

**EFFECTS OF VACCINATION OF HIGHLY PREGNANT COWS WITH ANTI BHV-1 VACCINES: I.
HUMORAL RESPONSE TO THE VACCINE "IBEROL PLUS"**

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The humoral immunological response was investigated in 33 highly pregnant cows vaccinated with anti bovine herpesvirus-1) vaccine ("Iberol-plus", Veterinary Department, Zemun) using the method of virus microneutralization were determined prior to vaccination and revaccination (3 weeks after vaccination) as well as during parturition. Titers of specific antibodies to BHV-1 in blood and colostrum serum. Before vaccination, the antibody titers ranged from 1:2 to 1:32, while at parturition they ranged from 1:32 to 1:512 in blood serum and from 1:256 to 1:2048 in colostrum serum. Antibodies against BHV-1 were also determined in calves from these cows before feeding with colostrum and afterwards at the ages of 2, 15, 30, 45, 60 and 90 days. Before feeding with colostrum, these antibodies were detected in 3 calves, but after feeding with colostrum all calves up to the age of 60 days were positive. The highest antibody titers were found in calves at 2 days of and the lowest values in 90 day old calves.

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

INTRODUCTION

In cattle herds where the infection with bovine herpesvirus-1 (BHV-1, IBR) is very widespread, many authors (Cirstet et al., 1982; Mohnty, 1982; Latarija, 1979; Bartha et al., 1977) performed or recommended vaccination of each animal and they especially 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 hyperimmunisation, which prevents spread of the virus in the herd and in highly pregnant cows leads to a significant increase of antibodies against BHV-1 in the colostrum. The authors recognize the vaccination of highly pregnant cows with anti BHV-1 vaccines as a possibility to prevent BHV-1 infection in calves. Moreover, Smith et al. (1978) suggest that neonatal diseases of calves, provoked by the IBR virus, could be prevented with colostrum antibodies, while, Kahrs (1985). Kahrs emphasize that maternal an-

antibodies against the IBR virus transferred in colostrum are of great importance for the protection of calves against infection with this virus.

The aim of our investigation was to vaccinate highly pregnant cows kept under intensive conditions with anti BHV-1 vaccine and to follow the humoral immune response in cows from the moment of vaccination, through calving to the period immediately after calving, as well as 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 been previously confirmed. A total of 33 highly pregnant randomly cows were vaccinated with an anti BHV-1 vaccine in the drying off period and revaccinated 3 weeks later. When vaccinated, the cows were between 207 and 231 days pregnant, namely 224 days on average. The investigations included 17 cows at the moment of parturition as well as their 18 calves (one cow calved twins). A total of 10 nonvaccinated cows, which were also randomly selected, as well as their 10 calves, were of the Holstein-Friesian breed and were between 4 and 5 years old.

Vaccine: The vaccine "Iberol-plus" (Veterinary Department, Zemun) is a bivalent oil vaccine that contains inactivated BHV-1 and the virus Pi-3. The dose of 5 ml contains min. $10^{7.8}$ CCID/50 of the inactivated IBR virus (immunogenic strain BPT-1 and $10^{6.3}$ CCID/50 of the virus Pi-3. The vaccine can be applied subcutaneously or intramuscularly.

Blood and colostrum sampling: Blood from cows and calves was sampled by puncture of the v. jugularis. Blood was taken prior to vaccination, 3 weeks after vaccination (prior to revaccination) and immediately after parturition, when colostrum was also sampled. Blood and colostrum was sampled from control cows immediately after parturition. Blood from calves of vaccinated cows was sampled immediately after birth, before feeding with colostrum, and afterwards at the ages 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 until analysed. The colostrum serum was separated using the lab-ferment and stored at -20°C .

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

RESULTS

The titers of specific antibodies against BHV-1 found in the blood serum of cows prior to vaccination and revaccination (3 weeks after vaccination) with "iberol-plus" are shown in Table 1.

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

Time	n	Titer 1:								GMV ⁺	p
		2	4	8	16	32	64	128	256		
prior to vaccination	33	4	8	10	9	2				7.52	
prior to revaccination	33					1	14	6	4	69.55	<0.001*

+ Geometric mean value

*GMV prior to vaccination: GMV prior to revaccination

Specific antibodies for BHV-1 were present in blood sera of all these cows. However, 3 weeks after the first vaccination, i. e. just prior to revaccination, the antibody titers were considerably increased, so that the difference between the geometric mean values for the two periods was highly significant.

