Duration of Maternally Derived Antibodies against Akabane Virus in Calves:
Survival Analysis

Toshiyuki TSUTSUI1)*, Takehisa YAMAMOTO1), Yoko HAYAMA1), Yasuhiro AKIBA1), Akiko NISHIGUCHI1), Sota KOBAYASHI1) and Makoto YAMAKAWA2)

1)Epidemiological Research Team, National Institute of Animal Health, 3–1–5 Kannondai, Tsukuba, Ibaraki 305–0856 and
2)Kyushu Research Station, National Institute of Animal Health, Chuzan, Kagoshima 891–0105, Japan

(Received 14 October 2008/Accepted 16 February 2009)

ABSTRACT. To prevent encephalomyelitis caused by Akabane virus, as observed in 2006, vaccination of calves is one of the most effective prophylactic measures. For vaccination of calves, the duration of the maternal antibodies need to be considered because these antibodies are an obstacle to the effectiveness of the vaccine. In order to estimate the age of antibody decay in calves and to find factors influencing the duration of passive immunity, we conducted survival analysis using data from nationwide sentinel surveillance for Akabane disease. The accelerated failure time model based on the presence of interval censored data was used. The best fit model with a log-logistic distribution indicated that the maternal antibodies of beef calves last 1.11 times longer (95% confidence interval [CI]=1.06–1.16) than those of dairy calves. Calves in the western part of Japan and on Kyushu island, Japan, maintained the maternal antibodies 1.17 times (95% CI=1.11–1.23 and 1.10–1.24, respectively) longer than those in the eastern part of Japan. The ages at which beef calves in the eastern part of Japan, western part of Japan and Kyushu loose the antibodies, with 90% probability, were estimated to be 4.1, 4.8 and 4.8 months, respectively, while the ages were 3.7, 4.3 and 4.3 months for dairy calves in the same regions. The duration of maternal immunity to Akabane virus was different for different types of cattle and among different regions. These differences need to be taken into account when a vaccination strategy is adopted for preventing epizootic encephalomyelitis in the future.

KEY WORDS: AFT model, akabane virus, maternal antibody, survival analysis.

Akabane virus (AKAV), of the genus Orthobunyavirus in the family Bunyaviridae, is known to cause Akabane disease, which is characterized by abortion, stillbirth, premature birth and congenital malformation when pregnant cattle, sheep and goats are infected [8]. This virus is transmitted by a hematophagous arthropod vector. In Japan, Culicoides biting midges are primarily responsible for transmitting AKAV [11, 24]. Akabane disease mostly occurs in the western and southern parts of Japan because the climates of those area are suitable for vector activity. However, AKAV occasionally spreads to the eastern and northern parts of Japan. In 1998, the disease spread widely, and more than 1,200 abnormal births were confirmed in Japan. After extended outbreaks, vaccination of breeding female cattle was promoted, and this has succeeded in reducing the number of Akabane cases in recent years. Despite this, in 2006, AKAV was the cause of epizootic encephalomyelitis in the southern part of Japan [10]. AKAV infected cattle do not normally show any clinical signs, but neurological disorders were observed particularly in young cattle during this epidemic. Although sporadic cases of encephalomyelitis caused by a similar AKAV strain have previously been reported [16], this was the first large outbreak of bovine encephalitis due to the AKAV infection in Japan. If such large disease outbreaks occur frequently, the vaccination of calves, in addition to breeding females, should be considered as a prophylactic measure for preventing spread of the disease.

Young calves are often affected by infectious diseases such as respiratory diseases and diarrhea. Therefore, vaccination is one of the most practical measures for protecting calves. Since the presence of maternally-derived antibodies is known to reduce the effectiveness of vaccines [18], it is important to consider the timing of maternal antibody decay when young calves are vaccinated [3]. Although vaccination is ideally carried out in consideration of a previously measured antibody titer, testing all calves on farms is unrealistic due to the cost and time required. It is therefore useful to determine the approximate time of maternal antibody decay to help decide the appropriate time of vaccination.

In regards to AKAV, field investigations on the decay of maternal antibodies have not been reported. Here, we estimated the age of antibody decay in calves using field data to find factors that influence the duration of passive immunity. For this purpose, we used survival analysis techniques, which model data of the time to events, to predict the duration. We expect that the information obtained will contribute to the proper timing of vaccination against Akabane disease in calves.

