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**SHORT COMMUNICATION**

# Maternally and Naturally Acquired Antibodies to *Pasteurella multocida* in Japanese Black Calves

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**A B S T R A C T**

We investigated the dynamics and duration of antibody titer against *Pasteurella multocida* in Japanese Black calves. Twenty unvaccinated calves from two Japanese Black breeding farms in Japan, were studied. Blood samples were obtained from all calves at 1, 4, 8, 12, 16 and 20 weeks after birth, and also obtained from their dams once at 1 week after calving. Antibody titer against *P. multocida* in calves at 1 week of age after birth was well correlated with that in their damsat 1 week after calving. Maternally derived antibody titer against *P. multocida* reached the lowest at 4 weeks of age. Calves began producing antibody against *P. multocida* by themselves between 4 and 8 weeks of age. These results might help designing a vaccination program against *P. multocida* for Japanese Black calves.



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## INTRODUCTION

*Pasteurella multocida* is common inhabitant of upper respiratory tract of healthy cattle (Allen *et al*., 1991; Abubakar *et al*., 2013), and *P. multocida* has been the most common isolate from bovine respiratory disease (Pardon *et al*., 2011; Kurćubić *et al*., 2014). Calves are naturally born hypogammaglobulinemic, because of the syndesmochorial character of the ruminant placenta, which prevents prepartum transfer of immunoglobulins from their dams. During the first 24hr of their life, calves must ingest and absorb colostral immunoglobulins from their seropositive dams in order to acquire passive immunity. The half-life of maternally derived antibody titer in the calves are about 2 weeks (Barrington and Parish, 2001). Antibodies in colostrum of cows are passively transferred to calves (Horimoto and Sakai, 1990). To our knowledge, there is no report about passive transfer of antibody to *P. multocida* in Japanese Black calves. Previous reports demonstrated that Hereford and Maine Anjou crossbred calves in the U.S.A, and Holstein calves in Sudan produced anti-*P. multocida* antibody following natural exposures to *P. multocida* (Prado *et al*., 2006). They further suggested that due to natural production of antibody, vaccinations against *P. multocida* actually induce an anamnestic rather than a primary antibody response. In Japanese Black calves, the duration and the maternal antibody titer against *P. multocida* have not been known, nor has been the spontaneous *P. multocida* antibody production following natural exposures. Therefore, understanding the dynamics and duration of anti-*P. multocida* antibody titer are important. The objective of this study was to investigate the dynamics and duration of antibody titer against *P. multocida* in Japanese Black calves in Japan.

## MATERIALS AND METHODS

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 left with their dams until 20 weeks of age. Neither group of calves and their dams was vaccinated against *P. multocida.* All calves and their dams remained clinically healthy during the study period.

Nasal swabs were collected from 10 clinically healthy calves of each group, one to five months of age, in March 2010. *Pasteurella multocida* was isolated from all calves of Group 1 and nine of ten calves of Group 2 using a method previously (Allen *et al*., 1991).

Blood samples were collected 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) after birth. Blood samples were also collected from their dams once at 1 week (7 days) after calving. Serum was isolated by centrifugation and kept at -20$^o$C until analysis.

Serum antibody to *P. multocida* was determined by ELISA. ELISA was performed as previously described (Otomaru *et al*., 2012). For the determination of the antibody titer to *P. multocida*, rabbit anti-*P. multocida* serotype A3 (strain BP165) serum was diluted 1/10000 with carbonate buffer and dispensed into wells of microtiter plate (NUNC, New York, USA) and incubated at 30$^o$C for 2 hours. After washing with buffer (PBS with 0.05% Tween 20 was used for all washings), blocking solution was added and washed. Capsular antigen of *P. multocida* serotype A3 was dispensed into wells of microtiter plate and incubated at 30$^o$Cfor 30 minutes. After washing, two-fold serially diluted serum samples (started from 1/100), were added into the wells and incubated at 37$^o$C. After washing, peroxidase conjugated anti bovine IgG was added and incubated at 30$^o$C for 30 minutes. After washing, o-phenylene diamine in citrate-phosphate buffer was added and incubated at 30$^o$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±SE. Data were log 10 transformed for statistical analysis. All analyses were performed using SPSS Statistics 21 (IBM, Tokyo, Japan). Data were analyzed by analysis of variance (One-way ANOVA) followed by the Tukey-Kramer multiple comparisons test to determine the difference between 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.

## RESULTS AND DISCUSSION

Figure 1 shows changes in antibody titers in calves. The antibody titer against *P. multocida* decreased by 4 weeks of age and increased from 4 to 20 weeks of age in both groups. The antibody titer against *P. multocida* in Group 1 at 4 and 8 weeks of age, and in Group 2 at 4 weeks of age were significantly lower than that at 1 week of age (P<0.05). The antibody titer against *P. multocida* at 20 weeks of age was significantly higher than that at 4, 8 and 12 week of age in both groups (P<0.05). 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 0.676 in Group 1 and 0.711 in Group 2.

*Pasteurella multocida* is considered as an opportunistic pathogen and can be isolated frequently from healthy calves (Allen *et al*., 1991). In order to prevent and treat respiratory disease, antimicrobial agents are used frequently. Recently there has been an increase in antimicrobial resistant *P. multocida* (Michael *et al*., 2012), thus vaccination has been considered more favorably than antimicrobial treatment for respiratory disease caused by *P. mulctocida* (Otomaru *et al*., 2012). The presence of maternal antibody titers reduces the effectiveness of the vaccine (Nonnecke *et al*., 2012; Woolums *et al*., 2013). The previous studies showed that antibody titers in neonatal calves were associated with that in their dams (Horimoto and Sakai, 1990; Hodgins and Shewen, 1994). In the present study, antibody titer against *P. multocida* in calves at 1 week of age was well correlated with that in their dams in both groups. Therefore, antibody titer against *P. multocida* in dams should be considered for programming vaccination to calves.