Immediately after parturition, which occurred 3 to 4 weeks after revaccination for the majority of the cows, the titers of specific antibodies for BHV-1 remained high as shown in Table 2.

Table 2. Titers of specific antibodies for BHV-1 in blood and colostral serum of vaccinated cows immediately after parturition

n = 17	Titer 1:							GMV	p
	32	64	128	256	512	1024	2048		
Blood serum	3	1	10	2	1			112.99	
Colostral serum				1	5	5	6	982.29	<0.001*

*GMV in blood serum: GMV in colostral serum

Antibody titers were especially elevated in the colostral serum, so that the difference of the geometric mean values of antibody titers between blood and colostral sera highly significant.

The antibody titers found for BHV-1 in blood and colostral serum of the control, nonvaccinated group of cows at parturition are shown in Table 3. The values were similar to those obtained before vaccination in the vaccinated group of cows. No specific antibodies were detected in blood and colostral sera from two cows.

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

n = 10	Titer 1:						GMV	p
	< 2	2	4	8	16	32		
Blood serum	2	1		2		5	9.19	> 0.05
Colostral serum	2		1	2		1	4	
							13.00	

*GMV in blood serum: GMV in colostral serum

The antibody titers for BHV-1 found in the 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	18	15		1	1	1						
2 days	18						1		7	7	3	195.36
15 days	18				1	2	4	6	3	2		54.95
30 days	18			1	1	5	5	4	2			25.46
45 days	18			1	2	6	7	2				20.97
60 days	18		1	3	6	5	1	2				10.85
90 days	18	3	6	6	2	1						2.95

The presence of specific antibodies for BHV-1 was detected in 3 calves at birth before feeding with colostrum. After feeding with colostrum, specific antibodies were found in all calves between the ages of 2-60 days, whereas at 90 days old no antibodies were found in 3 calves. The highest antibody titer occurred in 2 day old calves (GMV = 195.36) and the lowest in calves at the age of 90 days (GMV = 2.95).

The 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

Calf age	n	Titer 1:						CMV
		< 2	2	4	8	16	32	
0 days	10	10						< 2
2 days	10	2	2	1	2	1	2	5.30

Specific antibodies for BHV-1 were not present in any calf at birth. Two days after birth these antibodies were present in 8 calves. However no antibodies for

BHV-1 were detected in the 2 calves from the cows which had no specific antibodies for BHV-1 in the blood and the colostral serum.

DISCUSSION

The humoral immunological response in the highly pregnant cows vaccinated with "Iberol-plus", indicated that the immunological system of these cows was stimulated leading to high values for titers of specific antibodies against BHV-1.

The finding of specific antibodies for BHV-1 in the blood sera of highly pregnant cows prior to vaccination as well as in the blood and colostral sera of nonvaccinated cows at parturition points to the need and justice of carrying out immunoprophylaxis against BHV-1. Namely, out of 43 cows investigated experimental and 10 control cows, specific antibodies for BHV-1 were found in 41 (95.35%) cows. Such a high percent of cows infected with BHV-1 in a cattle herd confirms the results of many research workers (Cirstet et al., 1009; Soulebot, 1985; Latarija, 1979; Bartha et al., 1977), who recommended or applied vaccination of highly pregnant cows when the percent of infected individuals in a herd was high.

The antibody titers for BHV-1 found in the blood sera of cows during parturition were significantly higher than at the moment of revaccination, although the increase of the titer value was one serum dilution in 4 cows and the level of antibodies remained the same in one cow. Nevertheless the antibody titers significantly increased in 12 cows. These high values for antibody titers to BHV-1 in the blood serum of vaccinated cows confirm the results obtained by Cirstet et al. (1990), 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 obtained very high values of antibody titers (8,3 log 2.). Brun et al. (1982) obtained the highest values of antibody titers when the cows were vaccinated in the 8th month of pregnancy.

The finding of high titers of neutralizing for BHV-1 in the colostral sera certainly points to the characteristic of this animal species to provide its calves with an adequate passive immunological protection. The antibody titers found in colostral serum are twice as high as 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 immunoglobuline could accumulate in colostrum.

Besides the enormous differences in the mean antibody titers in blood and colostral sera between vaccinated and nonvaccinated cows, it is also interesting to mention that individual nonvaccinated cows had very different antibody levels. That was probably the result of the immunological response and of a 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 meant that they could not transfer them to the calves.

