in a suburb of the eastern Saitama Prefecture were examined for serum complement fixation (CF) antibody titers to bovine rotavirus (BRV). Six hundred and fifty-five of 852 animals (76.4%) were positive showing titers of 1 : 4 to 1 : 64. Calves less than one year of age showed lower positivity than adult cows. Antibody titers showed a tendency to rise in cooler seasons. The higher positivity rate was observed in the basin of the Tone River than in that of the Arakawa and Motoarakawa. No environmental or manage factors were revealed to be responsible for great differences in positivity rate among cities or towns.

Key words: Bovine rotavirus, CF antibody, Dairy cattle

The mass breeding in current dairy practice is based upon the enlarged scale and mechanization in breeding management which might result in difficulty in disease control. Particularly, the occurrence of calf diarrhea increased in the last 10 years, causing serious economical damages, because of high mortality and poor performance of recovered calves. This paper is to describe the results of sero-epizootiological surveys of calf diarrhea in a suburban dairy farming area of the eastern Saitama Prefecture [1] using complement-fixation (CF) test with bovine rotavirus (BRV).

Materials and Methods

SERA. Serum samples were collected from July to December 1985 from 852 Holstein dairy cattle raised in 91 farms in 14 cities or towns of the eastern Saitama Prefecture, located along the Arakawa, Motoarakawa or Tone Rivers. Fifty of 91 farms were randomly selected and investigated for animal introduction, breeding scale and systems, and barn conditions. These cities or towns showed a complicated pattern of land arrangement because of recent rapid urbanization. The sera were stored at −20°C and before testing, test serum samples were diluted 1:2 in saline and heated at 56°C for 30 min.

VIRUS. Lincoln strain of Nebraska calf diarrhea virus was obtained from Obihiro Univ., and it was maintained on LLC-MK_2 cell cultures grown in Eagle's minimum essential medium (MEM) (Nissui, Tokyo) containing fetal calf serum (5%) and 1 μg/ml of tripsin (Difco, U.S.A., 1:250). Before virus inoculation the cell cultures were grown without fetal calf serum. The infected culture fluid was concentrated by the method described by Bishop et al [2], and the CF antigen titers of the final preparation was 200–400 units in box titrations with hyperimmune rabbit serum showing a CF titer of 1:3200 [3].

COMPLEMENT-FIXATION (CF) TEST. The CF test was performed by the microtiter method with twofold serial dilutions of serum (0.025 ml), 4 units of antigen (0.025 ml), 2 full units of complement (0.05 ml), and 0.5% sensitized sheep erythrocytes (0.05 ml). Mixtures of serum, antigen

and complement were incubated overnight at 4°C before adding the sensitized cells. All the reagents were prepared with veronal-buffered saline with 0.1% gelatine. The serum samples used for the test were diluted 1:2 in saline and inactivated at 56°C for 30 min. The antibody titers were expressed as the reciprocal of the highest serum dilution showing 75% inhibition of hemolysis. Serum showing a titer of 1:4 or higher was recorded as positive.

Results

Of 852 sera 655 (76.9%) were positive for BRV antibody and mean most samples showed titers of 1:16 or lower varying from 1:4 to 1:64 with a mean of 6.63. Of 25 calves aged less than one year 48 (52.1%) were positive with a mean titer of 4.49. The data of younger calves were significantly lower than those of older ones ($p<0.01$) (Table 1, Fig. 1).

In cooler late September (18.5°C; 89.4%) and early October (18.6°C; 85.0%) higher positive rates were shown as compared with either hot late July (27.7°C; 60.9%), early (26.5°C; 69.6%) and middle September (21.2°C; 71.3%), or cold middle November (9.2°C; 76.7%) and middle December (2.4°C; 73.2%). The difference was $p<0.01$. No correlation was observed between the positive rate and precipitation or humidity.

Table 1 Age Distribution of Antibodies to BRV in the Eastern Saitama Prefecture

Age in years	Positivity rate (%)		Antibody titers						
			<4	4	8	16	32	64	Mean
<1	25/ 48*a)	(52.1)	23	7	9	5	4	0	4.49
1	66/ 88	(75.0)**	22	16	16	14	11	9	8.19
2	99/131	(75.6)**	32	29	43	18	7	2	5.98
3	101/135	(74.8)**	34	35	32	24	7	3	6.00
4	98/122	(80.3)**	24	29	32	28	4	5	6.90
5	83/102	(81.4)**	19	21	31	23	7	1	7.03
6	70/ 88	(79.5)**	18	18	30	15	5	2	6.67
7	47/ 58	(81.0)**	11	9	21	13	2	2	7.27
8	33/ 42	(78.6)**	9	8	10	10	4	1	7.37
9>	33/ 38	(86.8)**	5	10	13	4	4	2	7.71
Total	655/852	(76.9)	197	182	237	154	55	27	6.63

a) No. of positive (1:4 or higher)/No. of tested (%)
Values marked with asterisks differ significantly (chi-square test) from those less than 1 year: *$p<0.05$, **$p<0.01$.

