



NOTE

Virology

The estimation of duration of maternally-derived antibodies against Akabane, Aino, and Chuzan virus in calves by the receiver operating characteristic analysis

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ABSTRACT. The duration of maternally-derived antibodies against three arboviruses was investigated in calves, using the results of arbovirus serosurveillance performed in Kagoshima Prefecture during 2002–2016. The duration of maternally-derived antibodies against Akabane virus (AKAV), Aino virus (AINOV), and Chuzan virus (CHUV) was estimated to be 178 (sensitivity: 0.769, specificity: 0.730), 156 (sensitivity: 0.806, specificity: 0.791), and 156 days of age (sensitivity: 0.845, specificity: 0.814), by receiver operating characteristic analysis. The duration of maternally-derived antibodies against AKAV, AINOV, and CHUV differed 7–14, 22–28, and 20–31 days in the same calf types between the regions far from each other although it was similar between the adjacent regions. The dairy calves showed 6–29 days longer duration than the beef calves rearing in a similar region.

KEY WORDS: arbovirus, calf, duration, maternal antibody, receiver operating characteristic analysis

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Akabane virus (AKAV) and Aino virus (AINOV) of the genus *Orthobunyavirus* in the family *Peribunyaviridae*, and Chuzan virus (CHUV) of the genus *Orbivirus* in the family *Reoviridae*, are arthropod-borne viruses (arbovirus) transmitted by the blood-sucking arthropod vectors such as *Culicoides* biting midges. It has been believed that the infection of these viruses to the pregnant cattle induces the abortion, stillbirth, or congenital malformation of a fetus known as Akabane disease, AINOV infection, and Chuzan disease [1].

The three arboviruses have continued to impact the livestock industry for a long time in Japan. The large outbreak of Akabane disease occurred from 1972 to 1975 and induced the born of approximately 42,000 abnormal calves during the outbreak [17]. Moreover, the case of encephalitis caused by Iriki strain of AKAV reported in 1984 [11, 12]. Since 1998, the occurrence of Akabane disease has been reported every year until 2016 in Japan although the number of cases varied each year (https://www.maff.go.jp/j/syouan/douei/kansi_densen/attach/pdf/kansi_densen-164.pdf). The prevalence of AINOV was confirmed among the cattle in the summer season of 1995 and 1998 and a few hundred suspected cases of AINOV infection were reported in Chugoku and Kyushu district in 1995–1996 and 1998–1999 [14, 17]. Chuzan disease took place during 1985–1986 in Kyushu district, and it was believed that approximately 2,400 calves were born with a hydranencephaly-cerebellar hypoplasia syndrome characteristic of Chuzan disease [6, 7, 17]. Hence, these viruses have been paid attention to the prevalence among the cattle in Japan, especially in Kyushu district including Kagoshima Prefecture with high humidity and temperature in the summer season suitable for the activity of arbovirus vector, even though the occurrence of AINOV infection and Chuzan disease was not recently reported.

Vaccination is one of the effective strategies for the prevention of a viral disease, including Akabane disease, AINOV infection, and Chuzan disease. However, the maternally-derived antibodies in neonates have been known as one of the prevention factors of vaccination effectiveness because of interfering with the ability of vaccines to induce immunity [13], although it plays an important role in the prevention against pathogenic agents during younger periods [16]. Therefore, the successful vaccination strategy needs to estimate the timing of decay of maternally-derived antibodies in calves. The aim of this study is to estimate the duration of maternally-derived antibodies against AKAV, AINOV, and CHUV in a calf, using the results of arbovirus serosurveillance performed in Kagoshima Prefecture from 2002 to 2016.

Kagoshima Prefecture is located in the southernmost part of the Japanese archipelago and consists of one mainland, several large and numerous small islands (Fig. 1). As the southernmost island in Kagoshima Prefecture is located more than 600 km apart from the mainland of Kagoshima, Kagoshima Prefecture has a vast area and the geographical and climatic characterization differs

