

The logo features the words 'OUR ERGO' in a bold, black, sans-serif font, rotated 45 degrees counter-clockwise. The letters are thick and blocky. Behind the text, there are several parallel diagonal lines in a light gray color, creating a sense of motion or a stylized background. The overall design is clean and modern.

OUR ERGO

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LETTER FROM THE EDITOR

“Research is formalized curiosity. It is poking and prying with a purpose.”

Zora Neale Hurston

This year’s Ergo gave me the opportunity to learn many new things. The research papers were more varied than last year’s, and I found the topics that the students addressed fascinating. Even those papers that were not chosen for publication held my interest. It was very difficult to choose which papers to include in this year’s Ergo.

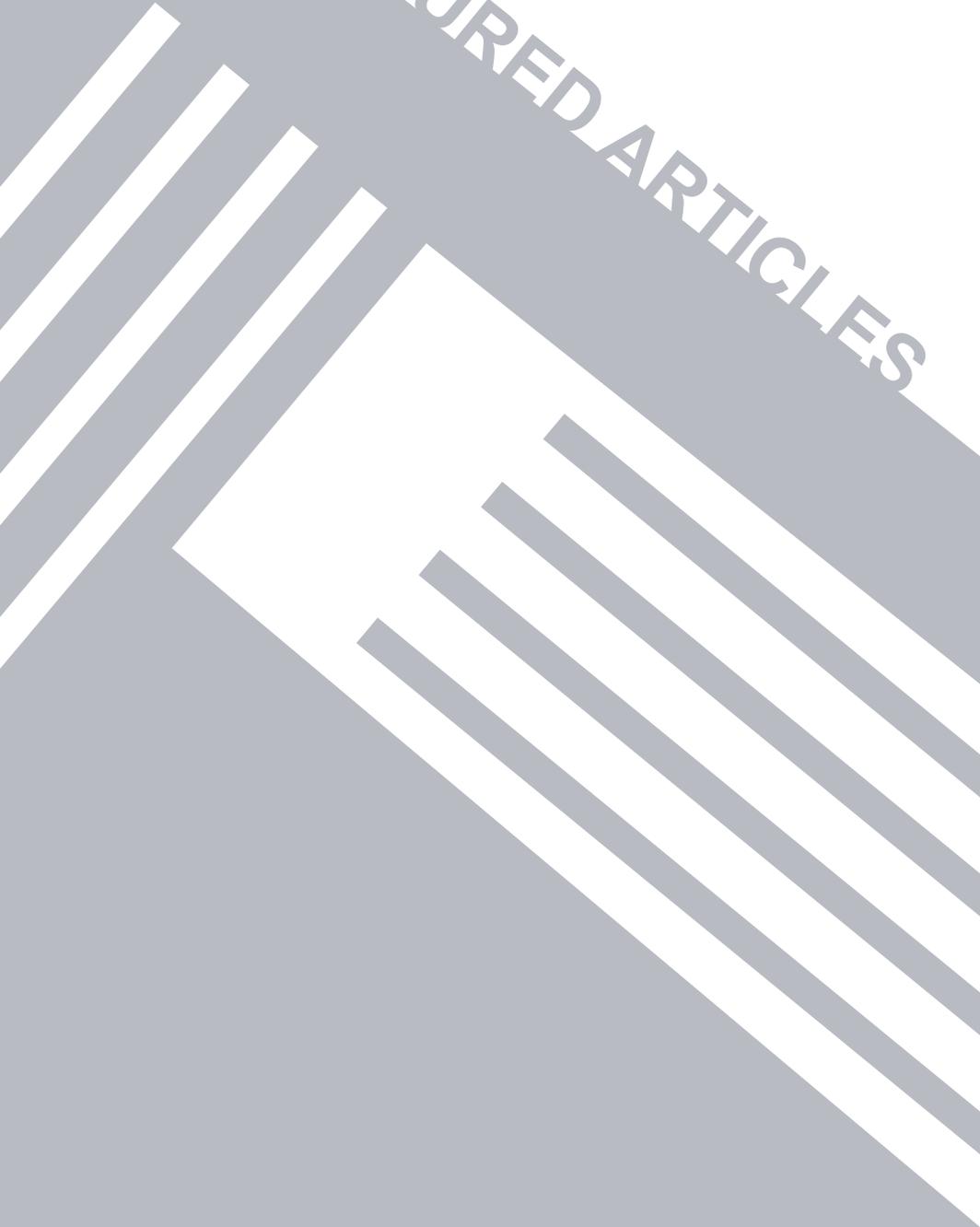
I was disappointed to be unable to include some excellent research papers because their length exceeded the maximum length requirement for submission. I can only hope those students will find other venues for publication, or can revise their papers to meet the Ergo submission length guidelines and resubmit them for next year’s journal.

I would like to give a special thanks to Beth Koford for putting up with my many requests for information during the course of the journal’s preparation. Her help was indispensable. I would also like to thank all the student and faculty reviewers. Without their knowledge of the specific fields of research, the submissions could not be properly edited and the journal would not be possible.

A handwritten signature in cursive script that reads "Kirsty Winkler". The signature is written in black ink on a light-colored rectangular background.

Kirsty Winkler
Editor-in-Chief

FEATURED ARTICLES



COLLEGE OF ARTS & HUMANITIES

PERFORMING ARTS

Beyond the Solo Singer: Tuning Group Pitch by Standardizing Individual Voices

Author: ShayLynne Clark

Mentor: Mark Henderson

ABSTRACT

Staying on the correct pitch in solo singing is difficult, but when in a multi-voice, multi-part choir, singing on pitch becomes an astronomical issue. Maintaining pitch in a group setting is difficult for many reasons, including the individual differences in vibrato pulses, as well as differences in the tone and physical structure of an individual's singing apparatus. Individuals participating in a small choral setting must learn to blend their individual voices with the 'group voice.' This is accomplished by having individuals understand their voice type, and observe whether they generally sing sharp or flat. Modifying individual voices to be on the correct pitch (give or take 10-15 cents) adds to the effectiveness of that individual within the choral setting. This study illustrates that by helping individual voices within the choir augment their own pitch tendencies with a chromatic tuner, the choir as a whole will progress toward an ideal group pitch. The use of a chromatic tuner enabled individuals to observe their own pitch in reference to the rest of the group, thus allowing individual voices to not only notice their natural tendencies with pitch, but to correct their pitch, which helps the group as a whole achieve accurate pitch. There is no literature on altering pitch of choral groups in this way, so this is primarily a pilot study, which may lead to more research regarding the delicate balance of individual voices in group choral settings.

INTRODUCTION

In choral music, no matter how technically perfect a performer may be, it is difficult to achieve optimum pitch efficiently. Although there are some people who possess perfect pitch, it is most definitely not a trait that the general population possesses. In order for a choir to be competitive in the performing world, pitch needs to be maintained and standardized within the group for every performance.

Weber State University's chamber choir director, Mark Henderson, has been trying to solve the problem of inaccurate pitch with multiple voices for many years. He has improved group dynamics as well as demanded more stringent vocal requirements for students who are members of the chamber choir. As the general population of students in WSU's chamber choir have a musical background, many issues that usually surround choirs (sight reading, memorizing, etc.) resolved themselves — everything, that is, except pitch. Henderson views the voice as a living instrument and, as such, saw it appropriate to correct pitch the way other instruments do, by using a tuner. Beginning in the fall semester of 2010, Henderson began requiring students in chamber choir to purchase chromatic tuners to be used every day during vocal exercises and piece rehearsals (Henderson, 2010).

The aim of this study is to illustrate to choir members as well as solo voices that, although accurate and efficient pitch is difficult to accomplish, it is not impossible. This research also illustrates that individual voices will have a general tendency to be sharp or flat, but if the individual understands what their tendency is through use of a tuner, they can compensate for their natural vocal variation.

LITERATURE REVIEW

There is very little information on techniques to alter group pitch, and even less on altering group pitch by the means of a tuner. Other literature regarding pitch correction was not found. It is the opinion of these researchers that this study is amongst one of the first of its kind, and we hope more research will be done on this topic in the future.

METHODS

All 31 auditioned members of WSU's chamber choir, directed by Mark Henderson, were required to purchase a Korg brand chromatic tuner (figure 1)

Chromatic tuners show pitch accuracy through with the measurement of cents. The musical term, 'cent' is a logarithmic unit of measure used for musical intervals. A cent represents a hundredth of a semitone (or "half step"). Twelve-tone equal temperament divides an octave into 12 semitones of 100 cents each. Typically, cents are used to measure extremely small finite intervals, or to compare the sizes of comparable intervals in different tuning systems. An interval of one cent is much too small to be heard with the human ear between successive notes.



Figure 1. Korg brand CA-40 chromatic tuner

to be used every day in choir rehearsal. Tuners were used to give students instant visual feedback on whether their voices are sharp or flat.

Every day at the beginning of class, students were asked to not sing or play anything on the piano, to help ensure that they did not have a 'fresh' pitch in their heads, which could potentially inflate the positive effect of the tuner usage. Students gifted with perfect pitch were asked to leave the room, again to prevent inflation of positive data. The students were then instructed by Henderson to sing, in unison, a particular note into their tuners on his cue. The goal of this exercise was to see how long it took the students to reach the correct sustained pitch within 10-15 cents sharp or flat. This exercise was administered at the beginning of every rehearsal day, equaling a total of 50 trials, 25 trials with students using tuners, 25 trials without. The data from every week was then averaged and plotted on a graph (figure 2) that compared the time it took to reach accurate pitch as a group with tuners and without.

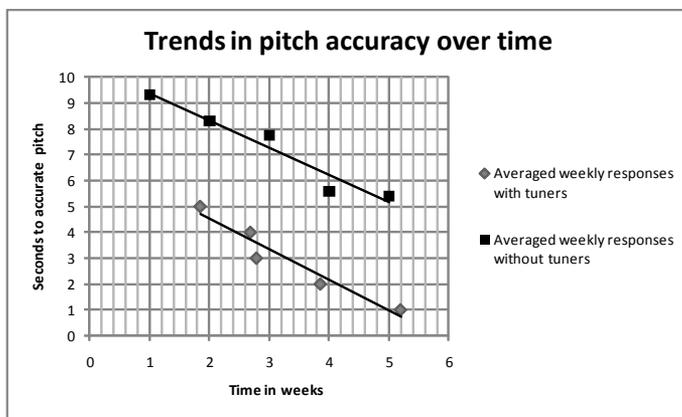


Figure 2. Average time to reach accurate pitch with and without tuners.

Consistently, even on the first day, correct pitches were achieved significantly faster with than without tuner use. In figure 2, averaged weekly responses with and without tuners were graphed to look for trends in the speed by which correct pitch was accomplished. The data on figure 2 represents an average of the five trials per week as indicated on the horizontal axis. As the graph indicates, the trend for average responses with and without tuners has a negative slope, indicating that with each trial, the students' speed improved. The slope for weekly responses with tuners, however, is much steeper than the slope seen without the tuners, indicating that students found their pitches markedly faster with each usage of tuners. Interestingly, the last point corresponding with week 5 for responses without the use of tuners was not as fast as the first point corresponding with week 1 and the tuners' first usage.

DISCUSSION

Even though much more work needs to be done in this field regarding pitch in choral settings, many positive conclusions can still be made from this pilot study.

The data in figure 2 represents an average of five trials for each week of testing. The study went on for five weeks, meaning that 50 trials were conducted, 25 with tuners and 25 without tuners. Even after the fifth week (25 trials), without the usage of tuners, the pitch was not reached as quickly as the first week (1-5 trials) of the students using tuners. This indicates that when students had a visual cue indicating whether they were sharp or flat, not only did their pitch improve immediately, but the learning and adjustments made by the singers themselves lasted longer and seemed to be more effective than practicing pitch without a visual indicator accompanying it.

After all of the trials had been administered, members of the choir were interviewed and asked what they did physically to adjust their individual pitch to the correct pitch on the tuners. 28 out of 31 students said they adjusted mouth and tongue positioning more than anything else. This gives added insight into pitch problems, suggesting that maybe most pitch problems are tied to a learned physical movement of the mouth and tongue, rather than something that just naturally happens with the voice itself. Theoretically, if tuner use was implemented with young singers in early training, it is possible that perfect pitch could be learned with the visual aid of the tuners, possibly eliminating most pitch problems related to mouth and tongue position.

CONCLUSION

Understanding individual tendencies in pitch is a powerful tool for any vocal performer, in a group setting or otherwise. If tuners were used in preliminary training, we believe that pitch problems could be considerably diminished, if not completely eliminated. The visual aspect of the tuner is thought to be one of the most important factors in this study's success. This study indicates that combining auditory musical cues with the visual cues of the tuner increases pitch learning more than just auditory cues alone. These results could potentially change the way that pitch is taught and learned within the whole vocal musical community.

REFERENCES

Henderson, D. M. (2010, October). Choral music professor. (S. Clark, Interviewer)

Statistically Low Prices: A Preliminary Study of Store-Brand Price Differences

Authors: Ryan Holt, Jill Donahue, Brayden Griffin & Maomao Cai
Mentor: Maomao Cai

ABSTRACT

It is commonly assumed that when comparing Wal-Mart and Target, Wal-Mart has lower prices. This paper intends to determine if this is a correct assumption. Using paired data consisting of store-brand products, a Wilcoxon Signed Rank Test is used to determine if the hypothesis that Wal-Mart prices are less than Target prices is true. The median price difference of Wal-Mart minus Target price is found to be negative and statistically significant, therefore it is concluded that Wal-Mart store-brand products are less expensive than Target store-brand products. An analysis of why nonparametric methods are used is included along with detailed steps for using the Wilcoxon signed rank test.

INTRODUCTION

Wal-Mart is the largest discount department store in the United States followed by Target (Barwise and Meehan 2004). These two stores are constantly competing to win the hearts and cash of consumers. The common perception among consumers is that Wal-Mart is less expensive than Target (Burrill 2009). For 19 years Wal-Mart's slogan was "Always Low Prices," and this slogan seems to have translated to "Always the Lowest Prices," for price savvy shoppers (Writer 2007). Recently Target has redesigned its store-brand, giving it a face lift and a new name, "Up and Up." Whether or not Target's redesigned store-brand products have a price tag that will sway consumers beliefs has yet to be seen. This article is a preliminary study to see if there are statistically significant differences between the prices of store-brand products at the two stores. By taking a random sample of several identical store-brand items it will be determined if the popular beliefs regarding Wal-Mart's low prices are fact or fiction. While this study ignores the prices of national brand products, it is justified when considering that most price conscious consumers will buy store-brand products to save money, and therefore store-brand prices are of interest in this study.

METHODS

Sampling

To test the belief that Wal-Mart is less expensive than Target a sample of forty store-brand products was selected from the inventory of Wal-Mart. These products were then matched to their store-brand counterpart at Target to give a paired sample. For simplicity, only products from the grocery and health/beauty departments were used. These two departments contain the majority of store-brand products carried at the stores. A list of all store-brand products can be found by searching each store's website. By numbering all of the store-brand products in the Wal-Mart health/beauty and grocery departments (1 to 396 and 1 to 1505 respectively), a random selection of 20 products was chosen from each of the two groups (appendix, table III). Observations that were not identical in terms of size were discarded to keep the paired sample unbiased.

After obtaining a random sample of items, price data was collected by visiting a Wal-Mart and a Target located in Layton, Utah. Data was collected on the same day in order to ensure there was no difference due to seasonal or weekly specials. Of the 40 chosen items, there were 3 products that did not have equivalent sizes or quantities and they were discarded from the sample. With the remaining data, price differences were calculated by taking the Wal-Mart price and subtracting the Target price. This sample of price differences (table 1) was used for testing the assumption that Wal-Mart store-brand products are less expensive than Target store-brand products.

*Table 1. Sample of Price Differences
(Wal-Mart price minus Target price)*

\$ 0.00	\$-0.25	\$ 0.69	\$-0.02
\$-0.01	\$-0.03	\$-0.11	\$ 0.65
\$-0.36	\$-0.39	\$-0.01	\$-0.09
\$-0.32	\$-0.04	\$-0.01	\$-0.03
\$-0.26	\$ 0.15	\$-0.01	\$-1.91
\$-0.01	\$-0.22	\$ 0.00	\$-0.02
\$-1.46	\$ 0.23	\$ 0.00	\$-0.06
\$ 0.09	\$-0.23	\$-0.03	\$-0.02
\$-0.09	\$-0.31	\$ 0.01	\$-0.01
			\$ 0.00

Normality Assumptions

Due to the small sample size of this study the non-parametric Wilcoxon signed-rank test was chosen over the traditional hypothesis test of a paired t-test. The main reason for this is that the assumption of normality was not met with such a small sample. In a paired t-test with a small sample, the estimate for the mean price difference can be biased due to extreme observations. A chi-squared goodness of fit test was used to verify if the sample data is normally distributed (Pearson, 1900). Using the mean and standard deviation from the sample of price differences, the null and alternative hypotheses for this test are as follows:

H_0 : the population of Wal-Mart and Target price differences comes from a normal distribution with mean $-.12$ and standard deviation $.4399$.

H_1 : the population of Wal-Mart and Target price differences does not have a normal distribution with mean $-.12$ and standard deviation $.4399$.

By dividing the normal distribution into four groups, the expected number of observations for each group can be determined by multiplying the area under the normal curve by the sample size. The groups chosen for this test were $(-\infty, -1]$, $(-1, 0]$, $(0, 1]$, and $(1, \infty)$. The corresponding areas for these sections of the normal distribution are approximately $.16$, $.34$, $.34$, and $.16$ respectively. By multiplying the sample size of 37 by the areas under the normal curve, the expected observations for the Chi-squared test are 5.92, 12.58, 12.58, and 5.92.

The chi-squared goodness of fit test compares expected counts to observed counts for different groups. A large chi-squared value indicates that the observed counts deviate from what would be expected for the assumed distribution. The chi-squared test statistic calculated for this sample was 19.12. With three degrees of freedom the p-value associated with this chi-squared value is $.000258$. Therefore the null hypothesis that the sample data of price differences are normally distributed is rejected (for a table showing expected and observed counts for the chi-squared statistic see table I in the appendix).

The Wilcoxon Signed-Rank Test

For nonparametric distributions of data, a Wilcoxon Signed-Rank test is one method to test a hypothesis (Wilcoxon 1945; Milton and Arnold 2002). When comparing two samples these can be written as:

$$H_0: M_d = 0 \quad H_1: M_d < 0 \quad \text{where } M_d = M_w - M_t \quad (1)$$

In this test, a sample size of n should be symmetric about an unknown median M , with $M_0 = 0$ being the median in question in this study. The difference between the prices of Wal-Mart and Target represents the values in our sample that are symmetric about the median in question. For a sample where the median is not zero it can be determined how far each observation is from the median by subtracting M_0 from each observation. In this sample, because the median in question is zero, the observed price differences represent the distance from the median.

To determine if the median in question is the true center of the data, observations must be enumerated by magnitude, from smallest to largest. A numbered ranking system, from 1 to n , is then used to measure the order of values. If there is ever a tie, the average rank between these values is used for each sample point. Each positive difference is assigned a “+” in front of its rank, while each negative difference is assigned a “-” in front of its rank. If H_0 , the hypothesis that $M = M_0$, is true, then each rank will be equally likely in being assigned a positive or negative sign. The test statistic for a Wilcoxon Signed-Rank Test is as follows:

$$\begin{aligned} W_+ &= \sum R_i \text{ (summation of all positive ranks)} \\ W_- &= \sum |R_i| \text{ (summation of magnitudes of all negative ranks)} \end{aligned} \quad (2)$$

The test statistic is defined as $W = \min\{W_+, |W_-|\}$ (Milton and Arnold 2002). In application to this study, a median difference that is less than zero would mean that on average Target prices are higher than Wal-Mart and a large $|W_-|$ would be expected. A median difference of zero would mean that on average the prices between Wal-Mart and Target are the same and W_+ and $|W_-|$ would be approximately equal. A median difference that is greater than zero would mean that on average Wal-Mart is more expensive than Target and a large W_+ would be observed. For this study it is of interest to see if Wal-Mart’s store-brand products are less expensive. Since the price differences are being calculated by subtracting the Target price from the Wal-Mart price, an alternative hypothesis with the median difference less than zero is used. If a small W_+ is observed then the null hypothesis in equation (1) can be rejected, and there would be sufficient evidence to suggest that Wal-Mart store-brand products are less expensive than Target store-brand products.

Calculation of the Wilcoxon Signed Rank Test Statistic

The first step in testing this hypothesis is to order the sample data from the smallest absolute difference to the largest as shown in table 2 on the next page.

Table 2. Values in Absolute Magnitude

0.00	0.01	-0.09	-0.32
0.00	-0.02	0.09	-0.36
0.00	-0.02	-0.11	-0.39
0.00	-0.02	0.15	0.65
-0.01	-0.03	-0.22	0.69
-0.01	-0.03	-0.23	-1.46
-0.01	-0.03	0.23	-1.91
-0.01	-0.04	-0.25	
-0.01	-0.06	-0.26	
-0.01	-0.09	-0.31	

Table 3. Signed-Rank

1	8	-21	-31
2	-13	21	-32
3	-13	-23	-33
4	-13	24	34
-8	-16	-25	35
-8	-16	-26.5	-36
-8	-16	26.5	-37
-8	-18	-28	
-8	-19	-29	
-8	-21	-30	

Values are then ranked from 1 to 37. These ranks keep the algebraic sign given when calculating the difference between prices. Therefore, if H_0 is true, a positive or negative rank would be equally likely. The ranking of the data is shown above in table 3.

The test statistic is a W_+ value (the sum of all the positive ranks) due to the alternative hypothesis (1). If the median price difference is less than zero we would expect the smaller of the two summed ranks to be the positive rather than the negative. The W_+ value returned from this sample is 158.5. The critical value obtained from the Wilcoxon signed-rank test table for a sample of size 37 is 183, for a significance level of .005. This critical value means that the smallest W_+ value that would be expected if the null hypothesis was correct would be 183. Because the test statistic W_+ is less than the critical value it can be concluded that it is too small to have occurred by chance. Therefore H_0 is rejected and there is sufficient evidence from this sample to conclude that Wal-Mart store-brand products are less expensive than Target store-brand products.

CONCLUSION

In gathering data it was found that prices between Wal-Mart and Target are very close. When all the items are considered together, the average price of Target store-brand items was 3.6% higher than the average price of Wal-Mart store-brand items. Looking at the data, 62% of the price differences fall between \$-0.10 and \$0.10 (see appendix table II for summarized data). Using the Wilcoxon signed-rank test the population median for differences of prices is estimated to be less than zero. The conclusion drawn from this preliminary study is that Wal-Mart store-brand products are less expensive

than Target store-brand products, though the questions of “by how much?” and “how often?” are left open for further review. Future studies should enable a larger sample size in order to estimate the mean price differences and confidence intervals for price difference data. Another area of interest is determining if these large discount department stores use a randomized pricing strategy as suggested by Varian’s “Model of Sales” (1980). Weekly or monthly data would need to be observed for several time periods to determine if price differences change from period to period or whether there is a consistent trend.