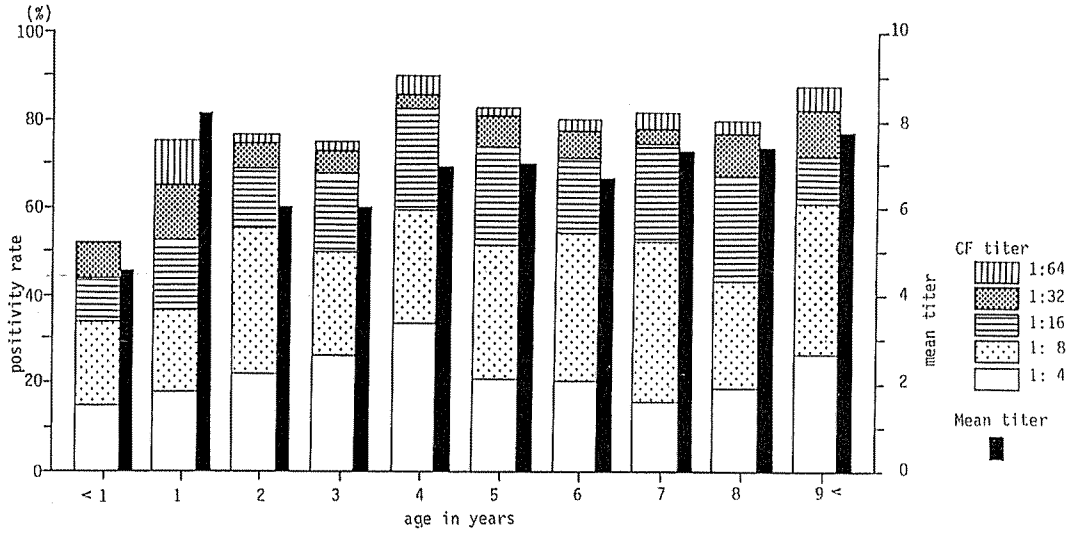

Fig. 1 Positivity Rates and Mean Titers of Antibodies to BRV in the Eastern Saitama Prefecture.

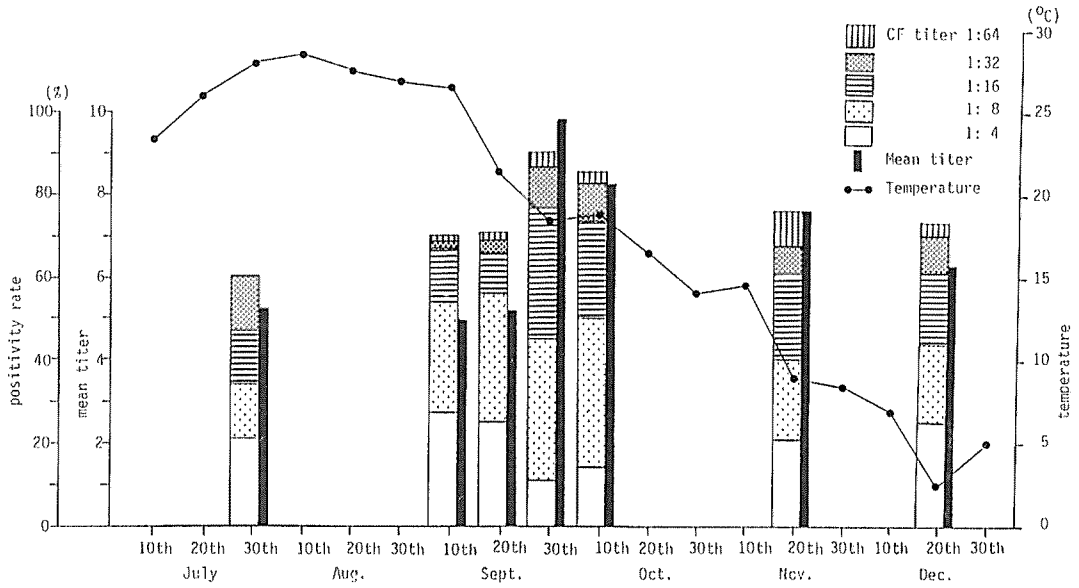

Fig. 2 Seasonal Changes in Positivity Rates and Mean Titers of Antibody to BRV.