The presence of specific antibodies for BHV-1 in calves before feeding with colostrum was detected in the blood serum of 3 (10.71%) of 28 calves investigated (18 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 indicated that the fetuses were infected with BHV-1 during intrauterine development, but this did not lead to the death of the fetus or to abortions. Calf fetuses are immunologically able to react to many infective agents including BHV-1 (Panjević et al., 1980; Kendrick and Osburn, 1973; Lazić et al., 1991; Rogan, 1987). However, the immunobiological response in the fetus should be accepted conditionally, because fetuses are not immunologically reactive enough to protect themselves and they lag behind immunologically developed organisms (Mihajlović, 1983).

Kendrick (1973) and Smith et al. (1978) showed that infection of fetuses with BHV-1 in a certain period of pregnancy induces death and abortions. The survival of the fetus and the immunobiological response to BHV-1 could, maybe, be explained also by the fact that the age and the vitality of the fetus as well as the virulence of the virus could be of crucial importance for survival.

There was a high increase of antibody titers to BHV-1 in blood serum of 2-day - old calves after feeding with colostrum from cows vaccinated with anti BHV-1 vaccine. Such a high increase of antibody titers two days after birth was expected, because very high antibody titers for BHV-1 were found in colostrum-serum of vaccinated cows. Thus, prompt feeding of calves with colostrum after birth in sufficient quantities may give very high values for antibody titers.

The antibody titers decreased significantly in calves by the age of 15 days, as the result of catabolism of passive antibodies transferred in the colostrum. Some authors (McGuire et al., 1976; Kimm and Schmidt, 1983; Perrereau, 1973) point out that colostrum antibodies decreased even more than 50% in the first 2-3 weeks after birth. Kahrs (1985) emphasized that colostrum passive immunity in calves rapidly disappears through metabolic decomposition. In our case, the antibody level in calves at the age of 15 days decreased by about 72% compared to the values determined in 2-day-old calves. However, in spite of this high rate of decomposition of colostrum antibodies, the antibody titer in calves at the age of 15 days was still high. The disappearance of colostrum antibodies for BHV-1 continued so that at the age of 90 days 3 (16.67%) calves did not have antibodies against BHV-1. The antibody titers found in calves of nonvaccinated cows could be compared only with those in calves from vaccinated cows at the age of 90 days.

The passively transferred immunological protection, observed through the values for antibody titers to BHV-1 obtained 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 the not adequately transferred passive immunological protection. In conditions of industrial cattle husbandry deviations

from physiological conditions that characterize the biology of ruminants are often present. The obtained results and the antibody titers for BHV-1 found can serve for further investigations on the prevention of infection of calves with BHV-1.

