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CELL-MEDIATED AND HUMORAL IMMUNITY IN BULLS INFECTED WITH IBR/IPV VIRUS (BHV1)

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Time-related changes in specific cell-mediated immunity (CMI) and in humoral immunity were monitored in 20 bulls, aged 12 to 16 months, divided into four groups each consisting of 5 animals. Group I was experimentally and group III – naturally infected with BHV 1 virus. Groups II and IV served as their respective controls. Tests for CMI included determination of delayed type hypersensitivity (DTH), leukocyte migration inhibition factor (LMIF) and granulocyte migration inhibition factor (GMIF) (LIF-buffy coat leukocyte migration inhibitory factor) in the presence of BHV 1 antigen, while humoral immunity was tested by serum induced neutralization of the virus (SN) and by estimation of serum IgG, IgG_1, IgG_2, IgGM and IgA levels. Immunologic, virologic and clinical examinations were performed two days before infection and 17 times within 91 days after the infection in bulls of group I and II and 7 times within 42 days after development of the disease in bulls of groups III and IV.

The results showed that positive CMI reactions appeared 3 to 4 weeks earlier than positive antibody titers in the SN test. The antibodies belonged most probably to subclasses IgG_1 and IgG_2.

Key words: cell mediated immunity, humoral immunity, bulls, BHV 1

INTRODUCTION

Among studies pertaining to cell-mediated immunity and humoral immunity in cattle infected naturally or experimentally with BHV 1 only a few have attempted to examine the kinetics of the response. Moreover, the examined time period did not exceed a few hours or a few days (cited by Deptula, 1988 and Krasnikov, 1989; Granatova and Psikal, 1989; Woronin, 1991; Compos et al., 1992; Wisniewski et al., 1993).

Our studies were aimed at determining the development of cell-mediated and humoral immunities in 12 to 16 month old bulls, infected experimentally or naturally with IBR/IPV virus.

MATERIALS AND METHODS

The studies were performed on 20 ncb strain bulls, aged 12 to 16 months, divided into four groups each including 5 animals. Group I consisted of bulls infected experimentally with BHV 1, group III – of bulls infected naturally with the virus while, groups II and IV included healthy bulls (control). Animals of groups I, II and IV originated from a herd of healthy cattle, maintained in conditions of high sanitary standards. Serological testing in the bulls was repeated three times, at 5 to 7 day intervals and detected no antibodies against IBR/IPV, VDMD, PI–3, adenoviruses 2 and 5, syncytial virus, leukemia virus, Chlamydia or Brucella. Bulls of group III were chosen from, a herd with frequent respiratory infections at the time when they usually began developing infection of the respiratory pathways.

Bulls of group I were experimentally infected with BHV 1 virus isolated from cattle with keratoconjunctivitis (collection of profesor Buczek, see Deptula 1990 and 1991).

The applied virus represented the 45 th passage of the strain in HKNC and showed a TCID titer of $10^{-6}/0,1$. Five mililitres of the agent was administered intramuscularly, 3 ml intravenously and 0,5 ml was placed into the conjunctival sacs. Animals of group II (control), obtained by the same routes corresponding amounts of medium from uninfected cultures of calf kidney cells. Bulls of group IV were not given any substances and served as a control group for the naturally infected animals of group III. In bulls of the latter group, respiratory infection was associated with isolation at a later stage of IBR/IPV virus and with detection by SN of serum antibodies against the virus, at titers higher than 1:2.

Clinical observations of the animals, as well as virological (cultural and serologic) and immunological tests in the bulls of groups I and II were started two days before and continnued on the day of infection and, then, at 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91 days thereafter. In animals of groups III and IV the studies were performed on days 1, 7, 14, 21, 28, 35, and 42 after detection of symptoms pointing to the disease process in bulls of group III. Swabs were taken from nasal mucosa and from conjunctivae of animals of each group for virologic (culture) studies. Blood for virologic (serologic) studies was taken from the jugular vein. Clinical studies were performed according to routine techniques while virologic (cultural and serologic) tests and serologic tests for Chlamydia and Brucella were conducted against antigens according to the techniques described before (Deptula 1991).

Specific cell-mediated immunity was determined by the delayed type hypersensitivity (DTH) skin test, and determination of the leukocyte migration inhibitory factor (LMIF) and granulocyte migration inhibitory factor (GMIF) (LIF-buffy coat leukocyte migration inhibitory factor). The first two tests (DTH and LMIF) were performed against antigens according to techniques described previously (Deptula 1990). The GMIF (LIF) test was was performed according to Aguilar-Setien as described by Deptula (1990), in which neutrophilic granulocytes were isolated as described by Nowacki, Naylor and Little (cited

by Deptula and Buczek, 1987). The IBR/IPV virus suspension used in the tests contained 400 to 600 mg protein/ml.