MATERIALS AND METHODS

Data source: In Japan, a serological survey using sentinel animals is conducted every year to monitor the circulation of AKAV. Each prefectural government selects around fifty sentinel calves that have not experienced summer, according to geographical location. Virus neutralization tests for
AKAV are performed on blood samples taken from each animal in June, August, September and November. AKAV circulation is assessed from evidence of seroconversion or an increase in antibody titer. Individual test results are collected by the Ministry of Agriculture, Forestries and Fisheries along with information about the sentinel animals, such as farm location, age (months), cattle type (beef or dairy), antibody titers and vaccination history of dams, for nationwide analysis of AKAV circulation. We defined a twofold antibody titer at the age of two months or younger as indicating the presence of maternal antibodies. We then retrieved data for 708 sentinel animals satisfying this criterion at the first test in June from a list of around 5,000 animals tested in 2004 and 2005. We used data for 581 animals that had complete information records and that were considered free from the infection with no increases in antibody titers at four sequential serological tests.

Statistical analysis: Blood sampling of each sentinel animal is performed in June, August, September and November, taking into account vector activity. Since the age of each animal is recorded in months, we could determine the age when the antibodies disappeared for each animal. However, the exact time of antibody decay is not specified because the age at which the maternal antibodies disappear falls in the interval between the last positive age and the first negative age. It is usual for the serological status of an animal to be measured at a specific interval. A common approach is to use the midpoints of these intervals or the upper bounds as approximations of the actual times, but this approach is to use the midpoints of these intervals or the upper bounds as approximations of the actual times, but this method is known to bias results [17, 20]. Therefore, we handled these data as interval censored data, where the event has occurred at time \( T \) within an interval of time \([L, U]\), \( L \) being the lower limit of the time and \( U \) being the upper limit, while \( L\leq T \leq U \). Age was recorded as a discrete variable, although this unit covers a specific length of time. For example, five months of age could include just over 5 months and just less than 6 months. For reflecting this width in statistical computing, we created interval censored data, adding 0.999 to the upper limit. For example, in the case of a positive test at three months of age and a negative test at five months of age, the interval censored data was set as \([3.0, 5.999]\). On the other hand, individuals with seropositive results at the fourth test were treated as right censored data. For example, a six-month-old animal with a positive result at the fourth test was recorded as \([6.0, \infty]\).

We included in the analysis the dams’ vaccination against Akabane disease, cattle type (beef or dairy), region (eastern part of Japan [East], western part of Japan [West] or Kyushu) and survey year (2004 or 2005) as factors potentially influencing the duration of maternal antibodies. The geographical regions are described in Fig. 1.

Univariate comparison was conducted using generalized Kaplan-Meier estimation [1]. The Kaplan-Meier method estimates the survival function without assuming any distributions for the form of the function. This non-parametric method is particularly useful for comparing the effects of different factors on survival. The Kaplan-Meier method was generalized to derive the maximum likelihood estimates under the presence of interval censored data using an iterative self-consistency algorithm [22]. The details of this algorithm have been described previously [7, 12]. The effects on the duration of maternal immunity between variables were compared by the estimated age of antibody decay at percentiles of 25, 50 and 75.

For multivariate analysis, we built parametric survival models taking into account interval censoring. An advantage of parametric survival models is that inferences are usually more precise, and there is a wider range of models with which to describe the data, including accelerated failure time (AFT) models [4]. The AFT model can specify a direct relationship between survival time and explanatory variables. Therefore, interpreting the model result is straightforward. In the AFT model, a ratio of survival times, called the acceleration factor, is used as a measure of association. For example, if an acceleration factor of two is derived when comparing subjects in two groups, then the median survival time for one group is double the median survival time of the other group. As is the nature of the parametric method, the AFT model requires the assumption of specific distributions for survival time, even though it is unknown in many cases. Therefore, the best fit distribution of the data is selected from several candidates, whereas AFT models are robust to misspecification because of their logarithmic form [17].