REFERENCES

1. Bartha A., Csontos L., Palfi V., 1977. Control of bovine viral respiratory and enteric diseases under different management systems, *Bull. Off. Int. Epizoot.*, 105-113.
2. Bommeli W., Kihm U., 1982. The IBR control programme in Switzerland, *XII World Congress on Diseases of Cattle, The Netherlands, Vol. I*, 153-156.
3. Brun A., Dubourget P. L., Soulebot J. P., 1982. Duree d'immunité, conferee par un vaccine inactive en adjuvant huileux de la rhinotracheite infectieuse bovine, *XII World Congress on Diseases of Cattle, The Netherlands, Vol. I*, 156-162.
4. Cristet J., Grigire C., Vlagioiu C., 1991: Aplicarea vaccinului inactivat anti IBR-IPV I la vacusi juninci gestuate din efective contaminate cu virusul IBR-IPV, *Lucrari J. C. V. B. Pasteur, Vol. XIX, Bucuresti*, 23-30.
5. Kahrs R. F., 1985; Viral diseases of cattle, Danmarks veterinær Jordbrugsbibliotek.
6. Kendrick W. J., 1973. Effects of the infectious bovine rhinotracheitis virus on the fetus, *J. A. V. M. A.*, 163, 7, 852-854.
7. Kendrick W. J., Osburn B. J., 1973. Immunologic response of the bovine fetus to inactivated infectious bovine rhinotracheitis - infectious pustular vulvovaginitis virus, *Vol. 34*, 1567-1571.
8. Kim W. J., Schmidt F. W., 1983. Zur Frage der Absorption von kolostralen Immunoglobulinen durch das Kalb, *Tierärztliche Umschau*, 90, 283-286.
9. Knežević N., Kosanović P., Rogan D., 1990. Imunoprofilaksa respiratornog oboljenja goveda inaktivisanim vakcinama. III. Ispitivanje imunogenosti bivalentne inaktivisane uljne vakcine IBR i Pi-3, *Veterinarski glasnik*, 44, 503-512.
10. Latarija N. T., 1979. Imunogenie svojstva virus-vakcini protiv infekcionava rinotraheita pri vakcinaciji staljnih karov, Problemi infekcionoj patologiji seljkohozjajstvenih životnjih, *Trudi, VIEV, Tom 49, Moskva*.
11. Lazić S., Mihajlović B., Đurišić S., Vidić Branka, Čekić N., 1991. Neutralizujuća antitela za goveđi herpesvirus-1 u krvnom serumu gravidnih krava, teladi i kolostrumu, *Veterinarski glasnik*, 45, 11-12, 791-797.
12. Lohrbach W., Forst J. W., Wachendorfer G., 1985. Investigation of the efficiency of a live attenuated and inactivated IBR/IPV vaccine in dairy herds, *Virology abstracts*, 18, 8.
13. Mc Guire C. T., Pfeiffer N. E., Weikel J. M., Bartsch R. C., 1976. Failure of colostral immunoglobulin transfer in calves during from infectious diseases, *J. A. V. M. A.*, 7, 169, 713-718.
14. Mihajlović B., 1983. Mikrobiologija I, OZID, Beograd
15. Mielke H., Schulz J., 1978. Darstellung zur Physiologie und Biochemie der Immunität des Rindes, *Mh. Vet. Med.*, 32, 98-106.
16. Mohntys S. B., 1982. Vaccination programs against infectious bovine rhinotracheitis, parainfluenza-3 and pasteurellae, *XII th World congress on Diseases of Cattle, The Netherlands*, 124-131.
17. Panjević Đ., Vasić B., Mihajlović B., Vukotić M., 1980. Imunološke reakcije fetusa, *Veterinarski glasnik*, 34, 3, 261-266.
18. Perreau P., 1973. Pneumopathies infectieuses des jeunes bovinis, *Recueil de Medicine Vet.*, 149, 9, 1147-1162.
19. Rogan D., 1987. Uppoređno ispitivanje humoralnog i ćelijskog imuniteta kao pokazatelja ostvarenja kontakta i perziistencije bovinog herpesvirusa-1 (IBR) u organizmu govečeta, *Doctoral Dissertation, Faculty of Veterinary Medicine, Beograd*

20. Scheldrake F. R., Husband A. J., 1985. Immune defences at mucosal surfaces in ruminants, *Journal of Dairy res.*, 52, 599-613.
21. Smith W. M., Miller R. B., Svodoba J., Lawson K. F., 1978. Efficacy of an intranasal infectious bovine rhinotracheitis vaccine for the prevention of Abortion in cattle, *Vet. J.*, 19, 63-71.
22. Soulebot P. J., 1985. Prophylaxe medicale des affections respiratoires des bovins de maladie des muquenses, *Rec. Med. Vet.*, 161, 12, 1271-1276.

**EFEKTI VAKCINACIJE VISOKOGRAVIDNIH KRAVA SA ANTI BHV-1 VAKCINAMA:
I. HUMORALNI ODGOVOR NA "IBEROL PLUS" VAKCINU**

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SADRŽAJ

Ispitivanje humoralnog imunološkog odgovora vršeno je na 33 krave u visokom stadijumu graviditeta vakcinisanih protiv bovinog herpesvirusa-1 (BHV-1) ("Iberol-plus", Veterinarski zavod, Zemun). U ispitivanju je korišćen metod virus mikroneutralizacije. Pre vakcinacije i revakcinacije (koja je izvršena 3 nedelje nakon vakcinacije) kao i u momentu porođaja određivane su vrednosti titra specifičnih antitela za BHV-1 u krvi i kolostralnom serumu.

Pre vakcinacije, vrednosti titra antitela iznosile su od 1:2 do 1:32, a u momentu porođaja njihove vrednosti u krvnom serumu bile su od 1:32 do 1:512 a u kolostralnom serumu od 1:256 do 1:2048. Kod novorođene teladi su vrednosti antitela za BHV-1 određivane pre hranjenja kolostrumom i nakon toga, u starosti od 2;15;30; 45; 60 i 90 dana. Pre hranjenja kolostrumom kod 3 teleta utvrđeno je prisustvo antitela. Nakon davanja kolostruma, antitela su pronađena kod svih teladi starosti do 60 dana. Najviše vrednosti titra antitela ustanovljene su kod teladi od 2 dana, a najniže vrednosti kod teladi uzrasta od 90 dana.