REFERENCES

- Barwise, P. & Meehan, S.N. (2004). Bullseye: Target’s Cheap Chic Strategy. Retrieved from <http://hbswk.hbs.edu/archive/4319.html>.
- Burritt, C. (2009). Wal-Mart Set to Repeat Share Gains, Grab Target Sales (Update3). Retrieved from <http://www.bloomberg.com>.
- Milton, J. & Arnold, J. (2002). *Introduction to probability and statistics: principles and applications for engineering and the computing sciences*. New York: McGraw-Hill Higher Education.
- Pearson, K. (1900). X. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. *Philosophical Magazine Series 5*, 50(302), 157-175.
- Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bulletin*, 1(6), 80-83.
- Writer, S. (2007). Wal-Mart Rolling out New Company Slogan. *Reuters via the New York Times*.
- Varian, H. (1980). A model of sales. *American Economic Review*, (September), 651-659.

APPENDIX: TABLES I, II, AND III

Table I. Intervals used for Chi-Squared Goodness of Fit Test with observed and expected frequencies

		ei	oi	(ei - oi) ²	(ei - oi) ² /ei
Group 1	(-∞, -.56]	5.92	2	15.3664	2.5957
Group 2	(-.56, -.12]	12.58	8	20.9764	1.6674
Group 3	(-.12, .32]	12.58	25	154.2564	12.2620
Group 4	(.32, ∞)	5.92	2	15.3664	2.5957
				X ²	19.1208
				p-value	0.000258

Table II. Summarized data for price differences

Mean	-\$0.12
Median	-\$0.02
Mode	-\$0.01
Standard Deviation	\$0.42
Minimum	-\$1.91
Maximum	\$0.69

Table III. Listing of sample items and quantities used

GROCERY (Market Pantry or Archer Farms, Great Value)	QUANTITY
Apple Cinnamon Fruit and Grain Bars (cereal bars)	10.4 oz
Applesauce (unsweetened in a jar)	50 oz
Black Beans (bag)	16 oz
Chewy Chocolate Chip Cookies	15 oz
Cranberry Juice Cocktail	64 oz
Distilled White Vinegar	64 oz
Fat Free Yogurt	6 oz Cup
Green Beans	14.5 oz
Half and Half	16 oz
Kosher Dill Spears (pickles)	24 oz
Long Grain Enriched White Rice	5 lb
Medium Pitted Olives	6 oz Can
Mild Green Chilies (easy to open)	4 oz
Mild Italian Sausage	5 links
Premium Ground Coffee 100% Columbian	13 oz
Rainbow Sherbet	1.5 qt tub
Small Curd Cottage Cheese	24 oz
Strawberry Orange Banana Drink Mix (6 tubs)	2.4 oz
Taco Seasoning	1.3 oz
Whole Tomatoes (canned)	14.5 oz
HEALTH/BEAUTY (Up and Up, Equate)	QUANTITY
Acetaminophen Pain Reliever 500 mg	100 ct
Anti-Diarrheal 2mg Tablets	48 ct
Antifungal Cream/Athletes Foot Cream	0.5 oz/1 oz
Centrum Silver MultiVitamin, Mature Adult Vitamin	220 ct
Childrens Ibuprofen (oral suspension) Bubble Gum	4 oz
Clear Liquid Soap (antibacterial)	7.5 fl oz
Cotton Swabs (Q-tips)	300 ct
Eye Itch Relief Drops	5 Ml
First Aid Triple Antibiotic Ointment	1 oz
Liquid Antacid/Anti Gas Original	12 fl oz
Men's Twin Blade Razors (sensitive skin, lubricated strip)	12 ct
Moisturizing Body Wash	23.6 oz
Multipurpose Solution for Contact Lenses	12 fl oz
Naproxen Sodium Tablets (pain reliever/fever reducer)	100 ct
Nicotine Gum (Polarcrilex) Cool Mint 2mg Nicotine	170 ct
Oxymetazoline Hydrochloride 0.05% Nasal Decongestant/Spray	1 oz
Regular Unscented Tampons	40 ct
Skin Protectant Petroleum Jelly	7.5 oz
Tussin Cough/Cold	8 oz
Waxed Dental Floss	55 yd

The Price Premium for Components of Organic Eggs: A Hedonic Analysis

Author: Parry Higginson

Mentor: Therese Grijalva

ABSTRACT

A fast rising sector of the poultry egg market has been the increase in the number and variety of organic eggs offered. This rise can be attributed to the increase in both consumer awareness into the harmful effects of eating foods with added chemicals or hormones and the animal welfare concerns for the treatment and care of the laying hens. Some of the components of an organic egg include whether the egg is cage free, free range, or free of added hormones or antibiotics. The purpose of this paper is to determine the premium consumers pay for these organic components of an egg. The hedonic model, which identifies the marginal cost of having a certain attribute within a good, is applied to a sample of 169 different eggs taken from Weber and Davis County, Utah. Results indicate that consumers do indeed pay a premium of \$.067, \$.062, and \$.053 per egg that is cage free, free range, and free of added antibiotics/hormones, respectively.

INTRODUCTION

The organic food market has shown significant growth in the last decade. Organic egg sales between the years 2000 through 2005 had an average growth rate of 19% per year, with an expected annual growth rate between 8 and 13% through the end of 2010 (Oberholtzer, 2006). This growth has been shown to evolve from a number of factors. Consumer awareness of the food production processes has created a higher demand for healthier foods. Moreover, the recent rise in the number of large corporate retail stores, such as Wal-Mart and Target, has given consumers an increase in the availability, selection, and variety of organic products. This increase has lowered search costs, which were previously a deterrent in the demand for organic products (Dimitri & Greene, 2002).

Consumers who purchase organic products place greater value on foods that are free of pesticides, hormones, and antibiotics (Dimitri & Greene, 2002). The perceived health benefits to the consumer and the reduced environmen-

tal impact are both significant in the demand for organic products, with the former being more influential on demand (Gracia & Magistirs, 2008). The purpose of this study is to establish the price premium for certain characteristics that make up the organic nature of an egg as well as the premium that consumers pay for other characteristics that affect the price of an egg.

Background

The United States Department of Agriculture (USDA) defines an organic egg to be from a hen that has “access to the outdoors, shade, exercise areas, fresh air, and direct sunlight suitable to their species and stage of production” and that producers “are not allowed to cage organic poultry” (Oberholtzer, 2006). It has been estimated that battery chickens, chickens kept only in confined cages, “are allotted 67 square inches of space at most, despite the fact that a hen needs 75 square inches to stand comfortably and 144 square inches to spread its wings” (Lusk, 2009; Dawkins & Hardie, 1989; Mason and Singer, 1990). Animal rights organizations as well as national and local media have been publicizing the treatment of laying hens, which has put pressure on producers and legislation to require more humane treatment of the hens. In 2008, the state of California passed Proposition 2 which requires eggs sold in California to be from hens that are not caged or unable to stand up, turn around, or spread out their wings.

LITERATURE

Karipidis et al. (2005) discussed the characteristics that affect the pricing structure of eggs sold in Athens and Thessaloniki, Greece. They hypothesized that there are certain characteristics and components of an egg that affect the price of the egg, and they set out to determine what those characteristics were by means of a hedonic pricing model, where certain characteristics of a particular good can be evaluated in terms of the price they contribute to that good. Data was first collected over the summer of 2004 by observing the characteristics of each egg from the egg labels. Using ordinary least squares (OLS) regression, Karipides et al. (2005) concluded that the most significant variables that affect price were: egg size, Omega enrichment, poultry feeding system, and package appearance.

Lusk (2010) analyzed the passage of Proposition 2 in California, which requires that any laying hen have the ability to move in such a manner that is currently not available in the traditional battery egg production, which requires the chicken to be caged and well confined. By analyzing the demand for eggs leading up to the vote on Proposition 2, Lusk (2010) showed that in the city of San Francisco, California, “demand for cage free and organic eggs increased by 180% and 20%, respectively” while overall demand for eggs did not change. This result shows that in the presence of information shocks to the egg market, demand for certain components of an egg may increase.

Hedonic Theory

The hedonic model is commonly used in identifying the value of certain characteristics that make up the pricing structure of a particular good. Rosen (1974) formulated the hedonic model by hypothesizing that consumers value a good based upon its utility bearing attributes. He indicated that any good is composed of n number of characteristics (Z_1, Z_2, \dots, Z_n). A good's price is a function of a vector of attributes represented by the equation:

$$P_i = f(Z_{1i}, Z_{2i}, \dots, Z_{ji}; u_i)$$

where P_i is the observed price of commodity i ; $Z_j, j = 1, \dots, j$ measures the certain quality characteristic for each of the i th commodity, and u_i is a disturbance term. A characteristic's parameter values may be estimated by means of OLS regression. In this paper each (Z_{ji}) characteristic represents a certain attribute of an egg.

METHODS

Eggs were sampled from stores in Davis and Weber County in Utah. Each county was also used as an experimental variable to see if the county had an effect on the price of eggs. In total, 169 observations were gathered over a span of four weeks in October 2010 that included twenty-two different brands of eggs. There were seven stores sampled in Davis county and eight in Weber county.

Empirical Model and Analysis

A description and the expected signs of the variables hypothesized in our study are explained in table 1 on the following page. It is hypothesized that as *EGGS/PKG* increases the price per egg will decrease. The hypothesis is based on potential price discrimination and possible economies of scale production for the producer. The following factors are all added costs to the producer and are hypothesized to drive up the cost per egg: *OMEGA*, *ORGANIC*, *FREE*, *CHOL_FAT*, *NO_HORMONES*, and *CAGE*. Egg packages came in two types of packaging material: cardboard and Styrofoam. It is not certain what the coefficient's sign will be on this variable. Based off of the theoretical framework given by Rosen's (1974) hedonic model, the following econometric model is tested:

$$\begin{aligned} PRICE_i = & \gamma + \beta_1 (ORGANIC_i) - \beta_2 (EGGS/PKG_i) + \beta_3 (LARGE_i) + \\ & \beta_4 (EXTRA_LARGE_i) + \beta_5 (JUMBO) + \beta_6 (OMEGA_i) + \\ & \beta_7 (CARD_i) + \beta_8 (NO_HORMONES_i) + \beta_9 (FREE_i) + \beta_{10} (CAGE_i) - \\ & \beta_{11} (UEP_i) + \beta_{12} (CHOL_FAT_i) + \beta_{13} (DAVIS_i) + \epsilon_i \end{aligned}$$

where $PRICE_i$ is the price per egg of the i th observation, ϵ_i is a random error term, and γ is an intercept term.

Table 1. Description of Variables

Dependent Variable	Expected Sign	Description
<i>PRICE</i>	n/a	Price per egg
Independent Variables		
<i>EGGS/PKG</i>	negative	Quantitative Variable
<i>MEDIUM</i>	positive	= 1 if medium size egg, = 0 otherwise
<i>LARGE</i>	positive	= 1 if large size egg, = 0 otherwise
<i>EXTRA_LARGE</i>	positive	= 1 if extra large size egg, = 0 otherwise
<i>JUMBO</i>	positive	= 1 if jumbo size egg, = 0 otherwise
<i>OMEGA</i>	positive	= 1 if Omega (Ω) enrichment, = 0 otherwise
<i>CARD</i>	uncertain	= 1 for cardboard package = 0 for Styrofoam package
<i>NO_HORMONES</i>	positive	= 1 if no added antibiotics or hormones = 0 otherwise
<i>ORGANIC</i>	positive	= 1 if USDA Certification
<i>FREE</i>	positive	= 1 if from a free range hen, = 0 otherwise
<i>CAGE</i>	positive	= 1 if from cage free hen = 0 if from battery hen
<i>UEP</i>	negative	= 1 if United Egg Producer certified = 0 otherwise
<i>CHOL_FAT</i>	positive	= 1 if Cholesterol or Fat reduced, = 0 otherwise
<i>DAVIS</i>	positive	= 1 if from a Davis County store = 0 if from Weber County

It is also hypothesized that the coefficients value will take the value of $MEDIUM < LARGE < EXTRA_LARGE < JUMBO$.

RESULTS AND ESTIMATION

The results from the first regression show the model to be a good fit with an adjusted R^2 of 88.1%. However, it is important to note that certain variables in the regression do have strong correlations present, especially referring to many of the organic variables, which creates some multicollinearity issues. By definition *ORGANIC* is a function of *FREE*, *CAGE*, and *NO_HORMONES*, which creates problems in interpreting the results of the model. Based on these findings and the strong correlations between *ORGANIC*, *FREE*, *CAGE*, and *NO_HORMONES*, we decided to drop *ORGANIC* as a variable in our OLS regression and proceed with the regression found in Table 2.

Goodness of fit measure indicates that regression 2 had an adjusted R^2 value of 86.3. A Koenker-Bassett (KB) heteroscedasticity test was conducted on the second regression. At the 5% level we calculated a t-statistic of 1.72 and failed to reject the Null Hypothesis and conclude that the error terms are ho-

Table 2. Estimated Regression Results Using Ordinary Least Squares
(Dependent Variable = PRICE)

Variable	Coefficient	Standard Error	T-statistic
Intercept	0.13178	0.01913	6.89***
EGGS/PKG	-0.00200	0.00093	-2.05**
LARGE	0.02013	0.00940	2.14***
EXTRA_LARGE	0.03308	0.01051	3.15***
JUMBO	0.02641	0.01265	2.09**
OMEGA	0.02027	0.01139	1.78**
CARD	-0.01684	0.01192	-1.41*
NO_HORMONES	0.05346	0.01114	4.80***
FREE	0.06193	0.01140	5.43***
CAGE	0.06654	0.00952	6.99***
UEP	-0.01169	0.00655	-1.78**
CHOL_FAT	0.05874	0.01255	4.68***
DAVIS	0.00233	0.00518	0.45
Adjusted R ²	86.3%		
R ²	87.3%		
# of Observations	169		
F-Statistic	89.49		

Notes: Statistical significance is noted by asterisks (*). Single, double, and triple asterisks denote statistical significance at the 10%, 5%, and 1% respectively. *MEDIUM* was treated as the omitted variable.

moscedastic. The results also show that a priori expectations on the sign and the value of the coefficients were met with the following exception: jumbo eggs were found to be cheaper than extra large eggs but more expensive than medium and large eggs.

Turning to some of the variables that explain the organic nature of the egg, we found that the variable *CAGE* and *FREE* were both significant at the 1% level and had associated coefficients of \$.067 and \$.06 per egg respectively. Lastly, we found consumers tend to pay \$.05 per egg for an egg that has no antibiotics or hormones added to it. Karipidis et al. (2005) also found the following variables to be significant: *EGGS/PKG*, size of the egg, *OMEGA*, and *FREE*.

The estimated coefficients in Table 2 represent both the consumers' willingness to pay for certain characteristics of an egg as well as the incurred cost by the producers to produce those kinds of eggs. Applying these results to

the hedonic model show that these premiums represent the consumer's perceived value of the characteristic and the additional charge in price they are willing to pay to obtain such a characteristic.

CONCLUSION

This study was conducted by using the hedonic framework to evaluate what premium consumers are willing to pay for components of an organic egg, which captures the value consumers are willing to pay for an egg that is free of added hormones/antibiotics, cage free and free range. Results indicate consumers are willing to pay \$.18 for an egg that has these three components.

Lusk (2010) shows that supply shocks like the passing of Proposition 2 in California increased consumer demand for organic and cage free eggs. With the passage of similar propositions in other states, producers who are currently not producing organic eggs can expect production costs to rise due to the increased burden of meeting the new regulations. With these increased production costs as well as an increase in consumer demand for organic and cage free eggs, consumers may expect to see prices for eggs increase substantially in the short run. However, in the long run, when better technology and process are developed for cage free eggs, the prices of eggs would decline.

REFERENCES

- Dawkins, M.S., and S. Hardie. (1989). Space Needs of Laying Hens. *British Poultry Science*, 30.02, 413–416
- Dimitri, C. and C. Greene. (2002). Recent Growth Patterns in the U.S. Organic Foods Market. *Agriculture Information Bulletin*, 777. Washington, D.C.: U.S. Department of Agriculture, Economic Research Service.
- Gracia, Azucena, and Tiziana Magistris. (2008). The Demand for Organic Foods in the South of Italy: A Discrete Choice Model. *Food Policy*, 33. 5, 386-96.
- Karipidis, Philippos, Efthimia Tsakirdou, Nikolaos Tabakis, and Konstantinos Mattas. (2005) Hedonic Analysis of Retail Egg Prices. *Journal of Food Distribution Research*, 36.03.
- Lusk, Jayson L. (2010) The Effect of Proposition 2 on the Demand for Eggs in California. *Journal of Agricultural & Food Industrial Organization*, 8.1.
- Lusk, Jayson L., and F. Bailey Norwood. (2009) Some Economic Benefits and Costs of Vegetarianism. *Agriculture and Resource Economic Review*, 38.2.
- McGuire, Kelly B., Nicole Ownes, and Nathalie B. Simon. (2004) The Price Premium for Organic Babyfood: A Hedonic Analysis. *Journal of Agriculture and Resource Economics*, 29.01.

- Oberholtzer, Lydia, Catherine Greene, and Enrique Lopez. (2006). Organic Poultry and Eggs Capture High Price Premiums and Growing Share of Specialty Markets. *United States Department of Agriculture*. Outlook Report from the Economic Research Service. September 4, 2010, from <http://www.ers.usda.gov/Publications/LDP/2006/12Dec/LDPM15001/ldpm15001.pdf>
- Rosen, S. (1974). Hedonic Prices and Implicit Markets. *Journal of Political Economy*, 82. 1, 34-55.
- Stanley, Linda R. and John Tschirhart. (1991). Hedonic Prices for a Nondurable Good: The Case for Breakfast Cereals. *Review of Economics and Statistics*, 73.03, 537-541.

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Antimicrobials vs. Homeopathic Remedies

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Mentor: Scott Wright

ABSTRACT

In recent years, essential oils and colloidal silver have become popular homeopathic remedies. The purpose of this study was to compare the antibacterial effectiveness of these alternative medicines to commonly prescribed antimicrobials. Colloidal silver and seven essential oils from dōTERRA International, LLC were diluted and tested against strains of Streptococcus pyogenes and Escherichia coli. Two regularly prescribed antimicrobials were used as a comparison. The in vitro antimicrobial activity of each treatment was tested by broth macrodilution. The minimum bactericidal concentration (MBC) was determined for each treatment. MBCs were found for the antimicrobials and six of the oils against S. pyogenes and E. coli. One oil and the colloidal silver did not inhibit either bacterium.

INTRODUCTION

Traditional medicine involves doctors, hospitals, and prescription drugs; however, there are many individuals who strongly believe in the benefits of homeopathic or natural remedies in conjunction with traditional medicine. Due to growing bacterial resistance, alternative therapies are also gaining interest in the research community (Ferrini et al., 2006). Research is being conducted to determine if these alternative therapies have any potential antimicrobial properties.

Alternative or homeopathic medicines can include essential oils and colloidal silver among other treatment options. Essential oils are distilled from plant roots, leaves, stems and flowers. Colloidal silver is a solution that contains particles of silver in distilled water (Tien et al., 2008).

There have been contradicting studies done on colloidal silver. An experiment by van Hasselt and Ahmad (2004) claimed that colloidal silver showed no antimicrobial effects, while studies conducted by Tien et al. (2008) and Lansdown et al. (1997) claimed that there were strong antimicrobial proper-

ties in colloidal silver. Tien et al. (2008) also claimed that the antimicrobial effect was related to the concentration of silver particles. In 1994, a study done by Clement and Jarrett found that colloidal silver has potential in topical treatments.

Research done on the antimicrobial activity of essential oils has focused on the individual components of each oil, with very little research done to compare whole essential oils to antimicrobials. Two studies tested the antimicrobial effects of melaleuca oil but did not compare them to antimicrobials (Carsen & Riley, 1994; Carsen et al., 1995). Two Australian studies tested antimicrobials against essential oils; Sartorelli (2007) compared different species of eucalyptus against nystatin and chloramphenicol, and Ferrini et al. (2006) compared melaleuca against ampicillin, erythromycin, chloramphenicol, gentamycin, kanamycin, and vancomycin. Both studies showed that melaleuca oil had bactericidal activity against the bacterial species *Staphylococcus aureus*.

The intention of this research project was to expand on previous research by comparing the antimicrobial effects of colloidal silver and seven essential oils to two commonly prescribed antimicrobials. The colloidal silver, essential oils, and antimicrobials were tested against two bacterial species to determine if the home remedies were as effective as antimicrobial medications in vitro. The essential oils used were clove, cinnamon, rosemary, eucalyptus, wild orange, melaleuca, and OnGuard. The antimicrobials used were cephalexin and azithromycin. The colloidal silver, essential oils, and antimicrobials were tested against *Escherichia coli* and *Streptococcus pyogenes*.

MATERIALS

The essential oils used in this research project were donated by dōTERRA International, LLC (Orem, Utah). The seven oils used were clove (*Eugenia caryophyllata*), cinnamon (*Cinnamomum zeylanicum*), rosemary (*Rosmarinus officinalis*), eucalyptus (*Eucalyptus radiata*), wild orange (*Citrus sinensis*), melaleuca (*Melaleuca alternifolia*), and OnGuard (a proprietary blend of five oils including clove, cinnamon, rosemary, eucalyptus, and wild orange). The listed concentration for all oils except eucalyptus was 60 µg /1 drop. Eucalyptus oil is not considered suitable for internal use by the manufacturer. The colloidal silver concentration used was 500 ppm, and was purchased online from Vita Springs (www.vitasprings.com). Single strains of *Streptococcus pyogenes* (ATCC 19615) and *Escherichia coli* (ATCC 25922) were used.

The antimicrobials cephalexin and azithromycin were purchased in pill form using a research prescription from the Weber State University pharmacy. Cephalexin was used against *E. coli*. Azithromycin was used against

S. pyogenes. The dosage of each pill of cephalexin was 500 mg and each pill of azithromycin was 250 mg. The pills were crushed and suspended in Columbia broth prior to use. The concentration of the stock solution prepared for each antimicrobial was 250 mg/mL. A Beckton Dickinson BBL Crystal-Spec nephelometer was used to determine bacterial concentration. Columbia Sheep Blood Agar (SBA) plates were used to quantitate bacterial growth. 1 μ L inoculating loops were used to transfer the suspension from the tubes to the SBA plates. Tween 80 was used as an emulsifier for the oils.