The AFT model is described as

\[
\ln(T) = \beta x + \ln t,
\]

where \( \beta x \) is a linear combination of explanatory variables and \( \ln t \) is an error term with an appropriate distribution [5]. The best fit distribution for the AFT model was chosen among the Weibull, log-logistic, log-normal, logistic, Gaussian and extreme distributions by comparison of the nega-
tive log-likelihood of the models with no variables [17]. Selection of variables for the final model was based on backward and forward procedures using a log-likelihood test (p<0.05). For selection of variables, the age at first test was always incorporated into models in order to adjust its effect. This is because we selected the data of seropositive animals at one and two months of age for the first test. This might neglect animals that changed to negative at one or two months of age, even though they had maternally derived antibodies at zero months of age. We considered that this could bias results towards a prolonged duration of maternal immunity and needed to be adjusted.

Finally, we predicted the probability of maternal antibody presence by age using the final model.

Data was stored and handled using Excel (Microsoft Corp.), and statistical analysis was performed using S-PLUS (Insightful Corp.).

RESULTS

The results of the generalized Kaplan-Meier estimation are showed in Table 1. The results indicate that calves born to vaccinated dams kept maternal antibodies slightly longer than those born to non-vaccinated dams. Similarly, maternal antibodies in beef calves lasted longer than those of dairy calves. Regarding the geographical regions, calves born in the West and Kyushu tended to maintain maternal antibodies longer than those born in the East. Conversely, the year of investigation seemed to not influence the duration of maternal antibodies.

For multivariate analysis, the AFT models with different distributions were compared to seek a best fit model. The model with a log-logistic distribution showed the lowest negative log-likelihood value of 497.7, while the second lowest value of 497.8 was observed for a log-normal distribution. Using the log-logistic model, significant variables were chosen using the likelihood ratio test. The variables included in the final model were cattle type and region. The estimated acceleration factors of these variables are shown in Table 2. These results indicated that the maternal antibodies of the beef calves lasted 1.11 times longer (95% CI: 1.06–1.16) than those of the dairy calves. Similarly, calves in the West and Kyushu maintained the maternal antibodies 1.17 times (95% CI: 1.11–1.23 and 1.10–1.24, respectively) longer than those in the East.

Figure 2 shows the probabilities of antibody presence by age for different types of calves in different regions. These probabilities were estimated using the final model by setting

<table>
<thead>
<tr>
<th>Items</th>
<th>Category</th>
<th>Number of animals</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination of dam</td>
<td>No</td>
<td>99</td>
<td>3.6</td>
<td>4.5</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Done</td>
<td>482</td>
<td>4.1</td>
<td>4.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Year of investigation</td>
<td>2004</td>
<td>268</td>
<td>4.2</td>
<td>4.6</td>
<td>5.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>313</td>
<td>3.7</td>
<td>4.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Type of cattle</td>
<td>Dairy</td>
<td>258</td>
<td>3.8</td>
<td>4.4</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Beef</td>
<td>323</td>
<td>4.2</td>
<td>4.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Region</td>
<td>East</td>
<td>208</td>
<td>3.5</td>
<td>4.3</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>West</td>
<td>209</td>
<td>4.1</td>
<td>4.8</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>Kyushu</td>
<td>164</td>
<td>4.4</td>
<td>4.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Age at first test(a)</td>
<td>0 months</td>
<td>18</td>
<td>2.6</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1 month</td>
<td>171</td>
<td>3.5</td>
<td>4.0</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>392</td>
<td>4.4</td>
<td>4.8</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Ages were estimated as infinity. The age estimated at the highest percentile at intervals of 5% was described as the value of the lower limit.

<table>
<thead>
<tr>
<th>Items</th>
<th>Category</th>
<th>AF</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>Dairy</td>
<td>1.00</td>
<td>1.06</td>
<td>1.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Beef</td>
<td>1.11</td>
<td>1.11</td>
<td>1.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Region</td>
<td>East</td>
<td>1.00</td>
<td>1.10</td>
<td>1.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>West</td>
<td>1.17</td>
<td>1.10</td>
<td>1.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Kyushu</td>
<td>1.17</td>
<td>1.11</td>
<td>1.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at first test(a)</td>
<td>0 months</td>
<td>1.00</td>
<td>1.10</td>
<td>1.46</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>1 month</td>
<td>1.27</td>
<td>1.34</td>
<td>1.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2 months</td>
<td>1.54</td>
<td>1.34</td>
<td>1.77</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>This item was forced into the model.
The curves of antibody decay varied with cattle type and region. The ages at which beef calves in the East, West and Kyushu loose the antibodies, with 90% probability, were estimated to be 4.1, 4.8 and 4.8 months of age, respectively, and 3.7, 4.3 and 4.3 months of age for dairy calves in the same regions.

