

EFFECTS OF VACCINATION OF HIGHLY PREGNANT COWS WITH ANTI BHV-1 VACCINES: 2. HUMORAL RESPONSE ON THE VACCINE "BORINAK"

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The humoral immunological response in 26 highly pregnant cows vaccinated with anti bovine herpesvirus-1 (BHV-1) vaccine ("Borinak", Mevak, Nitra, Slovak Republic) was investigated using the method of virus microneutralization. Titers of specific antibodies for BHV-1 were determined in blood and colostral serum prior to vaccination and revaccination (3 weeks after vaccination) as well as in the moment of parturition. Before vaccination, values of antibody titers ranged from 1:4 to 1:64. At the time of parturition they ranged from 1:64 to 1:1024 in blood serum and from 1:256 to 1:2048 in colostral serum. Antibodies for BHV-1 in calves from these cows were determined before feeding with colostrum and afterwards at the age of 2, 15, 30, 45, 60 and 90 days. Before feeding with colostrum, these antibodies were detected in 2 calves, and after feeding with colostrum in all calves up to the age of 60 days. The highest values for antibody titers were found in calves at 2 days age and the lowest values in 90 days old calves.

Key words: anti BHV-1 vaccine, BHV-1, calves, highly pregnant cows,

INTRODUCTION

In cattle herds where infection with bovine herpesvirus-1 (BHV-1, IBR) is very widespread, many authors (Cirstet *et al.*, 1990; Soulebot, 1985; Brun *et al.*, 1982; Mohny, 1982; Bartha *et al.*, 1977) performed or recommended vaccination of all cattle and emphasized the importance of vaccination of highly pregnant cows with anti BHV-1 vaccines. The cited authors emphasized that regular vaccination of cattle against BHV-1 leads to hyperimmunization, which prevents spread of the virus in the herd and, in highly pregnant cows significantly contributes to the increase of antibodies against BHV-1 in the colostrum. Vaccination of highly pregnant cows with anti BHV-1 vaccines was recognized as a possibility to prevent infection with BHV-1 in calves. Similarly, Smith *et al.* (1978) suggested that neonatal diseases of calves, provoked by the IBR virus, could be prevented with

colostral antibodies. Moreover, Kahrs (1985) emphasized that maternal antibodies against the IBR virus transferred in colostrum are of great importance for the protection of calves.

The aim of our investigations was to vaccinate highly pregnant cows with anti BHV-1 vaccine under conditions of intensive cattle raising, and to follow the humoral immune response in cows from the moment of vaccination up to calving as well as immediately after calving, and in their calves from birth to the age of 3 months.

MATERIAL AND METHODS

Experimental animals: The investigations were carried out in a cattle herd, where an infection with BHV-1 had previously been confirmed. Highly pregnant randomly selected cows ($n=26$) were vaccinated with an anti BHV-1 vaccine during the drying off period and revaccinated 3 weeks later. When vaccinated, the average pregnancy was 224 days ranging from 201 to 231. The investigations also included 20 vaccinated cows just after parturition, as well as their 20 calves and 10 randomly selected nonvaccinated cows, as well as their 10 calves as the control group. The chosen cows were of the Holstein-Friesian breed and were between 4 and 5 years old.

Vaccine: The vaccine "Borinak" ("Mevak", Nitra, Slovak Republic which contains inactivated BHV-1 emulgated in oil adjuvant was applied intramuscularly in the dose of 5 ml.

Blood and colostrum sampling: Blood from cows and calves was sampled by puncture of v. jugularis. Blood was taken prior to vaccination, 3 weeks after vaccination (prior to revaccination) and from 20 cows immediately after parturition, when colostrum was also sampled. Blood and colostrum were sampled from cows of the control group immediately after parturition. Blood was taken from calves of vaccinated cows immediately after birth, before feeding with colostrum, and afterwards at the age of 2, 15, 30, 45, 60 and 90 days. Blood from calves of nonvaccinated cows was sampled before feeding with colostrum and on the second day after birth. The separated blood serum was stored at -20°C up to examination. The colostrum serum was separated using the lab-ferment and stored at -20°C until required.