Humoral immunity was tested by estimating the serum content of IgM, IgG and IgA class immunoglobulins and IgG_1, IgG_2 subclasses, using cattle Ig as standards by radial immunodiffusion (plates of Miles, USA).

The results obtained in immunologic tests (except for those of the DTH)were subjected to statistical analysis using Student's test at $p=0,05$. They are presented together with the antibody titers for BHV 1 virus in Tables 1 and 4.

RESULTS AND DISCUSSION

In bulls experimentally infected with IBR/IPV virus (group I) the period of latency of the disease was 2–3 days and signs in the form of conjunctivitis, rhinitis and a cough were weakly expressed for 14 days. Similarly in naturally infected animals (group III), disease signs were registered only within the first 14 days of observation. They included cataral conjunctivitis, rhinitis and a cough.

IBR/IPV virus could be isolated only from animals of groups I and III. In group I the agent was isolated from conjunctivae and nasal mucosa on days 1, 14, 28 and 56 in two bulls, on days 2 and 42 from 4 bulls and on days 7 from three bulls. In animals of group III isolation of BHV 1 from conjunctivae was successful on days 1, 14, 28 in a single bull, and on days 35 and 42 in 3 bulls. From the nasal mucosa, BHV 1 could be isolated on days 1 and 35 from four bulls, on day 14 from two bulls and on day 28 from a single animal.

As compared to the control bulls, LMIF and GMIF tests in experimentally or naturally infected animals yielded lower values while the DTH test gave positive values (Tables 1 and 3), which appeared in bulls of group I between the third (GMIF) and the seventh day (DTH and LMIF) after administration of the virus, i. e. more than 4 weeks before BHV 1 seroneutralizing antibodies appeared. In bulls of group I the changes lasted for 7 weeks for GMIF and LMIF and for 11 weeks for DTH (Table 1). In naturally infected animals (group III) they lasted throughout the observation period (Table 3).

Serum antibody titers for BHV 1 were positive in the SN test (titers of 1:2 or higher are thought to confirm the disease according official national regulations) in all animals of group I between 35 and 77 days post infection (Table 2). The maximum titer of 1:16 was noted in a single bull on day 56. In animals of group III positive antibody titers were observed throughout the observation period, with a maximum titer of 1:8 detected in bulls on day 42 of the observation (Table 4).

The kinetics of serum immunoglobulin content in the bulls varied in animals of groups I and III (Tables 2 and 4). In animals of group I the increase in IgG_1 and IgG_2 contents started on day 2 after infection, IgA content began to increase from day 14 on, while an increase in IgAand IgG contents could not be observed until the 21 st and 28 th day respectively. For IgM and IgA the increased level in serum was maintained till day 70 and for IgG, IgG_1 and IgG_2 – till day 91

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Table 1. Indices of specific cell-mediated immunity in 12 to 16 months old bulls, experimentally infected with BHV1 (group I) and control (group II).

Migration inhibition test (mm²)		DTH skin test, readout after		Day of observation	-2	0	1	2	3	4	7	14	21	28	35	42	49	56	63	70	77	84	91
GMIF	LMIF			Group of animals																			
$\bar{X}$	$\bar{X}$																						
3,8	4,8	24h	1	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4,1	5,3	48h	1	I	–	–	–	–	–	+(4)	+(1)	–	–	+(3)	–	+(1)	–	+(1)	–	–	–	+(1)	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	+	+	+(2)	+(3)	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3,9	4,6	72h	1	I	–	–	–	–	+(4)	–	–	+	+	–	+(2)	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4,1	4,9	24h	1	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4,0	4,7	48h	1	I	–	–	–	–	–	+(4)	–	+	+	+	+	+	+	+	+	+	+	+	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4,3	4,6	72h	1	I	–	–	–	–	–	+(4)	–	+	+	+	+	+	+	+	+	+	+	+	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3,3	3,9	24h	1	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3,8	4,1	48h	1	I	–	–	–	–	–	+(4)	–	+	+	+	+	+	+	+	+	+	+	+	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3,9	4,1	72h	1	I	–	–	–	–	–	+(4)	–	+	+	+	+	+	+	+	+	+	+	+	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
			2	I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
				II	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/

Legend: I- BHV1 antigen; 2-control; ( ) number in brackets denotes number of animals; *-significant differences; +-positive DTH test; +-- doubtful result of DTH test; – negative DTH test.