METHOD

The NCCLS broth macrodilution method for aerobic bacteria was followed ("National Committee," 1990). Columbia broth was prepared to manufacturer's specifications, added to nephelometer tubes, and sterilized by autoclaving. Essential oils and colloidal silver were added to the tubes in concentrations of 0.03%, 0.06%, 0.12%, 0.5%, 1%, 2%, 4%, and 8% (v/v). Tween 80 was added in 0.5% (v/v) to all oil controls and test tubes to emulsify them into the broth. Antimicrobial stock solutions were prepared by suspending 250 mg of cephalexin and azithromycin in Columbia broth. Cephalexin was suspended in 25 mL of Columbia broth. Azithromycin was less soluble and required a suspension of 100 mL of Columbia broth. The antimicrobials were serially diluted in Columbia broth in concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μ g/mL. The controls for each assay included a broth control and broth with each of the following: each bacterium, colloidal silver, each essential oil and Tween 80, cephalexin and azithromycin.

E. coli and *S. pyogenes* were diluted in Columbia broth to 0.5 McFarland units. 10 μ L of the 0.5 McFarland units of each bacteria were then added to all appropriate tubes containing broth and either essential oils and Tween 80, colloidal silver, or antimicrobials. All tubes had a total volume of 6 mL.

These suspensions were mixed and incubated in a 37°C air incubator for 24 hours. Each tube was visually inspected for a minimum inhibitory concentration or clarity in the suspension. 1 μ L calibrated loops were used to plate the suspensions onto SBA plates, which were then incubated for 24 hours. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of treatment that eliminated all bacterial growth. Each bacterial assay was done in duplicate.

After the first assay, when colloidal silver did not prove effective against either type of bacteria, the concentration range of colloidal silver was increased to 10%, 20%, 30%, and 50% (v/v). The same method was used in this assay except for the increased concentrations. Increased concentrations of wild orange could not be done due to the inability of Tween 80 to emulsify the oil in broth past 8% without affecting results.

RESULTS

Six oils were able to inhibit the growth of *S. pyogenes* and *E. coli*. The MBC of cinnamon for both bacteria was 1,200 µg/mL or 0.12% (v/v). The MBCs of clove, melaleuca, and OnGuard for both bacteria was 5,000 µg/mL or 0.5% (v/v). Eucalyptus and rosemary had MBCs for *S. pyogenes* at 5,000 µg/mL and *E. coli* at 10,000 µg/mL or 1% (v/v). The MBC of cephalixin against *E. coli* was 64 µg/mL and the MBC of azithromycin against *S. pyogenes* was 16 µg/mL.

Colloidal silver and wild orange were ineffective in inhibiting the growth of *S. pyogenes* and *E. coli* and no MBCs could be determined from the initial concentration range. The increased concentrations of colloidal silver demonstrated MBCs against *S. pyogenes* and *E. coli* at 300,000 µg/mL or 30% (v/v) and 500,000 or 50% (v/v) respectively.

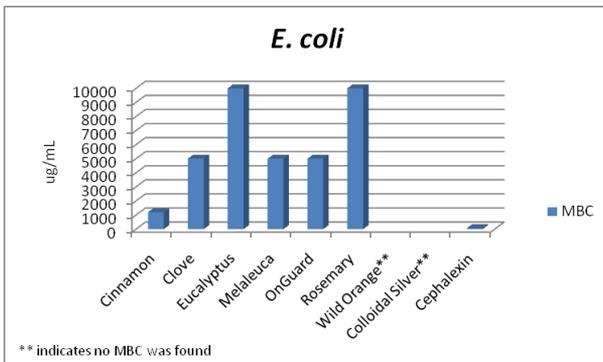


Figure 1. Minimum bactericidal concentrations that eliminated *E. coli*

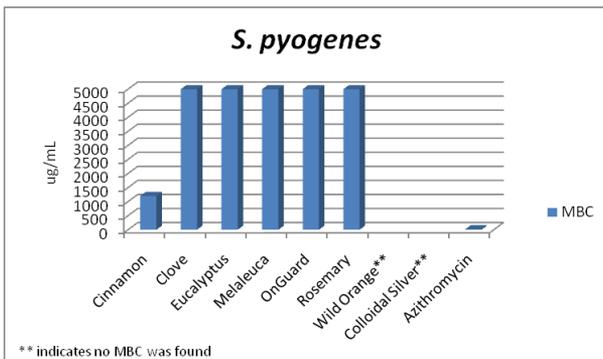


Figure 2. Minimum bactericidal concentrations that eliminated *S. pyogenes*

DISCUSSION

Six of the seven essential oils were effective *in vitro* against both *E.coli* and *S. pyogenes*. The effective oils were clove, cinnamon, melaleuca, eucalyptus, rosemary, and OnGuard. Wild orange and colloidal silver did not inhibit bacterial growth and proved ineffective against *S. pyogenes* and *E. coli*, which may eliminate them as treatment options. Previous research done at dōTERRA International measuring drops of essential oil concluded that one drop is 60 mg of oil, which is equal to 0.06 mL of oil. These calculations were used to convert the concentrations of the oils to the units of the antimicrobials ($\mu\text{g/mL}$).

CONCLUSION

Six oils showed significant antimicrobial properties *in vitro*. According to the MBCs, the essential oils were effectively bactericidal at higher concentrations than the antimicrobials. No MBC was determined for wild orange. The concentrations of colloidal silver were unable to inhibit either bacterium. The results of this study strongly support the hypothesis that homeopathic remedies have a place in medical research, especially as bacteria become increasingly drug-resistant. Further studies can be done to show whether using alternative remedies in conjunction with antimicrobials show a synergistic or antagonistic effect.

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REFERENCES

- Carsen, C., Hammer, K., & Riley, T. (1995). Broth micro-dilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Microbios*, 82, 181-185.
- Carsen, C., & Riley, T. (1994). Susceptibility of *Propionibacterium acnes* to the essential oil of *Melaleuca alternifolia*. *Lett Appl Microbiol*, 19, 24-25.
- Clement, J.L. and Jarrett, P.S. (1994). Antibacterial Silver. *Metal Based Drugs (BDM)*, 1.5-6, 467-482.
- Ferrini, A. M., Mannoni, V., Aureli, P., Salvatore, G., Piccirilli, E., Ceddia, T., et al. (2006). *Melaleuca alternifolia* essential oil possesses potent anti-staphylococcal activity extended to strains resistant to antibiotics. *Int J Immunopathol Pharmacol*, 19.3, 539-544.

- Lansdown, A., Sampson, B., Laupattarakasem, P., & Vuttivirojana, A. (1997). Silver aids healing in the sterile skin wound: experimental studies in the laboratory rat. *Br J. Dermatol*, 137, 728-735.
- Maudsley, F., & Kerr, K. G. (1998). Microbiological safety of essential oils used in complementary therapies and the activity of these compounds against bacterial and fungal pathogens. *Support Care Cancer*, 7, 100-102.
- National Committee for Clinical Laboratory Standards. (1990). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically - Second Edition. Vol. 10. Villanova: NCCLS.
- Sartorelli, P., Marquioreto, A. D., Amaral-Baroli, A., Lima, M. E., & Moreno, P. R. (2007). Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus. *Phytotherapy Research*, 21.3, 231-233.
- Tien, D.-C., Tseng, K.-H., Liao, C.-Y., & Tsung, T.-T. (2008). Colloidal silver fabrication using the spark discharge system and its antimicrobial effect on *Staphylococcus aureus*. *Medical Engineering & Physics*, 30, 948-952.
- van Hasselt, MD, PhD, P, Gashe, MSc, PhD, B.A., and Ahmad, MSc, PhD, J. (2004). Colloidal Silver as an antimicrobial agent: fact or fiction? *Journal of Wound Care (JWC)*, 13.4, 154-155.

Antinuclear Antibody Assay: Local Result Discrepancies in Testing Methods

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ABSTRACT

Antinuclear antibody (ANA) testing is used in assisting rheumatologists to diagnose and plan treatment regimens for patients with diseases such as systemic lupus erythematosus, rheumatoid arthritis, and scleroderma. To ensure accurate results for ANA testing at Davis Hospital and Medical Center, an in-house test by Enzyme Immuno-Assay (EIA) is run and all positive specimens are sent to an offsite laboratory for confirmatory testing by Immuno-Fluorescence Assay (IFA). Occasional discrepancies are found between these two methods. One contributing factor is the IFA's decreased sensitivity as compared to EIA's sensitivity. Of the total samples tested positive by EIA, 71% of them were negative by IFA. Fifty-four of these discrepant samples were sent to Bio-Rad for analysis on the Bio-Plex 2200. This multiplex analyzer tested 10 samples positive for the anti-SSa/Ro, anti-SSb/Lo, anti-RNP, and anti-dsDNA antinuclear antibodies with frequencies of occurrence at 9.26, 3.7, 3.7, and 1.8 respectively. A long-term study is recommended to reveal if these antibodies are indeed clinically significant. Of note is the manufacturer's post-study announcement of poor reagent stability for the EIA analyzer, which was used during most of this study, and may explain the increased quantity of discrepant results.

INTRODUCTION

Rheumatology is a field of study in which diagnosis for autoimmune diseases can be very difficult. Many of these disorders have a long list of diagnostic criteria that must be met. For example, a diagnosis of Lupus must meet 4 of 11 listed criteria (Sherer & Shoenfeld, 2007). Antinuclear Antibody (ANA) testing, as one criterion, is seen as a very beneficial tool in aiding the diagnosis of some rheumatic diseases. To establish a more accurate confirmatory system, it is common to test for ANAs by two or more methodologies. Because of the differences in sensitivity between the screening test and the titer test, in this case the EIA as screening method and IFA as titer, discrepancies

are common (i.e. positive through one method, negative through another). These discrepancies are usually assumed to be of little to no clinical significance.

It has been suggested that discrepancies might be due to specific antibodies which can be missed on the IFA either due to lack of antigens or low sensitivity for the given ANA (Dahle et al., 2009). The purpose of this study was to identify the occurrence and the frequency of these low fluorescence antibodies. This was done through a multiplexing immunoassay which can identify ANA type. The identification of these ANAs is important because “antibodies do not [all] have the same clinical significance: some are associated with both pathological and non-pathological conditions, while others are closely associated with [autoimmune disease]” (Desplat-Jego et al., 2009).

Previous research has established that some finer reactivities may show up on automated testing methods (i.e. EIA), when negative for fluorescence (IFA). In one instance there was a 17% increase in positive results for the automated methodology as opposed to IFA, at least 25% of which represented patients diagnosed with an autoimmune disease (Hoffman et al., 2002). Anti-SSA/RO, anti-SSB/La, and anti-Jo1 antibodies have all been implicated in similar negative fluorescence studies, as those were missed in the fluorescence assay in previous research (Hoffman et al., 2002). Despite these findings, the high discrepancy rate at Davis Hospital merits the need for this study. Knowledge of the cause of this discrepancy, which has the potential to occur in other hospital settings as well, will be important information for improved diagnosis of autoimmune disease.

MATERIALS AND METHODS

Serum samples were obtained from Davis Hospital and Medical Center Laboratory from December 2009 to March 2010. These samples had been sent in by local physicians for routine ANA testing. According to Laboratory procedure each sample was placed in a freezer and maintained at a temperature range of -20° to -30° C.

The samples were prepared for analysis on the EVOLIS analyzer according to the manufacturers directions found in the package insert (Bio-Rad Laboratories Inc., 2005). All supplies for the EVOLIS run were included in the Bio-Rad Autoimmune EIA ANA Screening Test Kit (catalogue #96AN).

Following testing, 150 μ L of serum was transferred into a new tube for all samples receiving a positive screen and labeled with a number that would connect it to the original sample. It was later found that 150 μ L was not adequate to account for dead volume needed for analysis on the Bio-Plex 2200

analyzer so a change to 400 μ L was necessary. Unfortunately, 87 samples were rejected as a result of this.

These tubes were then frozen in the same manner as mentioned above while the original tubes were sent to Labcorp (Burlington, North Carolina) for titer testing by IFA. Labcorp utilizes the epithelial (HEp-2) cells as substrate for their IFA testing at a cut off dilution of 1:80. Supplies for the IFA method came from DiaSorin Inc. (Stillwater, MN). Upon receiving the results from Labcorp, all samples confirmed positive by IFA were rejected from the study.

Fifty four samples were packed and sent frozen to Bio-Rad Laboratories (Hercules, California) to be run on their Bio-Plex 2200. The Bio-Plex 2200 analyzer utilizes heterogeneous sets of magnetic 8 μ m beads, which are infused with varying ratios of fluorescent dyes and ligand specific to a particular assay. It performs measurements of 13 autoantibodies in a single tube (Shovman et al., 2005). The multiplex detection technology reads each analyte a minimum of 150 times (Bio-Rad Laboratories, 2009).

RESULTS

Of the 54 samples analyzed on the Bio-Plex approximately 19% were positive for at least one ANA (table 1) (see figure 1 for frequencies). As anticipated, the multiplexing method showed stronger agreement with IFA, than with the EIA screening method.

Of all the samples analyzed at Davis Hospital over the 3 month duration of this study, 36% of them were screened positive by EIA. Of these positive samples, approximately 71% resulted as negative by IFA. Approximately 26% of total ANA screens sent to the hospital during this period demonstrated the apparent contradiction in test results (EIA to IFA) (table 2).

The Bio-Plex analyzer recovered ANAs in 10 specimens which had been missed by the IFA method. These were the SS-A/Ro (found in 4 samples), SS-B/La (found in 2 samples), RNP (found in 2 samples), and the dsDNA (found in 6 samples). Less clinically significant ANAs recovered were the Centromere B and Chromatin antibodies.

Table 1. Summary of test results among different methods

# of Specimens	Bio-Plex Result	EIA Result	IFA Result
10	Positive	Positive	Negative
44	Negative	Positive	Negative

Table 2. Results of ANA testing at Davis Hospital over a 3 Month Period

Date Run	Total Samples	Positive EIA	Negative EIA	Positive EIA & Negative EIA by IFA
12/23/2009	14	7	7	4
1/6/2010	39	18	21	13
1/11/2010	24	4	20	4
1/15/2010	29	10	19	10
1/19/2010	21	12	9	9
1/22/2010	22	8	14	8
1/30/2010	18	8	10	8
2/4/2010	26	8	18	7
2/11/2010	34	8	26	7
2/16/2010	15	6	9	3
2/22/2010	24	7	17	4
3/1/2010	35	19	16	8
3/8/2010	33	6	27	1
Totals	334	121	213	86

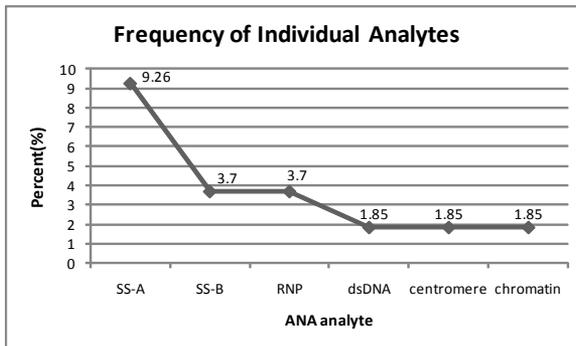


Figure 1. Frequency of ANA analytes among all samples analyzed*
 *Note: Ribosomal P, Sm, SmRNP, SCL-70, and Jo-1 were analyzed but not detected in any sample.

DISCUSSION

This study was done to identify possible causes of discrepant results found between EIA and IFA methods. Problems facing IFA include slide preparation, lot-to-lot variations, and type of substrate on the slide (Tonutti et al., 2004). Labcorp utilizes the HEp-2 cell for their IFA method, which has shown to have a confidence interval of 68-76% for low fluorescence auto-antibodies such as SS-a/RO and SS-b/La (Hoffman et al., 2002). Of inter-

est is the fact that stated ANAs are some of those missed frequently by IFA methodology.

The EIA is used as a screening method because it lacks specificity, meaning that it sometimes gives a positive result for normal sera. The EVOLIS analyzer is stated to yield a 92.8% specificity, which means it gives a negative result for normal sera 92.8% of the time (Bio-Rad Laboratories Inc., 2005). However, this study found the specificity of the EVOLIS ANA screen to be 67%. Furthermore, Labcorp's IFA only agreed with Davis's EIA 29% of the time. BioRad's EVOLIS instruction manual suggests it should agree with the IFA HEP-2 plate 86.1% of the time. These findings suggest possible reagent degradation, given the machine itself was operating according to the manufacturer's set standards.

On March 1, 2010 Bio-Rad issued a recall notification for certain lots of the 96 well test kits, which Davis Hospital uses. This letter stated that the integrity of the positive and negative controls was decreasing over a shorter range of time than current expiration dates suggested. It further suggested this may cause an increase in positive results for patient samples. This strongly supports why the EVOLIS may not have been operating according to the manufacturer's standards.

As previously stated, ANA testing is only part of diagnosing autoimmune diseases. Further study with larger sample sizes and a lengthy period of patient follow up to determine the significance of missed auto-antibodies should be done. Current research suggests some of these low fluorescent antibodies found by the Bio-Plex 2200 in this study may serve as markers for patients who could potentially develop an autoimmune disease (Shovman et al., 2005). Such studies may also facilitate standards and guidelines being set between manufacturers to decrease systematic errors.

CONCLUSION

The data in this study has shown that there is a significant number of false positives using the EIA method at Davis Hospital and Medical Center Laboratory. It further suggests the most likely reason for this may be the degradation of the EVOLIS ANA reagents before the printed expiration date. These types of errors are costly to the lab and to the patient. Davis and other institutions seeing these trends should evaluate them and take the steps necessary to decrease them by changing lot numbers of a current method or using a different manufacturer's product.

ACKNOWLEDGMENTS

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REFERENCES

- Bio-Rad Laboratories. (2009). *BioPlex 2200 System*. Hercules: Bio-Rad Laboratories, Inc.
- Bio-Rad Laboratories Inc. (2005). *Autoimmune EIA ANA Screening Test Instruction Manual*. Hercules: Bio-Rad.
- Check, W. (2009, September). *Cap Today: Making Sense of the ANA Hodgepodge*. Retrieved March 15, 2010, from CAP: <http://www.cap.org>
- Dahle, C., Skogh, T., Aberg AK., Jalal, A., Oleen, P. (2009). Methods of choice for diagnostic antinuclear antibody (ANA) screening: Benefit of adding antigen specific assays to immunofluorescence microscopy. *Journal of Autoimmunity*, 241-249.
- Desplat-Jego, S., Bardin, N., Larida, B., & Sanmarco, M. (2009). Evaluation of the Bioplex 2200 ANA screen for the detection of antinuclear antibodies and comparison with conventional methods. *Annals of the New York Academy of Sciences*, 245-55.
- Hoffman, I., Peene, I., Veys, E., & De Keyser, F. (2002). Detection of Specific Antinuclear Reactivities in Patients with Negative Anti-nuclear Antibody Immunofluorescence Screening Tests. *Clinical Chemistry*. Volume 48 , 2171-76.
- Sherer, Y., & Shoenfeld, Y. (2007). *Autoantibodies Guide in Systemic Lupus Erythematosus*. Hercules: Bio-Rad Laboratories.
- Shovman, O., Gilburd, B., Barzilai, O., Shinar, E., Larida, B., Zandman-goddard, G., Binder, R., & Shoenfeld, Y. (2005). Evaluation of the BioPlex 2200 ANA Screen. Analysis of 510 Healthy Subjects: Incidence of Natural/Predictive Autoantibodies. *Annals New York Academy of Sciences* , 380 - 388.
- Tonutti, E., Bassetti, D., Piazza, A., Visentini, D., Poletto, M., Bassetto, F., Caciagli, P., Villalto, D., Tozzoli, R., & Bizzaro, N. (2004). Diagnostic accuracy of Elisa Methods as an Alternative Screening Test to Indirect Immunofluorescence for the Detection of Antinuclear Antibodies. Evaluation of Five Commercial Kits. *Journal of Autoimmunity*. Volume 37, 171 - 176.
- Yilmaz, O., Karaman, M., Ergon, M., Bahar, I., & Yulug, N. (2004). Comparison of indirect immunofluorescence and enzyme immunoassay methods for the determination of antinuclear antibodies. *Mikrobiyoloji Bulteni* , 85-90.

Bone Specific Alkaline Phosphatase Reference Range for Children

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Mentors: William Roberts & Leonard Nielsen

ABSTRACT

Bone specific alkaline phosphatase (BSAP) is a glycoprotein found in the plasma membrane of osteoblasts. It is necessary for mineralization of bone and has been shown to be an indicator of bone turnover. Potential clinical applications for this assay include detection and monitoring of Paget's Disease, osteoporosis and bone related cancer (MacLaughlin, 2010). As more knowledge of this marker becomes available, testing for BSAP may become common among other disease states. Reference ranges have been established for women ages 7 and older and men ages 7-18 and 24 and older. We have established a reference range for children ages 6 months to seven years. To determine the reference ranges for children we used serum from the previously stated age groups and tested them on an analyzer using chemiluminescent immunoassay. We collected 70 samples from boys of each age group and 70 samples from girls of each age group. We then determined the appropriate reference range for clinical use by physicians, which will enhance the overall assessment and treatment of patients.

INTRODUCTION

A reference interval (RI) is often referred to as a normal range or reference range. RIs are established by assaying a large number of samples from healthy individuals and using the results to estimate where the central 95% of the population will fall. Estimating the central 95% of the population can seem simple but there are many important factors that must be taken into account: knowing what analytes are appropriate and what the cutoff should be, if sample participants are representative of the population the range will be used for, establishing it for the right subgroup, and obtaining enough samples to meet the recommendations of CLSI (ARUP Laboratories, 2009; CLSI document C28-A2).

Bone is a dynamic tissue in which formation and resorption continue throughout life by a process called remodeling. Remodeling is the function of a complex interaction between two types of bone cells: osteoblasts and osteoclasts. Osteoblasts are responsible for the formation of bone and osteoclasts for the resorption of bone. Under normal circumstances bone formation and resorption are kept in tight balance. This balance is important in maintaining the strength and organization of the skeletal system (Biegl-mayer & Kudlacek, 2009).

The level of BSAP in the serum is believed to reflect the metabolic status of the osteoblasts. An accurate level is important in assessing bone metabolism and determining the severity of metabolic bone disease. Total alkaline phosphatase measurements have previously been used to evaluate and diagnose bone disorders. Because the liver can also contribute to the total alkaline phosphatase level, BSAP would be the preferred method. Current methods have improved specificity and sensitivity to this enzyme, making it important in the clinical management of patients with bone disease.

The most widely used measurement of bone mineral density does not give enough information about the relationship between bone formation and resorption. BSAP is a very sensitive assay and along with other bone markers such as osteocalcin and procollagen type I N-terminal propeptide (PINP), a physician is able to closely monitor bone turnover (Avbersek-Luznik et al., 2007).

