Acta Veterinaria Hungarica 59 (1), pp. 77–86 (2011)
DOI: 10.1556/AVet.59.2011.1.7

IMMUNOMODULATORY EFFECT OF GLUCAN ON SPECIFIC AND NONSPECIFIC IMMUNITY AFTER VACCINATION IN PUPPIES

Eva HALADOVÁ*, Jana MOJŽIŠOVÁ, Peter SMRČO, Anna ONDREJKOVÁ, Boris VOJTEK, Marián PROKEŠ and Eva PETROVOVÁ

Department of Epizootiology and Preventive Veterinary Medicine, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 04181 Košice, Slovakia

(Received 22 June 2010; accepted 7 October 2010)

The objective of the study was to determine the immunostimulatory effect of β-(1,3/1,6)-D-glucan in puppies. The effect exerted on the efficacy of vaccination, especially against canine parvovirus and rabies infection, was studied. The application of vaccine and glucan leads to significant increases in the nonspecific immunological parameters (phagocytic ability of leukocytes, blastogenic response of lymphocytes, metabolic and chemotactic activity of polymorphonuclear cells). The level of antibodies against canine parvovirus (Ab CPV) and rabies infection reached the most statistically significant values on the 28th day after the application of vaccine and a syrup containing β-(1,3/1,6)-D-glucan (Group GV) as compared to the control group (Group V, puppies receiving only vaccine). Dogs without glucan supplementation did not produce such significant levels of antibodies. We can conclude that glucan has relevant immunostimulatory effects in dogs with altered immunity. The glucan product tested in this study (PleraSAN V, PLEURAN, Bratislava, Slovakia) could be used in the small animal clinical practice.

Key words: Dog, glucan, immunostimulation, vaccination, antibody titre

Canine parvovirus infection and canine distemper are infectious diseases characterised by high morbidity and mortality in puppies between 6 weeks and 6 months of age (Pollock and Coyne, 1993). Therefore, it is a common practice to immunise puppies with a modified live or inactivated vaccine. The vaccine usually provides satisfactory protection. Immunosuppressive conditions can influence the effect of vaccination, which has consequences on the protection of animals against infectious diseases (Maďar et al., 2003; Mojžišová et al., 2004). In recent years, one of the active ingredients responsible for the immunomodulatory effect of many herbs has been found to be a form of complex polysaccharides known as 'β-D-glucan', or simply called β-glucan (Ooi and Liu, 2000; Chang, 2002). The receptors and mechanisms of action of β-glucans have recently been unfolded by *in vitro* and *in vivo* animal experiments. β-glucans are ubiquitously

*Corresponding author; E-mail: haladova@uvm.sk; Phone: 00421 (905) 965-290

found in both bacterial or fungal cell walls and have been implicated in the initiation of antimicrobial immune response. Based on *in vitro* studies, β-glucans act on several immune receptors including Dectin-1, complement receptor (CR3) and TLR-2/6 and trigger a group of immune cells including macrophages, neutrophils, monocytes, natural killer cells and dendritic cells. As a consequence, both innate and adaptive response can be modulated by β-glucans, and they can also enhance opsonic and nonopsonic phagocytosis (Chan et al., 2009). Glucans are well known for their ability to stimulate the innate immunity and the cellular branch of immune reactions (Whistler et al., 1976). Of all ligniperdous mushrooms used in the food industry, the Oyster mushroom *(Pleurotus ostreatus)* has the most suitable nutritional values. The β-(1,3/1,6)-D-glucan isolated from this mushroom has considerable anticancer and immunomodulating effects (Mizuno, 1996). It is assumed that glucan application results in signalling processes that lead to the activation of macrophages and other cells and the subsequent secretion of cytokines and other substances initiating inflammation (e.g. interleukins IL-1, IL-2, IL-6, TNF-α) (Abel and Czop, 1992; Adachi et al., 1994; Vetvicka et al., 2002). Since β-glucans are inexpensive and have a good margin of safety based on historical track records, their potential therapeutic value deserves further investigation.