Neutralization test: Specific antibodies for BHV-1 were determined using the method of microneutralization of the virus. Double serum dilutions were prepared in microtiter plates ("Nunc", Denmark). The same quantity of the virus that contains 100 CCID₅₀/25 μl was added to each serum dilution. After incubation for 1 hour at 37°C , 50 μl of the suspension of MDBK cells was added. The plates were observed every day and the final reading of the results was on the 5th day. For each plate controls for the nutrient medium, the virus, positive and negative serum were included, as well as the investigated.

RESULTS

The titer values of specific antibodies for BHV-1 found in blood serum of cows prior to vaccination and revaccination (3 weeks after vaccination) with the vaccine "Borinak" are shown in Table 1.

Table 1 - Titers of specific antibodies for BHV-1 found in blood sera of cows prior to vaccination and revaccination

| Investigation period | n | 4 | 8 | 16 | 32 | 64 | 128 | 256 | GMV ⁺ | p |
|------------------------|----|---|---|----|----|----|-----|-----|------------------|---------|
| prior to vaccination | 26 | 4 | 5 | 14 | 2 | 1 | 8 | 6 | 12.58 | |
| prior to revaccination | 26 | | 1 | 1 | 10 | 4 | 9 | 1 | 57.68 | <0.001* |

⁺ Geometric mean value

* GMV prior to vaccination : GMV prior to revaccination

Specific antibodies for BHV-1 were present in blood sera from all cows. However, 3 weeks after vaccination, i.e. prior to revaccination, the antibody titers were considerably higher. The difference of geometric mean values between these antibody titers, obtained by the t-test, was highly significant.

The titer values of specific antibodies for BHV-1 immediately after parturition which occurred 3 to 6 weeks after revaccination for the majority of the investigated cows are shown in table 2.

Table 2 - Specific antibodies for BHV-1 found in blood and colostral serum of vaccinated cows immediately after parturition

| n = 20 | Titer 1: | | | | | | GMV | p |
|-----------------|----------|-----|-----|-----|-------|-------|----------|---------|
| | 64 | 128 | 256 | 512 | 1,024 | 2,048 | | |
| Blood serum | 2 | 5 | 8 | 4 | | | 230.72 | |
| Colostral serum | | | | 5 | 8 | 7 | 1,097.50 | <0.001* |

* GMV in blood serum : GMV in colostral serum

High values for antibody titers were found, especially in the colostral serum, as expected, so the difference between the geometric mean values of antibody titers was also highly significant in the vaccinated cows.

The values obtained for the control, nonvaccinated group of cows at the moment of parturition in blood and colostral serum are shown in table 3.

Table 3 - Titers of specific antibodies found in blood and colostral serum of nonvaccinated cows

| n = 10 | Titer 1: | | | | | | | GM V | p |
|-----------------|----------|---|---|---|----|----|----|-----------|-------|
| | <2 | 2 | 4 | 8 | 16 | 32 | 64 | | |
| Blood serum | 2 | 1 | | 2 | | 5 | | 9.19 | |
| | | | | | | | | | >0.05 |
| Colostral serum | 2 | | 1 | 2 | | 1 | 4 | 13.0 0 | |

* GMV in blood serum : GMV in colostral serum

No specific antibodies for BHV-1 were detected in two nonvaccinated cows either in blood or in colostral serum. The difference of the geometric mean values of antibody titers in blood and colostral serum of nonvaccinated cows was not statistically significant.

The antibody titers for BHV-1 in calves of vaccinated cows, for the period from birth (before feeding with colostrum) up to the age of 90 days, are shown in table 4.

Table 4 - Titers of specific antibodies for BHV-1 found in calves of vaccinated cows

| Age of calves | n | Titer 1: | | | | | | | | | | GSV |
|---------------|----|----------|---|---|---|----|----|----|-----|-----|-----|-------|
| | | <2 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | |
| 0 days | 20 | 18 | 1 | | 1 | | | | | | | |
| 2 days | 20 | | | | | | 3 | 4 | 5 | 8 | | 119.4 |
| 15 days | 20 | | | | 1 | 3 | 4 | 5 | 7 | | | 51.98 |
| 30 days | 20 | | | 1 | 6 | 5 | 5 | 7 | 1 | | | 16.56 |
| 45 days | 20 | | | 1 | 4 | 1 | 7 | 2 | | | | 19.03 |
| 60 days | 20 | | 2 | 3 | 4 | 5 | 6 | | | | | 11.31 |
| 90 days | 20 | 4 | 5 | 3 | 2 | 1 | | | | | | 4.14 |

The presence of specific antibodies for BHV-1 was detected at birth in 2 calves, before feeding with colostrum. After feeding with colostrum, specific antibodies were found in all calves at the age of 2-60 days, whereas these antibodies were not found in 4 calves at the age of 90 days. The highest antibody titer occurred in calves at the age of 2 days (GMV=119.43) and the lowest value in calves at the age of 90 days (GMV=4.14).