In healthy children bone is formed more readily than it is broken down. This reflects normal bone growth. In young adults the levels of bone formation and resorption are equal. In adults the rate of bone resorption is greater than the rate of formation. For these reasons it is important to have an established reference range for each stage of development.

MATERIALS AND METHODS

Samples were obtained from healthy children with no prior disease. Upon consent from the parents, a small amount of blood was taken from an IV that was already inserted, and was drawn into a serum separator tube. The serum was frozen prior to testing and relabeled for the individual groups to be tested. Grouping was differentiated by male or female for the ages 6 months, 1 year, 2 year, etc.... through 7 years of age. After calibration and quality control were performed and verified to be accurate, the samples were run on the Beckman-Coulter DxI 800. The DxI runs the samples by the following methods. A mouse monoclonal antibody specific to BSAP is added to a tube with paramagnetic particles coated with goat anti-mouse polyclonal antibody. Calibrators, controls, and specimens containing BSAP are added to the coated particles, and attach to the anti-BSAP monoclonal antibody. Fol-

lowing the formation of a solid phase/ BSAP complex, particles are separated in a magnetic field, and washed to remove materials not bound to the solid phase. A chemiluminescent substrate, Lumi-Phos 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the amount of BSAP in the specimen. The amount of BSAP in the specimen is determined by means of a stored, multi-point calibration curve (ARUP Laboratories, 2009). Upon completion of the sampling the results were processed by EP Evaluator 8.0. The transformed parametric (Gaussian) method was used, where 95% of the samples will fall within +/- 2 standard deviations (SD) of the mean. Outliers, values that were +/- 3 SD, were excluded from the reference range study. An outlier which is undetected and hence used in the calculation of the normal range will in general cause that range to be wider than it should be, and thus weaken the sensitivity of such a range as a predictor of disease (CLSI document C28-A2).

Method Limitations

1. Human anti-mouse antibodies (HAMA) may be present in specimens from patients who have received immunotherapy utilizing monoclonal antibodies (Hansen et al., 1993). Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient specimens (CLSI document C28-A2). This assay has been specifically formulated to minimize the effects of these antibodies on the assay.
2. Serum specimens with significant elevations of liver ALP activity may yield elevated results in the BSAP assay (Levinson, 1992).
3. Patients with metabolic bone disorders who have low levels of disease activity may have BSAP levels that fall within the expected values (Whyde, 1983).

RESULTS

Table 1. Male Mean

Age (years)	BSAP (µg/L)
0.5	72.24
1	74.02
2	60.36
3	57.27
4	58.96
5	65.2
6	61.37

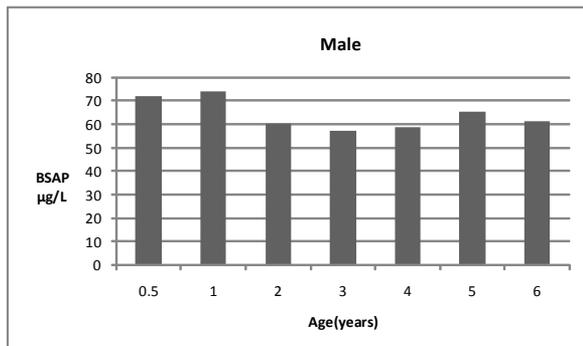


Figure 1. Male Mean

Table 2. Female Mean

Age (years)	BSAP (µg/L)
0.5	74.23
1	71.66
2	61.79
3	61.05
4	61.48
5	58.22
6	65.6

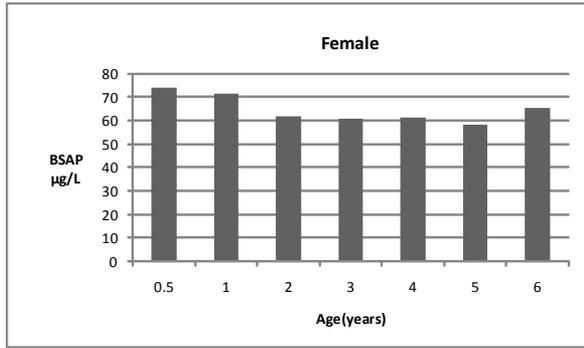


Figure 2. Female Mean

Table 3. New Reference Range

Age (years)	Male BSAP (µg/L)	Female BSAP (µg/L)
0.5	34.9-125.4	35.1-138.0
1	36.7-126.4	32.8-137.6
2	29.7-103.5	32.0-108.1
3	27.5-99.6	31.9-106.4
4	28.4-102.4	32.8-105.5
5	36.3-103.8	28.4-107.2
6	34.3-97.4	38.3-105.0

Table 4. Previously Established Reference Range (ARUP Laboratories, 2009)

Age (years)	Male BSAP (µg/L)	Female BSAP (µg/L)
8-9	48.6-140.4	36.3-159.4
10-12	48.8-155.5	44.2-163.3
13-15	27.8-210.9	14.8-136.2
16-17	15.3-126.8	10.5-44.8
≥ 25 yrs	6.5-20.1	4.5-22.4

DISCUSSION

A nonparametric method is preferred when there are at least 120 samples per group. A nonparametric method does not make the assumption that the results follow a Gaussian distribution, where the parametric method does. On average the sample groups measured had about 75 specimens, so the results were processed assuming they follow a Gaussian distribution. Certain groups may be combined due to the similarity of their ranges (CLSI docu-

ment C28-A2). Tables and figures 1 and 2 represent the means of the different age groups separated by sex. The data in Table 3 represents the newly established reference ranges for children six months through six years of age. Table 4 represents the previously established reference ranges that our data will be combined with.

CONCLUSION

BSAP is a recently discovered disease marker that physicians have begun to use for the diagnosis and monitoring of bone related diseases. As more is learned about the relationship of this marker with varying disease states, it will become more important to have a reference range for patient comparison. The reference ranges for BSAP that were generated from this study will serve as an important tool that physicians can use to detect and monitor young patients who are affected with bone related diseases. When used in conjunction with other bone markers, this assay has great potential.

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REFERENCES

- ARUP Laboratories "Bone Specific Alkaline Phosphatase (BSAP) on the Beckman-Coulter DxI 800" (April 2009)
- Avbersek-Luznik, Ivica, Tanja Gmeiner Stopar, and Janja Marc "Activity or mass concentration of bone-specific alkaline phosphatase as a marker of bone formation." *Clinical Chemistry & Laboratory Medicine* 45.8 (2007): 1014-1018. Academic Search Premier. EBSCO. Web. 30 Sept. 2009
- Bieglmayer C, Kudlacek S. The bone marker plot: an innovative method to assess bone turnover in women. *European Journal of Clinical Investigation* [serial on the Internet]. (2009, Mar), [cited April 12, 2010]; 39(3): 230-238. Available from: Academic Search Premier.
- CLSI document C28-A2. How to Define and Determine Reference Intervals in the Clinical Laboratory, Approved Guideline 2nd Edition 2000 Clinical and Laboratory Standards Institute Wayne, PA.
- Haliloglu B, Aksungar F, Ilter E, Peker H, Akin F, Ozekici U. Relationship between bone mineral density, bone turnover markers and homocysteine, folate and vitamin B12 levels in postmenopausal women. *Archives of Gynecology & Obstetrics* [serial on the Internet]. (2010, July), [cited April 12, 2010]; 281(4): 663-668. Available from: Academic Search Premier.

- Hansen HJ, et al. HAMA Interference with Murine Monoclonal Antibody-based Immunoassays. *J Clin Immunoassay* 1993; 16: 294-299
- Levinson SS. The Nature of Heterophilic Antibodies and the Role in Immunoassay Interference. *J Clin Immunoassay* 1992; 15: 108-115
- MacLaughlin E. Improving osteoporosis screening, risk assessment, diagnosis, and treatment initiation: Role of the health-system pharmacist in closing the gap. *American Journal of Health-System Pharmacy* [serial on the Internet]. (2010, Apr 3), [cited April 12, 2010]; 67S4-S8. Available from: Academic Search Premier.
- Schafer A, Vittinghoff E, Ramachandran R, Mahmoudi N, Bauer D. Laboratory reproducibility of biochemical markers of bone turnover in clinical practice. *Osteoporosis International* [serial on the Internet]. (2010, Mar), [cited April 12, 2010]; 21(3): 439-445. Available from: Academic Search Premier.
- Whyde MP. Alkaline Phosphatase and the measurement of Bone Formation, *Clinical Disorders of Bone and Mineral Metabolism*. Potts JT and Frame B, EDS., 1983, 12-125.

Interactions of *Lactobacilli* with Pathogenic *S. Pyogenes*

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Mentor: Travis M. Price

ABSTRACT

Lactobacillus bacteria produce lactic acid as a byproduct of metabolism, reducing the pH of surrounding areas to an acidic level in which few other organisms thrive. Nonpathogenic *Lactobacillus* species are the predominant flora in the vagina and minimize the opportunity for infection by common vaginal pathogens. Research has proven that various *Lactobacillus* species are capable of inhibiting the growth of bacterial and fungal pathogens, including *Candida albicans*, *Escherichia coli*, and *Neisseria gonorrhoeae*. Cases of bacterial vaginosis caused by *Streptococcus pyogenes* have increased over the last two decades. Since the rise in incidences is recent, the interactions of *S. pyogenes* with *Lactobacillus* bacteria have not been extensively studied. This research investigates whether a decreased concentration of *Lactobacillus* bacteria allows *S. pyogenes* to grow, or whether *S. pyogenes* is able to grow in the presence of healthy *Lactobacillus* concentrations. Understanding the interactions between *Lactobacilli* and *S. pyogenes* leads to a greater understanding of how to prevent and treat such infections.

INTRODUCTION

Lactobacillus bacteria (*Lactobacilli*) are large Gram positive rods that exist as nonpathogenic microbiota. *Lactobacilli* have been extensively studied due to their remarkable ability to inhibit the growth of other organisms through bactericidal activity and by producing lactic acid as a byproduct of metabolism (Hawes, 1996; Mårdh, 1983). Lactic acid production, production of bacteriocins, and production of hydrogen peroxide have led to an abundance of research involving the ability of *Lactobacilli* to inhibit pathogens. *Lactobacillus* species have proven highly effective at inhibiting the growth of bacterial and fungal pathogens which commonly cause vaginosis. *Lactobacillus* species, specifically *L. crispatus* and *L. jensenii*, are the predominant flora in the vagina, and thus minimize opportunities for infection (Athanasiou, 2006; Athanasiou, 2007; Boskey, 1999; Juárez Tomás, 2003; Larsen, 2001;

St. Amant, 2002). Several common pathogens that *Lactobacilli* inhibit are: *Candida albicans*, *Escherichia coli* (including *E. coli* O157:H7) and *Neisseria gonorrhoeae* (Athanasίου, 2006; Athanasίου, 2007; Hawes, 1996; Juárez Tomás, 2003; Mårdh, 1983; Ogawa, 2001; St. Amant, 2002).

Due to their ability to inhibit other organisms, *Lactobacilli* are commonly used for probiotic therapy to enhance intestinal microbiota, as well as to treat vaginosis. The purpose of this treatment is to increase the concentration of *Lactobacilli*, which will inhibit pathogens and allow the body's immune system to overcome the infection without the use of antimicrobials (Athanasίου, 2007; Ogawa, 2001).

Streptococcus pyogenes, often referred to as Group A strep, is a Gram positive coccus which tends to group together in chains. *S. pyogenes* causes the infection commonly known as "strep throat," and is the cause of 90% of bacterial pharyngitis cases. It can cause impetigo, erysipelas (cellulitis), toxic shock syndrome, and necrotizing fasciitis (also known as "flesh-eating strep"). Untreated infections may lead to acute glomerulonephritis, scarlet fever, or rheumatic fever. It has many virulence factors that contribute to its pathogenicity, such as: lipoteichoic acid, M protein, hyaluronidase, protease, streptokinase, DNase/RNase, C5a peptidase, and Streptolysins O and S. These allow the bacteria to hemolyze blood cells, spread throughout the body, adhere to surfaces, and necrotize tissues (Bauman, 2007) .

S. pyogenes can exist in the vagina (Larsen, 2001), but it was not previously considered a cause of bacterial vaginosis (Donald, 1991; Permar, 1917; Stricker, 2003). However, the recorded incidence of bacterial vaginosis caused by *S. pyogenes* has increased during the past two decades (Efstratiou, 2000; Petersen, 2006; Sweet, 2001). Studies regarding this increased prevalence suggest that the pathogen is introduced to the genital area by persons that carry *S. pyogenes* in their respiratory tract as either normal flora or as a pharyngeal infection (Donald, 1991; Funaro, 2007; Hansen, 2007; Morris, 1971; Meltzer, 2008; Stricker, 2003). In response to the increase in occurrence, many laboratories are beginning to make changes to protocols regarding the detection of *S. pyogenes*. Many protocols now include *S. pyogenes* as a potential vaginal pathogen that, in addition to other vaginal pathogens, needs to be identified when present.

Research indicates that in most cases of bacterial vaginosis, the *Lactobacillus* concentration is notably decreased (Levison, 1979; Meltzer, 2008; Ogawa, 2001), thus allowing an infection to take place. The decreased concentration of *Lactobacilli* is often due to the use of antimicrobials. This study addresses the following questions:

1. Does a decreased concentration of *Lactobacilli* allow *S. pyogenes* to grow?
2. Is *S. pyogenes* able to grow in the presence of healthy *Lactobacillus* concentrations?
3. Is *S. pyogenes* capable of inhibiting *Lactobacilli*?

Previous studies show that the average healthy vagina has a concentration of about 106 colony-forming units per milliliter (CFU/mL) of *Lactobacilli*. The average healthy vaginal pH is about 3.5-4.8 (Boskey, 1999; Larsen, 2001; Mania-Pramanik, 2008; Sweet, 2001). However, the pH can be as high as 8.0, depending on which part of the menstrual cycle is occurring (Levison, 1979; Marrazzo, 2002). Symptomatic cases of vaginosis usually have low concentrations of *Lactobacilli*, accompanied by an increased pH (≥ 7.0) (Larsen, 2001; Mårdh, 1983; Marrazzo, 2002; St. Amant, 2002).

MATERIALS AND METHODS

In order to minimize variation in *Lactobacillus* species that might be found in clinical specimens, strains of *L. crispatus* and *L. jensenii* were purchased from the American Type Culture Collection (ATCC). *L. crispatus* (ATCC 33197) and *L. jensenii* (ATCC25258) were mixed in sterile Columbia broth (Fisher Scientific, Pittsburgh, PA) to concentrations of 108, 106, 104, and 103 CFU/mL (Marrazzo, 2002). The 106 is representative of the average *Lactobacillus* concentration in a healthy female (St. Amant, 2002). A higher than average concentration was set up to represent the females who have more *Lactobacillus*, along with two lower concentrations to represent individuals who would be at a higher risk of infection. Columbia broth was chosen because it did not favor the growth of either organism, and it allowed the fluctuations in pH to be measured easily.

The source of bacterial vaginosis caused by *S. pyogenes* is suspected to be the throat, so 150 positive *S. pyogenes* throat screens were donated by Ogden Clinic in Ogden, Utah. Swabs in airtight tubes were transported in biohazard bags less than one mile to the university laboratory. Subcultures were performed in order to isolate and verify the identity of *S. pyogenes*. Research indicates that the average concentration of pathogens which cause vaginosis is about 103 CFU/mL (Petersen, 2006). However, the concentration of *S. pyogenes* in saliva has not yet been studied. A preliminary experiment was conducted to determine what concentration to use. For this preliminary experiment, concentrations of *S. pyogenes* at 102 and 103 CFU/mL were grown with *Lactobacilli* at 106 CFU/mL. A concentration of 103 CFU/mL was used, based on the colony counts from these concentrations.

A nephelometer, an instrument that measures the turbidity of liquid solutions, was used to measure the concentrations of the bacteria in the broth

preparations. This was then followed by serial dilutions to achieve the various desired concentrations. Three mL preparations of *S. pyogenes* at 103 CFU/mL were mixed with three mL of each of the four different concentrations of *Lactobacilli*. Each of these four mixtures was then plated on Columbia Sheep Blood Agar (SBA) using calibrated 1.0 μ L loops. The broth mixtures and SBA plates were incubated in a carbon dioxide incubator at 37 °C for 48 hours. After 48 hours, colonies were counted on the SBA plates, and pH measurements were taken from each broth mixture. Colonies were counted at 48 hours because the *Lactobacillus* colonies were larger and easier to count after 48 hours. There was no difference in colony counts or pH levels between 24 and 48 hours.

To validate the methodology used in this study, the same process was repeated using *Escherichia coli*, *Streptococcus agalactiae* (Group B strep), *Staphylococcus epidermidis*, and *Staphylococcus aureus*, which are known to be inhibited by *Lactobacilli*. Each of these was plated on an SBA plate before mixing with the *Lactobacillus* concentrations to compare to the growth in the presence of *Lactobacilli*. One hundred μ L were taken from the broth preparations before mixing with the *Lactobacilli* and were incubated along with the mixtures. The pH of the broth before mixing was taken after incubation to compare to the pH of the mixture. In each case, growth of the pathogens was inhibited, and pH was lowered. Utilizing these pathogens as controls proves that the conditions used in the methodology allowed the *Lactobacilli* to inhibit other organisms as they would in the body.

RESULTS AND DISCUSSION

In each of the samples, no inhibition of *S. pyogenes* by *L. crispatus* and *L. jensenii* was observed. Statistical tests (t-tests at an alpha level of $\alpha = 0.01$) showed no significant difference between the colony counts of *S. pyogenes* by itself and growth with the *Lactobacilli*. In the case of most pathogens, a higher concentration of *Lactobacilli* would cause more inhibition. However, statistical tests (ANOVA, $\alpha = 0.01$) also showed no significant difference between the colony counts of *S. pyogenes* in the four different concentrations of *Lactobacilli*. The difference in colony-forming units for each *S. pyogenes* isolate was most likely due to technical error during the serial dilutions and/or random error due to the differing strains of *S. pyogenes*.

The pH of the broth for each sample was reduced from the starting pH of 8.5 to a pH range of 4.5-7.0. The pH of the broth only containing *S. pyogenes* was on average 5.5-6.5, suggesting that the optimal pH of *S. pyogenes* is within the range considered asymptomatic for bacterial vaginosis (symptomatic pH is ≥ 7.0).

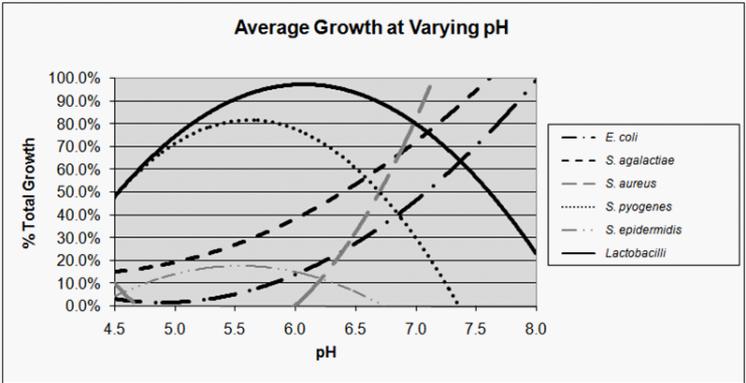


Figure 1. Percent of total growth of each organism, comparing growth with *Lactobacilli* to growth without *Lactobacilli*.

Table 1. Average growth and pH of the broth mixtures.

	10 ⁸ CFU/mL	10 ⁶ CFU/mL	10 ⁴ CFU/mL	10 ³ CFU/mL
Average colony counts of <i>Lactobacilli</i>	>100	>100	10	4
Average colony counts of <i>S. pyogenes</i>	13	14	13	11
Average pH	6.0	6.5	6.5	6.5

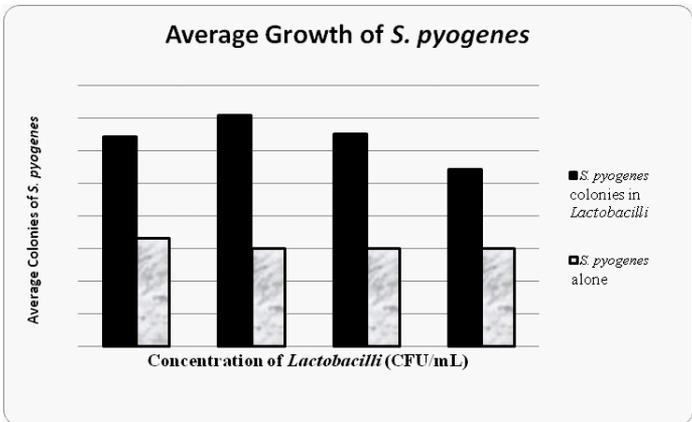


Figure 2. Average growth of *S. pyogenes*, suggesting that *Lactobacilli* may assist the growth of *S. pyogenes*

Figure 1 shows the growth patterns from data collected of the organisms used in this study. With the exception of *S. pyogenes* and *S. epidermidis*, the pathogens grew optimally in conditions where the *Lactobacillus* concentrations were decreased. *S. epidermidis* can also be normal vaginal flora, but is inhibited to nonpathogenic levels by the *Lactobacilli*. *S. pyogenes* grew optimally in the same conditions as the *Lactobacilli*. It is possible that higher concentrations of *Lactobacilli* may actually assist the growth of *S. pyogenes*. Limitations of this study included the elevated pH of the broth medium and technical error associated with serial dilutions. The elevated pH of the broth medium did not allow data at any pH lower than 4.5. The process of serial dilutions allowed the possibility of pipetting error which may have influenced the colony counts.

It is suggested that medical personnel treat vaginosis caused by *S. pyogenes* using antimicrobial therapy, as they would treat other *S. pyogenes* infections. It is also suggested that *Lactobacillus* probiotic therapy not be used as the sole means to treat bacterial vaginosis caused by *S. pyogenes*. The statistical data of the growth of these two organisms suggests that *Lactobacilli* did not inhibit the growth of *S. pyogenes*. Also, *S. pyogenes* did not inhibit the growth of *Lactobacilli*.

REFERENCES

- Athanasiou, S., Betsy, G. I., Falagas, M. E. (2006). Probiotics for Prevention of Recurrent Vulvovaginal Candidiasis: A Review. *Journal of Antimicrobial Chemotherapy*, 58(2), 266-272.
- Athanasiou, S., Betsy, G. I., Falagas, M. E. (2007). Probiotics for the Treatment of Women with Bacterial Vaginosis. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 13(7), 657-664.
- Bauman, R. W., Machunis-Masuoka, E., and Tizard, I. (2007). *Microbiology With Diseases by Taxonomy*: Pearson Education, Inc.
- Boskey, E. R., Telsch, K.M. Whaley, K.J., et al (1999). Acid Production by Vaginal Flora in Vitro is Consistent with Rate and Extent of Vaginal Acidification. *Infection and Immunity*, 67(10), 5170-5175.
- Donald, F. E., Slack, R. C. B., and Colman, G. (1991). Streptococcus pyogenes Vulvovaginitis in Children in Nottingham. *Epidemiology and Infection*, 106(3), 459-465.
- Efstratiou, A. (2000). Group A Streptococci in the 1990s. *Journal of Antimicrobial Chemotherapy*, 45, 3-12.
- Funaro, D., Kaplan, E. L., Sobel, J. D. (2007). Recurrent Group A Streptococcal Vulvovaginitis in Adult Women: Family Epidemiology. *Clinical Infectious Diseases*, 44(5), E43-E45.

