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Full Article

Study on duration of maternal antibodies in calves against Bovine Herpes virus type 1 (BHV-1)

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ABSTRACT

In order to estimate the mean of maternal antibody in calves against BHV-1 (Bovine Herpes virus type 1), this study was carried out in a population of calves from non-vaccinated dairy cattle at 2 livestock in Qazvin province. One hundred thirteen sera out of 512 were collected from 1-4 months unvaccinated calves. We used Blocking –Percentage of maternal antibodies against BHV-1, which obtained by Blocking ELISA assay in 1-4 months calves sera. The result of one way analysis of variance determined that there was a significant difference among blocking percentage of maternal antibodies against BHV-1 in 1-4 months ($P<0.001$). Comparing percentage of mean titer indicated a decreasing trend with respect to age i.e. from 84.4(a 95% CI: 78.1- 90.6) in 1 month to 57.6(a 95% CI: 47.1- 68.2) in 4 months, which was near to 55 as cut off point. Tukey's method showed a significant difference between the Blocking percent of mean titer between 1 and 4($P<0.001$), 2 and 4 ($P=0.034$) months. Chi-square test for independency showed a significant association between age and seroactivity ($P=0.005$).

Keywords: Bovine Herpes Virus, Blocking Elisa, calves, maternal antibodies

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) is a respiratory disease of cattle caused by Bovine Herpesvirus type 1 (BHV1). BHVI includes three sub types, 1 and 2a which are associated with respiratory disease (IBR), 2b is identified with reproductive disease (Infectious Pustular Vulvo-vaginitis, IPV) and 3 which is referred to as encephalitis (Wentink *et al* 1993). BHVI is a DNA virus in the genus Varicella virus in the family Herpetoviridae (Kahrs 2001).

BHV1 is readily transmitted and has worldwide distribution BHVI has been eradicated in Denmark, Austria, Finland Norway Sweden and some parts of France and Germany (Kahrs 2001) Immunity to BHV1 involves in complex interaction of humoral antibody and cell mediate immunity which are either activated following natural BHV1 infection or vaccination. Eliza is demonstrated to have superior sensitively for detecting of such antibodies in calves up to 9-11 months old (Cho *et al* 2002) .The pathogenesis of the disease includes the trigeminal nerve as a route of centripetal spread of the virus in acute infection

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(Homan & Easterday 1980) and also respiratory mucosae can be another route for infection (Winkler *et al* 2000). The objective of this study was to determine the duration of maternal antibodies against BHV1 in a population of calves from non-vaccinated dairy cattle.

MATERIALS AND METHODS

One Hundred thirteen sera out of 512 calves were randomly collected from unvaccinated 1-4 months dairy cattle originating from 2 industrial dairy farms in Qazvin province (90 km far from The Razi Institute) during 2009-2010.

Sample collection. Blood samples (3-5 ml) were collected in situ and sent it to the Pathology Department of Razi Institute. They were centrifuged at1500 g for 10 minutes at room temperature; the sera were collected and kept in -20 °C until tested.

Serological tests. Commercial Blocking Elisa kits developed by IDEXX, Switzerland were used to determine the presence of antibodies to BHV1 in bovine serum using IBR gB specific monoclonal antibody. The experiment was carried out according to the kit protocol. Briefly, serum samples 50 µl were diluted in reconstituted solution (1:1) and incubated for 2 hours at 37 °C. The plate was washed and 100µl of BHV, gB specific monoclonal antibody HRPO conjugate dispersed into each well and incubated for an hour. After washing100 µl of TMB substrate solution was added into each well and incubated 10 minute at room temperature (18° to 25 °C) in darkness. Finally 100µl of stop solution (HCL) dispersed into each well to stop the reaction. OD of samples and controls were measured at 450nm. Finally Blocking-Percentage for test sample was defined as: ((Negative Control mean – OD sample)/ (Negative Control mean)). 100 which interpreted as below:

<45%	45 to 55%	≥55%
Negative	Suspect	Positive

Data Analysis. A one way analysis of variance (ANOVA) was used to compare Blocking percentage of mean titer antibody against BHV-1 in different ages, and in order to detect the pair wise differences among the ages, Turkey's method was used. SPSS 13.0 and S-plus version 8 packages employed to data analysis and a value for P of less than 0.05 was considered to be significant.

RESULTS

Table 1 shows mean standard deviation, standard error and also a 95 percent confidence interval for blocking percentage of antibody of Bovine Herpes virus type 1 in 1-4 month. Comparing percentage of mean titer indicated a decreasing trend with respect to age i.e. from 84.4(a 95% CI: 78.1- 90.6) in 1 month to 57.6(a 95% CI: 47.1- 68.2) in 4 months, which was near to 55 as cut off point, however standard deviation gradually increased by the age (figure 1).