The aim of our study was to evaluate specific (antibodies after antirabies vaccination and after vaccination against canine parvovirus [CPV] infection) and nonspecific immunological parameters (the phagocytic ability of leukocytes, metabolic and chemotactic activity of polymorphonuclear cells, blastogenic response of lymphocytes to the mitogen phytohaemagglutinin [PHA]) after immunostimulation by β-(1,3/1,6)-D-glucan.

Materials and methods

Animals. Forty-eight dogs of different breeds and sexes were divided into two groups at the age of 4 months. Group 1 (GV): 24 dogs coming from a shelter for stray dogs were administered orally (at the day of vaccination and after vaccination) the veterinary syrup PleraSAN V (PLEURAN, Bratislava, kindly provided by the manufacturer) at a daily dosage of 2 ml/5 kg of body weight for a period of two months, followed by subcutaneous vaccination against canine parvovirus, distemper, parainfluenza, infectious hepatitis and leptospirosis using a commercially modified live vaccine (Eurican DHPPI^2 and DHPPI^{2}L, Merial, France) and against rabies with an inactivated vaccine (Rabisin, Merial, France). Group 2 (V): 24 dogs of different breeds and sexes obtained from a shelter, at the age of approximately 4 months, without the application of PleraSAN, were vaccinated against the same infectious disease as dogs in Group GV. Both groups of dogs (GV and V) were primo-vaccinated (sampling 1) and subsequently revaccinated at an interval of two weeks. On day 28 (sampling 3), the dogs were vacci-

nated against rabies. Immunosuppression was suspected because of the origin and healthy status of the experimental dogs, and it was later confirmed by immunological tests (sampling 1).

Blood collection. Peripheral blood samples were obtained by puncture of the cephalic vein and placed into plastic tubes containing heparin for immunological tests and into glass tubes for the evaluation of specific antibodies in serum. Sampling was carried out at the day of vaccination (sampling 1), and then on postvaccination days 14 (sampling 2), 28 (sampling 3, vaccination against rabies), 42 (sampling 4), 56 day (sampling 5) and 70 (sampling 6) and after the administration of β-(1,3/1,6)-D-glucan.

Blastogenic response of blood lymphocytes to mitogens. Lymphocytes were separated from venous blood on Ficoll density gradient (Pharmacia Biotech AB, Sweden). Cultivation (at 37 °C and 5% CO_2 in humidified air for 48 h), mitogen stimulation and measurement of the blastogenic response of lymphocytes were performed using a colorimetric BrdU cell proliferation enzyme-linked immunosorbent assay kit (Roche, Mannheim, Germany; Russmann et al., 1993). Phytohaemagglutinin (PHA-P, Sigma Chemical Co., USA) was used for stimulation in the optimum concentration of 20 μg/ml (Tajima et al., 1990). The level of the blastogenic response of lymphocytes was expressed as stimulation index (SI).

The phagocytic ability of blood leukocytes was assessed by the method described by Vetvicka et al. (1982). We used 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 μm, ARTIM, Prague, Czech Republic). The phagocytic activity (PA) of leukocytes was expressed as the percentage of cells phagocyting 3 and more MSHP, and as the phagocytic index (PI) representing the ingestion capacity of leukocytes (the ratio of the number of cells phagocyting MSHP and the number of all potentially phagocyting leukocytes). *Chemotactic activity* was measured by the method of chemotaxis of polymorphonuclear cells (PMNL) under agarose (Procházková et al., 1986*a*). The chemotactic index (CI) represents the ratio between the lengths of chemotactic and spontaneous paths of migration. The *iodonitrotetrazolium test (INT)* was carried out in accordance with the modifications made by Procházková et al. (1986*b*). Assessment of the functional ability of phagocytes is based on the ratio between spontaneous activity and the activity after stimulation by zymosan (Sigma, USA) i.e. the index of metabolic activity (IMA). The *antirabies antibody titres* were determined by an ELISA test developed in our laboratory with our own kit (Beníšek et al., 1989; Süliová et al., 1994). In this test, an anti-dog labelled antibody (RAD/IgG/Px, rabbit anti-dog IgG labelled with peroxidase, Sigma, USA) was used. The results were expressed in equivalent units (EU/ml) (Meslin et al., 1996) and were read from a calibration curve using a reference control positive serum with a known concentration of antirabies antibodies. A control negative serum was included in the test. *The level of specific antibodies against CPV* was evaluated by the haemagglutination inhibition test (HIT) using 1% pig erythrocytes. Before testing, the

serum was inactivated by heating to 56 °C. Serial twofold dilutions of serum in buffer were added in microtitre plates. Then 4 haemagglutination units (HAU) of virus were added into each well and the plate was kept at room temperature for 1 h. Results were read after adding the suspension of pig erythrocytes and incubation at 4 °C for 1 h. CPV antibody titre measured by the HIT was considered protective at a level of ≥ 64 (Carmichael et al., 1983).