- Hansen, M. T., Sanchez, V. T., Eyster, K., et al (2007). Streptococcus pyogenes Pharyngeal Colonization Resulting in Recurrent, Prepubertal Vulvovaginitis. *Journal of Pediatric Adolescent Gynecology*, 20, 315-317.
- Hawes, S. E., Hillier, S. L., Benedetti, J, et al (1996). Hydrogen Peroxide-Producing Lactobacilli and Acquisition of Vaginal Infections. *The Journal of Infectious Diseases*, 174, 1058-1063.
- Juárez Tomás, M. S., Ocaña, V. S., Wiese, B., et al (2003). Growth and Lactic Acid Production by Vaginal Lactobacillus Acidophilus CRL 1259, and Inhibition of Uropathogenic Escherichia coli. *Journal of Medical Microbiology*, 52, 1117-1124.
- Larsen, B., Monif, G. R. G. (2001). Understanding the Bacterial Flora of the Female Genital Tract. *Clinical Infectious Diseases*, 32, e69-77.
- Levison, M. E., Trestman, I., Quach, R., et al (1979). Quantitative Bacteriology of the Vaginal Flora in Vaginitis. *American Journal of Obstetrics and Gynecology*, 133(2), 139-144.
- Mania-Pramanik, J., Kerkar, S.C., Mehta, P.B., et al (2008). Use of vaginal pH in diagnosis of infections and its association with reproductive manifestations. *Journal of Clinical Laboratory Analysis*, 22(5), 375-379.
- Mårdh, P., Soltész, L. V. (1983). In Vitro Interactions Between Lactobacilli and Other Microorganisms Occurring in the Vaginal Flora. *Scandinavian Journal of Infectious Diseases*, 40, 47-51.
- Marrazzo, J. M., Koutsky, L. A., Eschenbach, D. A., et al (2002). Characterization of Vaginal Flora and Bacterial Vaginosis in Women Who Have Sex with Women. *The Journal of Infectious Diseases*, 185, 1307-1313.
- Meltzer, M. C., Schwebke, J. R. (2008). Lactational Amenorrhea as a Risk Factor for Group A Streptococcal Vaginitis. *Clinical Infectious Diseases*, 46(10), 112-115.
- Morris, C. A. (1971). Seasonal Variation of Streptococcal Vulvo-Vaginitis in an Urban Community. *Journal of Clinical Pathology*, 24, 805-807.
- Ogawa, M., Shimizu, K., Nomoto, K., et al (2001). Inhibition of in Vitro Growth of Shiga Toxin-Producing Escherichia coli 0157:H7 by Probiotic Lactobacillus strains due to Production of Lactic Acid. *International Journal of Food Microbiology*, 68, 135-140.
- Permar, H. H. (1917). An Analysis of the Vaginal Flora in Late Pregnancy. *The American Journal of Obstetrics and Diseases of Women and Children*, 75, 652-661.
- Petersen, E. E. (2006). *Infections in Obstetrics and Gynecology: Textbook and Atlas*: Thieme.
- St. Amant, D. C., Valentin-Bon, I. E., Jerse, A. E. (2002). Inhibition of Neisseria gonorrhoeae by Lactobacillus Species That are Commonly Isolated from the Female Genital Tract. *Infection and Immunity*, 70(12), 7169-7171.

- Stricker, T., Navratil, F., Sennhauser, F.H. (2003). Vulvovaginitis in Prepubertal Girls. *Archives of Disease in Childhood*, 88(4), 324-326.
- Sweet, R. L., Gibbs, R. S. (2001). *Infectious Diseases of the Female Genital Tract* (4 ed.): Lippincott Williams & Wilkins.

Lyme Disease: A Potential Deferral For Blood Donation

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ABSTRACT

Borrelia burgdorferi, a spirochete, is the etiological agent in Lyme Disease (LD). The National Bone Marrow Registry has a permanent deferral for donation if infected with LD. The AABB, formerly known as the American Association of Blood Banks, however, relies on non-specific screening questions to defer infected individuals. *B. burgdorferi* is viable in stored blood products and can be transmitted via transfusion (Badon et al., 1989). This study explored the prevalence of individuals infected with LD who donated blood. Surveys were sent to online state support groups. The survey was designed to gather information regarding the number of years it took to receive an accurate diagnosis of LD, whether individuals donated blood prior to diagnosis, and the states where infection was present. Five hundred and five surveys were completed from forty-five states. Of that number, 97% (486/503) of participants had LD. Two to five years was the prevailing time for correct diagnosis of LD. Of the total donations, 24.5% of individuals donated blood prior to diagnosis, and 8% had a co-infection of *Babesia microti*. The results highlighted incidences of infected people donating blood prior to a correct diagnosis of LD. This, coupled with the fact that *B. Burgdorferi* is sustainable in blood products, supports the need for either LD testing or a focused pre-donation screening question.

INTRODUCTION

Borrelia burgdorferi is the organism that causes Lyme Disease (LD). The vector that carries LD is the deer tick. There are estimated 160,000 to 320,000 reported and unreported cases of LD a year (Feder & Whitaker, 1995). Early symptoms of LD include fever, headache, stiff neck, and achiness. These early symptoms are often mistakenly diagnosed as a viral illness. In the early stage of LD about 70% of patients develop erythema migrans (EM), known as the classic bulls-eye rash (Lyme Disease symptoms, 2007). While EM is the

main presenting factor used for diagnosis, it is often missed even in endemic areas (Feder & Whitaker, 1995). The second stage of the disease can start a few weeks to months after the initial infection, with symptoms ranging from mild to severe; including facial paralysis, arm and leg numbness, and meningitis. The chronic (tertiary) stage includes symptoms of muscle pain, arthritis, cardiac, and neurological problems (Lyme Disease: A Patient's Guide; Lyme Disease, 2009). Misdiagnosis causes delays in treatment leading to chronic LD (Aucott et al., 2009). It is possible to be infected without showing outward symptoms or signs and/or have negative test results (Aucott et al., 2009).

The AABB regulates national standards for blood donor questionnaire requirements. Individuals planning on donating blood must answer a pre-screening questionnaire. Every donation center across the country must use the AABB questionnaire, while adding their own pre-donation questions that are referred to as “locality” questions. Locality questions are designed to identify endemic issues specific to local areas, such as LD. The Red Cross suggests, without requiring, that all blood donation centers should have a screening question for LD (Altman, 1989). Presently, the National Bone Marrow Registry asks all donors if they have chronic LD. If so, donors are permanently deferred (Bone Marrow Donors; Medical Guidelines, 1996). The New York Blood Donation Center has a six-month deferral if symptomatic for LD (Who Can Give Blood, 2009). Our research focused on discovering how many LD positive individuals donated blood prior to diagnosis to determine the need for testing or a focused screening question on the blood donor questionnaire.

MATERIALS AND METHODS

A survey conducted through www.surveymonkey.com, and included the following questions:

1. Do you have Lyme disease, and if so what tests were used to diagnose it?
2. How long do you believe you had Lyme disease prior to being diagnosed?
3. Did you ever donate blood during that time period, prior to your diagnosis?
4. Do you have any feelings about donating while infected?
5. If you donated blood, why did the questions “Do you have a fever” and “are you feeling healthy and well?” not dissuade you from donating?
6. All Answers will remain private, anonymous and secure; they will be used for research purposes in trying to get a Lyme disease

question added to the AABB blood donor questionnaire. Please indicate that you have read this statement. If you consent to the use of your information please type “Yes”. No signatures please.

7. What state do you live in?
8. Comments

The participation in the survey was recruited through online LD support groups listed at <http://www.lymenet.org/SupportGroups>. The group emailed a survey link to each director of support groups listed within those states. It was requested of each director to disseminate the email to support group members. The survey was left open for two months before compiling all the information on a designated date. The information from the survey was exported into an Excel spreadsheet and the data was analyzed by yes or no answers, numerical information, and state location.

RESULTS

There were 512 survey responses, of which, 17 were excluded because they reported not having LD. Six surveys from United Kingdom or Canada were not used. Three surveys were excluded because they were repeats. Out of 486 people who had LD, 119 (24.5%) stated they had donated blood while being infected prior to diagnosis.

The length of time an individual had the infection prior to diagnosis ranged from <1 to 50 years. The majority of participants were in the 2-5 year group, accounting for 23.8%, followed by 6-10 years at 17.4%, and <1 year at 16.8%. Of fifty states, 45 had support groups that could be contacted. Figure 1 shows the states that had at least 15 LD positive respondents and respective blood donors. Respondents reported other infectious diseases (23 *Bartonella*, 12 *Ehrlichiosis*, and 10 unidentified co-infections). One noteworthy disease and most reported co-infectious disease was *Babesia microti*. Forty-three individuals reported having *Babesia*. Of those, 33% donated blood unaware they were infected with *Babesia* prior to their LD diagnosis.

DISCUSSION

According to the CDC, LD is the most common vector borne disease in the United States (Lyme Disease --- United States, 2003--2005, 2007). When donating blood, all donors are given an AABB pre-donor questionnaire, which includes the question “Are you feeling healthy and well today?” When asked why this question did not dissuade people from donating, the most frequent responses were that they did not feel sick at the time or they had been told by doctors they were fine. The question from the AABB questionnaire does not adequately identify potential donors who do not experience symptoms associated with *Babesia*. In a transfusion transmitted *Babesia* study, “donors

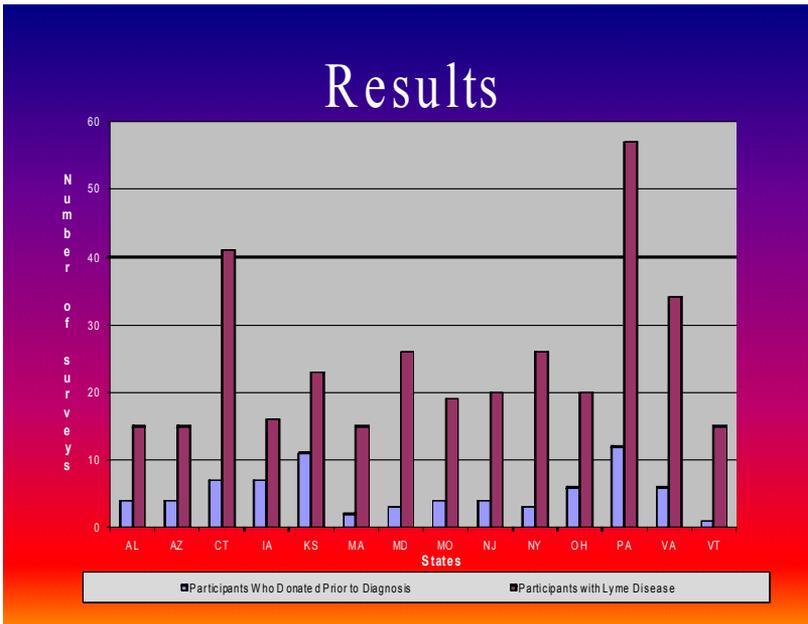


Figure 1. States that had at least 15 LD positive respondents & respective blood donors

did not recall any symptoms around the time of donation.” (Tonnetti et al., 2009). In reference to question #41 “Have you EVER had babesiosis?,” if a donor answers “yes” to this question they are indefinitely deferred, as it is transmissible through blood (Blood Donor History Questionnaire, 2010).

B. burgdorferi has been found viable in all four components of blood products and therefore can be transfused to three people receiving different transfusions (Badon et al., 1989). Consideration should be directed to the fact that these patients may already have compromised systems and receiving an infected unit could be deadly. Despite the fact that there is currently no documented case of LD being transmitted through transfusion, there is a real possibility that cases exist and are missed due to the complexity of diagnosing the disease (Cable et al., 2003).

Research has shown transmission in immune compromised mice and describes possible reasons why documented human cases have not been found (Gabitzsch et al., 2006). One conclusion is the spirochetemic phase is so short and transmission during that time is unlikely (Gerber et al., 1994). Looking at *Treponema palladium*, another spirochete, it is said that 25% of untreated syphilis carriers have recurring spirochetemic phases (Green & Goldman, 2009). This could be cause for concern, since 65.7% of respondents in our study were diagnosed more than 1 year after initial infection and the chance of a reoccurrence of the spirochetemic phase is high.

After concluding our survey, we discovered limitations that would need to be addressed in further research. Limitations include respondents that filled out one survey, answering one question for multiple family members, but did not follow through on all questions. This would have to be addressed in the directions if another survey were to be done. This study has no way of knowing whether all LD positive respondents are positive within CDC guidelines, opening the question for debate as to whether or not it is a “true” diagnosis. Any further surveys would specifically ask if individuals with LD also tested positive for *Babesia*.

The documentary “Under our Skin” clearly shows the lack of medical training leading to misdiagnosis and inadequate treatment of many LD infected individuals. It highlights that the two week antibiotic treatment guidelines are insufficient (Wilson, 2008).

While the probability of transmitting LD through blood products is still being researched, it is highly supported that people with LD donate blood prior to any diagnosis. The various stages of LD are perplexing and new testing practices, along with more research, needs to be done (Depietropaolo et al., 2005; Johnson et al., 1990). Our research clearly shows that current questions on the pre-donor questionnaire do not stop LD infected people from donating blood. Studies need to be done to determine the overall impact to the blood supply if a general tick exposure question is asked or a specific *B. burgdorferi* screening test is performed as part of the blood donor screening process.

REFERENCES

- Altman, L. (1989, Jul 18). The Doctor’s World; Lyme disease From a Transfusion? It’s unlikely, but Experts are wary. *New York Times*. Late Edition (East Coast); ProQuest Newsstand, ProQuest.
- Aucott, John; et al. (2009). Diagnostic challenges of early Lyme disease: lessons from a community case serious. *BMC Infectious Diseases* 9, 79.
- Badon, S., Fister, R., & Cable, R. (1989). Survival of Borrelia Burgdorferi in Blood Products. *Transfusion*, 29(7), 581-583.
- Blood Donor History Questionnaire*. (2010, Feb 4). Retrieved March 18, 2010, from AABB: <www.aabb.org/Content/Donate_Blood/Donor_History_Questionnaires/Blood_Donor_History_Questionnaire/dhq.htm>
- Bone Marrow Donors*. (n.d.). Retrieved Oct 7, 2009, from DKMS: <<http://www.dkmsamericas.org/category/bone-marrow-donors/eligibility>>
- Cable, Ritchard, G., & Leiby, D. (2003). Risk and prevention of transfusion-transmitted Babesiosis and other tick-borne diseases. *Current Opinion in Hematology*, 10(6), 405-411.
- Depietropaolo, D., Powers, J., & Gill, J. (2005). Diagnosis of Lyme Disease.

- American Family Physician*, 72(2), 297-303.
- Feder, H., & Whitaker, D. (1995). Misdiagnosis of Erythema Migrans. *The American Journal of Medicine*, 99(4), 412-419.
- Gabitzsch, Elizabeth; et al. (2006). Transfer of *Borrelia burgdorferi* s.s infection via blood transfusion in a murine model. *The Journal of Parasitology*, 92(4), 869-870.
- Gerber, M; et al. (1994). The risk of acquiring Lyme disease or Babesiosis from a blood transfusion. *The Journal of Infectious Diseases*, 170(1), 231-234.
- Green, L., & Goldman, E. (2009). *PRACTICAL HANDBOOK OF MICROBIOLOGY*. Taylor & Frances Group.
- Johnson, S., Swaminathan, B., Moore, P., Broome, C., & Parvin, M. (1990). Survival in Experimentally Infected Human Blood Processes for Transfusion. *The Journal of Infectious Diseases*, 162(2), 557-559.
- Lyme Disease --- United States, 2003--2005*. (2007, June 15). Retrieved March 18, 2010, from Centers for Disease Control and Prevention: <<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5623al.htm>>
- Lyme Disease*. (2009, Jan 30). Retrieved Oct 7, 2009, from The Arthritis Society: <<http://www.arthritis.ca/types%20of%20arthritis/lyme%20disease/default.asp?s=1&province=mb>>
- Lyme Disease symptoms*. (2007, Aug 9). Retrieved Sep 29, 2009, from Centers for Disease Control and Pervation: <www.cdc.gov/ncidod/dvbid/lyme/ld_human_disease_symptoms.htm>
- Lyme Disease: A Patient's Guide*. (n.d.). Retrieved Oct 7, 2009, from American College of Physicians: <http://www.acponline.org/clinical_information/resources/lyme_disease/patient/diagnosis.htm>
- Medical Guidelines*. (1996). Retrieved Oct 7, 2009, from National Bone Marrow Registry: <http://www.marrows.org/JOIN/Med_Guideline_Join/index.html>
- Tonnetti, L., Eder, A., Dy, B., Kennedy, J., Pisciotto, P., Benjamin, R., et al. (2009). Transfusion-transmitted *Babesia microti* identified through hemovigilance. *Transfusion*, 49(12), 2557-2563.
- Who Can Give Blood*. (2009). Retrieved Oct 7, 2009, from New York Blood Center: <http://www.nybloodcenter.org/files/Media/29/mediumFilename/Med_Condition.pdf>
- Wilson, A. (Director). (2008). *Under Our Skin* [Motion Picture].

Seed Removal in the Dwarf Bear-Poppy (*Arctomecon humilis*)

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Mentor: John Mull

ABSTRACT

The dwarf bear-poppy (Arctomecon humilis) is a federally endangered species that grows only on outcrops of gypsum rich soils in Washington County, Utah. The seeds of A. humilis have an attached elaiosome (a fat body employed to encourage dispersal of seeds by ants) and have been observed being handled by ants. We measured seed removal by ants and rodents at four population sites. Ants and rodents removed 21% of all seeds presented but there was no difference in the rate at which seeds were removed by these two agents. Rates of removal differed significantly among the four populations and between the two dates (late spring and early summer) used in the experiment. The fate of seeds removed by ants is likely dispersal, whereas rodents are typically seed predators. Rodents may also be seed dispersers through caching behaviors.

INTRODUCTION

Myrmecochory is the dispersal of seeds by ants, a mutualism that involves a reward for both plants and ants. Seeds of myrmecochorous plants bear elaiosomes, fat bodies that are attractive to ants (Bond & Breytenbach, 1985). Ants bring the seeds to the nest and feed the elaiosomes to their larvae, later discarding the seed bodies in refuse piles (Smith et al., 1989). Elaiosomes have a different chemical composition than the seed body that may provide essential nutrients to larvae. Ant larvae fed elaiosomes are more likely to become reproductives than workers (Fischer et al., 2007). This is beneficial to the ant colony because it increases reproductive potential.

Plants benefit from the dispersal of seeds away from parent plants (Turnbull & Culver, 1983). Seeds deposited in refuse piles are provided a nutrient-rich place for seed germination to occur (Smith et al., 1989; Turnbull & Culver, 1983), where they are less vulnerable to seed predators such as rodents (Heithaus, 1981).

The dwarf bear-poppy (*Arctomecon humilis*) is a perennial herb of the poppy family (Papaveraceae) that usually lives for about five years (Allphin et al., 1998). Its habitat is restricted to a 16 km radius of gypsum rich soils of the Moenkopi Formation, a geological layer of the Colorado plateau that is exposed at the edge of Mohave Desert in Washington County, UT (Nelson, 1989, USFWS, 1985). St. George is a rapidly growing city in Washington County, whose development threatens the species' already isolated populations. Off-highway vehicle use (OHV) is popular in this area and accelerates the compaction and erosion of the soil. These threats combined with its restricted range have led to the federal listing of *A. humilis* as an endangered species in 1979 (USFWS, 1985). The Bureau of Land Management has since designated and protected existing *A. humilis* populations (Allphin et al., 1998).

Favorable climatic conditions for *A. humilis* seeds to germinate are rare (Harper & Van Buren, 2004), and drastic fluctuations of established *A. humilis* populations have been observed (Allphin et al., 1998; Nelson, 1989). The soil seed bank preserves multiple generations of seeds awaiting favorable conditions, and is important to maintaining viable populations because germination events are rare, mortality is high, and only a fraction of plants survive to reproduce (Nelson, 1989). Higher levels of genetic variability have been observed in populations that are further from urbanization (Allphin et al., 1998). This suggests that the growth of St. George not only affects the habitat of *A. humilis*, but also its ability to maintain genetic diversity.

The seeds of *A. humilis* bear elaiosomes and have been observed being carried by ants (Nelson, 1989). Although these seeds are initially dispersed by the seed pod and attached flower in the wind, this plant may rely on myrmecochory for proper deposition in the soil (Nelson & Welsh, 1993). No research has examined the effects of ants on the removal of *A. humilis* seeds. Rodents may act as predators on the seeds of this myrmecochorous species, though no research has been conducted to analyze this either. The objective of this research was to quantify the effects that ants and rodents have on the removal of *A. humilis* seeds. Specifically, our aim was to understand potential differences in ant and rodent seed removal from late spring to early summer, as well as variation in removal among population sites that vary in their proximity to human population.

METHODS

Seeds were presented to ants and rodents on trays constructed from 1 cm thick pinewood. The trays were 12 cm x 12 cm x 3 cm. Some trays were accessible to ants only while others were accessible to rodents only. Ant-access trays were covered in a 1-cm wire mesh cage that fit tightly around the trays

and restricted the access of rodents. Openings were 2 cm x 2 cm and made in all four walls of the tray to provide access for ants. The sides of rodent-access trays were painted with full strength Fluon® paint, which is effective in restricting access to ants (Heithaus, 1981). All trays had four grooves in the bottom to hold the seeds.

One control tray was constructed for each site to exclude both ants and rodents. These trays were covered in wire mesh and the walls of the trays were painted with Fluon® paint. By restricting access to both rodents and ants, these trays allowed us to assess whether wind was responsible for the removal of seeds, as well as if the access restrictions were effective. The use of the control was limited due to the number of seeds that were allowed to be collected from this endangered species.

Four *A. humilis* population sites were chosen for the study. Seeds were collected at each of the four sites on 29 May 2010, and were only used at the site at which they were collected. Seeds were stored in a refrigerator during the time when the experiment was not being conducted.