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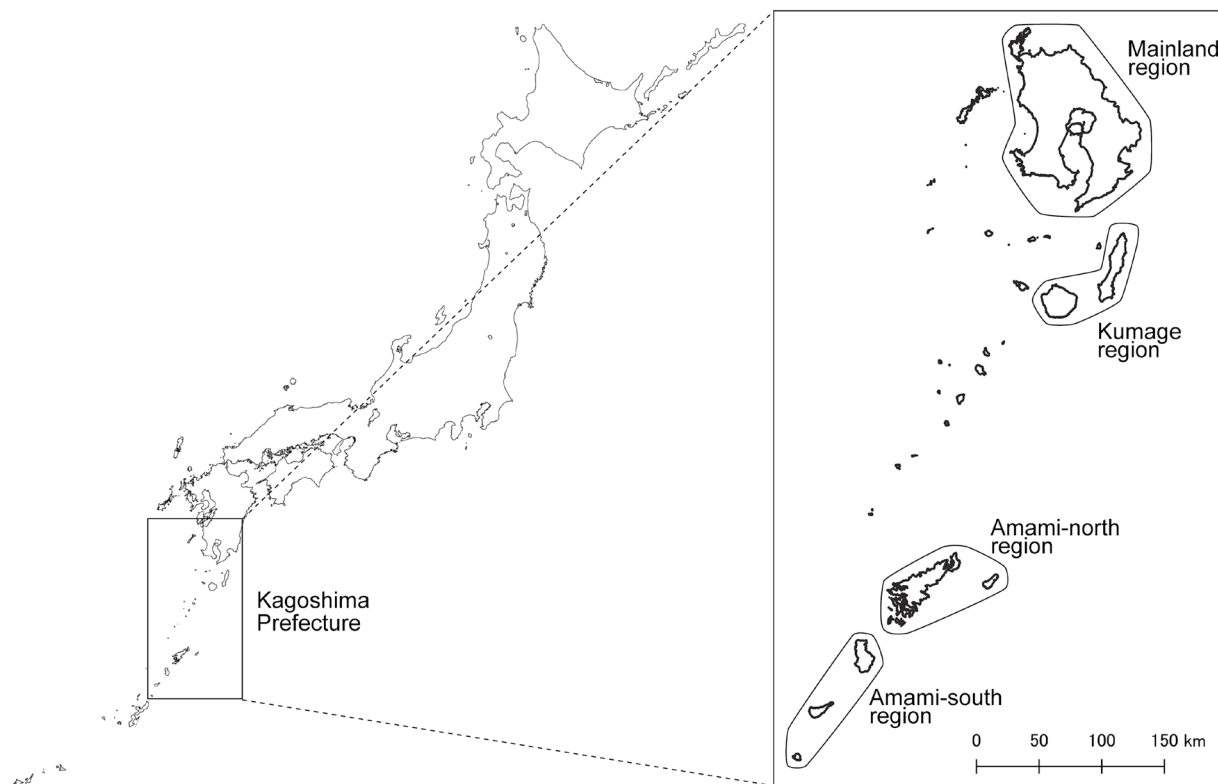


Fig. 1. The geographical location of four regions (Mainland, Kumage, Amami-north, and Amami-south), divided for the present study.

in every region in the Prefecture. Therefore, Kagoshima Prefecture was divided into four regions (Mainland, Kumage, Amami-north, and Amami-south) with close geographical relationships and similar climate conditions for the present study (Fig. 1).

In Kagoshima Prefecture, the serosurveillance of calves has performed every year to monitor the circulation of several arboviruses including AKAV, AINOV, and CHUV, according to our government policy. The approximately 120 young calves that have not experienced summer season were randomly selected from 40–50 farms as sentinel calves for the serosurveillance and the serum samples were collected for virus neutralization test at 5 times (June, August, September, October, and November) per a calf. The virus neutralization test was performed with established methods [14]. Briefly, the two-fold serial dilutions of serum samples were mixed with the equal volume of medium containing AKAV (JaGAR 39 strain), AINOV (JaNAr 29 strain), and CHUV (Chuzan 31 strain). After incubation for 1 hr at 37 degrees, the suspension of the HmLu-1 cell was added to the mixture. The mixture was incubated for seven days at 37 degrees. The maximum serum dilution which inhibits the elicitation of cytopathic effect was regarded as the antibody titer of serum. More than twofold increase and maintain of antibody titer against AKAV, AINOV, and CHUV was recognized as the evidence of the circulation of the arboviruses. A total of 1,866 calves were investigated in the serosurveillance from 2002 to 2016 and the seroconversion of antibodies against the three arboviruses was confirmed as shown in Table 1.