The predicted probability of antibody presence at 4.0 months of age for dairy calves in the West and Kyushu was 16%, while in the East it was 5%. The upper bounds of the 95% CIs of these estimates were 40% and 20%, respectively. The estimates are lower than those for the beef calves in the same regions. The probability of antibody presence at 4.0 months of age for beef calves in the West and Kyushu was 30% (upper 95% CI=60%), and the probability in the East was 11% (upper 95% CI=32%). The upper bound probability of the 95% CI becomes 8% for beef calves at 5.0 months of age in the East and at 6.0 months in the West and Kyushu.

DISCUSSION

By analyzing the age of maternal antibody decay for Akabane disease in calves, we found that beef calves in Kyusyu maintain maternal antibodies for the longest time. Around 4.8 months is the estimated age when the maternal antibodies decay with 90% probability. Although there are no previous reports on the age of maternal antibody decay for Akabane disease, several reports detail the ages for other bovine diseases. It has been reported that the age of maternal antibody decay is 5–6 months of age for bovine viral diarrhea (BVD) virus, 4 months of age for bovine herpes virus-1, 6 months of age for parainfluenza-3 virus and 6 months of age for bovine respiratory syncytial virus [6]. Other reports have suggested 3–7 months of age for BVD virus [9], and 6 months of age for BVD and IBR [15]. Our estimates for Akabane disease are slightly shorter than the reported ages for these diseases.

Maternal antibody decay is influenced by several factors, such as the level of dam antibodies and colostrum intake and absorption by calves [2, 6]. In our study, the age of antibody decay was influenced by region and cattle type. The spread of AKAV is often confirmed in Kyushu and the West by serological survey of sentinel animals. Conversely, evidence of AKAV spread is rarely detected in the East. This might be related to the higher temperatures and distribution of transmissible vectors in Kyushu and the West. More frequent exposure to AKAV means that dams in Kyushu and the West have higher levels of antibody titers against AKAV. Consequently, it is considered that calves born to dams in these regions receive higher levels of maternal antibodies and maintain them for a longer period compared with calves born in the East.
The influence of cattle type may be caused by breed differences or management practices, but the details of this are unknown. Beef cows generally have higher immunoglobulin concentration in their colostrum than dairy cows [2]. This may cause beef calves to take up maternal antibodies more effectively than dairy calves. Since the duration of passive immunity by colostrum depends on the amount of antibodies ingested and absorbed [6], beef calves may maintain immunity longer than dairy calves. Dam vaccination was considered to increase dam antibody levels and prolong the duration of maternal immunity in offspring. However, this variable did not remain in the final model, although the univariate comparison indicated a difference between calves born to vaccinated dams and calves born to non-vaccinated dams. Since dams of beef calves tend to receive vaccination more often than dams of dairy calves ($\chi^2$ test; p<0.001), this variable may not remain together with cattle type in the final model.

A twofold titer as determined by viral neutralization test indicated the presence of maternal antibodies. However, the relationships between antibody titer and the efficacy of vaccination for Akabane disease are unknown. It is possible that a low titer does not influence the efficacy of the vaccine [15, 25]. If this is the case, vaccination could be used at a younger age than at the time of maternal antibody decay. Further research would help to determine the appropriate time of vaccination more accurately.

We used data for sentinel animals from a nationwide serological survey for AKAV. There were some limitations in the recorded information, such as a small number of blood collections from each animal and rough measurement of ages. We tried to overcome these weaknesses by increasing the amount of individual data and using survival analysis methods for interval censored data. Although the limitations derived from the original data were unavoidable, we believe that the applied methods led to reasonable results. Our approach enabled us to effectively utilize the existing data, even if it was scarce.

In recent years, bluetongue has spread across Europe and affected a large number of farms in 13 countries [21, 23]. This unprecedented spread was caused by climate change in Europe [13, 14, 19]. Any increase in temperature results in an increase in the range, abundance and seasonal activity of Culicoides [19]. Global climate change possibly affects vector activity and AKAV spread in Japan as well. Whether or not the occurrence of epizootic encephalomyelitis caused by AKAV in 2006 was due to climate change is uncertain. However, given that the loss of calves due to AKAV infection causes serious economic damage to cattle industries, various protective measures including vaccination should be examined to minimize losses in the future.

REFERENCES


