

Evaluation of antibodies levels against *Escherichia coli*, rotavirus and coronavirus in the colostrum of non-vaccinated cows in southern Tehran, Iran.

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Key Words:

Colostrum antibodies; competitive ELISA; *E. coli*; rotavirus; coronavirus.

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Abstract

A competitive enzyme-linked immunosorbent assay (ELISA) kit was used for the evaluation of antibodies against *Escherichia coli K99*, rotavirus and coronavirus in colostrum samples of 240 non-immunized Holstein dairy cows in southern Tehran, Iran. Antibody levels against *E. coli K99*, coronavirus and rotavirus were higher than a 20% inhibition threshold in 76%, 99% and 100% of samples, respectively. From a total of 240 samples 14 cases (5.83%), 222 cases (92.5%) and 240 cases (100%) showed the strongest positive results (4⁺) for antibodies against *E. coli*, coronavirus and rotavirus, respectively. These colostrum samples were considered as high titre colostrum. The results showed that only a small number (5.83%) of colostrum samples had enough antibodies to protect the calves against diarrhea due to *E. coli K99* after passive transfer. In the cases of rotavirus and coronavirus it was concluded that the colostrum samples obtained from non-immunized, naturally infected cows contained enough antibodies to develop passive immunity against rotavirus and coronavirus in suckling calves.

Introduction

Diarrhea is one of the leading causes of death in calves aged less than one month. Rotavirus and coronavirus are the most important pathogens associated with gastroenteritis and diarrhea in young calves. Rota- and coronavirus are ubiquitous and as a result, most of the animals, including pregnant cows coming from intensive livestock farms, have specific antibodies against these pathogens. The enterotoxigenic *E. coli K99*, bearing the F5 attachment factor, can also be found in calves younger than three days, especially in animals that did not receive any colostrum or received colostrum without any specific antibodies against this pathogen. The antibodies produced by cows in response to natural immunization or vaccination are transmitted to the calf at birth via the colostrum (Radostits *et al.*, 2007). Passive immunization by using bovine colostrum is an area of increasing interest. The importance of colostrum in the transmission of immunity from mother to offspring was first mentioned by Ehrlich in 1892 (Silverstein, 1996). In cattle, passive transfer of maternal antibodies occurs in the first 20-48 hours after birth via the colostrum. During the immediate postnatal period the gastrointestinal tract

undergoes rapid growth with morphological changes and functional maturation related to colostrum ingestion (Xu, 1996). It has been postulated that many animal species have enhanced intestinal permeability to macromolecules, which may facilitate the absorption of colostrum immunoglobulins during the early neonatal period (Burin, 1997). Antigen recognition specificity of colostrum immunoglobulins is important in determining the protective power of colostrum. Deficiency of a specific antibody could explain why some calves, appearing to have sufficient total antibody concentration, die as a result of infections. In the case of passive immunization against coronavirus, there are few reports on oral administration of colostrum obtained from naturally infected cows. The aim of this study was to evaluate the level of antibody against bovine coronavirus (BCV), bovine rotavirus (BRV) and enterotoxigenic *E. coli K99* in the colostrum of non-vaccinated and naturally infected cows.

Materials and Methods

Samples

A total number of 240 colostrum samples was obtained randomly from selected populations of

Holstein dairy cows located in the south of Tehran, Iran. The samples were preserved at -20°C until further processing. A competitive ELISA test was carried out with clear colostrum whey which was obtained by centrifuging colostrum at 2000 g for 20 minutes at 4°C and the clear phase below the lipid phase was taken up by gentle pipetting.

The competitive enzyme-linked immunosorbent assay (ELISA) kits for the serodiagnosis of anti-BRV, anti-BCV and anti-*E. coli* K99 (F5) attachment factor antibodies in colostrum whey were obtained from Biox Diagnostics, Belgium.

Procedure

The colostrum whey samples as well as one positive and one negative control serum (control sera were available with each ELISA kit) were diluted 1:4 with dilution buffer and distributed in duplicates at a volume of 100 μ l per well. After a washing step, 100 μ l of the diluted (1:20) anti-bovine IgG peroxidase conjugate was added to each well and incubated for 1 hr at room temperature. The plates were rinsed three times and 100 μ l per well of the chromogen-substrate were added. After 10 min, stop solution was added and the optical densities (OD) of the wells were measured at a wavelength of 450 nm (Denley well scanner, UK). In this procedure the intensity of the color is inversely proportionate to the samples antibody concentration (Lockwood *et al.*, 1984; Paton *et al.*, 1991).

