

# A Randomised Double Blind Placebo Controlled Trial of a Nucleotide-Containing Supplement Nucell® on Symptoms of Participants with the Common Cold - A Pilot Study®

Isobel Davidson and Lorna Fyfe\*

Department of Nutrition and Biological Sciences, School of Health Sciences, Queen Margaret University, United Kingdom

\*Corresponding Author: Lorna Fyfe, Department of Nutrition and Biological Sciences, School of Health Sciences, Queen Margaret University, Edinburgh EH21 6UU8TS, United Kingdom.

Received: March 14, 2016; Published: May 20, 2016

## Abstract

**Objectives:** To ascertain whether a nucleotide containing nutritional supplement Nucell® attenuates self-reported symptoms of the common cold.

**Design:** A randomised controlled trial.

**Setting:** A University.

**Participants:** Participants with self-reported symptoms of the common cold but otherwise healthy individuals.

**Intervention:** Nucell® capsules containing a yeast-based nucleotide preparation or placebo were provided over a 28 day period.

**Outcome Measures:** Subjective ratings of symptoms were recorded by self-administered questionnaires using a nine-point scale. Salivary IgA concentrations were analysed from samples collected during the first 7 days and then at days 14,21 and 28 of supplementation. Total and white blood cell counts were also measured throughout the intervention.

**Results:** Thirty-six participants completed the study. Nineteen received Nucell® and 17 received the placebo. The mean age of participants was similar (29.8 + 2.5 in Nucell group v 30.7 + 2.7 in control group) and the time participants had been suffering from cold-related symptoms was not significantly different in each treatment group (2.5 + 0.40 days in Nucell® v 2.9 + 0.47 days in control group). Severity of self-reported symptoms was significantly attenuated in the Nucell® treated group in the first week of supplementation for questions asked with respect to taste, painful sinuses and earache ( $p < 0.05$ ). Supplementation with Nucell® did not adversely affect total or differential white blood counts.

**Conclusion:** These results suggest that Nucell® supplementation administered as a treatment for cold-related symptoms may reduce the severity of specific symptoms particularly in the early infective phase. In conclusion, Nucell® supplementation may provide subjective relief of some cold-related symptoms and may be of significant benefit administered as a treatment in participants where sinus pain, earache and diminished taste are common symptoms.

**Keywords:** Nucell® capsules; Nucleotides; Salivary IgA; DNA; RNA; Cyclosporine

## Introduction

Nucleotides are the structural units required for RNA and DNA synthesis and maybe considered conditionally essential when the body is under stress and can have beneficial effects mostly under metabolically increased demands such as during acute viral infections [1,2,3]. Nucleotides can be synthesized endogenously and are therefore not considered essential nutrients. They are involved in almost all biological processes like DNA and RNA synthesis as described above, energy metabolism and protein synthesis [4]. Nucleotides become essential

**Citation:** Isobel Davidson and L Fyfe. "A Randomised Double Blind Placebo Controlled Trial of a Nucleotide-Containing Supplement Nucell® on Symptoms of Participants with the Common Cold - A Pilot Study". *EC Nutrition* 4.1 (2016): 804-811.

when the endogenous supply is insufficient to meet the needs of rapidly proliferating tissues. A system which is dependent upon dietary nucleotides for normal host response is the immune system where studies have shown that dietary nucleotide deprivation enhances the immunosuppression induced by a sub-therapeutic dose of cyclosporine [5,6]. A nucleotide-free diet also suppresses the immune response by inhibiting the maturation of T-lymphocytes, the generation of T-helper cells and the induction of interleukin-2-responses in mice on a nucleotide-free diet [7]. Other studies indicate that dietary nucleotides are necessary for maintenance of the immune system [8,9].

In a trial with athletes, the post-exercise salivary IgA levels were consistently significantly higher in the groups receiving a nucleotide supplement compared with placebo and control groups [10,11]. Under such conditions of cellular stress, the rapidly proliferating cells of the immune system are not able to fulfil their nucleotides needs solely by *de novo* synthesis subsequently relying heavily on dietary intake [12,13].

A consequence of infection is an inflammatory response which up regulates *de novo* synthesis of nucleotides in tissues thus reducing the extracellular pool available for proliferating immune cells [9] such as macrophages and lymphocytes. Provision of nucleotides during viral infection therefore may provide additional substrates allowing for enhanced immunomodulation and reduce sequelae due to secondary effects of viral infections.

