

Maternally and Naturally Acquired Antibody to *Mannheimia haemolytica* in Japanese Black Calves

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ABSTRACT. We investigated the dynamics and duration of antibody titer against *Mannheimia haemolytica* in Japanese Black calves. Twenty unvaccinated calves from two Japanese Black breeding farms in Kagoshima Prefecture, Japan, were studied. The antibody titer against *M. haemolytica* reached the lowest level at 8 weeks of age after birth. Calves began producing antibody against *M. haemolytica* by themselves between 8 and 12 weeks of age. The results of this study might help designing a vaccination program against *M. haemolytica* for Japanese Black calves.

KEY WORDS: antibody titer, immunity, Japanese Black calves, *Mannheimia haemolytica*.

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Mannheimia haemolytica is common inhabitant of upper respiratory tract of healthy cattle and has been associated with enzootic bovine respiratory disease complex (BRDC) in housed calves [1, 7, 8]. *M. haemolytica* has traditionally been the most common isolate from severe BRDC cases of shipping fever [1, 7]. Calves are naturally born as hypogammaglobulinemic, because of the syndesmochorial character of the ruminant placenta, which prevents prepartum transfer of immunoglobulins from their dams [2]. During the first 24 hr of life, calves must ingest and absorb colostral immunoglobulins from their seropositive dams in order to acquire passive immunity [2]. The half-life of maternally derived antibody in the calf is between 11.5 and 16 days [2]. Antibody to *M. haemolytica* has been found in colostrum of Holstein cows, and these are passively transferred to calves [4]. However, little is known about passive transfer of antibody to *M. haemolytica* in Japanese Black calves. Previous reports demonstrated that Holstein and Hereford calves in the U.S.A. produced anti-*M. haemolytica* antibody following natural exposures to *M. haemolytica* [5, 14]. They further suggested that due to natural production of antibody, vaccinations against *M. haemolytica* actually induce an anamnestic rather than a primary antibody response. The duration and the titer of maternal anti-*M. haemolytica* antibody present in Japanese Black calves have not been known, nor has been the spontaneous antibody production to *M. haemolytica* as a result of natural infection. Understanding the dynamics of anti-*M. haemolytica* antibody titer with respect to duration needs to be studied. The objective of this study was to

investigate the dynamics and duration of antibody titer to *M. haemolytica* in Japanese Black calves from two herds in Kagoshima prefecture, Japan.

Japanese Black calves from two breeding farms born between December 2009 and March 2010 in Kagoshima Prefecture, Japan, were studied. Ten calves each from farms 1 (Group 1) and 2 (Group 2) were used. Group 1 calves were allowed to remain with the dams to suck colostrum freely for 5 days after calving. After that, calves were separated from their dams and housed in individual calf pens (with nose to nose contact with their peers) until about 12 weeks of age. Subsequently, they were moved to group pens. Group 2 calves were kept with their dams from birth to 20 weeks of age. Vaccine against *M. haemolytica* was not administered in both groups during this study. All calves did not exhibit clinical case of BRDC from birth to 20 weeks of age.

Blood samples were obtained from the jugular vein into the plain vacutainer tubes. All calves were bled at 1 week (7 days of age), 4 weeks (28–34 days of age), 8 weeks (56–62 days of age), 12 weeks (84–90 days of age), 16 weeks (112–118 days of age) and 20 weeks (140–146 days of age). Blood samples were also obtained from their dams once at 1 week (7 days) after calving. Serum was isolated by centrifugation and kept at –20°C until analysis.

