Original Article

The Potential Role of Antigen Priming In Increasing the Overall Antimicrobial Resistance in Captive Silver Foxes

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ABSTRACT

Objective: We hypothesized antigenic priming should stimulate the humoral and cell-mediated adaptive immunity, further improved by vegetal extracts, alleviating the immune suppression induced by captivity stress in silver foxes. **Material and Methods:** One of two groups of adult silver foxes was sc primed and boosted 7 days later with a 5% SRBC suspension. Serum antibody titers (hemagglutination test) and circulating immune complexes levels (4.2% PEG precipitation) were quantified and ln of the antibody titers were calculated seven days later. An *in vitro* blast transformation test was carried out on blood samples using alcoholic extracts of *C. officinalis, A. montana, S.officinale, Echinaceea spp.* and immune stimulating compounds and glucose consumption was evaluated. The significance of the differences was interpreted by Student's t-test. **Results**: Cells grew better in primed foxes versus the unprimed individuals (68.8 ± 9.88 and 17.49 ± 22.9). The primed animals reacted significantly ($p < 0.001$-$p < 0.01$) better to vegetal extracts (*C. officinalis, E. angustifolia*) and to thymus extract, selenium salts and bovine tuberculin. Anti-SRBC antibodies were highly variable (0.69 - 5.54). **Conclusion**: SRBC exerted a positive effect on both humoral and cell-mediated immune responses in silver foxes, supporting the enhancement of antimicrobial defense by booster vaccination and immune stimulating therapy.

Keywords: Silver Foxes, Antigenic Stimulation, Adaptive Immunity, Vegetal Extracts.

INTRODUCTION

The immune system links the individual to its microbial environment and the outcome of this interaction is vital for the survival. Environmental factors, including farming technology, act as stressors on the immune system, and the timeframe and degree of adaptation is highly dependant on the intensity and duration of the stressors' action. The impaired immune response in individuals subjected to various stress factors can cause: low protective responses following vaccination or concurrent infections/diseases due to lower protection, as well as economic losses. Modern immunology is seeking for alternative immune stimulating compounds for which plants could represent a source. Scientific data support the healing activity of plants indicating that, unlike conventional drugs, which sometimes aleviate the symptoms without removing the cause, natural remedies eliminate the cause.[1-8-13,14] Stimulating effects of plant extracts were scarcely studied in wild carnivores. At our best knowledge, there are no experiments carried out in Silver foxes aiming to prove the combined effects of antigen priming and medicinal plants on their immune system. We hypothesized that antigenic priming should stimulate the humoral and cell-mediated adaptive immune response, being further improved by vegetal extracts,

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diminishing the degree of immune suppression induced by captivity stress in silver foxes.

MATERIALS AND METHODS

The research was carried out on two groups of adult, antigen primed (n=16) and unprimed (n=14) silver foxes. The priming was performed by s.c. injection (day 0) and booster (day 7) with 0.5 ml a 5% SRBC suspension. On day 14, blood was sampled for serum and on heparin (50 IU/ml) from all animals.

The anti-SRBC antibody titers were assessed by a hem agglutination test according to the OIE[16] protocol, in 96 "U" shaped well plates, performing two fold dilution of the serum (1/2, ¼,…) in saline and adding 0.025 ml of a 5% SRBC suspension/well. After 2 h of incubation at 37°C, the last dilutions with positive results were calculated as ln of the titers.

To establish *the circulating immune complexes levels* (CIC) a 4.2% PEG precipitation test was applied, mixing 196.7µl of PEG and 3.3µl serum in 96 flat bottom plates, and followed by spectrophotometric reading at 450 nm, d=0.5 cm after 60 min of incubation. The CIC values in units (U) were calculated according to the formula: (U) CIC = (sample E- control E) x 1000.

Leukocyte blast transformation test.[6] The leukocyte blast transformation test measures the *in vitro* reactivity of mononuclear cells to sensitizing antigens. Commercial alcoholic extracts for human use of *Calendula officinalis, Arnica montana, Symphytum officinale, Echinaceea purpureea, Echinaceea angustifolia* (Plantextract, Romania) produced according to the German Homeophatic Pharmacopeia, were used to treat the cultures. Similarly, an aqeous thymus extract, commercial Seleretard, balsam of Peru and bovine tuberculin, as immunstimulators.