Individuals infected with viruses causing the common cold display a number of symptoms due to both the primary viral challenge and secondary bacterial challenges occurring as a consequence of compromised respiratory epithelia [14]. The presence of a local inflammatory response releases mediators capable of activating sneeze and cough reflexes as well as stimulation of local pain fibres [14]. Maintenance of immune function under such challenges is essential and the key metabolic roles recently identified for dietary nucleotides under such challenging conditions further strengthens the case for nucleotide supplementation.

We hypothesised that supplementation with a formula containing nucleotides (Nucell®) may improve symptoms associated with the common cold. To assess whether an improvement in mucosal immune function could be responsible for any changes, the dominant antibody isotype IgA responsible for protection of the common mucosal immune system [15] was determined in saliva. In order to reduce selection bias a randomised double blind control trial was the chosen design.

## **Materials and Methods**

### **Participants**

Participants employed or attending a University were informed initially of the study by e-mail and asked to come to the research laboratory where they were screened for entry in to the study. Participants who had been showing signs of the common cold within the previous 48 hours but otherwise healthy individuals aged 18-50 were identified as suitable for inclusion. Participants were excluded if any of the following were present - yeast intolerance, food allergies, pre-existing illness other than the common cold, any prescribed medication, smokers or pregnant women.

### **Piloting**

A questionnaire was designed to assess the self reported severity of common symptoms associated with the common cold and included earache and sinusitis (painful sinuses) which have recently been reported as having increased prevalence in clinical populations with this infection [16]. Participants were asked to rate on a nine-point scale the severity of their symptoms. After piloting the questionnaire on 6 individuals who were recovering from the common cold a number of questions were added which took account of additional symptoms not initially identified. The scales were altered so that the higher rating indicated increased severity of the symptoms.

### **Protocol**

At recruitment, participants were assigned a number for randomization. Participants were asked to complete the questionnaire in which they rated the severity of symptoms related to the common cold. Participants were also asked to give a baseline sample of saliva

(approximately 5 ml) for IgA determination. Participants were assigned by simple randomization to the placebo group or the intervention group to receive either placebo or Nucell®, a nucleotide containing supplement (see Table 1) and were provided with 7 days supply of capsules. The only difference in capsules was the nucleotide content. Neither subject nor investigator knew which was the placebo or the nucleotide supplement. In the first 7 days participants were asked to fill out a questionnaire and collect a further saliva sample and to freeze this at -20°C on a daily basis. Participants returned to the laboratory at the end of each supplement week to renew their supply of capsules. At this time participants were made aware of the need for further samples of saliva and questionnaires at the end of each week of the trial. In a sub-group venous blood samples were taken at recruitment at day 14 and day 28 of the trial for differential blood cell counts.

Vitamins	Per 500mg Capsules	%RDA
E	3.0 mg	30
C	24 mg	40
Folic acid	80 mcg	40
B12	0.3 mcg	30
Biotin	60 mcg	40
Pantothenic acid	2.4 mg	40
Nucleotides	135 mg	NA

**Table 1:** Composition of the Nucleotide Containing Supplement given to the Intervention Group.

**Analytical Procedures**

Saliva samples were used to assess salivary IgA (immunoglobulin A) concentration, a general marker of immune status. IgA samples were analysed using ELISA. Briefly, Dynex 4HBX (No 3855) 96 wellplates were coated overnight at 4°C with 100 µl of mouse mono clonal anti-human IgA diluted in coating buffer at a 1:500 dilution. The plates were then washed with 300 µl /per well of wash buffer. 200 µl of blocking buffer was added to each well and left for 30 minutes to block all non specific binding. IgA standard (Sigma Aldrich) was reconstituted with 1 ml of phosphate buffered saline and frozen at -80°C in 50 µl aliquots (5 mg/ml). A standard curve was constructed from 8 serial dilutions. 50 µl of saliva samples and standards were added to the plate and incubated at room temperature for 2 hours. The plates were washed, then 50 µl of horse radish peroxidase rabbit anti-human IgA was added to the plates and incubated for a further 2 hrs. Finally plates were washed 6 times to remove any unbond conjugate and 50 µl of polychlorinated biphenyl (PCB), containing the substrate O-phenylenediamine (4 mg/ml), was added to each well after activating the solution with 40 µl H<sub>2</sub>O<sub>2</sub>. The colour change was monitored over a 20 minutes period and stopped with 12.5% H<sub>2</sub>SO<sub>4</sub>. The plates were read at 490 nm (Dynex plate reader).