The records regarding the days of age and antibody titer of beef and dairy calves in serosurveillance were subjected to the present study. Of the 1,866 calves, 869, 554, and 744 calves as described below were extracted for the calculation of the duration of maternally-derived antibodies against AKAV, AINOV, and CHUV, respectively: the calves with no confirmation of more than the twofold increase of antibody titer against the objective viruses though a serosurveillance period (June to November), with ≥ 2 of antibody titer against the objective viruses in the serum collected at first sampling (at June) in a serosurveillance, and with complete information regarding the days of age and the antibody titer against objective viruses though a serosurveillance period (June to November).

The receiver operating characteristic (ROC) analysis was applied to estimate the duration of maternally-derived antibodies against the three arboviruses. The ROC analysis is one of the statistical tools that widely used in medicine to evaluate the accuracy of diagnostic tests with the dichotomous outcome (positive/negative) and to determine the optimal cut-off value of variable discriminating the test positive/negative [3, 8]. The calves with ≥ 2 and < 2 of antibody titer against the objective viruses were regarded as “antibody-decay negative” and “antibody-decay positive”, respectively. Using the days of age and the antibody-decay status in the calves, the ROC curve was illustrated to calculate the optimal cut-off value of days of age discriminating between the two antibody-decay status and the sensitivity/specificity in each virus, region, and calf type (beef and dairy). The optimal cut-off value was defined as the point on the ROC curve yielding the minimal value for $(1-\text{sensitivity})^2 + (1-\text{specificity})^2$ [3] and was regarded as the duration of maternally-derived antibodies against the three arboviruses in a calf. The area under the ROC curve

Table 1. The overview of the results of arbovirus serosurveillance performed in Kagoshima Prefecture during 2002–2016

Virus	Region	Number of times and the years that the seroconversion was observed in sentinel calves
Akabane virus	Mainland	6 times (2002, 2003, 2006, 2008, 2013, 2015)
	Kumage	4 times (2002, 2008, 2013, 2015)
	Amami-north	3 times (2002, 2013, 2014)
	Amami-south	3 times (2007, 2014, 2016)
Aino virus	Mainland	2 times (2002, 2005)
	Kumage	4 times (2002, 2003, 2004, 2005)
Chuzan virus	Mainland	1 time (2013) ^{a)}

a) It was found that the D'Aguilar virus, which is the Palyam serogroup virus similar to the Chuzan virus and has a cross antigenicity with the Chuzan virus, spread through Kagoshima Prefecture in 2013. Therefore, the seroconversion in 2013 was probably caused by the D'Aguilar virus, not by the Chuzan virus.

(AUC) and the 95% confidence interval (95%CI) of AUC were also calculated to evaluate the accuracy of the outcome as follows: AUC ≥ 0.9 , High accuracy; AUC 0.9–0.7, Moderate accuracy; AUC 0.7–0.5, Low accuracy; and AUC < 0.5 , Chance result [3]. All of the statistical analyses were performed using R (version 3.6.1), free software distributed by R Project (<https://www.r-project.org/>).

The duration of maternally-derived antibodies against AKAV, AINOV, and CHUV was 178 (sensitivity: 0.769, specificity: 0.730), 156 (sensitivity: 0.806, specificity: 0.791), and 156 days of age (sensitivity: 0.845, specificity: 0.814) in calves rearing in Kagoshima Prefecture (Table 2). The AUC showed 0.8–0.9 (moderate-high accuracy) in each virus, region, and calf type in the present study (Table 2).

The duration of maternally-derived antibodies against AKAV and AINOV was similar in the adjacent two regions, Mainland and Kumage region. The duration of maternally-derived antibodies against AKAV and AINOV was 179 (sensitivity: 0.809, specificity: 0.683) and 161 (sensitivity: 0.840, specificity: 0.764) days of age in the Mainland region whereas that is 176 (sensitivity: 0.805, specificity: 0.731) and 160 (sensitivity: 0.789, specificity: 0.815) days of age in the Kumage region. On the other hand, the duration of maternally-derived antibodies against CHUV in the Mainland (172 days of age, sensitivity: 0.815, specificity: 0.855) is 16 days of age longer than in the Kumage region (156 days of age, sensitivity: 0.803, specificity: 0.838). The similarity of the duration was also observed between Amami-north and Amami-south regions. The duration of maternally-derived antibodies against AKAV, AINOV, and CHUV was 165 (sensitivity: 0.778, specificity: 0.785), 132 (sensitivity: 0.864, specificity: 0.805), and 136 (sensitivity: 0.893, specificity: 0.833) days of age in the Amami-north region whereas that was 169 (sensitivity: 0.756, specificity: 0.771), 136 (sensitivity: 0.872, specificity: 0.785), and 135 (sensitivity: 0.882, specificity: 0.868) days of age in the Amami-south region. The maternally-derived antibodies against AKAV, AINOV, and CHUV in beef calves rearing in the Amami-north and Amami-south region showed the 7–14, 22–28, and 20–31 days shorter duration than in the Mainland and Kumage region (Table 2).