Statistical analysis. The data were expressed as mean ± standard deviation. The significance of difference was checked by one-way analysis of variance (ANOVA), Mann-Whitney U-test, and Student's unpaired test. Statistical analyses were done with the programme GraphPad Instat V2.04. P values less than 0.05 were considered significant.

Results

All results and the statistical analyses of differences between Group GV and Group V are presented in Table 1. Statistical comparisons between sampling 1 and the other samplings in the particular groups before and after vaccination are presented in Table 2. The dogs examined came from a shelter for stray dogs, so we can presume that poor nutrition and stress contributed to the alteration of activity of the immune system. On the day of vaccination (sampling 1), most of the selected immunological parameters were lower than in healthy dogs examined in our laboratory (Mojžišová, 1997; Hromada et al., 2003; Mojžišová et al., 2004).

The *phagocytic activity of the leukocytes* (*PA Le %*) showed an increasing tendency in both groups, and the most noticeable value was observed at the last sampling (day 70). Phagocytic activity was not significantly higher in Group GV than in Group V.

On day 56 after vaccination, the *phagocytic index of the leukocytes (PI Le)* was significantly different ($P < 0.05$) in Group GV as compared to Group V, and a significantly lower value ($P < 0.05$) was observed after the third blood sampling in puppies administered glucan. In Group GV this parameter was higher at day 56 than at day 1. PI Le was equal in the two groups when sampling 1 was compared with the last sampling. The values of *IMA* in vaccinated animals administered glucan were not significantly different from those found in dogs vaccinated only; however, the index significantly increased in both groups when comparing day 1 vs. day 56 and day 1 vs. day 70. The IMA slightly (but significantly) changed in Group V already after the third blood sampling ($P < 0.001$) as compared to the first blood sampling.

The *CI* significantly increased ($P < 0.05$) only after the second blood collection in Group GV.

The most significant increase of *SI* ($P < 0.0001$) in puppies of Group GV as compared to Group V was observed on day 14. A significant increase ($P < 0.05$) in SI was found between day 1 and day 28 in Group GV.

Table 1

Nonspecific immunological parameters and titres of antirabies and CPV antibodies before and after the application of vaccine. Statistical comparison of Groups GV vs. V on the same days