The vaccination response of calves is affected by the levels of maternal antibody present in the calves at the time of vaccination (Nonnecke *et al*., 2012; Woolums *et al*., 2013). It is important to know the duration of passively acquired antibody titer when designing an effective

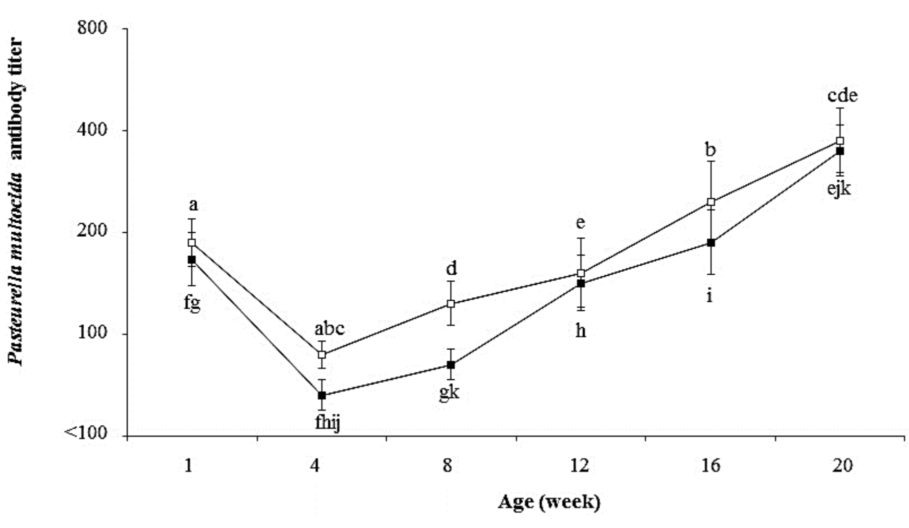


**Fig. 1:** Changes in ELISA antibody titer to*Pasteurella multocida.* Group 1 (n=10, dark square) and Group 2 (n=10, empty square). Data are shown as geometric mean±SE. Same letters indicate significant difference sampling points within the same group (P<0.05).

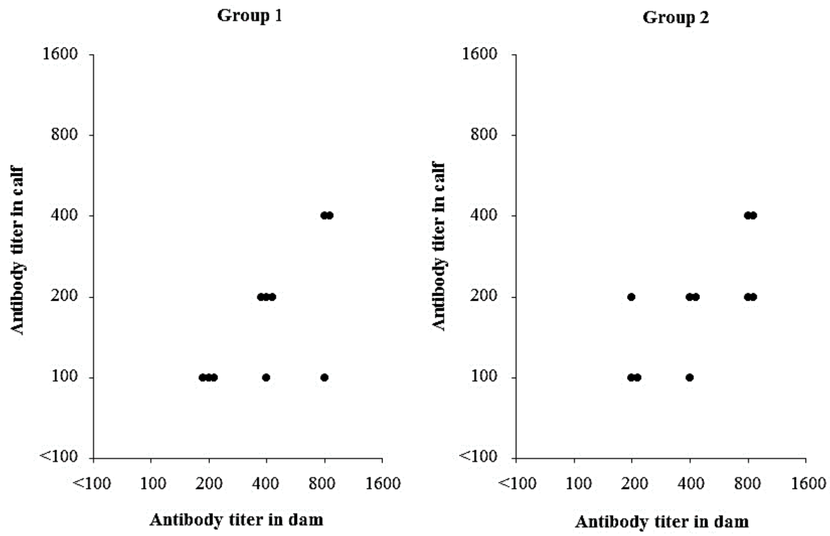


**Fig. 2:** Correlation of antibody titer between claves at 1 week of age and their dams at 1 week after caving.

vaccination program. The ideal time for vaccination to calves is the period when calves have less influence of maternal antibodies. The previous studies showed that maternally acquired antibody titer against *P. multocida* with Hereford, Maine Anjou crossbred and Holstein calves decreased by 90 days, 60 days and 4 weeks of age, respectively (El-Eragi *et al*., 2001; Prado *et al*., 2006). In the present study, maternally acquired antibody titer in calves decreased by 4 weeks of agein both groups. Based on the results of this study, it might be beneficial to vaccinate Japanese Black calves against *P. multocida* at around 4 weeks of age.

Calves in both groups showed autogenous antibody production against *P. multocida* starting between 4 and 8 weeks of age, however, no clinical cases of respiratory diseases were reported during the study period. *Pasteurella multocida* was isolated from clinically healthy calves of each farm. Therefore, spontaneous seroconversion might have been associated with colonization of *P. multocida* as normal flora through the nasal passage. These observations were in agreement with the previous study by Prado *et al*. (2006). They showed that autogenous antibody production against *P. multocida* in Hereford and Maine Anjou crossbred calves, which did not show clinical respiratory diseases, started from 90 days and 60 days of age, respectively.

**Conclusion:** We demonstrated that maternally acquired antibody against *P. multocida* in clinically healthy Japanese black calves decreased by 4 weeks of age. Autogenous production of antibody against *P. multocida* started between 4 and 8 weeks of age. Therefore, it might be beneficial to vaccinate Japanese Black calves against *P. multocida* at around 4 weeks of age. However, further studies are needed to determine the best time of *P .multocida* vaccination to Japanese Black calves.

**Author's contribution:** KO conceived and designed the review. KO and MT executed the experiment. SK analyzed the serum. KO analyzed data. All authors interpreted intellectual contents and approved the final version.

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