The percentage inhibition for each tested sample and for the positive control was calculated by dividing the OD of each sample by the OD of the negative control multiplying by 100. The degree of positivity for each sample was determined according to the percentage of inhibition, using the scale 1⁺, 2⁺, 3⁺ and 4⁺, if their percentage of inhibitions was equal or higher than 20%, 40%, 60% and 80%, respectively (Callebaut *et al.*, 1989).

Results

Anti-*E. coli* K99

Out of 240 samples, 14 cases (5.83%) showed more than 80% inhibition (4⁺) in the competitive ELISA, which were considered high titers associated with *E. coli* K99. Twenty-eight samples were 3⁺ and 142 samples were positive (1⁺ and 2⁺), while the rest showed no anti-*E. coli* antibodies (Table 1.) The OD values of the negative and positive control sera obtained were 1.230 and 0.232, respectively. The lowest OD among the 14 samples that showed more than 80% inhibition was 0.123 with 90% inhibition, and the highest OD was 0.125 with 82% inhibition

Anti-BCV

Out of 240 samples, 222 cases (92.5%) showed inhibition levels higher than 80% (4⁺). Sixteen samples

Table 1: The frequency levels of antibodies against *E. coli*, BRV and BCV in colostrum samples, according to percent of inhibition in competitive ELISA.

| Antibody type | Percentage of inhibition/category | | | | |
|--------------------------|-----------------------------------|-----------|-----------|-----------|-----------------|
| | >80% (4+) | =60% (3+) | >40% (2+) | >20% (1+) | <20% (Negative) |
| Anti- <i>E. coli</i> k99 | 14 | 28 | 50 | 92 | 56 |
| Anti-BRV | 240 | 0 | 0 | 0 | 0 |
| Anti-BCV | 222 | 2 | 8 | 6 | 2 |

(6.6%) showed inhibition levels 1⁺ to 3⁺, and two samples were considered negative as the percentage of inhibition was less than 20% (Table 1). The OD values of negative and positive control sera obtained were 1.195 and 0.034, respectively.

Anti-BRV

All of the 240 samples (100%) showed inhibition levels of 4⁺, as the lowest OD was 0.021 with 98.6% inhibition, and the highest OD was 0.106 with 93% inhibition in competitive ELISA.

Discussion

The passive transfer of immunoglobulins via colostrums is of critical importance in the defense against many diseases in neonatal calves (Banks, 1982). Immunoglobulins specifically recognize viral and bacterial agents, enhancing their elimination by direct opsonization, phagocytosis, or activation of the complement cascade (Tizzard, 2004). The presence of antibodies against *E. coli* with titers of 640 IU had been shown in colostrum of non-vaccinated cows (Lissner *et al.*, 1996). The presence of anti-shiga-like toxin antibodies was also shown in 84% of colostrum by capture ELISA (Pirro *et al.*, 1995).

The present study showed that only 5.83% of colostrum samples had high titers anti-*E. coli* K99 antibodies that would enable protection through passive transfer against diarrhea due to *E. coli* K99. Therefore, the vaccination of pregnant cows against *E. coli* K99 is recommended to enhance the antibody titers. In contrast, all of the colostrum samples from non-immunised cows were seropositive with high titers for anti-BRV antibodies. This indicates that BRV is spread widely among cattle in southern Tehran. Cows immunized with BRV vaccine have been shown to produce 12-fold higher neutralization antibody titres against rotaviruses than non-immunized control cows (Tsunemitsu *et al.*, 1989). Many investigators have reported that frequent ingestion of colostrum with high titers of anti-BRV can protect suckling calves from enteric viral infection (Snodgrass *et al.*, 1980). This immunogenic mechanism has been described as lactogenic immunity. Lactogenic immunity seems to be effective in the protection of newborn calves against BRV infection. Saif *et al.* (1983) have shown that passive immunity to BRV in newborn calves that were fed colostrum from immunized or non-immunized

cows allows protection of calves against diarrhea. In contrast, it was reported that in non-immunized, naturally infected animals low levels of antibody to the antigen(s) such as rotavirus (Bogstedt *et al.*, 1996) and enteropathogenic *E. coli* and its colonization factor antigen (Facon *et al.*, 1995) were found.

BCV is a well-recognized agent of neonatal diarrhea in calves, causing severe dehydration and often death by infecting the enterocytes of the small intestine and colonic epithelium (Hackert *et al.*, 1991). BCV contains 4 major structural glycoproteins including the peplomer protein (E2) and the hemagglutinin (E3) that elicit virus-neutralizing antibodies (King *et al.*, 1985; Deregt *et al.*, 1987) and could be detected through ELISA.

Our findings showed high anti-BCV antibody titers in 92.5% of the colostrum samples. In this study we found that the pools of colostrum obtained from non-immunized, naturally infected cows had high enough antibody levels against BRV and BCV to develop a passive immunity in suckling calves.

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