Table 2. Indices of nonspecific humoral immunity in 12 to 16 months old bulls, experimentally infected with BHV1 [group I] and control [group II].

Day of observation		-2		0		1		2		3		4		7		14		21		28		35		42		49		56		63		70		77		84		91	
Group of animals		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Antibody titer in SN test		<1:2		<1:2		<1:2		<1:2		<1:2		<1:2		<1:2		<1:2		<1:2		<1:2		1:2		<1:2		1:4		<1:2		1:4 [7]	1:8 [4]	1:16 [3]	1:4 [3]	<1:2		1:4		<1:2		1:4 [7]	1:2 [4]	<1:2		1:2		<1:2		<1:2		1:2 [7]	<1:2 [4]	<1:2	
Immunoglobulins [g/l]																																					
G	$\bar{x}$	23,5	23,0	24,5	22,6	26,5	24,5	26,0	24,0	26,5	25,0	25,0	22,0	25,0	23,0	26,0	25,0	25,0	23,0	28,0	24,0	26,0	24,0	30,0	23,0	30,0	25,0	28,0	26,0	28,0	23,0	28,0	23,0	29,0	23,0	30,0	25,0
G_1	$\bar{x}$	11,2	14,2	13,1	14,5	11,1	9,5	12,1	14,0	11,0	14,0	9,8	14,8	14,0	14,0	15,0	14,0	9,8	14,0	14,6	9,0	14,5	8,5	14,0	8,5	14,6	11,1	14,5	7,5	12,3	6,5	14,5	9,0	14,5	11,0	14,4	8,0	17,2	17,2	16,0	6,0
G_2	$\bar{x}$	5,6	6,2	4,8	5,3	4,9	4,7	6,3	4,2	7,2	5,6	7,6	5,6	8,0	4,6	6,3	4,0	4,5	2,5	9,3	4,5	7,5	4,5	7,0	5,5	9,0	7,5	7,5	4,5	6,5	4,1	7,4	5,2	7,6	6,0	6,6	4,2	6,3	4,6
M	$\bar{x}$	4,3	4,9	5,2	4,9	4,4	5,0	5,1	5,3	3,9	4,1	4,3	4,7	2,9	2,8	5,6	4,2	5,4	3,2	5,9	3,5	5,3	3,9	6,4	4,2	4,4	2,6	5,6	3,9	4,5	2,9	6,0	4,0	4,5	3,5	3,8	2,9	3,5	4,5
A	$\bar{x}$	4,5	4,0	5,0	4,5	4,5	3,5	4,5	3,0	2,0	2,0	2,0	1,0	2,0	1,0	2,0	2,0	2,0	1,0	2,0	2,0	2,0	2,0	2,0	1,0	1,5	1,0	1,5	1,0	1,5	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Legend: [ ]-figure in brackets denotes number of animals;
x - significant difference

of observationion (Table 2). On the other hand, in bulls of group III increased serum Ig levels were observed throughout the testing period, with the most pronounced increase noted, in sequence, in IgM, IgG, IgG_2, IgA, IgG_1 (Table 4). The clinical signs in experimentally and naturally infected animals resembled those described earlier (Deptula, 1988; Krasnikov, 1989; Voronin, 1991; Campos et al, 1992; Wisniewski et al., 1993). Also the results pertaining to the disease period in which the virus was isolated from animals of groups I and III as well as the easier and more frequent successful isolations of BHV virus in experimentally or naturally infected bulls correspond to literature data (cite by Deptula 1988). On the other hand, the isolation of BHV 1 from naturally infected animals manifesting signs of inflammation in respiratory pathways which was more facile from their nasal mucosa than from conjunctivae, at least in the first stage of the disease, indicates that the nasal mucosa should serve as the sampling site for routine diagnostic virologic tests.

Table 3. Indices of specific cell-mediated immunity in 12 to 16 month old bulls naturally infected with BHV 1 (group III). and control (group IV).