A 100-m, linear transect was chosen at each of the four sites in an area of relatively constant topography. Five trays of each treatment were interspersed every 10 m along the transect and located 5 m to each side of the transect. The control tray was placed in the middle of each transect.

The experiment was conducted over two four-day periods during May and June of 2010. Twenty-five seeds were placed in each tray. The sites were visited each day and the number of seeds removed from each tray was recorded. The trays were then replenished to 25 seeds on days 2 and 3 in trays where seeds had been removed.

SPSS version 17 was used to perform two-way ANOVA tests on data with equal variances (date and site) and a t-test assuming unequal variances was performed on data that was heteroscedastic (treatment). Although the t-test is parametric and is sensitive to errors of hypothesis rejection concerning heteroscedastic data, the t-test assuming unequal variances is adequate to analyze heteroscedastic data (Ruxton, 2006). The dependent variable was the number of seeds removed for each treatment per site over each four-day period. All data was log-transformed ($\log + 1$) to meet the assumption of normality.

RESULTS

Ants and rodents removed 21% of all seeds presented, but there was no difference in the rate at which seeds were removed by these two agents ($t = 1.047$, $df = 62$, $P = 0.300$)(Fig. 1). There was no difference between rodent seed removal and the control ($t = 1.257$, $df = 38$, $P = 0.230$)(Fig. 1),

or between ant seed removal and the control ($t = 2.126$, $df = 38$, $P = 0.061$) (Fig. 1). The 2-way ANOVA revealed that there was a significant difference between seed removal during the late spring and early summer ($F = 3.954$, $df = 1,56$, $P = 0.052$) (Fig. 2) and among the four sites ($F = 5.588$, $df = 3,56$, $P = 0.002$) (Fig. 3). It also revealed a significant interaction between date and site ($F = 5.379$, $df = 3,56$, $P = 0.003$).

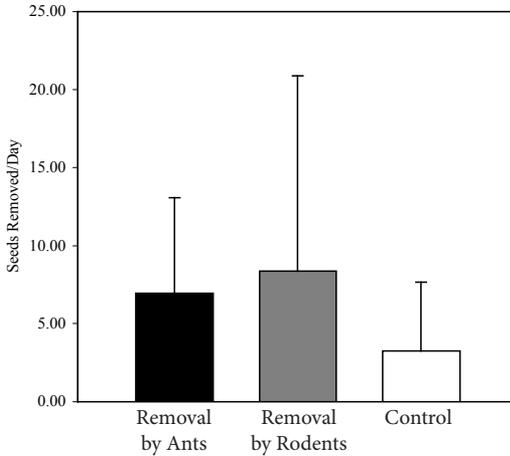


Figure 1. Mean (+1 S.D) number of seeds removed by ants ($n=32$), rodents ($n=32$), and in the control ($n=8$).

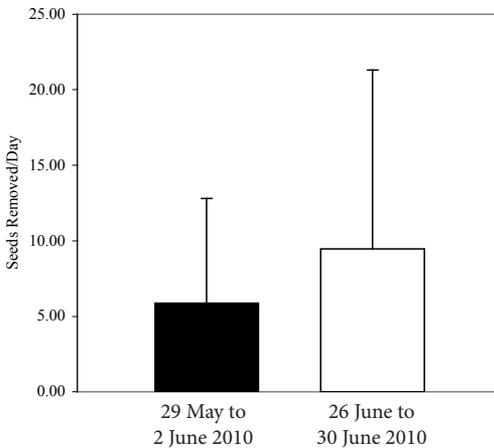


Figure 2. Mean (+1 S.D) number of seeds removed in the late spring ($n=32$) and early summer ($n=32$).

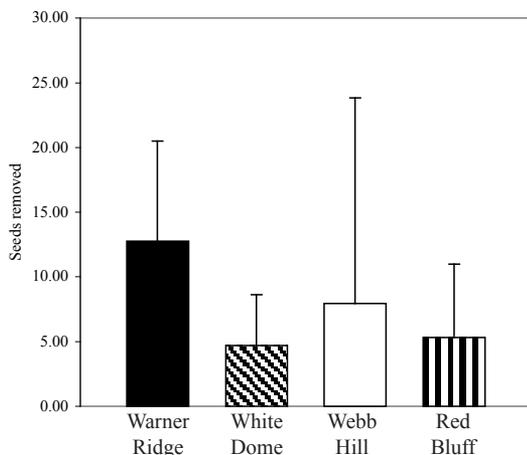


Figure 3. Mean (+1 S.D) number of seeds removed at the four study sites, Warner Ridge ($n=16$), White Dome ($n=16$), Webb Hill ($n=16$), and Red Bluff ($n=16$).

DISCUSSION

We found that ants and rodents have an equal influence on the removal of *A. humilis* seeds. Rodent exclusion was effective because none of the wire mesh cages were removed during the study. Urine stains were found on seven trays from both treatments during the study indicating regular visits by rodents. When urine was found on rodent-access trays all or most of the 25 seeds were removed though this did not occur when urine was found on ant-access trays. No ants were observed attempting to access rodent-access trays. Some seeds in the ant and rodent treatments may have been removed by wind, however due to seed collection limits and only one control tray at each site the importance of seed removal by wind is unclear.

Seed removal may have been higher in the early summer because both ants and rodents are more active then. Nelson and Welsh (1993) state that the flowering of this plant peaks in early May, therefore it is more likely that seed removal corresponded with the later maturity and availability of seeds.

Differences in seed removal may be due to the proximity of sites to human population, as Warner Ridge and White Dome are further from people than Webb Hill and Red Bluff. The growing human population of the St. George area continues to affect *A. humilis* populations (Harper & Van Buren, 2004). It is possible that human populations also have an influence on the activity of ants and rodents, though this is speculative.

We documented high spatial variation in seed removal, a pattern commonly found in such experiments (Smith et al., 1989). Elaiosome-bearing seeds handled by ants are more likely to be dispersed than those handled by rodents. Longland (2001) found that Indian ricegrass seeds cached by rodents were more likely emerge as seedlings than those collected by ants, although these seeds do not bear elaiosomes. However, Turnbull and Culver (1983) found that ants consistently removed more seeds of the elaiosome bearing species *Viola nuttallii* than rodents, suggesting that ants are much more interested in elaiosomes than rodents. Heithaus (1981) stated that the removal of seeds by ants is important to maintaining genetic variability, because rodents will consume most of the seeds beneath a parent plant, thus counteracting the efforts of sexual reproduction.

This was the first study to quantify the removal of *A. humilis* seeds by ants and rodents. Knowledge of the dispersal ecology of *A. humilis* may be beneficial to its conservation and protection. Future studies on *A. humilis* should focus on measuring the fate of ant-handled and rodent-handled seeds. This would clarify the role that ants have on the germination of *A. humilis* seeds and determine whether interactions with ants benefit this plant.

REFERENCES

- Allphin, L., Windham, M.D., and Harper, K.T. 1998. Genetic diversity and gene flow in the endangered dwarf bear poppy, *Arctomecon humilis* (Papaveraceae). *American Journal of Botany* 85:1251-1261.
- Bond, W.J. and Breytenbach, G.J. 1985. Ants, rodents, and seed predation in Proteaceae. S. *African Journal of Zoology* 20:150-154.
- Fischer, R.C., Richter, A., Hadacek, F., and Mayer, V. 2008. Chemical differences between seeds and elaiosomes indicate an adaptation to nutritional needs of ants. *Oecologia* 155:539-547.
- Harper, K.T. and Van Buren, R. 2004. Dynamics of a dwarf bear-poppy (*Arctomecon humilis*) population over a sixteen-year period. *Western North American Naturalist* 64:482-491.
- Heithaus, E.R. 1981. Seed predation by rodents on three ant-dispersed plants. *Ecology* 62:136-145.
- Longland, W.S., Jenkins, S.S., Vander Wall, S.B., Veech, J.A., and Pyare, S. 2001. Seedling recruitment in *Oryzopsis hymenoides*: Are desert granivores mutualists or predators? *Ecology* 82:3131-3148.
- Nelson, D.R. 1989. Demographic and seed bank biology of *Arctomecon humilis* (Papaveraceae), a short lived perennial. Master's thesis, Brigham Young University, Provo, UT.
- Nelson, D.R. and Welsh, S.L. 1993. Taxonomic revision of the *Arctomecon* Torr & Frem. *Rhodora* 95:197-213.

- Ruxton, G.D. 2006. The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test. *Behavioral Ecology* 17:688-690.
- Smith, B.H., Forman, P.D., and Boyd, A.E. 1989. Spatial patterns of seed dispersal and predation of two myrmecochorous forest herbs. *Ecology* 70:1649-1656.
- Turnbull, C.L. and Culver, D.C. 1983. The timing of seed dispersal in *Viola nuttallii*: Attraction of dispersers and avoidance of predators. *Oecologia* 59:360-365.
- U.S. Fish and Wildlife Service. 1985. *Dwarf bear-poppy recovery plan*. U.S. Fish and Wildlife Service, Denver, Colorado. 26 p.

Isolation of Novel Phage from the Great Salt Lake that Infect *Idiomarina*

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Mentor: Craig J. Oberg

ABSTRACT

Characterization of euryhalophilic bacteria from the South Arm of the Great Salt Lake (GSL) revealed a number of Idiomarina-like organisms. Since bacteriophages play a role in controlling bacterial populations and facilitate lateral gene transfer, GSL water samples were tested against four Idiomarina GSL isolates. Initial screening showed a large number of plaques with a wide range of sizes. Plaques were tentatively grouped into four categories: plaques with a 10 mm clear zone with a much larger opaque halo extending around each plaque, two mm plaques, one mm plaques, and a fourth group with variable shapes. A host comparison performed with purified phage isolates against the Idiomarina strains indicated five different phage types. One type only infected Idiomarina S3. A second phage type could infect two hosts, Idiomarina S3 and S11. The third phage type infected both Idiomarina S3 and S21, while a fourth type only infected Idiomarina S21. The fifth phage type only infected Idiomarina S11. The 16S rRNA gene sequences of the Idiomarina-like isolates had less than 0.5% difference over 1,300 bp indicating phage typing may provide greater strain resolution than 16S sequencing. There also appears to be some correlation between plaque morphology and host range.

INTRODUCTION

The Great Salt Lake (GSL) is a naturally occurring hypersaline environment containing a wide diversity of euryhalophilic to extremely halophilic microorganisms (Baxter, 2005). High concentrations of sodium chloride (NaCl) and nutrients, constant sunlight exposure, and wind-generated aeration allow the GSL to support a dense population of halophilic microorganisms (Ventosa, 2006). These microorganisms contribute significantly to the ecological balance in this saline lake because they form the base of the food

chain, act as decomposers, and participate in the recycling of basic nutrients (Dyall-Smith, 2005).

Most characterized species of the genus *Idiomarina* are from thalassohaline (seawater-derived) environments including species isolated from deep-sea vents (4000-5000 meters) (Ivanova et al., 2000). Several species have been isolated from hypersaline environments and exhibit a NaCl growth range of 1-15% w/v with optimum growth at 5% w/v NaCl (Kwon et al., 2006; Yeon et al., 2005). The GSL isolates appear most closely related to *Idiomarina loihiensis* which grows optimally in salt concentrations of 7.5 to 10% w/v, similar to the salinity of the GSL's South Arm, the location where they were isolated (Donachie, 2003). The *Idiomarina* genus has a unique iso-branched fatty acid composition, rather unusual for Gammaproteobacteria, which could play a role in adaptation to high salinity (Brettar et al., 2003). They are gram-negative rods that grow aerobically and are motile by a single polar or sub-polar flagellum (Donachie, 2003). Genome comparison indicates that this bacterium has maintained its amino acid transport and degradation systems, but has lost sugar transport and certain sugar metabolic genes, suggesting it lives on amino acids rather than sugars.

Bacteriophages (phage) are viruses that infect bacteria and are thought to help maintain equilibrium between bacteria and nutrient resources, and between related bacterial species. Phages play an important role in recycling nutrient biomass in their environment. Phage can also mediate transduction, lysogenic conversion, species successions, and help maintain microbial diversity (Jiang, 2004). Bacteria predation by phages may be a significant source of bacterial mortality in hypersaline environments (Dyall-Smith, 2005), but there are few reports of bacterial predation by phage in the unique microbial environment found in the GSL (Kauri, 1991). One reason for this may be the lack of phage-host model systems expressly isolated from the GSL.

Previous studies characterizing halophilic bacteria from the South Arm of the GSL revealed a number of *Idiomarina*-like isolates. There are very limited reports of bacteriophage specific to this genus, with only one phage isolate reported from a halophilic environment (Jiang, 2003). Sampling Mono Lake (only 70-85 g•L⁻¹ NaCl as compared to the GSL South Arm at 120 g•L⁻¹ NaCl) researchers isolated phage FMono1 with an *Idiomarina* host closely related to *Idiomarina baltica*. Currently, there is no report of phage isolated or identified in the GSL that infect *Idiomarina*. In this study, we sought to determine if GSL-isolated *Idiomarina* strains are susceptible to phage predation from phage present in the GSL.

METHODS

Media

An oligotrophic Halophilic Medium (HM) was prepared with modifications from Halophilic Broth using the following formulation per liter: NaCl, 80 g; MgSO₄·7H₂O, 25 g; casamino acids, 5 g; yeast extract, 5 g; protease peptone, 2.5 g; KCl, 2 g; trisodium citrate, 3 g (Atlas, 1993). HM agar (HMA) was supplemented with 1.5% agar for agar plates and HM soft agar (HMSA) was supplemented with 0.5% agar for plaque assays. A HM broth was prepared using the same formulation without the addition of agar. HM 2X broth contained twice the amount of nutrients of halobacteria medium, but only 8% NaCl. The final pH was adjusted to 7.2 using 1 N NaOH.

Culture Isolation

Samples were collected along the north shore of Bridger Bay on Antelope Island located in the south arm of the Great Salt Lake. Sediment and water samples were collected three to five meters from the shoreline, and samples of suspended exoskeletons were collected within 1.5 meters of the shoreline. Samples were incubated with halophile broth to enrich organisms and then isolated on halophile agar. Colonies with unique morphology or color were chosen for chitin analysis.

Bacterial Cultures

Four isolates of *Idiomarina*-like bacteria, originally isolated and purified from the South Arm of the Great Salt Lake, were used for this phage isolation/characterization study. All bacterial cultures were allowed to grow for 18 hours at 30°C in HM broth or until an optical density of 0.1-0.2 at 600 nm was reached.

16S rRNA Gene Sequencing

DNA was extracted from the isolates using a modified bead beater-lysis method with phenol-chloroform purification. The 16S rRNA gene was amplified using bacteria specific primers (27F 5' AGA GTT TGA TCM TGG CTC AG 3' / 1492R 5' ACG GYT ACC TTG TTA CGA CTT 3') (Lane, 1991). The reaction mixture contained 200 nM of each primer, 250 µm of the dNTPs, 0.2 mg•ml⁻¹ bovine serum albumin, 1U DNA Taq Polymerase and the diluted reaction buffer (Promega, Madison, WI). The amplification parameters were 94°C for 3 min., followed by 25 cycles of 94°C for 45 sec., 57°C for 1 min., 72°C for 2 min., and a final extension step at 72°C for 7 min. Sequencing was done either by Genewiz, Inc. (South Plainfield, NJ) or by the Idaho State University Molecular Research Core Facility. The sequences were compared to the GenBank database using the BLAST search tool. All matches were greater than 98% similar.

Isolation of Phage from GSL Water Samples

Water samples from the South Arm of the GSL at Bridger Bay were collected and centrifuged at 3000 rpm for 15 minutes. The supernatant was filtered through a 0.45 µm CA filter) followed by a 0.20 µm CA filter. One mL of 0.2 µm GSL water filtrate and 500 µL of host *Idiomarina* isolate were combined with 4 mL of HMSA then poured over HMA plates (soft agar overlay method). These plates were incubated for 24 hours at 30°C before examining for plaques (clear zones in the soft agar overlay indicating a phage infection of the host bacteria).

Enrichment Method of Isolation of Phage

Phage enrichment cultures were made by combining 5 mL HM 2X, 5 mL of GSL filtrate, and 1 mL of the specific *Idiomarina* host culture in a sterile 100 ml flask, and incubated at 30°C for 24 hours. Phage enrichment cultures were compared to a control containing only HM and *Idiomarina* host, but no GSL filtrate. A positive result for a phage enrichment culture was observed as reduced turbidity when compared to the control. Phages from the enrichment cultures were harvested by centrifuging at 5,000 rpm for 5 minutes. The supernatant was filtered using a 0.45 µm filter, followed by a 0.2 µm filter to remove remaining bacteria. The filtrate was serially diluted in 8% sterile saline and a titer was done using the soft agar overlay method as previously described to look for plaques.

Isolation of Phage from Plaques

Individual phage were isolated by harvesting agar plugs from the center of a plaque using a sterile Pasteur pipette. Three to five agar plugs were harvested from each plaque. The agar plugs were placed in 1 mL of an 8% NaCl sterile solution and the phage were allowed to diffuse out of the agar for 12 hours. The solution was then filtered through a 0.45 µm filter to remove any remaining bacteria.

Host Range Determination

Method 1 – Plaque Morphology. Five hundred microliters of each *Idiomarina* GSL isolate was combined with 500 µL of a purified phage isolate and incubated for 15 minutes to allow for phage attachment. Following incubation, the phage/host mixture was serially diluted in 8% sterile saline and 500 µL of each phage/host dilution was added to 4 mL of molten HMSA (52°C) and poured over HMA plates.

Method 2 – Spot Test. Five-hundred microliters of each *Idiomarina* GSL isolate was combined with 4 mL of HMSA, poured over HMA plates, and allowed to solidify. Three microliters of each purified phage isolate was applied to the inoculated soft agar in defined locations across the Petri plate.

Method 3 – Streak Test. *Idiomarina* host isolates were diluted 1:10 in sterile 8% w/v NaCl. HMA plates were prepared by applying four streaks of diluted *Idiomarina* host across the length of the agar, one streak for each host isolate. A 2 μ L droplet of a purified phage isolate was applied to the center of each streak. Petri plates were incubated at 30°C for 24 hours.

RESULTS

Genetic analysis of the 16S rRNA for the GSL isolates revealed that all four strains were closely related to *Idiomarina loihiensis*, originally isolated from a submarine volcano off the coast of Hawaii (appendix, Figure 1).

Initial screening showed that a large number and variety of plaques developed on several of the GSL *Idiomarina* isolates (appendix, Figure 2). One group of plaques formed a 10 mm clear zone with a much larger opaque halo extending around each plaque. A second group of plaques were small (two mm diameter). A third plaque morphology was the smallest with only a one mm diameter, while a fourth group of plaques had variable morphologies. Repeated plating of phage with variable sized plaques was repeatable, even when a single plaque was harvested and purified before plating. Aside from the last group of phage, plaque morphologies consistently appeared on their respective host strains as individual phage were isolated, purified, and propagated.

Initial host range results have revealed phage that can be tentatively placed into five different groups (appendix, table 1). One group of phage (isolates 11, 12, 15, and 16) could only infect *Idiomarina* S3. A second group of phage (isolates 2, 3, 6, 10, 13, and 14) could infect two different hosts, *Idiomarina* S3 and S11 (appendix, figure 2a). The third group of phage (isolate 9) infected both *Idiomarina* S3 and S21 (appendix, figure 2b), while a fourth phage group (isolate 8) only infected *Idiomarina* S21 (appendix, figure 2c). The fifth group of phage (isolates 1, 4, 5, and 7) was found to infect only *Idiomarina* S11 (appendix, figure 2d).

CONCLUSION

Genetic characterization of *Idiomarina* isolates from the GSL suggests they are more closely related to species found in deep-sea vents than to species isolated from hypersaline environments (Choi & Cho, 2005; Ivanova et al., 2000). This may be due to the unusual chemical environment of the GSL, particularly in respect to the concentration of different ions in the water besides NaCl, which may be more similar to the water chemistry found near deep-sea diffuse-flow hydrothermal vents.

Results suggest possibly five unique phage types have been isolated that infect *Idiomarina* bacteria present in the Great Salt Lake. Initial screening

showed a large number and variety of plaques developed on the four GSL *Idiomarina* isolates. A comparison of plaque morphologies suggested that a number of the phage isolates could be grouped together based on the diameter and turbidity of the plaques they produced. Although it is not a definitive taxonomic tool, plaque morphology is usually unique and, in our observations, reproducible with each host making it a valuable screening tool.

A more definitive grouping resulted when a host comparison was used to identify which phage isolates infected each of the four *Idiomarina* host strains. Host analysis indicated at least five different phage types might be present in the GSL that can infect GSL-isolated *Idiomarina* strains. There appears to be some correlation between plaque morphology and host range for the five groups.

Given the number of *Idiomarina* isolates from the GSL and the number of unique phage isolates that infect specific strains, there may be a role for phage in controlling *Idiomarina* populations when abundant bacterial growth occurs seasonally. One possible reason for such a large number of phage types may be the lack of protozoal grazers in the GSL (Post, 1977) necessitating a more robust population of phage that can respond to dramatic changes in bacterial populations.

This is the first report of *Idiomarina* strains being isolated from the GSL and the first report of phage isolated from the GSL that infect these halophilic *Idiomarina* isolates.

