AgroLife Scientific Journal - Volume 11, Number 2, 2022
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

COULD VEGETAL EXTRACTS ENHANCE THE CELL-MEDIATED IMMUNITY AND ALEVIATE VACCINATION STRESS IN DAIRY CATTLE?

Paul-Adrian BOR[1], Gheorghița DUCA[2], Carmen Dana ȘANDRU[1, 2], Diana OLAH[1], Marina SPÎNU[1, 2], Emoke PALL[1, 2], Constantin CERBU[1], Emilia UNGUREANU[1], Adrian POTÂRNICHE[1], Aurel VASIU[1]

[1]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania
[2]Institute of Research and Development for Montanology Cristian- Sibiu, 147 Street XIII, Cristian, Sibiu, Romania

Corresponding author email: aurel.vasiu@usamvcluj.ro

Abstract

This study investigates influence of the anti-anthrax vaccination on blast transformation capacity of the leukocytes and also the possible in vitro influence of two, taxonomically different, autochthonous plants, Calendula officinalis and Vaccinium mirtyllus in promoting post-vaccinal proliferation. For that, blood was sampled on heparine (50 IU/ml) before and two weeks after the anti-anthrax vaccination (R 1190 Stamatin strain) of twenty-three dairy cows and 11 calves raised extensively. Blood samples were subjected to the in vitro blast transformation test, where glucose consumption was read after 72 hours of incubation at 37°C by the o-toluidine test. The experimental variants included samples treated with alcoholic extracts of the plants (7.5 µ/ml). There was a statistically significant increase in all the stimulation indices after the vaccination of the animals in cows, including those for Vaccinium myrtillus (48.57±13.27 to 53.53±12.49, p=0.02) and Calendulla officinalis (56.39±12.57 and 61.51±13.85, p=0.01). The increase induced in the growth of calf lymphocytes by the two plants was non-significant post vaccination.
Both extracts could be used to enhance cell mediated immunity but there was an age dependent improvement of their in vitro response.

Key words*: dairy cows, anthrax, vaccination, blast transformation, extensive raising.*

INTRODUCTION

For both small and industrial farmers, cattle represent an important financial resource worldwide. Cattle farming proved to have a broad economic impact, also influencing the health and habitat of people (Thornton, 2010). Cattle farming was blamed for increase in CO_2 concentrations in the atmosphere and also for the green-house effect, which lead to the decrease of the ozon layer around the planet (Herrero et al., 2013; Rotz et al., 2013). Nevertheless, cow milk and products obtained from it along with beef, provide important nutritional resources to humans all over the world, supporting the development of local markets and rural communities.
The herd health status is utmost important for obtainment of adequate productions from the farming process. To intensify the production potential of bovine and also provide a healthy environment to the animals and protection towards diseases, improved nutrition strategies and perfected preventive measures were applied to farmed animals.
Cattle-farming is one of the main branches of animal production, which provides people and the processing industry with particularly valuable products and by-products such as milk and meat as food products, and hides, bones, hooves, horns and manure as by-products (Pawlak and Kołodziejczak, 2020), especially in developing countries.
Within the epidemiological triangle represented by the pathogen, host and the environment for their interaction, the close proximity of people and bovine has enhanced the progression of a new host-pathogen relationship and jump over species for various microbes, mainly viruses but also zoonotic bacteria (Schiffman et al., 2002).

Although non-contagious, anthrax is considered to be a zoonotic disease, *Bacillus anthracis* being transferred mainly from the soil but also from the diseased individual to healthy susceptible ones to direct contact. Anthrax infection from animal sources is mainly "targeting" risk categories such as farmers, veterinarians, slaughterhouse workers, caretakers, or personnel involved in handling carcases or by-products (Rume et al., 2020).

Vaccination represented one of the important veterinary operations to provide protection against microbial diseases (Turnbull, 1991; Misra, 1996). Numerous diseases were eradicated due to vaccination from the affected territories, anthrax included in the category (OIE Manual). In most countries, where the epidemiological pressure is still existing (Africa, Asia, etc.) the anti- anthrax vaccination is involved in preventive protocols designed by the governments. Depending on the epidemiological situation, the vaccination protocols applied in cattle farms are diversified, some of them being included in the national strategic programs.

In spite of the positive impact on health and economy, vaccinations have revealed lately adverse effects, especially those which needed repeated shots; allergic mechanisms underlie the hypersensitivity, allergy or anaphylactic shock caused either by the included antigenic structures or adjuvants used in the vaccine to increase its immunogenicity (Fasanella et al., 2001).

The immune system, with a high variety of structures and mechanisms involved in providing protection against microbes, mainly relies on the intervention of antigen presenting cells, T helper lymphocytes and plasma cells in producing specific antibodies (Cesaretti et al., 2020).