Day of observation			1		7		14		21		28		5		42	
Group of animals			III	IV	III	IV	III	IV	III	IV	III	IV	III	IV	III	IV
DTH skin test readout after	24h	1	+	—	+	—	+	—	±	—	+	—	+	—	±	—
		2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	48h	1	+	—	+	—	+	—	+	—	+	—	+	—	+	—
		2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	72h	1	+	—	+	—	+	—	+	—	+	—	+	—	+	—
		2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Migration inhibition test (mm2)	LMIF	$\bar{x}$	.2*	6.0	.2*	5.0	.8*	6.2	4.4*	7.2	4.5*	6.2	2.8*	4.9	4.5*	5.9
	GMIF	$\bar{x}$	4. *	5.1	2.9*	4.8	.9*	5.0	.9*	5.7	2.9*	4.	4.0*	5.0	4.0*	5.0

Legend: 1-BHV1 antigen; 2-control, (+)- positive DTH test;
(+ -)- doubtful result of DTH test, (-) - negative DTH test;
x- significant difference

Table 4. Indices of specific humoral immunity in 12 to 16 month old, bulls, naturally infected with BHV1 (group III) and control (group IV).

Day of observation			1		7		14		21		28		5		42	
Group of animals			III	IV	III	IV	III	IV	III	IV	III	IV	III	IV	III	IV
Antibady titer in SN test			1:2	<1:2	1:2	<1:2	1:4 ( ) 1:2 (2)	<1:2	1:2	<1:2	1:4 ( ) 1:2 (2)	<1:2	1:4 ( ) 1:2 (2)	<1:2	1:8 (2) 1:4 ( )	<1:2
Immunoglobulins ŠgIIC	G	$\bar{x}$	24.5*	19.0	26.5*	20.0	6.0*	26.0	2.5*	26.0	1.0*	2.0	.0*	24.0	5.0*	24.5
	G1	$\bar{x}$	16.8*	14.8	17.6*	15.2	14.5*	1.6	16.2*	15.2	14.7*	11.	14.8*	9.1	12.8*	7.0
	G2	$\bar{x}$	4.4*	.4	5.4*	2.1	4.9*	2.5	5.5*	2.	7.6*	.8	6.4*	4.0	6. *	4.5
	M	$\bar{x}$	4.6*	.6	5.2*	2.5	5.5*	2.6	4.2	.2	4.0*	2.8	4.0*	2.7	4.2*	2.
	A	$\bar{x}$	0.65*	0,45	0.49*	0.29	0.51*	0.24	0.4 *	0.26	0.48*	0. 1	0.98*	0.27	0.78*	0.24

Legend: ( ) figure in brackets denotes number of animals,
x - significant diference

The results concerning specific cell-mediated and humoral immunity were analysed. Independently of the type of IBR/IPV virus infection (experimental or natural) in bulls, changes in the studied indices were quite similar and correlated

with the clinical pattern and with the results of virologic tests. The latter, despite slight discrepancies, were similar in bulls of group I and III. In the experimentally infected bulls, on the other hand, positive specific cell-mediated immunity reactions were detected earlier (by 4 weeks) as compared to positive SN tests, which confirmed the general opinion on the role of T lymphocyte conditioned immunity in viral diseases in animals and humans (Deptula, 1988; Aguilar-Setien et al., 1978. and 1979; Brochier et al., 1984; Krasnikov, 1989; Campos et al., 1992; Wisniewski et al., 1993). Although the bulls already demonstrated augmented IgG_1 and IgG levels on the second day post infection, an increase in IgM was not detected until the 14 th day and increases in IgA and IgG until the 21 st–28 th days, while positive titers of seroneutralizing antibodies were not observed until the 35 th day post infection. This confims the dominating role of specific cell-mediated immunity in combating herpes virus infections also in cattle. It should be added that, according to the literature on the subject, (Guy, 1973; Irvin, 1976; Rossi, 1976; Marschang et al., 1983; Mishra, 1983; Rodak, 1983; Guy, 1985 a, b; Deptula, 1988; 1991; Krasnikov 1989; Deptula and Buczek, 1990; Voronin, 1991), anti-BHV 1 antibodies in the animals belong first of all to subclass IgG_2, IgG_1 and/or IgG_{2b} and then to the IgM and IgA classes, independently of the infection type and animal age even if participation and, therefore, the levels of iso-and allotypic Ig varieties can change in the course of the infection (Deptula, 1988).