One ml of each blood sample was diluted with four times the amount of RPMI 1640 supplemented with 5% FCS and antibiotics, at pH 7.4 (Sigma-Aldrich, USA). The mixture was distributed in duplicate, in 96-sterile-well plate (200 µl per well). Twelve *in vitro* experimental variants were tested for each individual animal, namely (1) untreated control culture, (10) phytohaemagglutinin-M (PHA)(1µ per well), (3) thymus extract, (4) 70° alcohol and (5–9) alcoholic vegetal extracts of *Calendula officinalis*, *Arnica montana*, *Symphytum officinale*, *Echinacea purpurea* and *Echinaceea angustifolia*, Balsam of Peru (11), bovine tuberculin (12) and Seleretard (1.5 µl/well).

The aliquots for all additions were established when using the same technique during preliminary studies as being the most effective *in vitro*. Subsequent to an incubation of 48 h at 37.5°C in a 5% CO_2 atmosphere, glucose consumption was evaluated.[11,12] For this, 12.5 µl of the cultural supernatant were transferred to 0.5 ml of orto-toluidine reagent, boiled for 8 min, cooled suddenly in cold water and read in a spectrophotometer at 610 nm wavelength (SUMAL PE2, Karl Zeiss, Jena, Germany), using the reagent as a blank. The stimulation/inhibition index (S/I) was calculated as: S/I %=[(IG–GR) /IG]´100, where S/I =blast transformation index, IG= the initial glucose concentration in the culture medium and GR=glucose residue in the sample after incubation.

Statistical analyses

Average values and standard error were calculated by use of Excel program. Student's t test was applied to evaluate the statistical significance of the differences.

The black line indicates the average value of the group, showing that 62.5% of the animals have antibody values lower than the average (Figure 1).

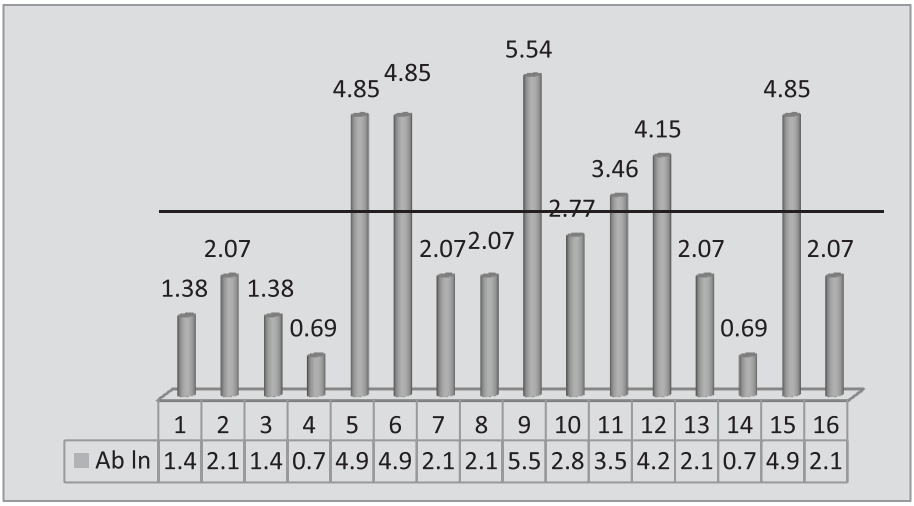

Figure 1: Individual variation of anti SRBC-antibody titres in primed foxes (ln).

Table 1: Stimulation/inhibition indices in the leukocyte blast transformationtest (x±s)		
Variant	**Unprimed group (n=14)**	**Primed group (n=16)**
Control	17.49 ± 22.9	66.8 ± 9.88
PHA M	-12.61 ± 10.37	6.67 ± 29.0
Thymus extract	0.05 ± 3.55	28.37 ± 23.64
Alcohol	-10.92 ± 32.0	27.63 ± 23.46
Calendula officinalis	-14.71 ± 10.73	12.92 ± 21.00
Arnica montana	-100.01 ± 69.55	-101.01 ± 58.70
Symphytum officinale	-13.58 ± 28.05	-14.88 ± 19.74
Echinaceea purpureea	-78.64 ± 35.53	-71.10 ± 49.48
Echinaceea angustifolia	20.13 ± 13.10	20.28 ± 21.27
Balsam of Peru	-140.89 ± 121.77	-162.84 ± 58.44
Bovine tuberculine	35.41 ± 19.76	51.43 ± 25.28
Seleretard (barium selenite)	37.0 ± 15.79	29.38 ± 24.20