The response of such cells could be enhanced, thus providing an increased antibody protection by chemical but also natural products, including plant extracts.

This research aimed at investigating the *in vitro* response of the leukocytes from anti-anthrax vaccinated animals to various mitogens, including the alcoholic extracts of *Vaccinium myrtillus* and *Calendula officinalis*, to estimate their immune enhancing potential and eventual possibility to use those as immune stimulating products.

MATERIALS AND METHODS

Biological material. Thirty-three cattle (23 dairy cows and 11 calves, both males and females), reared extensively were selected for the experiment. The animals were vaccinated according to the Strategic Program, with a live R 1190 Stamatin strain vaccine, which is acapsulogenic but edematogenic and showed an increased immunogenicity.

Blood samples were collected from all the animals before and two weeks after the vaccination on heparine (50 UI/ml) and subjected to the *in vitro* blast transformation test for evaluating the mitogenic capacity of the extracts.

Plant extracts (Dacia Plant, Romania). The two extracts used *in vitro* during the evaluation of the blastogenic capacity of bovine leukocytes were commercial ones for human use. Both were prepared from dried plant parts, leaves in case of blueberries and flowers for the marigold extract, according to the techniques described by the German Pharmacopoeia.

The leukocyte blast transformation test (Khokhlova et al., 2004)

The leukocyte blast transformation test measures the *in vitro* reactivity of mononuclear cells to sensitizing (*in vivo* encountered) antigens. Similarly, it can be used to identify the effects of various substances, including plant extracts on immunogenesis (Hume & Weidemann, 1979).

Cell growth was quantified by means of the glucose consumption technique, using orto-toluidine.

Blood samples were diluted in a proportion of 1:4 with RPMI 1640 culture medium (Sigma, USA). The amont of blood+culture medium mixture was calculated according to the number of envisaged cultural variants, performed in duplicate.

The mixture was distributed in wells of a 96-well sterile plate (200 µl per well). Six variants were tested for each individual animal, namely (1) untreated control culture, (2) 70º alcohol (2.5 µl per well), (3) Con A treated culture (1 µl per well), (4) phytohaemagglutinin-M (PHA) (1 µl per well) treated culture, (5-6) alcoholic extracts of *Vaccinium myrtillus* and *Calendula officinalis* (2.5 µl per well) treated cultures. The quantities of both PHA and antigens were

established when using the same technique during preliminary studies as being the most effective *in vitro* for the tested species. The cultures were incubated for 72 h at 37°C and 5% CO_2.

The cell growth was estimated by quantifying the glucose consumption in the wells. Glucose concentrations were measured in the initial medium and at the end of the incubation period, using a standard glucose solution (100 mg %), by means of an orto-toluidine colorimetric test. For that, 12.5µl of the cultural supernatant were transferred to 0.5 ml of orto-toluidine reagent, boiled for 8 min, suddenly cooled in water and read in 96-well plates at 610 nm wavelength in a spectrophotometer (SUMAL PE2, Karl Zeiss, Jena), using the o-toluidine reagent as a blank. The stimulation index (SI %) was calculated as follows: SI%=[(MG–SG)/MG]×100, where TI=blast transformation index, MG=glucose concentration in the initial RPMI and SG=glucose concentration in the sample after incubation.

Statistical processing of the data. Averages of SI% for both samplings, prior and after the vaccination, along with the standard deviations were calculated.

The Excel program was used to calculate the significance of the differences between the SI in vaccinated animals during the two samplings by means of the t- Student test.

RESULTS AND DISCUSSIONS

Eradication of numerous communicable diseases was achieved by injecting the susceptible hosts with antigens processed as vaccines. The method is beneficial in general, but side effects (vaccinoses) have been mentioned along time for various antigens. This led to implementation of modern antigen processing methods (genetic engineering, deleted antigens, etc.).

The analysis of the host's protective response to pathogens indicated that mostly T and B lymphocytes are accountable for the cell-mediated immunity and are also responsible for antigenic information and antibody synthesis.

The proliferative response of bovine lymphocytes following stimulation with various alcoholic extracts of a vegetal nature was demonstrated by means of the blast transformation test, according to the protocol proposed by Ghergariu et al. (2000).

The technique has been used over time in the evaluation of bovine blastogenesis (Ishikawa & Shirahata, 1986) in the evaluation of proliferating cell metabolism (Hume and Weidemann, 1979) but also more recently for the evaluation of the energy balance of circulating mononuclear cells (Schwarm et al., 2013).

The results obtained in the experiment related to SI% pre- and post-vaccination against anthrax of adult and young cattle are presented in Figure 1 and Figure 2.