It should also be mentioned that among the parameters of specific cell-mediated and humoral immunities studied in the four groups of animals only the values of the DTH test and serum Ig levels and then only in experimentally infected bulls (group I) were comparable to the observations of other authors (Guy, 1973; Aguilar-Setien et al., 1978; 1979; Brochier et al., 1984; Guy 1985, a;b; Wisniewski et al., 1993). The results of testing specific cell mediated immunity using the DTH test (Aguilar et al., 1978; 1979; Brochier et al., 1984; Wisniewski et al., 1993) confirm not only the superior specificity and sensitivity of the test as compared to the SN test but demonstrate that it is of higher value than blast transformation tests. It should be added that, in contrast to the results of Brochier et al., (1984), positive DTH tests were first observed not between the 2 nd and the 4 th days after infection of bulls with IBR/IPV but on the 7 th day after the infection, i. e. 4 days after detection of positive results in the GMIF test. Also the levels of serum igM, IgG and of IgG_1 and IgG_2 subclasses as well as their changes in bulls of group I only in part confirmed the results of Guy (1973; 1985). Guy showed that in the first period after experimental infection of calves aged up to two years with BHV 1, an increase was noted mainly in IgM and IgG and, later, in IgG, IgG_2 and/or IgG_{2b} and IgG_1. He also argued that each increase in iso and allotypic varieties of Ig was associated with virus release from the cells with concomitant stimulation of the organism. Thus, the increase in IgG_1, IgG_2, IgM from days 2 to 14 and the

increase in IgA on days 21 to 28 registred in bulls experimentally infected with IBR/IPV (Group I) results most probably from a specific interaction of the virus with B lymphocytes.

No other comparative material was available for the bulls of group I. Nevertheless, the results of LMIF and GMIF tests as well as for all studied parameters of cell-mediated and humoral immunities in 12 to 16 months old animals of group III confirmed the general tendencies observed in naturally or experimentally virus infected cattle of various ages (Deptula, 1988; Deptula and Buczek, 1987; Deptula 1990; 1991; Krasnikov, 1989; Voronin, 1991; Wisniewski et al., 1993). Positive SN titers of anti-BHV 1 antibodies were not detected in the sera of group I bulls until day 35 post infection, i. e. later than the increase in serum Ig classes and sublasses, which points to the lower sensitivity of SN tests in the detection of low levels of antibody against IBR/IPV virus. The obtained titers of anti-BHV 1 seroneutralizing antibodies and timing of their appearance in groups I and III resemble closely those obtained by other authors (Aguilar-Setien, 1978; Marschang et al., 1983; Deptula and Buczek, 1990; Deptula et al., 1991). The only difference compared to the cited papers involves the somewhat higher SN titers in our naturally infected (Group III) bulls. It should also be added that in our bulls of group I and III, SN titers were correlated best with the results of the DTH test and with in;creased serum IgG_1 and IgG.

CONCLUSION

In 12 to 16 month old bulls infected experimentally or naturally with IBR/IPV, the clinical, virologic and immunologic changes paralleled each other. Moreover, the results of immunologic tests showed that positive cell-mediated immune reactivity, as measured by DTH, LMIF and GMIF tests, appeared 3 to 4 weeks earlier than positive SN antibody titers. Increase in the latter, in turn, was preceded 2 to 4 weeks by an increase in serum Ig levels. Seroneutralizing antibodies in the studied animals seemed to belong, first of all, to the IgG_1 and IgG_2 subclasses, but also to the IgM and IgA classes. The results demonstrated that, the extreme sensitivity and specificity of DTH, LMIF and GMIF tests, as compared to SN tests, and the technical ease of performing them (DTH and LMIF in particular), favours their introduction as soon as possible into routine veterinary diagnostic procedures. This conclusion has already been made earlier (Deptula, 1990; Deptula and Buczek, 1990; Wisniewski et al., 1993) not only for adult cattle (Deptula, 1990; Deptula and Buczek 1990) but also for 2 to 4 month old bulls (Deptula 1990; Wisniewski et al., 1993) which indicates the high potential practical value of the tests.

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ĆELIJSKI I HUMORALNI IMUNI ODGOVOR U BIKOVA INFICIRANIH VIRUSOM IBR/IPV i BHV_1

WIESLAW DEPTULA

SADRŽAJ

U radu su ispitivani ćelijski i humoralni imuni odgovor kod 20 bikova starih 12 – 16 meseci. Bikovi su bili podeljeni u 4 grupe sa po 5 bikova. Grupa I je bila eksperimentalna, III grupu su predstavljali bikovi inficirani prirodnim putem sa BH_1 virusom a bikovi iz II i IV grupe su bili kontrole.

Imunološka, virusološka i klinička ispitivanja su vršena dva dana pre infekcije i 17 puta u vremenu od 91 dana posle infekcije kod bikova I i II grupe i 7 puta u vremenu od 42 dana posle pojave oboljenja kod bikova II i IV grupe.

Rezultati su pokazali da se pozitivna CMI reakcija pojavljuje 3–4 nedelje ranije od pojave pozitivnog titra antitela u CN testu.