REFERENCES

- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Parks, L.C. (ed.) CRC Press, Boca Raton, FL.
- Baxter, B. K., C. D. Litchfield, K. Sowers, J. D. Griffith, P. A. Dassarma, and S. Dassarma. (2005). Microbial diversity of Great Salt Lake. In *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. Gunde-Cimerman, N., Oren, A., Plamenitas, A. (ed.) Springer Publishing, Netherlands.
- Brettar, I., R. Christen, and M. G. Höfle. (2003). *Idiomarina baltica* sp. nov., a marine bacterium with a high optimum growth temperature isolated from surface water of the central Baltic Sea. *International Journal of Systematic and Evolutionary Microbiology* 53:407-413.
- Choi, D. H., and B. C. Cho. (2005). *Idiomarina seosinensis* sp. nov., isolated from hypersaline water of a solar saltern in Korea. *International Journal of Systematic and Evolutionary Microbiology* 55:379-383.
- Donachie, S. P., S. Hou, T. S. Gregory, A. Malahoff, and M. Alam. (2003). *Idiomarina loihiensis* sp. nov., a halophilic γ -Proteobacterium from the

- Lō'ihi submarine volcano, Hawai'i. *International Journal of Systematic and Evolutionary Microbiology* 53:1873-1879.
- Dyall-Smith, M. L., D. G. Burns, H. M. Camakaris, P. H. Janssen, B. E. Russ, and K. Porter. (2005). Haloviruses and their hosts. In *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. Gunde-Cimermann, N., Oren, A., Plamenitas, A. (ed.) Springer Publishing.
- Ivanova, E. P., L. A. Romanenko, J. Chun, M. H. Matte, G. R. Matte, V. V. Mikhailov, V. I. Svetashev, A. Huq, T. Mauhel, and R. R. Colwell. (2000). *Idiomarina* gen. nov., comprising deep-sea bacteria from the Pacific Ocean, including descriptions of two species, *Idiomarina abyssalis* sp. nov. and *Idiomarina zobellii* sp. nov. *International Journal of Systemic and Evolutionary Microbiology* 50:901-907.
- Jiang, S., G. Steward, R. Jellison, W. Chu, and S. Choi. (2003). Abundance, distribution, and diversity of viruses in alkaline, hypersaline Mono Lake, California. *Microbial Ecology* 47:9-17.
- Kauri, T., H-W. Ackermann, U. Goel, and D. J. Kushner. (1991). A bacteriophage of moderately halophilic bacterium. *Archives of Microbiology*, 156:435-438.
- Kwon, S-W., B-Y Kim, H-Y Weon, Y-K Baek, B-S. Koo, and S-J. Go. (2006) *Idiomarina homiensis* sp. nov., isolated from seashore sand in Korea. *International Journal of Systemic and Evolutionary Microbiology* 56:2229-2233.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*. E. Stackebrandt and M. Goodfellow (ed.) John Wiley & Sons.
- Post, F. J. (1977). The microbial ecology of the Great Salt Lake. *Microbial Ecology* 3:143-165.
- Ventosa, A. (2006). Usual microorganisms from unusual habitats: hypersaline environments. In *Prokaryotic Diversity: Mechanisms and Significance*. Logan, N. A., H. M. Lappin-Scott, and P. C. F. Oyston (ed.) Cambridge University Press.
- Yeon, S-H., W-J. Jeong, and J-S. Park. (2007). The diversity of culturable organotrophic bacteria from local solar salterns. *The Journal of Microbiology* 43:1-10.

APPENDIX

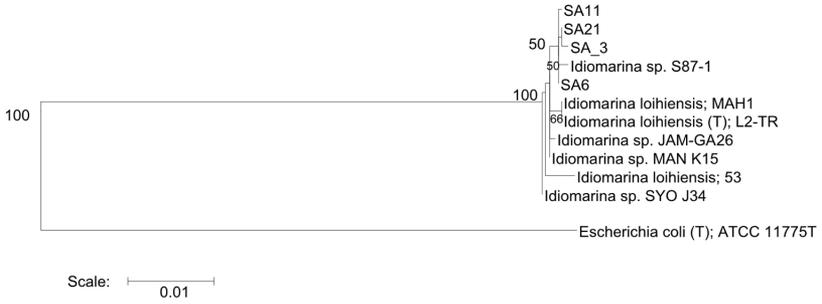


Figure 1. Taxonomic tree comparing *Idiomarina* strains isolated from the Great Salt Lake with known *Idiomarina* species (scale indicates changes per base divided by changes per 100 bases).

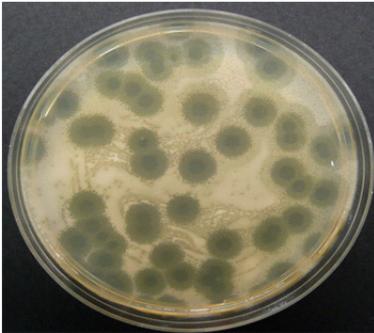


Figure 2a. Phage Isolate 13, Group 2

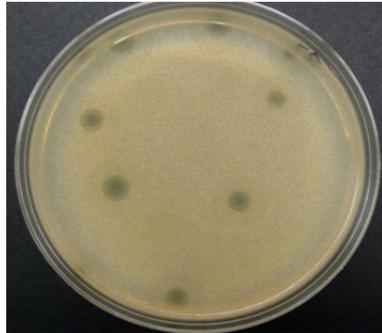


Figure 2b. Phage Isolate 9, Group 3

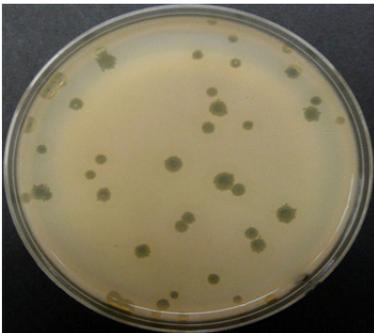


Figure 2c. Phage Isolate 8, Group 4



Figure 2d. Phage Isolate 7, Group 5

Figure 2. Plague morphologies for *Idiomarina* bacteriophage isolated from the Great Salt Lake.

Table 1. Host range of *Idiomarina bacteriophage* isolated from the Great Salt Lake

Phage Isolates	Host Strains (<i>Idiomarina</i> GSL Isolates)			
	S3	S6	S11	S21
Group 1				
11	X			
12	X			
15	X			
16	X			
Group 2				
2	X		X	
3	X		X	
6	X		X	
10	X		X	
13	X		X	
14	X		X	
Group 3				
9	X			X
Group 4				
8				X
Group 5				
1			X	
4			X	
5			X	
7			X	

ERGO



The image features a dark gray background with a white diagonal band running from the bottom-left towards the top-right. In the upper-left corner, there are several parallel, light gray diagonal stripes. The word "ABSTRACTS" is written in a bold, light gray, sans-serif font, positioned within the white diagonal band and following its angle.

ABSTRACTS

My Mother, My Friend?: A Thematic Analysis of Turning Points in Mother-Daughter Relational Deterioration

Author: Leah Garrett
Mentor: Colleen Garside

While the relationship between a mother and daughter can be a source of comfort, understanding and intimacy, for many daughters this is not the case. What was once a warm loving relationship turns into one of distrust, manipulation and an overall lack of intimacy. Studies suggest that turning points contribute to these relational changes. The purpose of this study is to explore relational deterioration in the context of mother-daughter relationships. This study is grounded in the theoretical foundations of turning point theory. A thematic content analysis of ten transcripts from in-depth interviews and focus group transcripts provide the methodological framework for this study. The findings reveal that there is a connection between a lack of self disclosure and no perceived turning points; where there is no perceived turning point, the relationship remains consistent for better or worse due to censoring communication; and high self disclosure, while resulting in higher perceived turning points, either deteriorates the relationship, or enhances it depending upon the personality of the individuals involved. These findings contradict extant research, suggesting that some relationships may not experience turning points as the impetus for relational deterioration.

This research was presented at the National Conference on Undergraduate Research in Missoula, Montana, April 15–17, 2010.

Bilateral Femoral Shaft Stress Fractures in an 18-Year-Old Collegiate Female Distance Runner: A Case Report

Authors: Benjamin Leishman, BreAnne Perkes & Chris Barber

Mentors: Shannon Hansen & David Berry

Background: We present the case of a collegiate long distance runner who suffered bilateral femoral shaft stress fractures and her gradual return to athletic participation. An 18-year-old Caucasian female (body mass=61 kg, height=170 cm) reported to the athletic trainer complaining of right, high anterior hip pain which radiated down into the groin, as well as right sided low back/gluteus pain during long distance training runs. The athlete denied any previous history of serious injuries or illness to hip, groin, and lower extremity. She reported training through the pain for approximately two-three weeks before beginning to cross-train on her own in order to decrease joint loading and reduce her pain level. However, the pain returned immediately once she began running long distances again. It was not until she was unable to compete in distance race that she sought out medical attention. On physical examination the athlete presented anterior inguinal pain on the right side elicited with extremes of external and internal rotation of the hip. The athlete also demonstrated weakness with right hip adduction, abduction, and hip flexion. She was initially treated for iliopsoas tendonitis, however, she failed to progress and was eventually referred to the team physician who ordered an MRI and bone scan.

Differential Diagnosis: Iliopsoas tendonitis, groin injury, femoral neck stress fracture, pubic rami stress fracture, osteitis pubis, and sacroiliac joint injury.

Treatment: Upon medical examination, the athlete complained of right, anterior inguinal pain, exacerbated with extremes of external and internal hip rotation. She also complained of tenderness upon palpation of pubis and pubic rami, but with no pain with palpation to the femoral shaft. A DEXA essential bone density scan demonstrated femoral stress fractures in the proximal shaft of the both femurs (with increased signal activity on the right), even though the athlete's pain was only reported on right side. The athlete was immediately removed from all weight bearing activities, referred to a dietitian, and initiated a cross-training program of aquatic, elliptical, and stationary bike therapy for 18 weeks after the initial diagnosis. The athlete

continues to improve, running on a treadmill every other day to progressively increase her mileage and intensity, and on an elliptical on the off days.

Uniqueness: Femoral stress fractures (to all parts of the femur) account for approximately 10 percent of all stress fractures sites in general population. In the athletic population, the prevalence of femur fractures decreases, with approximately 2.8 percent-7 percent of stress fractures isolated to the femur and typical only to one leg. Many of the stress fractures reported in the athletic population are typically located in femoral neck. Femoral shaft stress fractures are considerably more uncommon, and only a few cases of bilateral femoral shaft stress fractures have been reported to date. Tenderness to palpation is also typically present at the injury site and is the hallmark of a stress fracture, however, in this case the athlete only presented with complaints of pain in the groin and anterior hip (typical presentation) but only in one leg.

Conclusion: Bilateral femoral stress fractures are rare in athletics and difficult to diagnose, and if not treated correctly have high rates of reoccurrence and complication (internal fixation for tension-type fractures). Proper management includes early identification of the etiology, unloading of the limb through non-weight bearing activities such as aquatic exercise and cross-training, and implementing a nutrition program to improve bone density when warranted.

This research was presented at the Rocky Mountain Athletic Trainers' Association Clinical Symposium and Business Meeting in Denver, Colorado, April 8-11, 2010.

Effects of Kinesio® Tape on Ankle Proprioception in Healthy Individuals

Author: Danielle S. Burningham

Mentor: David C. Berry

Context: Kinesio® tape has reportedly been used by healthcare providers in reducing edema, strengthening muscle, decreasing pain, and relieving abnormal muscle tension. Product manufacturers also advertise the tape's ability to increase proprioceptive awareness. However, research examining this property is conflicting.

Objective: To determine the efficacy of Kinesio® tape on ankle joint proprioception. *Design:* A single blind, counter-balanced, cross-over model.

Participants: Healthy subjects (age=23.68±4.65 years, mass=76.69±16.32 kg, height=175.43±9.73 cm) meeting the following inclusion criteria (1) 18-45 years of age, (2) right foot dominant, (3) without foot or ankle abnormalities, and (4) without foot or ankle injury in the past 9 months.

Intervention: Subjects were randomly allocated into a tape first or no-tape first group. Ankle proprioception was determined by subject ability in active reproduction of joint position sense (RJPS) for 26 degrees dorsiflexion and 20 degrees plantar flexion, after moving into and holding these positions 3 times for 5 seconds. Data was collected using a Kin-Com 125AP. The subject's forefoot was strapped to the footplate, and subjects were blindfolded. Subjects were instructed to move into 26 degrees dorsiflexion and hold their foot in that position for 5 seconds and then to move into 20 degrees plantar flexion and hold their foot in that position for 5 seconds. This series was repeated three times, after which subjects were passively moved to neutral (0 degrees). From neutral subjects were instructed to move their foot into dorsiflexion where they believed their foot was when the machine stopped them and then signal researchers when there, their angle was then recorded. Subjects were then instructed to move their foot into plantar flexion where they believed their foot was when the machine stopped them at 20 degrees and signal the researchers when there, their angle was then recorded. This series of dorsiflexion to plantar flexion RJPS was then repeated a second time. Subjects were then allotted a 3-minute window wherein they were either taped or untaped before repeating the test sequence.

Main Outcome Measures: Pre- and post-RJPS angle measurements were recorded for ankle dorsiflexion and plantar flexion. Dependent variables were relative and absolute error for ankle joint dorsiflexion and plantar flexion joint angles using the means of the two trials.

Results: Data was analyzed using a repeated t-test. Testing revealed no significant difference ($p=0.05$) in the relative and absolute error for ankle joint proprioception in reproduction of 26 degrees dorsiflexion (relative ($M=2.68\pm 1.12$), absolute ($M=1.68\pm 1.06$) and 20 degrees plantar flexion (relative ($M=0.27\pm 1.39$), absolute ($M=0.36\pm 1.04$)).

Conclusions: Findings revealed no significant differences in relative or absolute error between the tape and no-tape conditions in either 26 degrees of dorsiflexion or 20 degrees of plantar flexion. These findings support previous work suggesting that Kinesio® tape does not likely enhance proprioceptive awareness in active ankle joint repositioning in healthy adults.

This research was presented at the 2010 Rocky Mountain Athletic Trainers' Association Clinical Symposium and Business Meeting in Denver, Colorado, April 8-11, 2010.

It was also presented at the National Athletic Trainers' Association Annual Business Meeting and Symposium in Philadelphia, Pennsylvania, June 22-25, 2010.

Effects of Relaxation and Imagery Script Interventions to Reduce DHEA Levels in Competitive Athletes: A Pilot Study

Authors: Landon Deru & Jordan Hamson-Utley

Mentor: Rodney Hansen

Context: The purpose of this original research was to test the effects of relaxation and imagery on the stress levels of athletes. This was quantitatively measured through salivary DHEA levels. DHEA is a steroid whose concentrations inversely reflect that of the stress hormone cortisol (a key regulator in the physiological stress response).

Objective: To answer the following question through quantitative methods: Will relaxation and imagery scripts reduce both mental and physical stress as reflected in salivary DHEA levels?

Design: 40 samples were tested in a pre versus post single blind study. Each sample was tested in duplicate for a total of 80 sample readings.

Setting: Subject saliva collection and relaxation/imagery took place in the Weber State University Stress Lab. Saliva samples were stored and tested in the Weber State University Nutritional Biochemistry Lab.

Participants: 40 collegiate athletes (31 females, 9 males) were recruited from university and club sports teams at Weber State University (age 25.5 ± 7.5 years).

Interventions: The participants were asked to spit into a previously labeled vial both pre and post intervention. They were then given an iPod and instructed to sit in a recliner and listen to the relaxation/imagery script and follow its direction. Testing for the salivary enzyme DHEA was completed using a standard DHEA-S sandwich ELISA enzyme immunoassay.

Main Outcome Measures: Pre- and post-DHEA levels were observed and recorded in ng/mL.

Results: No statistically significant increases between pre and post DHEA levels were evident. A high amount of individual variability between subjects may have resulted in a high standard deviation (pre=1.8, post=3.18, $p < 0.05$).

Conclusions: Though pre and post levels were not significant, it was observed that 30 out of the 40 subjects did have an increase in DHEA levels. There was no significant difference between DHEA levels and treatment intervention. Further study with a larger subject sample and tighter circadian control are necessary to determine the effects of imagery and relaxation on DHEA levels.

This research was presented at the Rocky Mountain Athletic Trainers' Association Clinical Symposium and Business Meeting in Denver, Colorado, April 8-11, 2010.

Radial Collateral Ligament Ruptures of the 4th and 5th Metacarpophalangeal Joints in a Collegiate Male Cheerleader: A Case Report

Author: Danielle S. Burningham

Mentors: David C. Berry & Shannon Hansen

Background: The purpose of this case report is to document the treatment and outcome of a complete rupture of the 5th metacarpophalangeal joint (MCPJ) radial collateral ligament (RCL) and partial rupture of the 4th MCPJ RCL of the left hand in a 23-year-old male collegiate cheerleader. During stunting practice a female cheerleader performed a stunt where she was tossed too high in the air, causing her to over rotate in a back flip. Trying to compensate for the over rotation, she came out of the tuck early and subsequently landed on the 4th and 5th phalanges of the left hand of a male cheerleader as he attempted to catch her. The 4th and 5th phalanges hyperextended and abducted beyond normal joint limitations. A popping sensation, pain, and immediate swelling were noted by the athlete. Upon examination by the athletic trainer the following day, the athlete was placed in a Plastalume® finger splint to reduce further joint movement. The athletic trainer referred the athlete to the team physician for further evaluation.

Differential Diagnosis: Metacarpophalangeal RCL sprain, metacarpophalangeal joint dislocation/subluxation, metacarpal/phalangeal fracture, strain, and contusion.

Treatment: Upon examination by the team physician, pain and swelling along the outside of the hand were noted. Decreased flexion of the 4th and 5th phalanges, extreme laxity with abduction of the 5th phalange, and moderate laxity with abduction of the 4th phalange of the left hand were also noted. The physician's diagnosis was a complete rupture of the 5th MCPJ RCL, and a partial rupture of the 4th MCPJ RCL. The athlete was fitted with a Velcro buddy tape device to secure the 4th and 5th phalanges to the 3rd phalange. This method of stabilization allowed for flexion and extension of the MCPJs while limiting abduction. The athlete was cleared for continued participation, using pain as his guide. Upon follow-up examination, ten weeks post-injury, the 4th and 5th MCPJ RCLs were intact and the athlete no longer complained of pain. The athlete's range of motion in MCP flexion, PIP flexion, and DIP extension of the 5th phalange; and, MCP extension,

PIP extension, PIP flexion, and DIP flexion of the 4th phalange were severely limited (>10 degrees), as compared bilaterally. There was also a significant decrease in grip strength of the left hand (M=11 kg) in comparison to the right (M=38.67 kg). A rehabilitation protocol was developed to restore strength and flexibility. The protocol consisted of targeting the extrinsic and intrinsic muscles of the hand by: (1) passively flexing the IP while extending the MCP, (2) passively extending the IP while flexing the MCP, (3) individualized active flexion of the IP while maintaining MCP extension, (4) individualized active flexion of the MCP while maintaining IP extension, (5) MCP extensions off a flat surface, (6) hook grip to fist grip exercises, and (7) tennis ball gripping exercises. The strengthening protocol was followed for four weeks, and produced a grip strength increase to 78 percent and ROM for DIP extension of the 5th was 25 degrees less than the right hand. All other joint movements were restored within 8 degrees or better as compared bilaterally. Abduction of the 4th MCP remains limited.

Uniqueness: Metacarpophalangeal RCL sprains are rare in athletics with no known reported cases in cheerleading. In professional football the incidence of any finger MCPJ sprain occurring is approximately 2 percent of all hand injuries. The athlete maintained his practice routine while wearing the Velcro buddy tape device as the only means of support.

Conclusion: The nature of this injury is highly uncommon, and while treatment is conservative it was effective and allowed the athlete to continue to competitively compete.

This research was presented at the Rocky Mountain Athletic Trainers' Association Clinical Symposium and Business Meeting in Denver, Colorado, April 8–11, 2010.

Running Through a Fibula Fracture: A Case Report

Authors: L Deru, R Stagg, C Allen & S Hansen

Mentor: David Berry

Background: We present the case of a collegiate cross country runner who suffered a distal fibula fracture and consequently continued to train for 3 weeks before being properly diagnosed, eventually returning to unrestricted athletic competition 3 weeks later. An 18-year-old Caucasian male long distance runner (body mass= 61 kg, height=172 cm) reported to the athletic training room with a chief complaint of left posterior lateral ankle pain. The runner was an otherwise healthy active male who denied any previous history of left lower leg and ankle injuries. He stated that the pain began while running in a cross country race where the athlete believed he twisted his ankle, and consequently did not finish the race secondary to ankle pain. Upon medical examination; the athlete, presented with decreased ability to plantar flex and evert the left foot. No palpable defects were detected and the athletic trainer diagnosed the injury as a peroneal muscle strain.

Differential Diagnosis: Lateral ankle sprain, syndesmotic injury, peroneal tenonopathy, fibula fracture.

Treatment: The athlete was treated conservatively for nine days using cryotherapy and light walking on a treadmill at 2 mph, and over the next sixteen days progressively increased to a jogging speed of 7-8 mph. The durations of these episodes increased each day over the 16 days. Twenty-one days after initial onset of symptoms the athlete was able to jog 60 minutes on a treadmill. At this time he also noted a protrusion along the distal fibula approximately 8-9 cm from the lateral malleolus. This protrusion was reported by the athlete to be abnormal, but not painful. Concerned with the apparent growth, the athlete was referred to the team physician for further evaluation. Radiographs of the lower left leg revealed a callus formation 7 cm from the ankle mortise, which indicated the presence of a healing fibula fracture. The athlete was placed into a walking boot for two weeks. While in the boot the athlete was allowed to continue pain-free training through the use of a cross-training program which included: (1) swimming, (2) aquatic jogging, (3) biking, and (4) the elliptical machine. Three weeks later follow-up radiographs demonstrated sufficiently callus formation over the fibula fracture site. The physician cleared the athlete to participate in unrestricted pain free physical activity 6 weeks after the sustaining a fibula fracture.

Uniqueness: Fractures of the fibula themselves are not uncommon. They can occur anywhere along the length of the fibula, and are often seen in combinations with severe ankle sprains. In fact, the fibula only bears 17 percent of the body weight so these types of fractures are not considered as severe as weight bearing bone fractures. However, in this case, even though the fibula only bears a small percentage of the body weight, the athlete managed to engage in physical activity and activities of daily living for three weeks without considerable pain, and he returned to unrestricted pain free physical activity in 6 weeks despite being initially treated for a peroneal strain.

Conclusions: While fibula fractures are common and considered less severe than other weight bearing bones, athletic trainers need to remain cognizant of and ensure completion of a thorough history and physical examination in order to prevent exacerbation of the injury, while allowing athletes to return to sports participation as quickly as possible.

This research was presented at the Rocky Mountain Athletic Trainers' Association Clinical Symposium and Business Meeting in Denver, Colorado, April 8–11, 2010.

Spontaneous Tension Pneumothorax in a Female Collegiate Tennis Player: A Case Report

Author: Tyson Salley, David Berry & Nancy Weir

Mentors: David Berry

Objective: We present the case of a female collegiate tennis player with a right spontaneous tension pneumothorax and the clinical decision making necessary in the evaluation, management, and surgical intervention of the athlete.

Background: An 18-year-old female (body mass=59.4 kg, height=180.3 cm, Body Composition=17 percent) collegiate tennis player presented with the signs and symptoms typical of a costochondral separation during a 90 second tennis match medical timeout. The athlete, in conjunction with the athletic trainer decided to resume the match. Approximately 2 ½ hours after the presentation of right rib pain, the athlete became acutely short of breath with increased costochondral pain. She was referred to the emergency room where a medical examination demonstrated markedly diminished right lung breath sounds compared to the left lung during auscultation. Emergency room radiographs demonstrated a tension pneumothorax and a tear at the apex of the right lung. No history of blunt trauma, infection, or illness was noted.

Differential Diagnosis: Intercostal strain, costochondral separation, pulmonary embolus, and asthma.