Serum antibody to *M. haemolytica* was determined by ELISA. ELISA was performed as previously described [12]. *M. haemolytica* serotype 1 (HL2 strain) was grown in RPMI medium at 37°C for 14–18 hr. The supernatant was concentrated 100 times. For the determination of antibody to *M. haemolytica*, concentrated supernatant was diluted to 1/25,000 with carbonate buffer and dispensed into wells of microtiter plate (NUNC, New York, NY, U.S.A.) and incubated at 4°C overnight. After washing with buffer (PBS with 0.05% Tween 20 was used for all washings), blocking solution was added and washed. Two-fold serially diluted serum samples (started from 1/100) were incubated in the wells at

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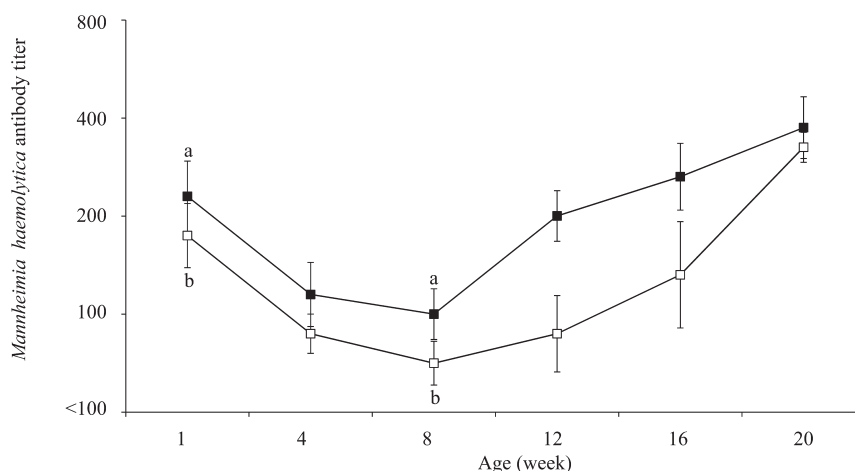


Fig. 1. Changes in ELISA antibody titer to *Mannheimia haemolytica*. Group 1 (dark square) and Group 2 (empty square). Data are shown as geometric mean \pm SE. Same letters indicate significant difference between 1 week and the other sampling points within the same group ($P < 0.05$).

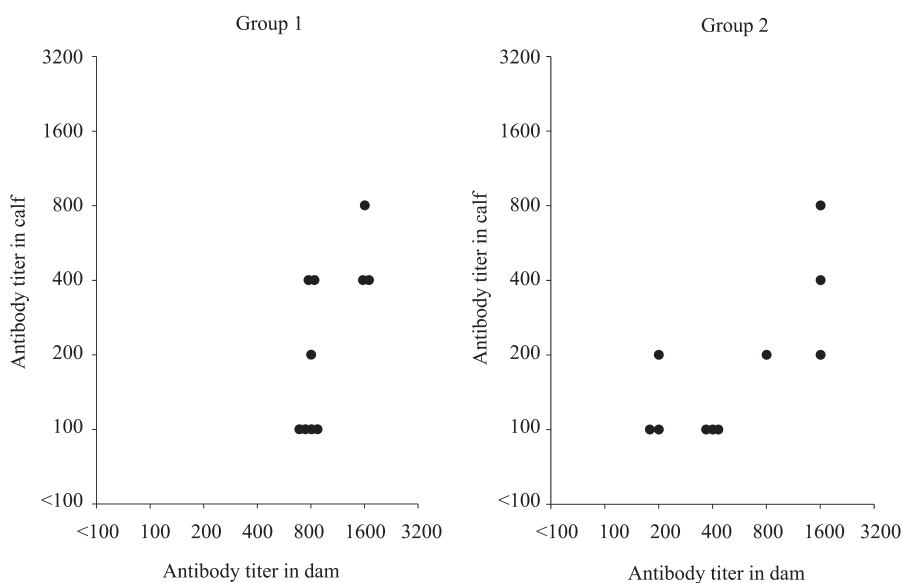


Fig. 2. Correlation of antibody titer between calves at 1 week of age and their dams at 1 week after calving.

37°C for 1 hr. After washing, peroxidase conjugated anti bovine IgG was added and incubated at 37°C for 1 hr. After washing, o-phenylene diamine in citrate-phosphate buffer was added and incubated at 30°C for 30 min. After stopping the reaction, optical density was read at 492 nm using 630 nm as a reference. The highest dilution, which showed optical density higher than 0.4, was used as an antibody titer. Antibody titer more than 100 was considered antibody positive.