**Statistical Analysis**

Repeated measures analysis of variance was used to determine significant differences between salivary IgA concentration with time as a within subject factor and treatment as between subject measure. The 5% level was taken as significant. Questionnaire subjective symptom ratings were analysed using Mann-Whitney where the level of significance was 5%. All data were stored and analysed using SPSS Version 15 for Windows.

**Ethical Approval**

Ethical approval for the project, as well as approval for all risk assessments were granted by the Research Ethics Sub Committee of Queen Margaret University. All subjects were informed of the nature of the project, each received an information sheet and signed a letter of consent. Subjects knew they could withdraw from the study at any time in accordance with QMU regulations and procedures.

**Results**

Thirty-six participants completed the trial, with 18 allocated to the subgroup analysis for differential blood counts. Subject characteristics are given in Table 2 which shows that those individuals recruited and randomised to the nucleotide supplement and control groups

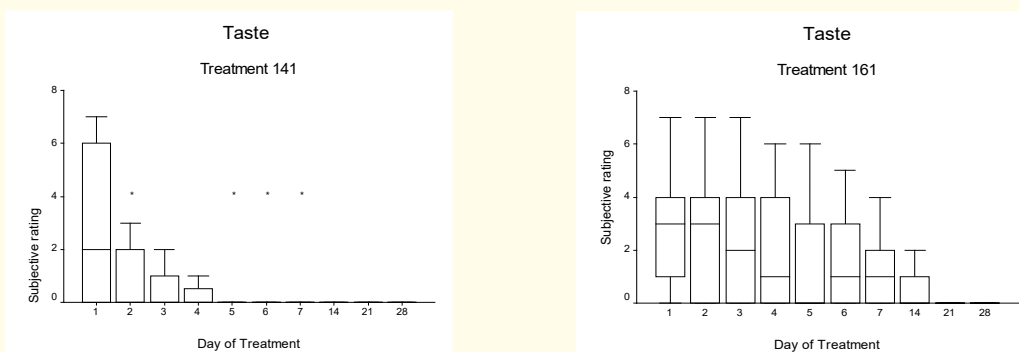
had been experiencing cold-related symptoms for a similar time period prior to inclusion.

	<b>Treatment</b>	<b>Control</b>
Number recruited	19	17
Gender (M:F)	6:13	8:10
Age (years)	29.8 + 2.5	30.7 + 2.7
Length of Symptoms on Recruitment (days)	2.5 + 0.40	2.9 + 0.47

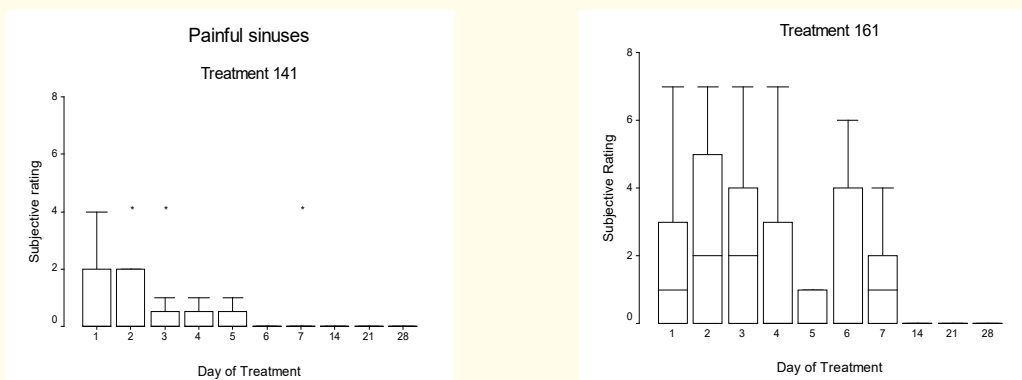
Values are mean + sem.

**Table 2:** Subject Characteristics of Treatment Groups.

When rating scores from the questionnaire relating to the severity of cold-related-symptoms were analysed for significant differences between treatment groups at days 1-7, day 14, day 21 and day 28 a number of differences were found. Subject ratings in response to the question whether their sense of taste had diminished showed no difference at baseline but at days 2, 5, 6 and 7 the treatment group showed significantly less alteration of taste ( $p < 0.05$ ). In addition after day 5 no taste symptoms were reported by the treatment group, however the control group continued to rate symptoms related to diminished taste until day 14 (Figure 1).