Treatment: A 28-French chest tube was inserted into the apex of the right lung for decompression and was connected to an underwater suction device to create a negative pressure for lung re-inflation. The re-expansion of the right lung was successful. During the insertion of the chest tube, apical pleural blebs and a secondary air leak into the lateral aspect of the right upper lobe next to a rather large fibrotic plaque was noted. Over the next several days the athlete experienced bouts of air leakage from the chest tube. Several attempts were made to reestablish a negative pressure between the chest tube and the suction device; however, this resulted in an increase in the size of the pneumothorax. For this reason the athlete underwent a right thoracoscopy and resection of the extreme apex of the right upper lobe and small area in the lateral aspect of the upper lobe where an obvious air leak was noted. A

talc pleurodesis was also performed to prevent recurrent pleural effusion. Following the second procedure the athlete was released to drive, not fly home; due to the pressure changes accompanying airline travel. Follow-up radiographs 3 weeks later demonstrated complete re-inflation of all but one small section near the apex of the right lung. Her activities were limited to activities of daily living in order to prevent overexertion and a reoccurrence of the pneumothorax. Radiographs 5 weeks later demonstrated complete inflation of the right lung. At this point the athlete was cleared for unrestricted physical activity.

Uniqueness: A pneumothorax is an collection of air or gas in the pleural cavity that occurs because of trauma, diseases or other naturally occurring defects. The prevalence of a spontaneous pneumothorax with no significant trauma are very rare, with only a few know cases in the athletic population participating in low impact sports like tennis. Pneumothorax ranks only second to only to rib fracture as the most common sign of chest injury. The frequency of occurances in women is 1.2-6 cases per 100,000 population per year. Complicating this case was the unknown presence of the blebs located on the apex of the right lung and potentially the change in altitude experienced by the athlete. Normally the athlete trains at an elevation of 2,578 feet, however, on this occasion the match took place at an altitude of 4,785 feet. As many as 10 percent of patients with a spontaneous pneumothorax may be asymptomatic at time of initial evaluation; most cases of spontaneous pneumothorax are not associated with exertion. The rate of repeated episodes of spontaneous pneumothorax after the first episode places the athlete at an increased risk for reoccurrence.

Conclusions: Increased clinical awareness is necessary when assessing thoracic pain and breathing difficulties during medical time outs. While the lack of time can influence the ability to complete a thorough assessment, every attempt to adequately assess the athlete must be made. In the case of chest pain and difficulty breathing, auscultation of the lungs should be a standard component of the assessment in order to properly diagnosis the athlete and determine the appropriate medical intervention(s).

This research was presented at the Rocky Mountain Athletic Trainers' Association Clinical Symposium and Business Meeting in Denver, Colorado, April 8-11, 2010.

C-Reactive Protein and Alpha 1-Antitrypsin Levels in Tears of Extended-Wear Contact Lens Patients

Authors: Aaron Barrett, Derek Gnehm & Jordan Jones

Mentor: Gary Nielsen

The purpose of this work is to evaluate tear levels of C-Reactive Protein (CRP) and $\alpha 1$ -antitrypsin as indicators of ocular surface inflammation in the extended wear (EW) of silicone hydrogel contact lenses. Ten current wearers of lotrafilcon B and seven wearers of senofilcon A were used in a cross-over study. Tear samples were collected after one week of non-contact lens wear to establish a baseline value. A second sample was collected after a week of EW to establish the test value. High sensitivity ELISA screenings were used to quantify the tear samples. $\alpha 1$ -antitrypsin was significantly increased in tears of lotrafilcon B wearers. Senofilcon A wearers displayed a rise in $\alpha 1$ -antitrypsin values that were not statistically significant. CRP, previously undetected in the tear film, was discovered but did not show a significant increase in levels. Clinical observations for inflammation correlated with the quantified protein levels to a fair degree of accuracy. Biochemical analysis of $\alpha 1$ -antitrypsin in tears could be used as a reliable indicator of ocular surface inflammation. Further research could prove useful in determining the values of CRP and $\alpha 1$ -antitrypsin in ophthalmic disease.

This research was presented at the Contact Lens Association of Ophthalmologists Research Symposium in Las Vegas, Nevada, September 23–25, 2010.

Identification of Respiratory Pathogens in Hospitalized “Non-Influenza” Utah Patients Using xMAP Technology

Authors: Joshua Clark & Shannon Reighard

Mentor: Scott Wright

The Unified State Laboratories: Public Health (USLPH) receives specimens for molecular Influenza testing from hospitalized patients with Influenza-like illness. Approximately half of the samples are negative for seasonal and non-seasonal Influenza virus strains. The procedure at USLPH is to test negative samples using a viral culture technique that is labor intensive and unreliable if the virus is dead or fastidious. Polymerase chain reaction (PCR) is more sensitive, specific, and rapid than traditional testing methods and some are capable of detecting multiple pathogens. ResPlex PCR panels and xMAP technology (Qiagen), which simultaneously targets eight viruses and five bacteria common to respiratory infections, was used in this research. Results show that of the 108 negative samples tested in the month of June 2009, 81 were sent to virology for a back-up culture and direct fluorescence antibody. Out of the 81 samples, only 3 (4 percent) respiratory pathogens were isolated from cultures. The ResPlex II panel found 19 of 81 (23 percent) samples positive for viruses. In conclusion, the cost of the ResPlex panel is higher than traditional viral cultures; however, our research concludes that multiplex PCR panels have a faster turn-around-time and greater sensitivity than traditional methods leading to rapid identification and precise treatment.

This research was presented at the 2010 Annual Meeting of the American Society for Clinical Laboratory Science in Anaheim, California, July 27–31, 2010.

Lyme Disease: A Potential for Deferral

Authors: Sherri Gagnon, Crystal Nelson Miller, Sarah Pierce & Kaelyn Udy

Mentor: Janet Oja

Borrelia burgdorferi, a spirochete, is the etiological agent in Lyme Disease (LD). The National Bone Marrow Registry has a permanent deferral for donation if infected with LD. The AABB however, relies on nonspecific screening questions to defer infected individuals. *B. burgdorferi* is viable in stored blood products and can be transmitted via transfusion. This study explored the prevalence of individuals infected with LD who donated blood. Surveys were sent to online state support groups. The survey was designed to gather information regarding: number of years it took to receive an accurate diagnosis of LD, individuals who donated blood prior to diagnosis, the states where infection was present, and incidentally learned of numerous co-infections.

Five hundred and five surveys were completed from 45 states. Ninety-seven percent (489/505) of the participants had LD, 24.3 percent donated blood prior to diagnosis, two to five years was the prevailing time for a correct diagnosis of LD, and 8 percent had a co-infection of *Babesia microti*. The results highlighted the incidence of infected people donating blood prior to a correct diagnosis of LD. This, coupled with *B. burgdorferi* being sustainable in blood products, supports the need for either LD testing or a focused pre-donation screening question.

This research was presented at the 2010 Annual Meeting of the American Society for Clinical Laboratory Science in Anaheim, California, July 27–31, 2010.

Perceived Emotional Aptitude of Clinical Lab Science Students Compared to Students in other Healthcare Profession Majors

Authors: Austin Adams, Cassandra Zundel, Kristin McCabe & Corey Dahl

Mentor: Travis Price

Emotional aptitude can be defined as the ability to recognize and manage one's own emotions and interpret the emotions of others. It has been speculated that Clinical Laboratory Science students may lack the emotional skills to most effectively interact with patients and other health care professionals. While this has been a topic of discussion in healthcare, a lack of research has been conducted to validate this assumption. This study will help assess the perceived emotional aptitude of Clinical Laboratory Sciences students compared to students of other healthcare majors in the Dr. Ezekiel R. Dumke College of Health Professions at Weber State University. The perceived emotional aptitude of the health care students was determined by completion of a self evaluation questionnaire that included questions about one's emotions, their understanding of others' emotions, and how they manage conflict. 401 questionnaires were completed, compiled, and analyzed. Although minor differences were seen in the responses, statistical analysis found these differences to be insignificant. Clinical Laboratory Science students perceive their emotional aptitude as equal to that of students of other health care majors at Dr. Ezekiel R. Dumke College of Health Professions.

This research was presented at the 2010 Annual Meeting of the American Society for Clinical Laboratory Science in Anaheim, California, July 27-31, 2010.

Observed Relationships Between Water Chemistry and Plant Communities in Groundwater-Dependent Ecosystems, Ashley National Forest, Uinta Mountains, Utah

Authors: Marek Matyjasik, Michael W Hernandez, James D Arnold, Sonya B Welsh, Richard L Ford, Lee M Bartholomew, Michele L Sanders, Alice J Shurtz & Darlene Koerner

A variety of groundwater-dependent ecosystems make up the montane wetlands of the Uinta Mountains, northeastern Utah. Unlike other ranges of the Rocky Mountains, these systems have been relatively unstudied. The Department of Geosciences at Weber State University partnered with the U.S. Forest Service, Ashley National Forest, to conduct a multidisciplinary baseline study of the relationships between plant communities and water chemistry within these alpine fens. The drainage basin of Reader Creek, located near the crest of the range on the south slope, was selected for detailed study because of its variety of wetland plant communities, minimal human impact, and homogenous bedrock geology.

Water chemistry data was collected from more than 100 locations over two summer seasons in 2008 and 2009. NAIP CIR orthoimagery from 2006 were used to map 13 plant-community classes by performing a maximum-likelihood supervised classification, based on selected training sites in the field. Representative water samples were also collected for the nine plant classes associated with standing water. This digital-imagery-based classification was also tested in the neighboring Dry Fork basin.

A significant geomorphic characteristic of these wetlands is their compartmentalization by a system of flarks and strings, oriented perpendicular to the dominant surface flow. Deeper portions of the peat work as highly isolated flow cells that store water for extended periods of time. The study area is characterized by relatively narrow range of pH values (5.1 to 7.6), with higher values corresponding to sloping fens with greater topographic relief and lower values observed in valley-bottom fens. Basic parameters including pH, dissolved oxygen, oxidizing-reducing potential, and concentration of phosphates and nitrates were divided into classes ranging from low to

high values. Preliminary observations indicate that each of the plant classes displays a unique combination of these parameters, with very little overlap between classes. The relationships between measured chemical parameters and the image-classified plant communities are currently being examined to determine if strong correlations can be identified and subsequently used to map varying groundwater conditions based on wetland types across the Uinta Mountains.

This research was presented at the 2010 GSA Denver Annual Meeting in Denver, Colorado, October 31–November 3, 2010.

Survey of Chitinase Activity in Halophilic Microorganisms from the Great Salt Lake

Author: Jared Phelps

Mentors: Craig Oberg & Michele Culumber

Chitin, a polymer of N-acetyl-D-glucosamine (GlcNAG), is the second most common biopolymer in nature. Its abundance in the Great Salt Lake (GSL) suggests that it is a significant source of carbon and nitrogen in that ecosystem. To determine how halophilic microorganisms in the GSL use chitin, a fluorescent assay was used that measures chitinase activity when the fluorescent tag 4-MUG is released from GlcNAG residues as they are metabolized. Six GSL bacterial isolates were incubated with the monomer, dimer, and trimer of 4-MUG GlcNAG and fluorescence was measured with a spectrophotometer to determine chitinase activity for each substrate. Results indicated that, of the six GSL isolates, *Halomonas* sp. and two *Salinivibrio* sp. were able to metabolize chitin, while one *Salinivibrio* sp. and two *Idiomarina* sp. were unable to metabolize chitin. Most isolates cleaved the chitin oligomers giving a unique degradation profile with the three 4-MUG GlcNAG substrates. *Halomonas* sp. metabolized 4-MUG GlcNAG initially, then cleaved the 4-MUG GlcNAG dimer and 4-MUG GlcNAG trimer after about two hours. The two chitinase utilizing *Salinivibrio* sp. cleaved the 4-MUG GlcNAG dimer before the other two GlcNAG substrates. From these results we conclude that each organism expresses a unique set of enzymes for chitin degradation. We suspect that a symbiotic relationship may exist between GSL halophilic species to facilitate a complete breakdown of the chitin polymer.

This research was presented at the 110th General Meeting of the American Society for Microbiology in San Diego, California, May 23–28, 2010.

Production of Tannin Binding Proteins in Prairie Voles (*Microtus orchrogaster*)

Authors: Mike Coombs & Michele M. Skopec

Mentor: Michele M. Skopec

Tannins are polyphenolic compounds produced by plants that readily bind protein. Binding of protein in the saliva decreases the amount of lubrication in the mouth and leads to an astringent sensation, which is thought to deter herbivores. Prairie voles (*Microtus orchrogaster*) are small herbivorous rodents that have variable responses to tannins. Prairie voles readily consume diets with tannic acid (TA), a hydrolysable tannin, but will refuse to consume diets containing quebracho, a condensed tannin. We hypothesized that prairie voles lack tannin binding proteins (TBPs) in their saliva capable of binding to condensed tannins like quebracho to decrease the astringency. We therefore surveyed voles for the presence of TBPs and the ability of their TBPs to bind to either TA or quebracho. Voles were either given a tannin free control diet, a control diet and isoproterenol injections to stimulate TBP production, or a 4 percent TA diet. Saliva and salivary glands were collected and the gel shift assay using native gel electrophoresis and silver staining was used for TBP detection. There were no treatment differences in the salivary gland weights of the voles but there were treatment differences in TBP production. Voles fed the control diet did not produce any TBPs, while voles given isoproterenol injections or 4 percent TA produced TBPs that bound to both TA and quebracho. We conclude that prairie voles are able to produce TBPs that bind to quebracho but hypothesize that TBP production is not stimulated by consumption of quebracho.

This research was presented at the Society for Integrative and Comparative Biology Annual Meeting in Salt Lake City, Utah, January 3–4, 2010.

Cognitive processes in Gambling Judgments: A Test of Dual Process Theory

Authors: Clint Norseth & Amy Trevethan

Mentors: Aaron Ashley & Eric Amsel

The present study gathered data regarding the time it takes college freshmen to choose between one of two gambles: One with a higher ratio (e.g., 2/10) and one with more winners (e.g., 16/100) or fewer losers (1/7). Only the former gamble offers a better chance of winning and so is the optimal response on the task. Dual process theory (Amsel et al., 2008, 2009) proposes that optimal responses on judgment and decision-making tasks require that participants inhibit default experiential (automatic, heuristic, effortless) processes which encodes and compares the absolute number of winners or losers and rely instead on analytical (conscious, algorithmic, and effortful) processes which encodes and compares ratios. It was predicted that additional time is required to inhibit default suboptimal responses and to respond optimally on the task. As a result a positive correlation was predicted between the frequency of optimal responses and reaction time. Although consistent with Dual Process theory, this prediction is inconsistent with work on intelligent or expert cognitive performance which predicts that intelligence or expertise would be associated with more optimal responses and faster reaction time. From this perspective those who respond optimally more often would do so more quickly than others because of their greater intellectual or expert abilities.

To test these predictions, college students were presented with 16 pairs of gambles to evaluate. Eight of the pairs of gambles were labeled as Conflict Items and required a choice between one gamble with more winners or fewer losers and the other had a higher ratio of winners. Each item was presented with a visual depiction of two jars containing white and black jellybeans. Underneath each jar was listed the total number of jellybeans in the jar. The number of jellybeans in each jar was different but easily recognized as a multiple of each other (20 vs. 80; 90 vs. 10). On half the trials (N=4) the number of black winning jellybeans was listed along with the total number of jellybeans and on the other half of the trials, the number of white losing jellybeans was listed. The optimal jar was counterbalanced on the left (labeled Jar A) and right side (labeled Jar B) of the page over trials. The presentation order of the trials was randomized but presented in a fixed order for all participants.

The frequency of optimal responses on the 4 “winning” and 4 “losing” tasks were summed and compared. Participants performed as well on the winning items ($M = 2.75$) as the losing ones ($M = 2.34$). There was no relation between sex or age on RBJ optimal responses or latency. As expected, math performance (simple computations and comparisons of probabilities expressed as ratios and fractions) was positively related to math performance ($r = .57$) and to negatively related to latency ($r = -.25$). Overall, there was a positive relation between average latencies and frequency of optimal responses ($r = .32$). The results provide support for the Dual Process theory and the claim that optimal judgments and decisions may involve inhibiting automatic processes.

This research was presented at the Rocky Mountain Psychological Association in Denver, Colorado, April 15-17, 2010.

Nutrition and Mental Health in the College Age Population

Authors: Shannon E. Tortolano & Amanda Kwok

Mentors: Theresa Kay & Dianna Abel

College students' nutritional status, with an emphasis on self-reported Omega-3 and folate intake, was correlated with depression and anxiety. It was hypothesized that those who exhibited more symptoms of anxiety and depression would also report poorer nutritional status. Results indicate a significant relationship between all aspects of nutrition and mood disorder; most marked were the correlative findings between anxiety and omega-3 status [$F(177) = -.285, p < .001$].

This research was presented at the Rocky Mountain Psychological Association's Annual Conference in Denver, Colorado, April 15–18, 2010.

Religiosity, Personality, and Religious Denomination

Authors: Michael Brown & Amanda Allen

Mentors: Aaron Ashley & Todd Baird

Previous research examining the relationship between religiosity and personality has found that different personality traits correlate differentially with separate aspects of religiosity. The current research replicates and extends these findings by examining the relationships between personality and religiosity across different religious denominations.

This research was presented at the Rocky Mountain Psychological Association Meeting in Greenwood Village, Colorado, April 15–17, 2010.

The Effects of Aerobic Exercise Intensity and Duration on Serum Levels of Brain-Derived Neurotrophic Factor in Healthy Human Adult Males

Authors: David Webb, Rodney Hansen & Matthew Schmolesky

Mentor: Matthew Schmolesky

Brain-derived neurotrophic factor (BDNF) is known to be involved in cellular development and growth, mood regulation, and cognitive functions such as learning and memory. Acute physical exercise temporarily elevates levels of serum BDNF (s-BDNF) in animals and in humans. This study examined for the first time the combined effects of exercise intensity and duration on s-BDNF levels in healthy human adult males age 18-25. Forty participants were randomly assigned to one of six stationary bike exercise conditions based on varying intensities (80 percent or 60 percent of heart rate reserve, or control) and durations (20 or 40 min). Compliance with vigorous (80 percent heart rate reserve, "Vig") and moderate (60 percent heart rate reserve, "Mod") exercise assignment was verified by heart rate monitor. Control subjects did not exercise but were treated identically to exercise subjects in all other ways. Pre- and post-exercise blood draws were conducted. Physical exercise caused a significant increase in s-BDNF levels relative to baseline (35.17+52.94 percent, $p < .05$) and compared to control conditions in which s-BDNF levels actually decreased (-15.57+22.73 percent, $p < .05$). The percentage of subjects that attained a substantial s-BDNF increase (≥ 10 percent post-pre) was significantly different across the vigorous (Vig40=100 percent, Vig20=85.7 percent), moderate (Mod40=62.5 percent, Mod20=80 percent) and control conditions (Con40=25.5 percent, Con20=0.0 percent) (one-way χ square $p < .05$). A two-way ANOVA for Δ s-BDNF across the four exercise conditions revealed no main effect for intensity (Vig=28.53 percent, Mod=42.84 percent, $p > .05$), duration (40 min=29.35 percent, 20 min=42.94 percent, $p > .05$), or the interaction thereof ($p > .05$). The mean s-BDNF integrals for Vig40 (340.84+134.00 ng ml-1) and Mod40 conditions (333.22+301.22 ng ml-1) were approximately double the mean of the Vig20 condition (156.32+92.27 ng ml-1). Collectively, these findings demonstrate that any of the exercise conditions may be sufficient to increase s-BDNF, but that long duration, vigorous exercise is most likely to yield benefits when considering both the probability for substantial BDNF increase and the value of the BDNF integral.

This research was presented at the Faculty for Undergraduate Neuroscience Meeting in San Diego, California, November 14-17, 2010.

The Role of Mathematics and Metacognition in Optimal Gambling Judgments: A Dual Process Account

Authors: Christopher Malan & Mike Young

Mentor: Eric Amsel

The present study addresses the role of mathematical and metacognitive skills on making optimal gambling judgments and decisions. Previous research has shown that suboptimal gambling decisions on the ratio bias task and actual gambling behavior are often made by otherwise rational college students because of their reliance on experiential (automatic, heuristic, and effortless) processes over analytical (conscious, algorithmic, and effortful) ones (Amsel et al., 2009). The tendency to rely on experiential and analytic processes was related to both situational factors (e.g., task variables) and metacognitive competence to regulate one's own thinking (Amsel et al., 2009). Amsel et al., (2008, Study 1) found that general mathematics ability as measured by the ACT Math scores were related to metacognitive competent status but not directly to gambling decisions. The present study more directly tests the role of both specific mathematical ability to understand probabilities and metacognitive skills to distinguish between analytic and experiential cognitive processes in optimal performance on a gambling task. Introductory Psychology (60 percent female and 60 percent freshmen) students completed four trials of the ratio bias judgment (RBJ) task which assesses their tendency to express "no preference" between two equal gambles. Participants were additionally assessed for their a) mathematical knowledge by testing RBJ task-relevant skills of converting and comparing fractions and percentages and b) metacognitive status by measuring their certainty that each RBJ response was analytically-based (logical, reflective, and mathematically sound). For every participant on each trial, the metacognitive task immediately followed participants' completion of the RBJ task. The 16 item mathematics task was completed prior to the four RBJ and metacognitive trials for half the participants and preceding the RBJ trials for the other half. As in previous studies (Amsel et al., 2009) most participants (92 percent) could be reliably categorized over the 4 trials as competent (certain that only "no preference" judgments are analytic) or non-competent (any other reliable response pattern) in metacognitive status. Participants were further categorized as Mathematically Competent (a score at or above the median split score of 94 percent) or Mathematically Non-competent (a score less

than the median split). A 2 (Order of Math Task) by 2 (Metacognitive Status) by 2 (Mathematics Knowledge) ANCOVA was computed, controlling for participants' age, sex and year in school. The analysis revealed an effect of Metacognitive Status (M Competent = 3.69 vs. M Non-competent = 1.74) and Math Order (M Math Task First = 2.94 vs. M Math Task Second = 2.12) on optimal "no preference" RBJ responses. Although mathematics performance did not predict RBJ performance, the data suggest the mathematics task primed optimal RBJ performance, providing evidence of the role of situational factors in analytic reasoning. A second 2 (Order of Math Task) by 2 (Mathematics Knowledge) ANCOVA on metacognitive status revealed a Mathematics effects (Above Median = 76 percent metacognitive competent status vs. Below Median = 40 percent metacognitive competent status). Although the mathematics ability was related to metacognitive status, there was no priming of metacognitive status by mathematics performance, attesting to the stability of metacognitive status in light of task variability. The results were interpreted as evidence of the role of unconscious (priming) and conscious (metacognitive competence) regulatory processes on optimal responding. The discussion highlights the theoretical importance of regulatory processes (Klaczynski, 2007) in dual processing theories of judgment and decision-making.

This research was presented at the Rocky Mountain Psychological Association Meeting in Denver, Colorado, April 15–17, 2010.