Data were expressed as geometric mean \pm SE. Data were log 10 transformed for statistical analysis. Student's *t*-test was used to determine the difference between calves at 1 week of age and calves at other weeks of age within the same groups. Spearman's correlation coefficients were used to evaluate the correlation of antibody titers between calves

at 1 week of age and dams at 1 week after calving. Values of $P < 0.05$ were considered to be significant.

Figure 1 shows changes in antibody titers in calves. The antibody titer against *M. haemolytica* decreased gradually by 8 weeks of age and increased from 8 to 20 weeks of age in both groups. The antibody titer against *M. haemolytica* at 8 weeks of age was significantly lower ($P < 0.05$) than that at 1 week of age in both groups. Figure 2 shows correlation of antibody titers between calves and their dams. Significant correlations ($P < 0.05$) in antibody titers were detected in both groups with correlation coefficient of 0.689 in Group 1 and 0.638 in Group 2.

M. haemolytica is considered as an opportunistic pathogen and can be isolated frequently from healthy calves [1, 8]. We

previously investigated changes in antibody titer against *M. haemolytica* in Japanese Black calves starting from 3 to 5 months of age, when they were introduced to a farm and received or non-received vaccination against *M. haemolytica* for 12 weeks. Non-vaccinated calves increased antibody titer against *M. haemolytica* gradually due to natural infection, whereas vaccinated calves exhibited faster and higher antibody production compared to non-vaccinated calves, and it effectively reduced the incidence of BRDC [11]. The presence of maternal antibody titers reduces the effectiveness of the vaccine [3, 13, 15]. Thus, understanding the duration of passively acquired antibody titer is important for designing an effective vaccination program. The previous studies showed that antibody titers in neonatal calves were associated with that in their dams [4, 6]. In the present study, antibody titer against *M. haemolytica* in calves at 1 week of age was well correlated with that in their dams in both groups. Therefore, antibody titer against *M. haemolytica* in dams should be considered for programming vaccination to calves. In general, young calves have immature immune system demonstrated by low antibody response [3, 13]. Especially, Japanese Black calves have lower number of MHC-II+ antigen presenting cells and B cells, which are associated with antibody production, compared to Holstein calves [10]. Previous studies reported that Holstein calves, which were vaccinated against *M. haemolytica* at 1 month of age or at 2 and 4 weeks of age, did not show increase in antibody titer within 1 month after the first vaccination [5, 9]. On the other hand, Japanese Black calves which were vaccinated against *M. haemolytica* at 3 to 5 months of age or Holstein calves which were vaccinated against *M. haemolytica* at 6 and 8 weeks of age exhibited increased antibody production within 1 month after the first vaccination [5, 11]. The vaccination response of calves is affected by the age of the calves as well as the levels of maternal antibody present in the calves at the time of vaccination. Therefore, the ideal time for vaccination to calves is the period when calves have less influence of maternal antibodies, as well as they have sufficient ability to produce antibodies by themselves. Previous studies with Holstein and Hereford calves without vaccination against *M. haemolytica* showed that maternally acquired antibody titer against *M. haemolytica* decreased by 5 weeks and 60 day of age, respectively, followed by an initiation of autogenous antibody production [5, 14]. In the present study, the antibody titer against *M. haemolytica* decreased by 8 weeks of age in both groups. Calves started producing antibody between 8 and 12 weeks of age. Based on the results of this study, it might be beneficial to vaccinate Japanese Black calves against *M. haemolytica* at around 8 weeks of age. We previously reported that vaccination against *M. haemolytica* to Japanese black calves at 3 to 4 months of age when they had antibody titer 100 (analyzed in the same method as in the present study) could induce antibody production in calves [12]. There are various unknown factors regarding the antibody levels at the time of vaccination, which might induce antibody production or induce vaccination break. Further studies are needed to determine the best time of *M. haemolytica* vaccination to Japanese Black calves.

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