**Figure 1:** Subjective ratings of symptoms related to diminished taste represented by nucleotide treatment group (141 top graph) compared to the placebo control group (161 lower graph)  $*p \leq 0.05$  as calculated by the Mann-Whitney test.



**Figure 2:** Subjective ratings of symptoms related to painful sinuses represented by nucleotide treatment group (141 top graph) compared to the placebo control group (161 lower graph)  $*p \leq 0.05$  as calculated by the Mann-Whitney test.

In response to the question of having painful sinuses, baseline ratings were similar in each treatment group. However at days 2, 3 and 7 the treatment group rated significantly lower symptoms related to painful sinuses than the control group ( $p < 0.05$ ), this is illustrated in Figure 2. Subjective symptoms rated in response to the question of whether participants had earache are summarised for both treatment groups in Figure 3. Baseline ratings were similar, as they were on day 2, however on day 3 and 4 significantly lower ratings were seen in the treatment group than in the control group. Subsequent days showed no further differences between groups.

Further differences in subjective rating scores were evident between groups in other questions asked in the questionnaires, however these were limited to a single time point and are summarised in Table 3.

Question/Time point	Rating	Significance
Do you feel as though you have a temperature? Day 1 - Baseline	treatment = 1 + 3 control = 3 + 3	$P < 0.05$
Do you feel like the glands in your neck are swollen? Day 1 - Baseline	treatment = 1 + 3 control = 3 + 4	$P < 0.05$
Do you have a sore throat? Day 7	treatment = 0 + 0 control = 1 + 2	$P < 0.05$
Do you have a dry mouth? Day 21	treatment = 0 + 1 control = 3 + 4	$P < 0.05$

Values are represented as median + IQR.

**Table 3:** Differences in Subjective Rating Scores Obtained from the Questionnaire for the Treatment and Control Group.

Concentration of IgA in the saliva samples (data not shown) was found to be very variable (0-8.5 mg/ml treatment group v 0.05-6.4 mg/ml placebo). Results of statistical comparisons between groups by repeated measures ANOVA revealed no significant differences.

Cell Type	Treatment	Baseline	Day 14	Day 28
White cell count (x10 <sup>9</sup> )	Control	8.0 + 0.3	8.4 + 0.6	6.5 + 0.5*
	Nucell	7.4 + 0.8	8.1 + 1.1	6.5 + 0.6
Neutrophils (x10 <sup>9</sup> )	Control	5.8 + 0.8	5.7 + 0.9	3.3 + 0.4**
	Nucell	4.4 + 0.4	4.9 + 0.6	3.9 + 0.5
Lymphocytes (x10 <sup>9</sup> )	Control	1.5 + 0.2	1.9 + 0.2	1.8 + 0.3**
	Nucell	3.0 + 0.8	3.8 + 1.2	3.2 + 1.2*
Monocytes (x10 <sup>10</sup> )	Control	5.4 + 0.6	5.0 + 0.6	4.4 + 0.6
	Nucell	5.6 + 0.4	5.6 + 0.4	4.7 + 0.3
Eosinophils (x10 <sup>10</sup> )	Control	2.1 + 0.4	1.9 + 0.4	1.7 + 0.3
	Nucell	2.9 + 0.6	2.0 + 0.4	1.7 + 0.2*
Basophils (x10 <sup>10</sup> )	Control	0.2 + 0.0#	0.2 + 0.1#	0.3 + 0.1#
	Nucell	0.4 + 0.1	0.4 + 0.1	0.4 + 0.1

\* $p < 0.05$ ; \*\* $p < 0.01$  v baseline values.

# $p < 0.05$  v treatment group.

**Table 4:** Summary of Changes in Differential White Blood Cell Counts over 28 days of Intervention between the Treatment and Control Group.

In the subgroup analysis of individuals where blood was taken (n= 9 in each group) no significant differences with regard to treatment for total white cell count was evident. However within group factor analysis showed a significantly lower WBC count in the control group only between baseline (Day 0) and day 28 (p< 0.05). No such reduction in WBC between baseline and day 28, the final day of intervention was evident with treatment group. No significant differences were seen between groups was evident for lymphocyte differential counts. However within group factor analysis showed that in both treatment groups a significantly higher lymphocyte count was evident at day 28 when compared with baseline values. Statistical comparisons of neutrophil counts revealed no significant differences between the treatment groups. However in the control group a significant reduction in neutrophil count was evident at day 28 when compared with values obtained at baseline and day 14. Differences in differential blood counts are summarised in Table 4.