It can be seen that in the case of adult animals, the blastisation indices are increased post-vaccination, both in the case of spontaneous and mitogen-induced responses, except for blueberry extract, which, although leads to increased post-vaccination indices, compared to the alcohol control has a weaker effect. *Calendula* extract has a stimulating effect.

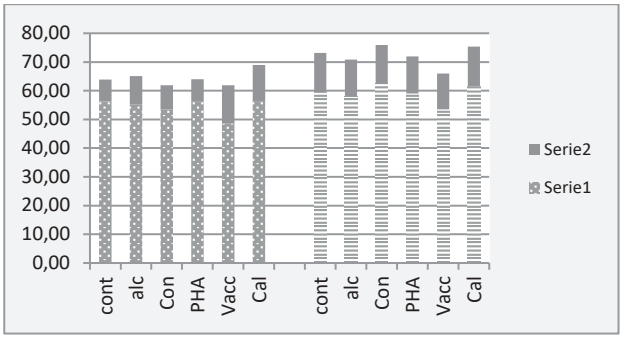

Figure1. SI% calculated for the two samplings: prior and post vaccination in cows (x±s), with an anti-anthrax vaccination history

Legend: (Series 1 before, Series 2 - after the vaccination, cont=control, alc-alcohol, Con-concanavaline A, PHA-phytohaemagglutinine, Vacc-*Vaccinium myrtillus* alcoholic extract, Cal - *Calendula officinalis* alcoholic extract)

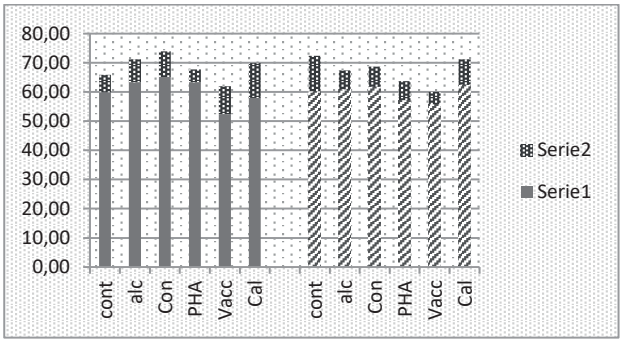

Figure 2. SI% calculated for the two samplings: prior and post vaccination in calves (x±s), with an anti-anthrax vaccination history

Legend: (Series 1 before, Series 2- after the vaccination, cont=control, alc-alcohol, Con-concanavaline A, PHA-phytohaemagglutinine, Vacc-*Vaccinium myrtillus* alcoholic extract, Cal - *Calendula officinalis* alcoholic extract)

In the case of the group of young animals, the *in vitro* cell-mediated response is weaker post-vaccine (Figure 2) for all variants, except for the one treated with marigold extract, which proves to be stimulating in this case as well.
A comparison of the values obtained post vaccination in cows and calves (Figure 3) indicated that the blueberry extract was more efficient in the calves while the marigold extract improved the blastogenesis in the adult animals. This stands for different active principles in the two taxonomically different plants and also for a difference in reactivity in different age categories of animals, although from the same species.

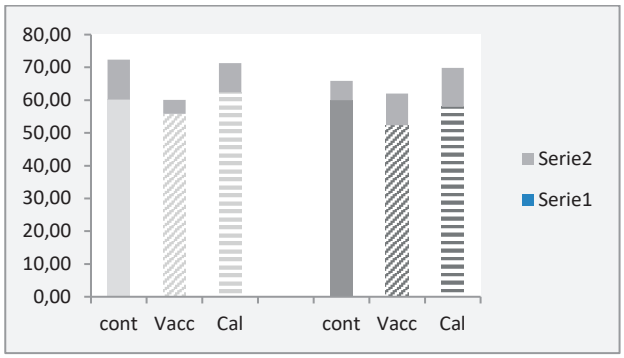

Figure 3. Changes in SI% subsequent to vaccination (x±s) in cows and calves subject to vaccination

Legend: (Series 1 before, Series 2- after the vaccination, cont=control, Vacc- *Vaccinium myrtillus* alcoholic extract, Cal - *Calendula officinalis* alcoholic extract)

Table 1 shows the statistical significance of the stimulation observed after the vaccination, while calculating the differences between the two groups. All cultural variants used encountered an enhanced growth in cows, with higher SI% indices than those observed in calves. This phenomenon is probably due to the presence of the memory cells in the adult animals, subjected during their history to multiple vaccinations against anthrax.