Group GV	Sampling 1 Day 1 (application of DHPPI^2 vaccine)	Sampling 2 Day 14 (application of vaccine DHPPI^{2}L)	Sampling 3 Day 28 (application of antirabies vaccine)	Sampling 4 Day 42	Sampling 5 Day 56	Sampling 6 Day 70
PA Le%	32.0 ± 7.2	35.8 ± 15.1	33.75 ± 13.36	36.75 ± 10.37	42.83 ± 14.35	54.46 ± 3.59
PI Le	5.25 ± 0.9	6.21 ± 1.65	5.23 ± 1.12*	6.54 ± 0.89	7.72 ± 1.51*	8.88 ± 1.83
IMA	1.25 ± 0.13	1.25 ± 0.12	1.39 ± 0.22	1.512 ± 0.248	1.56 ± 0.254	1.97 ± 0.375
CI	1.43 ± 0.47	1.796 ± 0.24*	1.41 ± 0.54	1.432 ± 0.227	1.164 ± 0.460	1.25 ± 0.16
SI	1.4 ± 0.5	3.6 ± 1.17***	2.125 ± 0.521	1.2 ± 0.258	1.7 ± 0.62	1.13 ± 0.497
CPV Ab	52.0 ± 67.24*	195.0 ± 202.4	1200.0 ± 788.63	1648.0 ± 755*	1600.0 ± 692**	912.0 ± 636.57
Ab Rabies UE/ml	0.003 ± 0.002	0.50 ± 0.308	0.66 ± 0.432	1.52 ± 0.3***	1.7 ± 0.2***	1.57 ± 0.3***
Group V	Sampling 1 Day 1	Sampling 2 Day 14	Sampling 3 Day 28	Sampling 4 Day 42	Sampling 5 Day 56	Sampling 6 Day 70
PA Le%	29.3 ± 6.7	30.67 ± 23.37	32.16 ± 8.45	36.83 ± 13.30	36.54 ± 19.55	57.83 ± 16.03
PI Le	5.31 ± 1.5	8.02 ± 3.14	7.71 ± 2.22	7.66 ± 2.34	5.57 ± 1.545	9.35 ± 2.602
IMA	1.16 ± 0.13	1.28 ± 0.123	1.25 ± 0.18	1.56 ± 0.147	1.58 ± 0.228	2.24 ± 0.531
CI	1.29 ± 0.15	1.41 ± 0.148	1.36 ± 0.182	1.42 ± 0.212	1.15 ± 0.288	1.11 ± 0.109
SI	1.4 ± 0.8	2.02 ± 0.9	2.18 ± 0.756	0.9 ± 0.283	1.57 ± 0.89	1.4 ± 0.389
CPV Ab	25.0 ± 33.68	65.0 ± 68.3	736.0 ± 529.52	1088.0 ± 741.36	693.0 ± 524.8	443.0 ± 584.3
Ab Rabies UE/ml	0.002 ± 0.002	0.451 ± 0.070	0.459 ± 0.039	0.669 ± 0.346	0.640 ± 0.302	0.488 ± 0.277

PA Le – phagocytic activity of blood leukocytes; PI Le – phagocytic index of leukocytes; IMA – index of metabolic activity of phagocytes; CI – chemotactic index; SI – stimulation index of lymphocytes; Ab Rabies – antirabies antibody, UE/ml; CPV Ab – titre of antibody against canine parvovirus, mean ± standard deviation. Statistical comparison between Groups GV vs. V: $^{***}P < 0.0001$, $^{**}P < 0.01$, $^{*}P < 0.05$; ns – nonsignificant

Table 2

Statistical comparison of the first blood collection (sampling 1) vs. other blood samplings in the particular groups before and after vaccination

Comparison	PA Le	PI Le	IMA	CI	SI	Ab CPV	Ab Rabies
Group GV							
Day 1 vs. 14	ns	ns	ns	ns	$^{***}P < 0.0001$	ns	ns
Day 1 vs. 28	ns	ns	ns	ns	$^{*}P < 0.05$	$^{**}P < 0.01$	ns
Day 1 vs. 42	ns	ns	ns	ns	ns	$^{***}P < 0.0001$	$^{***}P < 0.0001$
Day 1 vs. 56	ns	$^{***}P < 0.0001$	$^{*}P < 0.05$	ns	ns	$^{***}P < 0.0001$	$^{***}P < 0.0001$
Day 1 vs. 70	$^{***}P < 0.0001$	$^{***}P < 0.0001$	$^{***}P < 0.0001$	ns	ns	ns	$^{***}P < 0.0001$
Group V							
Day 1 vs. 14	ns	$^{*}P < 0.05$	ns	ns	ns	ns	ns
Day 1 vs. 28	ns	ns	ns	ns	ns	$^{***}P < 0.0001$	ns
Day 1 vs. 42	ns	ns	$^{**}P < 0.01$	ns	ns	$^{***}P < 0.0001$	$^{*}P < 0.05$
Day 1 vs. 56	ns	ns	$^{**}P < 0.01$	ns	ns	$^{***}P < 0.0001$	$^{*}P < 0.05$
Day 1 vs. 70	$^{***}P < 0.0001$	$^{***}P < 0.0001$	$^{***}P < 0.0001$	ns	ns	ns	ns

For explanations, see footnotes in Table 1

Antibodies against rabies. In Group GV, the level of rabies antibodies reached the protective level (> 1 UE/ml) at all blood sampling dates from the beginning of the immunoprophylaxis, whereas in Group V it did not reach the level required for protection at any of the blood samplings. The highest antibody level ($P < 0.0001$) was observed on day 28 after vaccination against rabies (sampling 5) in Group GV (administered glucan) as compared to Group V (without glucan). Significant differences ($P < 0.0001$) were found between values measured on days 14 and 42 as compared to the first blood sampling (day 1) in Group GV.