## Discussion

The results from this trial indicate subjective improvements in specific self-reported symptoms (earache, diminished taste and painful sinuses) in the treatment group over the control. None of these self-rating scores were significantly different at baseline. Attenuation of these symptoms was evident in the first 2-7 days of supplementation. This would be expected as both groups of participants would be recovering over the course of the supplementation and severity of symptoms would decline naturally with time. These results indicates that supplementation with yeast derived nucleotides may confer significant benefit in attenuating severity of earache, diminished taste and painful sinuses where these are predominant symptoms.

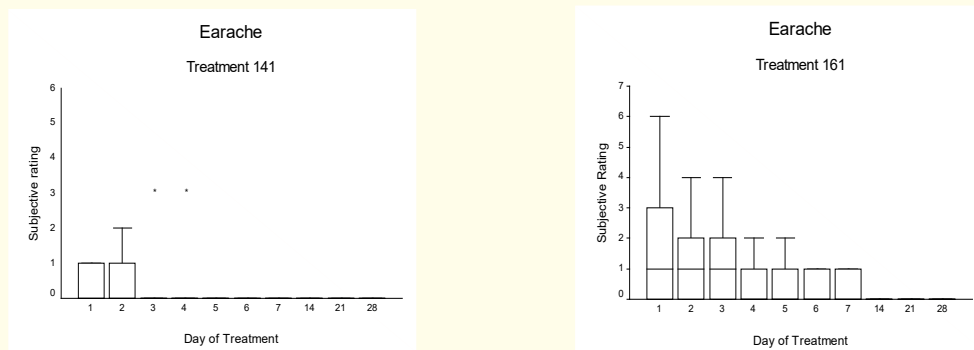
In addition to those symptoms described above where improvement in severity of self-reported symptoms was evident a number of other differences were evident in the treatment group. Baseline differences were recorded for questions asked regarding the presence of swollen glands (neck) and whether participants had a raised temperature. This suggests that the two groups may not comparable at baseline at least in regard to these symptoms perhaps due to the presence of secondary bacterial infection.

In addition significant differences were evident between groups at day 7 of supplementation. Reported symptoms of a sore throat were lower in the treatment group than the placebo. Likewise at day 21 the treatment group scored their symptoms in relation to having a dry mouth significantly lower than placebo.

Since participants were randomised to each group and the design of the trial was one in which both observer and subject were blind to the nature of either supplementation, this dictates that no known bias was introduced to reporting of self-reported symptoms. As a placebo controlled trial there was no 'placebo' bias either. It may be argued that participants were recruited at different stages of their infective state. However in the groups as a whole there was no significant difference in the length of time participants had been suffering cold-related symptoms prior to the introduction of the supplement. The treatment group had symptoms occurring 2.5 + 0.40 days prior to receiving the supplement and the placebo group 2.9 + 0.47 days, these were not significantly different and would tend to favour recovery from self-reported symptoms in the placebo group rather than the treatment group. Any effect of age in relation to the differences seen in these self-reported symptoms is also unlikely as again the ages in each group were similar.

The presence of symptoms such as sinus pain, earache, sore throat and taste changes imply that a degree of inflammation is present particularly in the oral-nasal passages. The symptoms themselves may also be indicative of a secondary bacterial infection developing after the initial viral insult. However from the sub-group analysis where blood the differential counts showed no difference at baseline in neutrophil or lymphocytes counts which may be expected in the presence of infection. Likewise total white cell counts did not differ between groups although in the placebo group a significantly lower count was evident at day 28 of supplementation than at baseline. Throughout the trial white cell counts remained within normal levels.

Times when bloodletting took place do not correspond exactly to those when subjective self-rated improvements of symptoms were recorded. Thus it is not possible to track in parallel the subjective symptoms and corresponding differential blood counts. Since most of the improvement occurred in the treatment group between days 2-7 it may have been more prudent to collect bloods at these times.



**Figure 3:** Subjective ratings for symptoms related to earache represented for the nucleotide treatment group (141 top graph) compared to the placebo control group (161 lower graph) \* $p \leq 0.05$  as calculated by the Mann-Whitney test.

Although the statistical analysis did reveal an overall effect of treatment for basophil counts, with the treatment group the mean counts at baseline were considerably higher than placebo. There was no effect of time when analysed as a within subject factor and basophil counts in both groups did not alter throughout supplementation. This overall difference appears to be due to difference at recruitment and not an effect of the supplement. This is unlikely to be due to any allergic reaction as would normally be indicated in a basophil count because of these baseline differences and in addition because of the lack of inter-group differences in the eosinophil counts.