Table 1. The statistical significance of the comparison between cows and calves and the stimulation induced by the vegetal extracts

t test BV - AV	Contr	Alc	Con	PHA	Vacc	Cal
Cows	0.009	0.032	$2x10^{-6}$	0.009	0.02	0.01
calves	0.48	0.22	0.16	0.01	0.15	0.17

Legend: (BV- before vaccination, AV-after vaccination, cont=control, Con – concanavalin A, PHA - phytohaemagglutinine, Vacc- *Vaccinium myrtillus* alcoholic extract, Cal - *Calendula officinalis* alcoholic extract)

Similarly, the results obtained in this category were statistically ensured for both plant extracts. IN calves, only the standard mitogen PHA induced a sufficient increase post vaccination to be statistically supported, all the other variants, in spite of the recorded growth, proved to stimulate lesser the bovine immune cells.

CONCLUSIONS

The results supported the hypothesis that multiple vaccination, continuously triggering immune cell-mediated activity, could be responsible for the enhanced response to mitogens, but also to plant extracts. Therefore, further attempts should be made to identify the role not only of the age and plant taxonomy but also of the dosage in inducing immune stimulation at highest levels. Both extracts could be used to enhance cell mediated immunity but there was an age dependent improvement of their *in vitro* response.

ACKNOWLEDGEMENTS

This research work was carried out within the PhD study program of Paul Bor, logistically supported by the Doctoral school of USAMV Cluj-Napoca.

REFERENCES

Cesaretti, A., Montegiove, N., Calzoni, E., Leonardi, L., Emiliani, C. (2020). Protein hydrolysates: from agricultural waste biomasses to high added-value products (minireview). *AgroLife Scientific Journal*, Vol. 9, Number 1, ISSN 2285-5718, 79-87.

Fasanella, A., Losito, S., Trotta, T., Adone, R., Massa, S., Ciuchini, F., Chiocco, D. (2001). Detection of anthrax vaccine virulence factors by polymerase chain reaction. *Vaccine*. 19(30): 4214-4218.

Herrero, M., Havlík, P., Valin, H., Notenbaert, A., Rufino, M. C., Thornton, P. K., Blümmel, M., Weiss, F., Grace, D. and Obersteiner, M. (2013). Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. *Proc. Natl. Acad. Sci.* 110: 20888-20893.

Khokhlova, I. S., Spinu, M., Krasnov, B. R., Degen, A. A. (2004). Immune response to fleas in a wild desert rodent: effect of parasite species, parasite burden, sex of host and host parasitological experience. *J Exp Biol.,* 207(Pt 16): 2725-2733.

Pawlak, K., Kołodziejczak, M. (2020). The role of agriculture in ensuring food security in developing countries: Considerations in the context of the problem of sustainable food production. *Sustainability*, 12, 5488.

Rotz, C. A., Isenberg, B. J., Stackhouse-Lawson, K. R., and Pollak, E. J. (2013). A simulation-based approach for evaluating and comparing the environmental footprints of beef production systems. *J. Anim. Sci.* 91(11): 5427-5437.

Rume, F. I., Karim, M. R., Ahsan, C. R., Yasmin, M., Biswas, P. K. (2020). Risk factors for bovine anthrax in Bangladesh, 2010–2014: a case-control study. *Epidemiology and Infection* 148, e67, 1–6.

Schiffman, J., Beer, T., Yonghong, W. (2002). The emergence of global disease 7-882 control priorities. *Health Policy and Planning*, 17: 225-234.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. P*hilos Trans R Soc Lond B Biol Sci.* 2010 Sep 27, 365(1554): 2853-2867.

Turnbull, P. C. (1991). Anthrax vaccines: past, present and future. *Vaccine*, 9(8): 533-539.

Misra, R. P. (1991). Manual for the Production of Anthrax and Blackleg Vaccines. Food and Agriculture Organisation of the United Nations (FAO) Animal Production and Health Paper 87, FAO, Rome, Italy.

Gherghariu, S., Pop, A., Kadar, L., Spînu, M. (2000). *Manual de laborator clinic veterinar*. Ed. All, București, 299-381.

Ishikawa, H, Shirahata, T. (1986). Application of glucose consumption test for evaluating blastogenesis in bovine lymphocytes. *Nihon Juigaku Zasshi*., 48(1): 111-115.

Hume, D. A., Weidemann, M. J. (1979). Role and regulation of glucose metabolism in proliferating cells. J *Natl Cancer Inst.*, 62(1): 3-8.

Schwarm, A., Viergutz, T., Kuhla, B., Hammon, H. M., Schweigel-Röntgen, M. (2013). Fuel feeds function: Energy balance and bovine peripheral blood mononuclear cell activation. *Comp Biochem Physiol A Mol Integr Physiol.*, 164(1): 101-10.

***OIE Terrestrial Manual 2018, Section 3.1, Multiple species, Chapter 3.1.1. *Anthrax,* 311-32.