The development of *antibody titre against CPV* after vaccination was delayed by one week in Group V as compared to Group GV. At samplings 1 and 4 ($P < 0.05$) and sampling 5 ($P < 0.01$), the titre of antibodies in Group GV treated with glucan was significantly higher than in Group V. The titre of antibodies in Group V decreased rapidly until the end of observation but remained on the protective level.

Discussion

The common vaccination procedure in dogs can cause changes in nonspecific immunity. Vaccination against canine distemper and canine parvovirus was reported to cause enhanced lymphocyte blastogenesis and decreased lymphocyte and leukocyte count in puppies (Miyamoto et al., 1992; Miyamoto et al., 1995). The positive effect of an inactivated CPV vaccine on lymphocytes in puppies was used to improve immunosuppression after surgery (Taura et al., 1995). Other authors described a mild, short-lasting suppressive effect of vaccination on lymphocyte blastogenesis (Mastro et al., 1986). Phagocytosis and intracellular killing were not significantly modified by vaccination (Pratelli et al., 2000). However, as already mentioned above, the major finding of this study was that the observed decline in cell-mediated immunity after vaccination was accompanied by a simultaneous increase in various components of humoral immunity. Our results show that phagocytosis tended to increase in both groups. The most noticeable value was observed at the last sampling (on day 70). We demonstrated a stimulatory effect of antirabies and CPV vaccination on the proliferative response of lymphocytes, which was significant in both groups but was more prominent on day 14 in Group GV. Unlike previous studies (Van Loveren et al., 2001; Henry et al., 2001), in which no significant difference was observed in antibody response after vaccination between immunosuppressed and immunocompetent animals, our results demonstrate a considerable difference in the production of antirabies antibodies in dogs with altered immunological parameters. The titre of antirabies antibodies measured by ELISA test is considered protective at the level of > 1.0 EU/ml (Meslin et al., 1996; Cliquet et al., 2000). Such a titre was not detected in dogs without the immunostimulating effect of glucan, as compared to puppies of Group GV whose immunity was supported by β-(1,3/1,6)-D-

glucan. The CPV antibody titre measured by the HIT was considered protective at a level of ≥ 64 (Carmichael et al., 1983). Such a titre was detected one week later in non-stimulated puppies than in dogs stimulated with glucan. Subsequently, the level of antibodies in Group V decreased rapidly until the end of the observation period but remained on a protective level.

As shown in animal experiments, after oral administration the specific backbone 1→3 linear β-glycosidic chain of β-glucans cannot be digested. Most β-glucans enter the proximal small intestine and some of them are captured by the macrophages. They become internalised and fragmented within the cells, then transported by the macrophages to the bone marrow and the endothelial reticular system. Small β-glucan fragments are eventually released by the macrophages and taken up by other immune cells, leading to various immune responses. However, β-glucans of different sizes and branching patterns may have significantly variable immune potency. Careful selection of appropriate β-glucans is essential for the clinical investigation of the effects of β-glucans (Chan et al., 2009). The β-glucan of high purity selected by us (PLEURAN, Bratislava) had a positive effect on the immunological parameters. β-(1,3/1,6)-D-glucan administered at a dose of 2 ml/ 5 kg body weight caused, besides a marked improvement of phagocytic functions, a significant increase in CPV and antirabies antibody titres that persisted until the end of the experiment. Similarly, the proliferation activity of lymphocytes was higher for a longer time in the group of dogs receiving glucan. In an attempt to limit the use of antibiotics in animal husbandry, spent brewer's yeast is added to the feed in order to increase natural resistance. This effect is attributed to β-glucans, which are present in yeast cell walls, and are supposed to interfere with, and enhance, immunity (Kogan and Kocher, 2000). The possible mechanism of action is the ability of β-glucans to bind to cell receptors, which are known to include dectin-1, CR3, LacCer, and scavenger receptors. This event then leads to the activation of multiple signal pathways which, in turn, promote immune responses in the affected cells (Chen and Seviour, 2007). The exact immunological actions and signalling pathway(s) induced by β-glucan are still unclear and have to be further defined.