Table 2 Comparison of BRV Antibody-Positivity Rates in Different Parts of the Eastern Saitama Prefecture

Arakawa basin :	Okegawa (60.9%)							
Motoarakawa basin :	Hasuda (73.2%)							
	5%	Iwatsuki (56.5%)						
	NS	NS	Koshigaya (65.9%)					
	NS	5%	NS	Yashio (85.0%)				
Tonegawa basin :	Gyoda (90.2%)							
	5%	Hanyu (79.6%)						
	5%	NS	Kitakawabe (77.1%)					
	NS	NS	NS	Ohtone (83.3%)				
	NS	NS	NS	NS	Kurihashi (86.7%)			
	NS	NS	NS	NS	NS	Satte (85.5%)		
	1%	NS	NS	NS	NS	5%	Sugito (69.4%)	
	1%	5%	NS	NS	5%	1%	NS	Matsufushi (66.3%)
	1%	NS	NS	NS	NS	5%	NS	NS Yoshikawa (69.2%)

Antibody titers ran parallel with positivity rates (Fig. 2).

In the basin of the Tone River, the positivity rate (79.6%) and titers (7.06) were significantly higher ($p<0.01$) than those in the basin of the Arakawa or Motoarakawa. In Gyoda and Satte cities along the Tone, the highest positivities of 90.2%, and 85.5%, respectively, were shown (Table 2).

Of 50 farms surveyed, 31 and 16 were rearing 20 or less and 21 to 40, respectively, while 3 were rearing 40 or more. No correlation was

Table 3 Environmental Factors and BRV Infection in the Eastern Saitama Prefecture

Factor	Antibody positivity rates and titers	BRV infection
Age	Elevated at 1 year of age	Latent infection 1 year of age or younger
Climate	peaking at the end of September	Markedly decreased incidence in lower temperature
District	Higher positivity rates and titers in Tonegawa basin, especially in Gyoda and Satte citis	Hygienic counterplan effective
Feeding	Not correlative with keeping size, types of feeding and management, reproductive performance, and barn location	Social foctor not involved

observed between the antibody positive rate and breeding scales. As for the breeding system, 39 farms were of barn system throughout the year, and 11 were of a combination system of barn and padock grazing. Fourty-one farms were located in the agricultural district, while 4 and 5 were in the urban and midtype districts. No difference was observed between antibody positive rates and breeding systems nor farm locations.

Discussion

Environmental conditions can definitely predispose animals for diarrheal diseases. Since calf diarrhea is frequently due to a mixed infection with bovine coronavirus or enterotoxigenic *Escherichia coli*, the exact pathogenesis is difficult to analyze [4–10]. Some efforts were made to elucidate causative agents and management factors involved in calf diarrhea, and we have focussed on the role of BRV, one of the causatives of infections diarrhea [11], frequently occuring all over Japan since the first outbreak in 1978 [12–18] and showing incidence increased with the popularization of intensive breeding system. The present survey revealed that the eastern Saitama Prefecture was heavily contaminated with the virus. The antibody positivity was higher in calves one year of age than others, suggesting that the youngers could tolerate BRV infection. Preventive measures against BRV should be taken immediately after birth [11].

Calf diarrhea is known to have a tendency to occur frequently between the fall and spring [19], probably because of an avalanche of births and to breeding plan and/or cold environments in winter [20, 21]. In the present survey, a single curve of antibody positivity rate and titers was observed peaking at late September. The environmental temperature markedly decreased by 5.3°C between early and middle September, and by 8.0°C between early and late September. In comparison with antibody positivity rates or titers, the temperature can be estimated to predispose the animal for the viral infection as well as stress of loading.

The antibody positivity rate was higher in the basin of the Tone River than in that of the Arakawa or Motoarakawa, suggesting that environmental factors might be important for BRV distribution as well as concentrated outbreaks of diarrhea due to BRV [20]. There was, however, no significant correlation between the positivity rate and any of managing factors, such as the breeding scale and system, and circumstances of barns. Further study on wider areas is needed to see the relationship between the positivity rate and environmental factors.

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都市近郊酪農地域である埼玉県東部のホル種系乳牛852頭の，牛ロタウイルス（BRV）に対する補体結合（CF）抗体陽性率は 76.4%，平均抗体価は 6.63 であり，抗高価は 1：4～1：64 に分布した。CF 抗体陽性率の分布状況から，当地域の乳牛は BRV 感染に対して1才齢までに耐過し，気温の急激な低下が BRV 感染を誘発する傾向が推察された。利根川流域の CF 抗体陽性率は，荒川流域および元荒川流域に比べて高かく，しかも CF 抗体陽性率の高い市町村が認められたが，その原因に環境要因の関与を明らかにすることはできなかった。