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Short Communication

Assessment of Serum Neutralizing Antibody Titers against Foot-and-Mouth Disease Virus A and O in Young Colostrum-Fed Dairy Calves

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Foot-and-mouth disease (FMD) is an acute and highly contagious viral disease caused by a picornavirus that infects cloven-hoofed animals (1). The causative agent of this disease belongs to the *Aphtovirus* genus in *Picornaviridae* family (2). Clinical signs of FMD are fever, anorexia and vesiculation, mostly in the mouth, feet and teats (3).

Although the mortality rate of this disease is low (except in young animals), FMD is a major worldwide animal health problem because of the highly communicable nature of the disease, and severe losses in the production of animals. Therefore, FMD is a perpetual menace to animal health of FMD-free-countries and disease management programs are very important, whereas regular large-scale vaccination is the current strategy for endemic areas, such as Middle East countries, Africa and South America.

Maternal passive antibodies transmit to a calf by its dam during colostrum feeding (4). These colostorally derived antibodies that present in the beginning stage of animal life, block replication of FMD virus and protect against infection during the first months of life. Ipso facto clostoral antibodies are also important factor related to primary vaccine failure. The rate of passively acquired clostoral antibodies has a critical role in duration of passive protection. As clostoral antibody titers wane, susceptibility of the infection increase (5, 6). Therefore determination of the earliest age at which high rates of immune responses can be obtained, is very important. Some studies indicated range of level of clostoral antibodies and proposed strategies of FMD vaccination. To date, however, there are no studies in this field on Iranian foot-and-mouth disease virus (FMDV) vaccines. The aim of this study was to identify the best age of vaccination with Iranian FMDV vaccine containing aluminum hydroxide adjuvant, by identifying the serum-antibody titer of young animals and their dams.

The vaccine was produced by Razi Vaccine and Serum Research Institute (Tehran, Iran). The vaccine was supplied as bivalent containing $O_{pan\ asia}$ and A_{87} strains and aluminum hydroxide adjuvant. FMD viruses, strains $O_{pan\ asia}$ and A_{87} were grown in heteroploid hamster kidney (BHK-21) cells. The viruses were assayed by TCID50 method and used in virus neutralization (VN) test.

The study conducted on three dairy cattle farms (farms 1, 2 and 3) situated in the karaj area in Iran. Two experiments (A and B) were performed to compare serum neutralizing-antibody titer of young animals and dams. Blood samples were taken from the newborn calves and their dams immediately after birth. Calves were allowed to suckle their dams, and were bled from the jugular vein every month

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Table 1. Serum anti-FMD A and O titers in 12 individual immunized dams.

Farms	Cows	VNA titer (Log_{10})	
		$O_{pan\ asia}$	A_{87}
1	1-27	< 0.9	< 0.9
	1-903	< 0.9	< 0.9
	1-69	1.65	2.1
	1-39	0.9	0.9
	1-22	< 0.9	< 0.9
	1-59	< 0.9	< 0.9
2	2-052	1.05	1.35
	2-841	< 0.9	< 0.9
	2-059	< 0.9	< 0.9
	2-915	< 0.9	< 0.9
3	3-103	1.5	1.5
	3-125	1.35	1.5

for five months. Bovine whole blood samples were collected in tubes without anticoagulant, and then centrifuged at 1500 rpm for 15 minutes to separate the sera. The sera carefully removed and transferred to sterile labeled vials and stored at -20°C until used in the VN tests. Sera were incubated at 56°C for 30 min for complement inactivation and the serial dilutions of sera ranging from 1:8 to 1:256 were established, then an equal volume of the virus contained the 100 log_{10}TCID50/ml was added to each dilution. Subsequently, the samples incubated at 37°C for 45 min to allow the formation of antigen-antibody complex. Each dilution was placed into four wells; 100 µl of each virus-serum mixture were inoculated into preformed monolayer of BHK-21 cells in a cultured plate. Then, the cultures were maintained at 37°C, and they were examined for cytopathic effect (CPE) after 48-72 h. The media were removed from the culture plates, the cells fixed in ethanol for 5 min, and stained by crystal violet. Antibody titers are reported as the reciprocal of the highest dilution that showed complete viral CPE inhibition in 50% of the wells. The sample neutralizing antibody titers were calculated by the method of Reed and Muench (7). The geometric mean titers were calculated for each group.

The protective antibody titers of dams are summarized in table 1. Total of 12 dams tested by neutralization test (NT) were seropositive. Most of the dams (67%) in this study had protective antibody titers below 0.9 $log_{10}SN_{50}$ against two strains. Four dams (# 1-69, 2-052, 3-103 and 3-125) had suitable protective antibody titers against to the two $O_{pan\ asia}$ and A_{87} strains. There was no FMDV virus neutralizing antibody (VNA) detectable titer in sera of newborn calves (before colostrum feeding). Fig 2 shows the change in mean VNA titers in sera of calves from 15th day to 5th month in three different farms. The geometric mean of the VNA titer for all colostrum-fed calves was high during the first days after birth. The highest titer was reached 15 days after birth. The $O_{pan\ asia}$ and A_{87} mean titers then gradually declined to 0.64 and 0.76 $log_{10}SN_{50}$ at 5 month after birth, respectively.