RESULTS & DISCUSSION

The main mechanisms of defense against microbes involve antibodies directly blocking the pathogenic agent before it takes action against the host, promoting phagocytosis by micro- and macrophages and also involving T lymphocytes in the process. These mechanisms could be induced by microbial challenge by either natural infection or vaccination, as part of disease prevention measures. Effects of antigen priming in Silver foxes were less studies.[2-7-9]

Farmed animals of wild origin are even more exposed to stress factors that interfere with their immune responses leaving them exposed to disease. All factors connected to farming lead to stress and subsequent failure of immunization.

Extracts from numerous plants were tested for their therapeutic and disease preventing potential. Plant extracts, as products with increased bioavailability and little or no side effects, could provide maximal stimulating and adjuvant activity;[3] thus, vegetal extracts that could enhance the immune response to vaccines or during therapy are of utmost importance.[15] Economic aspects on investments in vegetal adjuvants and drugs should be evaluated based on their therapeutic and immune active potential.[4,5-10]

The results of the Student t-test indicated significance of the differences between primed and unprimed groups for the blast transformation test at different levels ($p < 0.001$-$p < 0.010$), only for the control, thymus extract and *C. officinalis* extract. The test also indicated that the effect of the extracts are strongly dependant on the plant genus, but also differ from species to species (Table1) *Calendula officinalis* and *Echinaceea angustifolia* leading to positive results.

The antibody synthesis induced by antigen priming did not lead to expected results. The antibody levels were highly variable, standing for the individual differences in the immune reactivity but also for the different perception of stress by these animals. In order to better protect these animals, antigen priming combined with plant extracts that positively acted *in vitro* could be of use. Necessarily, individualized protocols are required. The increased concentration of anti-SRBC antibodies also led to a non-significantly increased complexation (109 ± 4U and 41 ± 1U, t= 1.709) in primed versus unprimed foxes.

CONCLUSION

SRBC exerted a positive effect on both humoral and cell-mediated immune responses in Silver foxes, supporting the enhancement of antimicrobial defense by booster vaccination and immune stimulating therapy, represented by vegetal extracts selected by host and plant species.

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CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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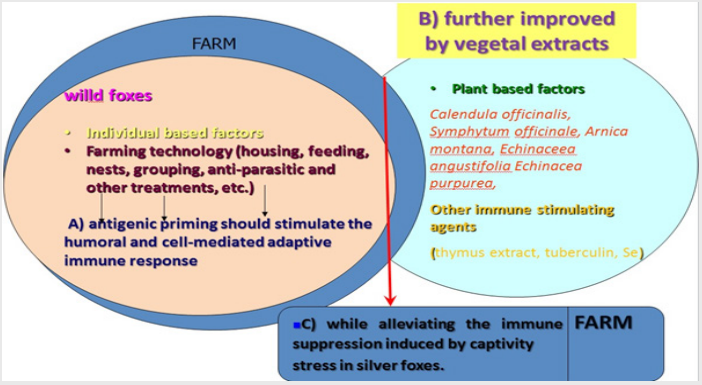
PICTORIAL ABSTRACT



SUMMARY

- This study investigated the influence of several ethanolic vegetal extracts and 5% SRBC priming on *in vitro* cell-mediated reactivity, circulating immune complexes and antibody levels in farmed foxes (n = 16 primed and n = 14 unprimed).
- The *in vitro* leukocytes blast transformation test was carried out using blood samples from foxes, while for antibody titers and circulating immune complexes sera were used.
- The effect of the vegetal extracts were strongly dependant on the plant genus, but also differed from species to species. *Calendula officinalis* (12.92 ± 21.00%) and *Echinaceea angustifolia* (20.28 ± 21.27%) lead to stimulation of the cellular immunity in the SRBC-primed group.
- The antibody levels were highly variable (from ln 0.69 to ln 5.54), standing for the individual differences in the immune reactivity in these animals.
- SRBC and vegetal extracts, selected by plant species, exerted a positive effect on both humoral and cell-mediated immune responses in Silver foxes, supporting the enhancement of antimicrobial defence by booster vaccination and immune stimulating therapy.

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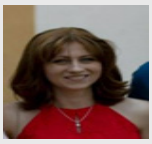

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