# *EVALUATION OF IMUNO-2865® ON GENERAL REHABILITATION OUTCOMES AND CYTOKINE PROFILE ANALYSIS IN ABANDONED NEONATAL HARBOR SEALS (Phoca vitulina) AND MALNOURISHED WEANLING ELEPHANT SEALS (Mirounga angustirostris)*

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## ABSTRACT

The immune system is the interface between an individual's health, pathogens, and antigens present in the environment[1]. Young stranded pinnipeds with health problems and challenged immune systems[2] offer an opportunity to examine factors affecting immunity and survival. Clinically, a dietary supplement that enhances the immune response in these patients would likely be beneficial in their medical management. Imuno-2865® (Animal Necessity LLC, New York, NY, 10001) is a new immune supplement that shows encouraging findings in enhancing human lymphocyte activation and interleukin activity[6-7]. Imuno-2865® is a form of beta-glucan, a polysaccharide, that is delivered as a microparticle, which helps promote improved absorption[3].

A pilot study was performed in the spring of 2013 to determine the effects of Imuno-2865® on the weight gain, survival, and cytokine concentrations of stranded neonatal harbor seals (*Phoca vitulina*) and weanling northern elephant seals (NES, *Mirounga angustirostris*) at The Marine Mammal Center in Sausalito, CA (TMMC). The study was comprised of two groups; weaned NES with a working diagnosis of malnutrition and newborn harbor seals that were brought in for rehabilitation. Each species had 7-9 study and 7-9 control animals selected by admit chronology into alternate groups. Study animals received 500 mg of Imuno-2865®, orally, twice daily for the duration of rehabilitation. Control animals were not given the supplement. Serum collected during admission and pre-release clinical examinations was tested for cytokine analysis after storage at -70°C.

Cytokines were quantified using the Millipore Canine Cytokine 13-plex test kit, which has previously been validated for measuring important pro- and anti-inflammatory cytokines in several pinniped species[4]. Cytokine concentrations (pg/ml) were measured using the Luminex xMAP 200 system and Bio-Rad Manager 5.0 software. Paired t-tests were used to compare cytokine levels in the serum, at the two time points, admission and release, within each study group. Only animals that were released at the end of the study were used in the statistical analysis.

Mortality rate and weight gain in rehabilitation did not differ between treated and control groups. For harbor seals, IFN-γ was significantly lower in the treated animals, but not in the control group, at the time of release, compared to admission. IFN-γ is a cytokine involved in immunity against viral and intracellular bacterial infections and an important activator of macrophages. It is reasonable to expect lower concentrations of IFN-γ in animals no longer combatting a potential intracellular infection. In treated NES, TNFα was higher at release, but did not change in the control group. TNFα is a cytokine that helps induce fever, apoptotic cell death, inflammation, as well as inhibiting viral replication. Elevations in TNFα in treated NES could indicate active/enhanced response to a pathogen. In contrast, KC-like cytokines were significantly lower in treated NES at the time of release. KC-like is a cytokine involved in the processes of inflammation and wound healing. It is possible that as the health of individuals improved (e.g. reduced inflammation) during rehabilitation, KC-like would be reduced at the time of release.

Further analysis is needed, including further testing with a larger sample size, which will allow refinement and further assessment of these results.

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**LITERATURE CITED**

*Journal article*

[1]Beineke, A., Siebert, U., Wohlsein, P., Baumgatner, W., Immunology of whales and dolphins, Veterinary Immunology Immunopathology, February 2010; 133 (2-4): 81-94.

[2]Chavoustie S, Perez P, Fletcher M, Maher K, Mitrani A, Thomas R. Pilot study: Effect of PDS-2865 on natural killer cell cytotoxicity. *Journal on Nutraceuticals and Nutrition*, Volume 6, No. 2: pp.39-42, 2003.

*Clinical Study*

[3]Chavoustie S, Perez P, Fletcher M, Maher , Mitrani a, Thomas R, Initial Clinical study on Natramune (PDS-2865) at The Clinical Immunology-University of Miami School of Medicine, 2002.

*Conference proceedings*

[4]De Guise, S, Mori, C, Levin, M, Trailsmith, M, Morsey, B, Leibrecht, H., Dunn, L. 2006. Diagnostic and prognostic tools in stranded marine mammals, IAAAM 37th *Annual Conference Proceedings, Nassau, Bahamas;* p.141

[5]Levin,M., Romano, T.A., Matassa, K., De Guise, S. 2013. Validation of commercial cytokine assay to help measure the health of stranded, IAAAM 44rd *Annual Conference Proceedings,* Sausalito, CA;

[6]Fonfara,S., Siebert, U., Prange, A., Colijn, F. 2006. Variations of cytokine expression and the impact of stress and infection in harbor seals (Phoca Vitulina) of the North Sea, IAAAM 37th *Annual Conference Proceedings*, Nassau, Bahamas; p.8

[7]Mejia, J**.,** Rodriguez, M., Blanchard, M., Stott, J., Pestano, N., Perez, P. 2008**.** Natramune™ (IN-2865®), An Immunostimulator Supplement in Cetaceans. IAAAM 39th *Annual Conference Proceedings*, Pomezia, Italy;p.26

[8]Rodriguez, M.,* Mejia, J., Blanchard, M., Stott, J., Pestano, N., Perez, P. 2007. Pilot Study: The Affects of Natramune™ (IN-2865®), A New Immunostimulator Supplement on Different Cetacean Species; *Tursiops truncatus, Lagenorhynchus obliquidens,* and *Orcinus orca.* IAAAM *38th Annual Conference Proceedings*, Orlando, Florida;pp.44-45

Book Chapter
[9]Mejia-Fava, J., Colitz, C. Supplements in Exotic Pets. (2014) Veterinary Clinics of North America – Exotic Animal Practice in press.