The values of antibody titers for BHV-1 in calves of nonvaccinated cows immediately after birth (before feeding with colostrum) and at the age of 2 days are shown in table 5.

Table 5 - Titers of specific antibodies for BHV-1 found in calves of nonvaccinated cows

| Investigation period | n | Titer 1: | | | | | | GMV |
|----------------------|----|----------|---|---|---|----|----|------|
| | | <2 | 2 | 4 | 8 | 16 | 32 | |
| 0 days | 10 | 10 | | | | | | 5.30 |
| 2 days | 10 | 2 | 2 | 1 | 2 | 1 | 2 | |

Specific antibodies for BHV-1 were not present in any calf before feeding with colostrum. Two days after birth these antibodies were present in 8 calves. Antibodies for BHV-1 were not detected in the 2 calves which originated from the cows which did not have specific antibodies for BHV-1 in their blood or colostrum serum.

DISCUSSION

Investigating the humoral immunological response in highly pregnant cows given the vaccine "Borinak", we can say that the immunological system of cows was stimulated, and as a final effect of the humoral immunological response higher values for titers of specific antibodies for BHV-1 were determined in cows after vaccination.

The finding of specific antibodies for BHV-1 in blood sera of highly pregnant cows prior to vaccination as well as in blood and colostrum sera of nonvaccinated cows at the moment of parturition points to the need and justice of carrying out immunoprophylaxis against BHV-1. Namely, specific antibodies for BHV-1 were found in 34 (94.44%) out of 36 investigated cows (26 experimental and 10 control). Such a high percent of cows infected with BHV-1 in a cattle herd confirms the results of many research workers (Cirstet *et al.*, 1990; Soulebot, 1985; Latarija, 1979; Bartha *et al.*, 1977), who recommended or applied vaccination of highly pregnant cows when the percent of infected cattle in a herd was high.

The values of antibody titers for BHV-1 found in blood sera of cows at the moment of parturition are significantly higher than at the time of revaccination, although in 2 cows the level of antibodies remained the same as at revaccination. However, the antibody titers in the other 18 cows (90%) significantly increased after revaccination. The high antibody titers for BHV-1 in blood serum of vaccinated cows confirm the results of Cirstet *et al.* (1990), where the titer was 6-9 log. 10. Knežević *et al.* (1990) vaccinated cows in the 7th month of pregnancy with an inactivated oil anti BHV-1 vaccine and they also determined very high values of antibody titers of 8.3 log 2. Brun *et al.* (1982) obtained the highest values of antibody titers after vaccination of cows in the 8th month of pregnancy.

The finding of high titers of neutralizing antibodies for BHV-1 in the colostral sera of cows certainly confirms the characteristic of this animal species to provide its calves with adequate passive immunological protection. The antibody titers in colostral serum were a couple of times higher than in blood serum (Table 2). Many authors (Sulebot, 1985; Mielke and Schulz, 1978; Sheldrake and Husband, 1985) emphasize that the most abundant accumulation of immunoglobulin in colostrum happens in the last 3 weeks of pregnancy and that this is a period when the organism should be immunologically stimulated, so that the highest possible level of immunoglobulin accumulates in colostrum.

Besides the enormous differences in antibody titers found in blood and colostral sera of vaccinated and nonvaccinated cows, it is also interesting to mention that nonvaccinated cows had very different antibody levels. That is probably the result of the immunological response and of the different time of infection with BHV-1 in natural conditions. Cows that had no antibodies in blood serum did not have them in colostrum either, which means that they could not transfer them to their calves.