It is interesting to note the differences in VNA titers between young animals and their dams. The VNA titers of all of the samples of calves (experiment A) were found to be higher than the corresponding dams sera (experiment B) and differences in VNA titers between the colostrum-fed calves vs. their dams were statistically significant. These suggest that the VNA titer of colostrum of the dams is higher than that in the sera, possibly due to active pumping of antibody into colostrum after parturition. Therefore colostrum feeding during 24 h after birth might completely protect neonatal calves against FMD infection.

The timing of FMD vaccination depends on the level of antibodies to the FMDV, which proffers the period time between the clearance of colostral antibodies and the transmission of virus to susceptible calves (5, 8). Titers of antibodies to FMD neutralizing antigens below 1.14 are strongly associated with the presence of sensitivity to FMD infection (9) and titers > 1.14 were considered to be protective. Suitable VNA titers for vaccination in calves were 1.5 $log_{10}SN_{50}$.

A study that conducted in FMD endemic regions (10) have shown that most colostrum-fed calves of well-vaccinated farms become susceptible to FMD infection after 30-60 days of age (timing of vaccination). Other investigations also confirmed this finding and suggested that 2-3 months of age is the best time of vaccination (5, 11).

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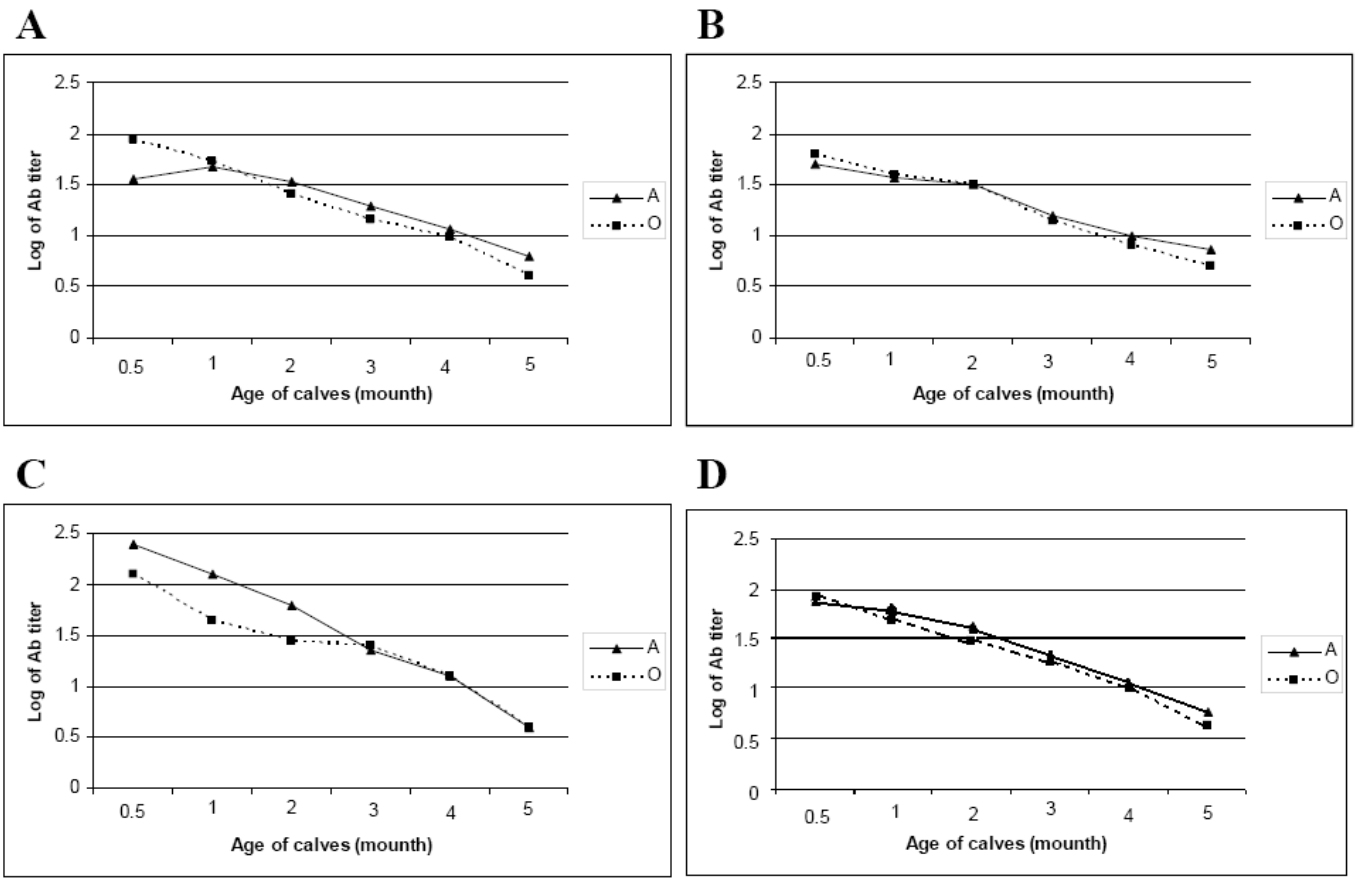

Fig. 1. Change in virus neutralization antibody (VNA) anti-FMD A (▲) and O (■) titers in colostrum-fed calves in different farms during 5 months.
(A) farm 1; (B) farm 2; (C) farm 3; (D) mean of tree farms.

In conclusion, these results are in agreement with finding of others that the best time of FMD vaccination is 2-3 months after birth, when interference of colosteral antibodies cease.

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