The duration of maternally-derived antibodies against three arboviruses differed in the calf type. The duration of maternally-derived antibodies against AKAV, AINOV, and CHUV was 208 (sensitivity: 0.690, specificity: 0.792), 182 (sensitivity: 0.750, specificity: 0.798), and 172 (sensitivity: 0.822, specificity: 0.780) days of age in the dairy calf in the Mainland region whereas that was 179 (sensitivity: 0.799, specificity: 0.695), 158 (sensitivity: 0.852, specificity: 0.774), and 166 (sensitivity: 0.852, specificity: 0.843) days of age in the beef calf in a similar region. Thus, the duration in the dairy calf was 6–29 days longer than in the beef calf (Table 2).

The result of the present study showed the duration of maternally-derived antibodies against AKAV, AINOV, and CHUV in calves rearing in Kagoshima Prefecture, with high-moderate accuracy. Tsutsui *et al.* estimated by a statistical model that the duration of maternally-derived antibodies against AKAV was 4.8 months and 4.3 months, with 90% probability, in beef and dairy calf rearing in Kyushu district including Kagoshima Prefecture [15]. Also, the previous report described that the maternal antibodies against the Schmallenberg virus maintained for approximately 6 months in a calf [5]. Besides, the maternally-derived antibodies against respiratory viruses were believed to be kept for 4–6 months for bovine viral diarrhoea virus (BVDV)–1, 3–6 months for BVDV–2, 5–7 months for bovine respiratory syncytial virus, 2–4 months for bovine herpesvirus–1, and 5–6 months for bovine parainfluenza virus–3 [4, 9, 10]. The maternally-derived antibodies against foot and mouth disease virus decreased below the protective level at 27–28 weeks (above 6 months) of age [2]. Although the longevity of the duration of maternally-derived antibodies varied in each virus, it was assumed that the maternally-derived antibodies against a virus are substantially maintained for 4–7 months in a calf.

The AUC, shown in Table 2, revealed that the duration of maternally-derived antibodies calculated by ROC analysis has high-moderate accuracy. On the other hand, each sensitivity and specificity of the duration of maternally-derived antibodies suggested the possibility of missing false-positive cattle, which is evaluated to decay the antibody despite possession of antibody, and false-negative cattle, which is interpreted to possess the antibody despite the decay of antibody, when adopting the days of age shown in Table 2 as the duration of maternally-derived antibodies. Also, the maximum days of age detected the maternally-derived antibodies against AKAV, AINOV, and CHUV was 316, 315, and 275, respectively, in the present study. Several previous studies

Table 2. The duration of maternally-derived antibodies against the three arboviruses in each region and calf type, estimated by receiver operating characteristic analysis