Specific antibodies for BHV-1 were detected in blood serum of 2 (6.67%) out of 30 investigated calves before feeding with colostrum (20 calves of the experimental group and 10 calves of the control group). The presence of specific antibodies for BHV-1 in the blood serum of calves before feeding with colostrum indicates that the fetus was infected with BHV-1 during intrauterine development, without death of the fetus or abortion. The calf fetus is immunologically capable to react to many infectious agents and in that way to BHV-1 too as observed by many authors (Panjević *et al.*, 1980; Kendrick and Osburn, 1973; Lazić *et al.*, 1991; Rogan, 1987). However, an immunological response in the fetus should be accepted conditionally, because they are not immunologically reactive enough to give protection and they lag behind immunologically developed organisms (Mihajlović, 1983). Kendrick (1973) and Smith *et al.* (1978) showed that the infection of the fetus with BHV-1 in a certain period of pregnancy induces death and abortion. Survival of the fetus and an immunological response to BHV-1 could be explained also in relation to the age and the vitality of the fetus as well as the virulence of the virus, which could be of crucial importance for survival of the fetus.

A large increase of antibody titers for BHV-1 occurred in blood serum of 2-day-old calves after feeding with colostrum of cows vaccinated with anti BHV-1 vaccine. Such a great increase of antibody titers in calves two days after birth was expected, because very high values of antibody titers for BHV-1 were found in colostral serum of the vaccinated cows. Prompt feeding of calves with colostrum after birth in sufficient quantities enables very high antibody titers to be reached in their blood serum.

The antibody titers had decreased significantly in calves at the age of 15 days. This is the result of catabolism of passive antibodies transferred in colostrum. Other authors (McGuire *et al.*, 1976; Kimm and Schmidt, 1983; Perereau, 1973) point out that colostral antibodies are decomposed by more than 50% in the first 2-3 weeks after birth in calves. Kahrs (1985) emphasized that colostral passive immunity in calves rapidly disappears through metabolic decomposition. In our case, the antibody level in calves at the age of 15 days decreased through metabolic decomposition by about 56% comparing to the values in 2-day-old calves. However, in spite of this degradation of colostral antibodies, the antibody titer in calves at the age of 15 days was still high. The disappearance of colostral antibodies for BHV-1 was progressive. At the age of 90 days 4 (20.00%) calves did not have antibodies for BHV-1. Antibody titers in 2 day old calves of nonvaccinated cows were low and at the level of antibody titers obtained at the age of 90 days.

The passively transferred immunological protection, observed through the values of antibody titers for BHV-1 in cows and calves, show that the functioning of the immunological system of calves is very complex. A more complete understanding of the immunological system of newborn calves should also have practical use. Many diseases of calves that arise in the first weeks of life could be etiologically connected with passive immunological protection that was not adequately transferred. In industrial conditions of cattle raising, deviations from physiological conditions that characterize the biology of ruminants are often present. The obtained results and the antibody titers found for BHV-1 can serve for further investigations and contribute towards prevention of infection of calves with BHV-1.

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EFEKTI VAKCINACIJE VISOKO STEONIH KRAVA ANTI BHV - 1 VAKCINOM : 2. HUMORALNI IMUNOLOŠKI ODGOVOR NA VAKCINU "BORINAK"

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SADRŽAJ

U ovom radu izneti su rezultati ispitivanja humoralnog imunološkog odgovora metodom mikroneutralizacije kod 26 visoko steonih krava vakcinisanih sa antiherpesnom BHV - 1 vakcinom ("Borinak", Mevak, Nitra, Slovačka). Titar antitela protiv BHV - 1 određivan je u krvnom serumu pre vakcinacije i tri nedelje posle vakcinacije a na dan teljenja i u kolostrumu. Pre vakcinacije, titar antitela u krvnom serumu se kretao u opsegu od 1:4 do 1:64, na dan teljenja u opsegu od 1:64 do 1:1024 dok je u kolostrumu ova vrednost iznosila 1:256 do 1:2048. Antitela na BHV 1 određivana su kod teladi ovih krava pre unošenja kolostruma a zatim i u uzrastu od 2, 15, 30, 45, 60, i 90 dana. Pre unošenja kolostruma antitela su otkrivena samo kod dva teleta a posle toga kod sve teladi do uzrasta od 60 dana. Najveće vrednost titra su utvrđene kod teladi u uzrastu od dva dana, a najmanje kod teladi stare 90 dana.