Age	Number of cattle	Mean	SD	SE	95% CI	
					Lower Bound	Upper Bound
1	28	84.4	16.0	3.0	78.1	90.6
2	26	75.3	24.7	4.8	65.3	85.2
3	30	71.9	24.6	4.5	62.7	81.1
4	29	57.6	27.7	5.1	47.1	68.2
Total	113	72.1	25.3	2.4	67.4	76.8

Table 1. Mean standard error (SE) and a 95% confidence interval (CI) for Blocking Percentage of maternal antibody of BHV-1 in 1-4 month aged cattle.

The result of one way analysis of variance determined a significant difference among the average titers of maternal antibodies in 1-4 month calves ($P<0.001$). Tukey's method used to compare pair wise difference among ages and showed a significant difference between 1 and 4 ($P<0.001$), 2 and 4 months calves ($P=0.034$). Table 2 shows the number of calves based on seroactivity (seropositivity /seronegativity) in different ages. As it is illustrated, out of 113 subjects, 89 were seropostive.

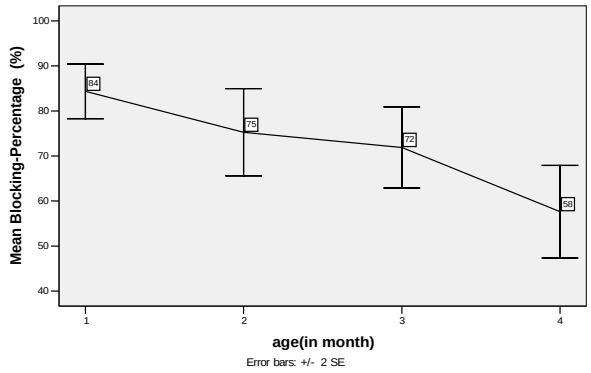

Figure 1. Blocking-percentage of maternal antibody of BHV-1 in 1-4 month aged calves.

Chi-square test for independency showed a significant association between age and seroactivity (P=0.005). Also the result of this table and figure 2 showed the percentage of seropositivity decrease as age of calves increase. Percentage of seropositivity gradually decreased from 96.4 in 1 month to 58.6 in 4 month calves.

Table 2. Number of calves based on seroactivity in different ages

Age (in month)	Seroactivity		Total
	negative	positive	
1	1	27	28
2	4	22	26
3	7	23	30
4	12	17	29
Total	24	89	113

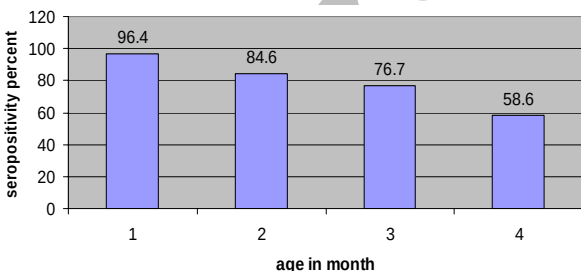

Figure 2. Percentage of Seropositive cales in 1-4 months of age

DISCUSSION

The bovine fetus produces antibody to viruses beginning at 90-120 days and by third trimester of gestation it responds to a variety of viruses including BHV1 and bacteria. In calves passive immunity is occurred by the new born nursing its dams and ingesting enough colostrums containing IgG, IgM and IgA (Radostits *et al* 2007). The value of maternal antibody transferred to new born calf by nursing of colostrums is well known and is recognized as an essential management practice (Menanteau-Horta et al 1985) . The presence of antibody indicates current or recent exposure to the virus (Martin & Bohac 1986). Colostrally acquired antibody is important in vaccination programs and resistance to disease. This study estimated mean titre that was 84.35 in 1 month diminished to 57.6 in 4 months .The reduction process of the titre in our study showed the amount of maternal standard antibodies in 4 months approaching to 55 as cut off point and also the percentage of seropositive gradually decreased from 96.4 in 1 month to 58.6 in 4 month calves. In fact colostral antibody appears in the serum of calves that suckle immune dams on the first day of life diminishes rapidly via metabolic degradation .The titres of colostrally acquire antibody vary from calf to calf depending on antibody levels in colostrums . Some calves lose maternal antibody as early as one month of age and a few may still have titers up to six months of age (Kahrs 2001). This interpretation was in accordance with our study. BHV-1 seropositive cow may reflect the proportion of BHV-1 carrier. In fact the virus as a few shedding latent in neural ganglions or respiratory mucosae for long periods shows seronegative titre after the disappearance of material antibodies (Persistent Immunity). Therefore under passive immunity, a cell mediate immune response is developing (Lemaire *et al* 2000) .Transportation of cattle with latent infection can reactivate the virus and the existence of seronegative latent carrier are a threat for cattle husbandry (Nandi *et al* 2009) .Therefore in order to find out the PI in cows (seronegative and seropostive cows) vaccination plans in the herds are necessitated (Akerman *et al* 1990, Fulton *et al* 2004). Inactivated vaccines and modified live virus vaccines are used although the former may not provide complete

protection due to a low antigen load (Monaco 1992) and the possibility of latency in the latter should be pay attention.

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