Virus	Region	Calf type	Duration estimated (days of age)	Sensitivity	Specificity	AUC ^{a)}	95%CI ^{b)} of AUC
Akabane virus							
	Total of all regions	Total of all calf types	178	0.769	0.730	0.819	0.807–0.831
	Mainland	Total of all calf types	179	0.809	0.683	0.819	0.803–0.834
		Dairy calf	208	0.690	0.792	0.819	0.784–0.855
		Total of dairy calf	208	0.690	0.792	0.819	0.784–0.855
		Beef calf	179	0.799	0.695	0.819	0.801–0.837
	Kumage	Beef calf	176	0.805	0.731	0.830	0.798–0.863
	Amami-north	Beef calf	165	0.778	0.785	0.845	0.815–0.875
	Amami-south	Beef calf	169	0.756	0.771	0.842	0.81–0.875
		Total of beef calf	178	0.757	0.743	0.820	0.807–0.833
Aino virus							
	Total of all regions	Total of all calf types	156	0.806	0.791	0.869	0.855–0.884
	Mainland	Total of all calf types	161	0.840	0.764	0.873	0.854–0.892
		Dairy calf	182	0.750	0.798	0.851	0.803–0.9
		Total of dairy calf	182	0.750	0.798	0.851	0.803–0.9
		Beef calf	158	0.852	0.774	0.879	0.859–0.899
	Kumage	Beef calf	160	0.789	0.815	0.875	0.841–0.909
	Amami-north	Beef calf	132	0.864	0.805	0.898	0.865–0.931
	Amami-south	Beef calf	136	0.872	0.785	0.896	0.857–0.936
		Total of beef calf	154	0.810	0.798	0.874	0.859–0.889
Chuzan virus							
	Total of all regions	Total of all calf types	156	0.845	0.814	0.911	0.901–0.92
	Mainland	Total of all calf types	172	0.815	0.855	0.917	0.905–0.929
		Dairy calf	172	0.822	0.780	0.876	0.843–0.91
		Total of dairy calf	172	0.822	0.780	0.876	0.843–0.91
		Beef calf	166	0.852	0.843	0.927	0.915–0.939
	Kumage	Beef calf	156	0.803	0.838	0.892	0.864–0.92
	Amami-north	Beef calf	136	0.893	0.833	0.942	0.925–0.959
	Amami-south	Beef calf	135	0.882	0.868	0.946	0.924–0.968
		Total of beef calf	156	0.839	0.839	0.917	0.908–0.927

a) AUC: Area under the receiver operating characteristic curve. b) 95%CI: 95% confidence interval.

reported the influence of antibody concentration in colostrum that the calf ingested [4], region, and calf type [15] to the duration of maternally-derived antibodies. It appeared that the duration of maternally-derived antibodies against the three arboviruses widely varies between individual calves. The detection test of antibody against the objective virus such as virus neutralization test should be applied for confirmation of vaccination effect even if the vaccination is performed after the days of age shown in Table 2.

The result of the present study showed the similarity of the duration of maternally-derived antibodies against the three arboviruses in the adjacent regions and the difference of the duration between the regions far from each other (Fig. 1 and Table 2). That was suggested that the duration of the maternally-derived antibodies would be influenced by the geographical factors, as described in the previous study [15]. On the other hand, the duration of maternally-derived antibodies against AKAV, AINOV, and CHUV in dairy calf was 29, 24, and 6 days longer than in beef calf in the present study. The result in AKAV differed from that of the previous report which suggested the longer duration in the beef calf breeding in Kyushu district including Kagoshima Prefecture [15]. Although it was unknown why the present and previous study revealed the converse result, the difference in the frequency of exposure to the antigen in dams might influence the results of each report. Because it appeared that there is a correlation between the duration of maternally-derived antibodies in each region and the frequency of seroconversion in the corresponding region. For instance, the present study showed that the maternally-derived antibodies against AKAV maintained for 179, 176, 165, and 169 days in beef calf in Mainland, Kumage, Amami-North, and Amami-south region, respectively (Table 2). On the other hand, the frequency of seroconversion against AKAV, which suggests the evidence of the prevalence of the virus in Kagoshima Prefecture, was 6, 4, 3, and 3 times in the corresponding regions (Table 1). Also, the maternally-derived antibodies against AKAV, which has been prevalent even in recent years and has a greater frequency of seroconversion than AINOV and CHUV (Table 1), showed the longest duration of the three arboviruses in the present study (Table 2). Thus, it appeared that the greater frequency of the prevalence of the virus, the longer duration of maternally-derived antibodies. The duration of maternally-

derived antibodies might be more influenced by the acquired factors such as the frequency of exposure to the antigen in dams and the region that the calf was born, rather than congenital factors such as calf type. Especially, because of the history of numerous damages to the livestock industry caused by the arbovirus, the breeding cattle in Kagoshima Prefecture has a high vaccination coverage level, which might influence the higher antibody concentration in colostrum contributed to the longer duration of maternally-derived antibodies [4]. Further analysis including the consideration regarding the artificial exposure to the virus such as vaccination history of dams would be needed.

In 2013, it was found that the D'Aguilar virus, which is the Palyam serogroup virus similar to the CHUV and has serologically cross antigenicity with the CHUV, spread through Kagoshima Prefecture (Table 1). Thus, it is hard to deny the possibility of the influence of other viruses serologically close to AKAV, AINOV, and CHUV in the results of surveillance subjected to the present study. The further verification would be required for a correct comprehension of the duration of maternally-derived antibodies against AKAV, AINOV, and CHUV in calves.

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