Other objective markers that were measured at the same time-points as the self-reported symptoms were salivary IgA and glutathione measurements. At baseline IgA concentrations were similar and despite concentrations in the treatment group tending to be higher throughout the rest of the supplementation period no statistical significance between values was evident. This may reflect the fact that both groups are responding to a similar infective event and the absolute amount of IgA produced cannot be upregulated to a level where a significant difference between groups would be evident.

### Conclusion

In conclusion, participants who were supplemented with yeast derived nucleotides reported a lower severity of some symptoms related to the common cold during the first 7 days of supplementation. Such supplementation may provide subjective relief of some cold-related symptoms and may be of significant benefit administered as a treatment in participants where sinus pain, earache and diminished taste are common symptoms.

### Acknowledgement

We are grateful for the support of Wyreside Products, Lancashire United Kingdom and Pro Bio of Switzerland. We declare there is no conflict of interest. This publication has not been submitted elsewhere. This manuscript is dedicated to the memory of Professor Isobel Davidson.

### Bibliography

1. Carver JD. "Dietary Nucleotide Effects on the Immune and Gastrointestinal System". *Acta Paediatrica Supplement* 88.430 (1999): 83-88.
2. Pickering LK., *et al.* "Modulation of the Immune System by Human Milk and Infant Formula Containing Nucleotides". *Paediatrics* 101.2 (1998): 242-249.



3. Webb CJ, *et al.* "Effects of DksA and Clp Protease on Sigma S Production and Virulence in Salmonella Typhimurium". *Molecular Microbiology* 34.1 (1999): 112-123.
4. Van Buren R. "Dietary Nucleotides: A conditional Requirement". *Nutrition* 13.5 (1997): 470-472.
5. Van Buren R, *et al.* "The Influence of Dietary Nucleotides on Cell Mediated Immunity". *Transplantation* 36.3 (1982): 350-352.
6. Kulkarni AD, *et al.* "The Role of Dietary Sources of Nucleotides in Immune Function. *Journal of Nutrition* 124 (8 Suppl) (1994): 1442S-1446S.
7. Kulkarni SS, *et al.* "Functional Impairment of T-Lymphocytes in Mouse Radiation Chimeras by a Nucleotide-Free Diet". *Experimental Hematology* 12.9 (1984): 694-699.
8. Jyonouchi H. "Nucleotide Action on Humoral Immune Responses". *Journal of Nutrition* 124 (1 Suppl) (1994): 138S-143S.
9. Kulkarni AD, *et al.* "The Role of Dietary Sources of Nucleotides in Immune Function: A Review" *Journal of Nutrition* 124 (8 Suppl) (1994): 1442S-1446S.
10. McNaughton L, *et al.* "The Effects of a Nucleotide Supplement on the Immune and Metabolic Response to Short Term, High Intensity Exercise Performance in Trained Male Subjects". *Journal of Sports Medicine and Physical Fitness* 47.1 (2007): 112-118.
11. McNaughton L, *et al.* "The Effects of a Nucleotide Supplement on Salivary IgA And Cortisol after Moderate Endurance Exercise". *Journal of Sports Medicine and Physical Fitness* 46.1 (2006): 84-89.
12. Gil A, *et al.* "Modulation of the Immune Response Mediated by Dietary Nucleotides". *European Journal of Clinical Nutrition* 56 (3 Suppl) (2002): S1-S4.
13. Hess J, *et al.* "The Role of Nucleotides in the Immune and Gastrointestinal Systems: Potential Clinical Applications". *Nutrition in Clinical Practice* 27.2 (2012): 281-294.
14. Brownlee JW and Turner RB. "New Developments in the Clinical Spectrum and Epidemiology of Rhinovirus Infections". *Current Opinion in Pediatrics* 20.1 (2008): 67-71.
15. Mestecky J. "Saliva as a Manifestation of the Common Mucosal Immune System". *Annals of the New York Academy of Sciences* 694 (1993): 184-194.
16. Revai K, *et al.* "Incidence of Acute Otitis Media and Sinusitis Complicating Upper Respiratory Tract Infection: The Effect of Age". *Pediatrics* 119.6 (2007): e1408-e1412.

**Volume 4 Issue 1 May 2016**

**© All rights reserved by Isobel Davidson and L Fyfe.**