On the basis of our results we can certify that the application of β-(1,3/ 1,6)-D-glucan increases specific (antirabies and CPV antibodies) as well as non-specific (IMA, PI, SI) immunological responses. The use of glucan in small animal clinical practice is recommended for prevention as well as for the immunopotentiation of vaccination. We would recommend repeated antirabies vaccination, because primo-vaccination does not guarantee adequate protection.

Acknowledgement

This work was supported by the Slovak Grant Agency VEGA 1/0615/09 of the Slovak Republic.

References

Abel, G. and Czop, J. K. (1992): Stimulation of human monocyte beta-glucan receptors by glucan particles induces production of TNF-α and IL-1β. Int. J. Immunopharmacol. **14,** 1363–1373.

Adachi, Y., Okazaki, M., Ohno, N. and Yadomae, T. (1994): Enhancement of cytokine production by macrophages stimulated with (1->3)-beta-D-glucan, Grifolan (GRN), isolated from *Grifola frondosa.* Biol. Pharm. Bull. **17,** 1554–1560.

Beníšek, Z., Süliová, J., Švrček, Š. and Závadová, J. (1989): ELISA test for rabies antibodies titration (in Slovak). Veterinárske správy **III,** 25–27.

Chan, G. C., Chan, W. K. and Sze, D. M. (2009): The effects of β-glucan on human immune and cancer cells. J. Hematol. Oncol. **2,** 25. doi:10.1186/1756-8722-2-25.

Chang, R. (2002): Bioactive polysaccharides from traditional Chinese medicine herbs as anticancer adjuvants. J. Altern. Compl. Med. **8,** 559–565.

Chen, J. and Seviour, R. (2007): Medicinal importance of fungal β-(1→3), (1→6)-glucans. Mycol. Res. **111,** 635–652.

Carmichael, L. E., Jouber, J. C. and Pollock, R. V. H. (1983): A modified live canine parvovirus vaccine. II. Immune response. Cornell Vet. **73,** 13–29.

Cliquet, F., Sagné, L., Schereffer, J. L. and Aubert, M. F. A. (2000): ELISA test for rabies antibody titration. Vaccine **18,** 3272–3279.

Henry, C. J., McCaw, D. L., Brock, K. V., Stoker, A. M., Tyler, J. W., Tate, D. J. and Higginbotham, M. L. (2001): Association between cancer chemotherapy and canine distemper virus, canine parvovirus, and rabies virus antibody titers in tumor-bearing dogs. J. Am. Vet. Med. Assoc. **219,** 1238–1241.

Hromada, R., Mojzisova, J., Paulik, S., Hipikova, V., Bajova, V. and Cizek, M. (2003): Immunological changes in dogs with malasseziosis. Ind. Vet. J. **80,** 1109–1112.

Kogan, G. and Kocher, A. (2007): Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livestock Sci. **109,** 161–165.

Maďar, M., Švrček, Š., Franka, R., Závadová, J., Ondrejka, R., Beníšek, Z., Paulík, Š., Pošiváková, S., Ondrejková, A., Mojžišová, J. and Hromada, R. (2003): Epizootiological and epidemiological importance of the feline AIDS immunosuppression (in Slovak). Agriculture **49,** 81–87.

Mastro, J. M., Axthelm, M., Mathes, L. E., Krakowka, S., Ladiges, W. and Olsen, R. G. (1986): Repeated suppression of lymphocyte blastogenesis following vaccination of CPV-immune dogs with modified-live CPV vaccines. Vet. Microbiol. **12,** 201–211.

Meslin, F. X., Kaplan, M. M. and Koprowski, H. (eds) (1996): Laboratory Techniques in Rabies. WHO, Geneva. pp. 353–397.

Miyamoto, T., Taura, Y., Une, S., Yoshitake, M., Nakama, S. and Watanabe, S. (1992): Changes in blastogenic responses of lymphocytes and delayed type hypersensitivity responses after vaccination in dogs. J. Vet. Med. Sci. **54,** 945–950.

Miyamoto, T., Taura, Y., Une, S., Yoshitake, M., Nakama, S. and Watanabe, S. (1995): Immunological response after vaccination pre- and post- surgery in dogs. J. Vet. Med. Sci. **57,** 29–32.

Mizuno, T. (1996): A development of antitumour polysaccharides from mushroom fungi. Food Ingredients J. (Japan) **167,** 69–85.

Mojžišová, J. (1997): Effect of immunomodulatory substances on selected parameters of immunological profile and their use in viral and parasitic diseases of animals. PhD Thesis, UVL in Košice (in Slovak).

Mojžišová, J., Hromada, R., Paulík, Š., Ondrašovič, M. and Bajová, V. (2004): Immune response and immunomodulatory effect of levamisole in immunosuppressed dogs vaccinated against parvovirosis. Bull. Vet. Inst. Puławy **48,** 93–97.

Ooi, V. E. and Liu, F. (2000): Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. Curr. Med. Chem. **7,** 715–729.

Pollock, R. V. H. and Coyne, M. J. (1993): Canine parvovirus. Vet. Clin. North Am.: Small Anim. Pract. **23,** 555–568.

Pratelli, A., Altamura, M., Buonavoglia, D., Pepe, M., Tafaro, A., Lembo, A., Cirone, F., Tempesta, M., Jirillo, E. and Buonavoglia, C. (2000): Evaluation of the natural immunity in pups inoculated with a modified-live canine parvovirus type 2b (CPV-2b) strain. Immunopharmacol. Immunotoxicol. **22,** 451–464.

Procházková, J., John, C. and Mareček, D. (1986*a*): Chemotaxis under agarose. In: Selected Diagnostic Methods in Medical Immunology (in Czech). Avicenum, Praha. pp. 211–215.

Procházková, J., John, C. and Mareček, D. (1986*b*): Micro-INT test. In: Selected Diagnostic Methods in Medical Immunology (in Czech). Avicenum, Praha. pp. 219–222.

Russmann, E., Sieber, K., Doppler, C. and Hinzpeter, M. (1993): Cell proliferation ELISA, BrdU (chemiluminescence). In: Coligan, J. E., Bierer, B. E., Margulies, D. M., Shevach, E. M., Strober, W. and Coico, R. (eds) Current Protocols in Immunology. Volume 1. John Wiley & Sons, New York. Chapter 7.10. Colloquium Roche Molecular Biochemicals **4,** 1–4.

Süliová, J., Beníšek, Z., Švrček, Š., Ďurove, A. and Závadová, J. (1994): Quantification of antirabies antibody level in serum of vaccinated humans (in Slovak). Bratislavské Lekárske Listy **95,** 73–77.

Tajima, M., Fujinaga, T., Okamoto, Y., Otomo, K. and Koike, T. (1990): Relationship between mitogen receptors in peripheral blood lymphocytes and blastogenic responses to mitogen. Res. Vet. Sci. **48,** 1–5.

Taura, Y., Ishii, K., Nagami, M., Mikasa, N., Nakaichi, M. and Nakama, S. (1995): Changes in lymphoproliferation and DTH responses after vaccination immediately before surgery in puppies. J. Vet. Med. Sci. **57,** 899–904.

Van Loveren, H., Van Amsterdam, J. G., Vanderbriel, R. J., Kimman, T. G., Rumke, H. C., Steerenberg, P. S. and Vos, J. G. (2001): Vaccine-induced antibody responses as parameters of the influence of endogenous and environmental factors. Environmental Health Perspective **109,** 757–764.

Vetvicka, V., Fornusek, I., Kopeček, J., Kamínková, J., Kašpárek, I. and Vránová, M. (1982): Phagocytosis of human blood leukocytes. A simple micromethod. Immunol. Lett. **5,** 97–100.

Vetvicka, V., Terayama, K., Mandeville, R., Brousseau, P., Kournikakis, B. and Ostroff, G. (2002): Pilot study: orally-administered yeast beta-1,3-glucan prophylactically protects against anthrax infection and cancer in mice. JANA **5,** 1–6.

Whistler, R. L., Bushway, A. A., Singh, P. P., Nakahara, W. and Tokuzen, R. (1976): Noncytotoxic, antitumor polysaccharides. Adv. Carbohydr. Chem. Biochem. **32,** 235–275.