ANOVA: Comparing More Than Two Treatments

Sergey Tarima, PhD

Sponsored by the Clinical and Translational Science Institute (CTSI)
and the Department of Population Health / Division of Biostatistics

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Outline

- Refreshment: t-test
- Multiple Comparisons
- One-way ANOVA
- Two-way ANOVA

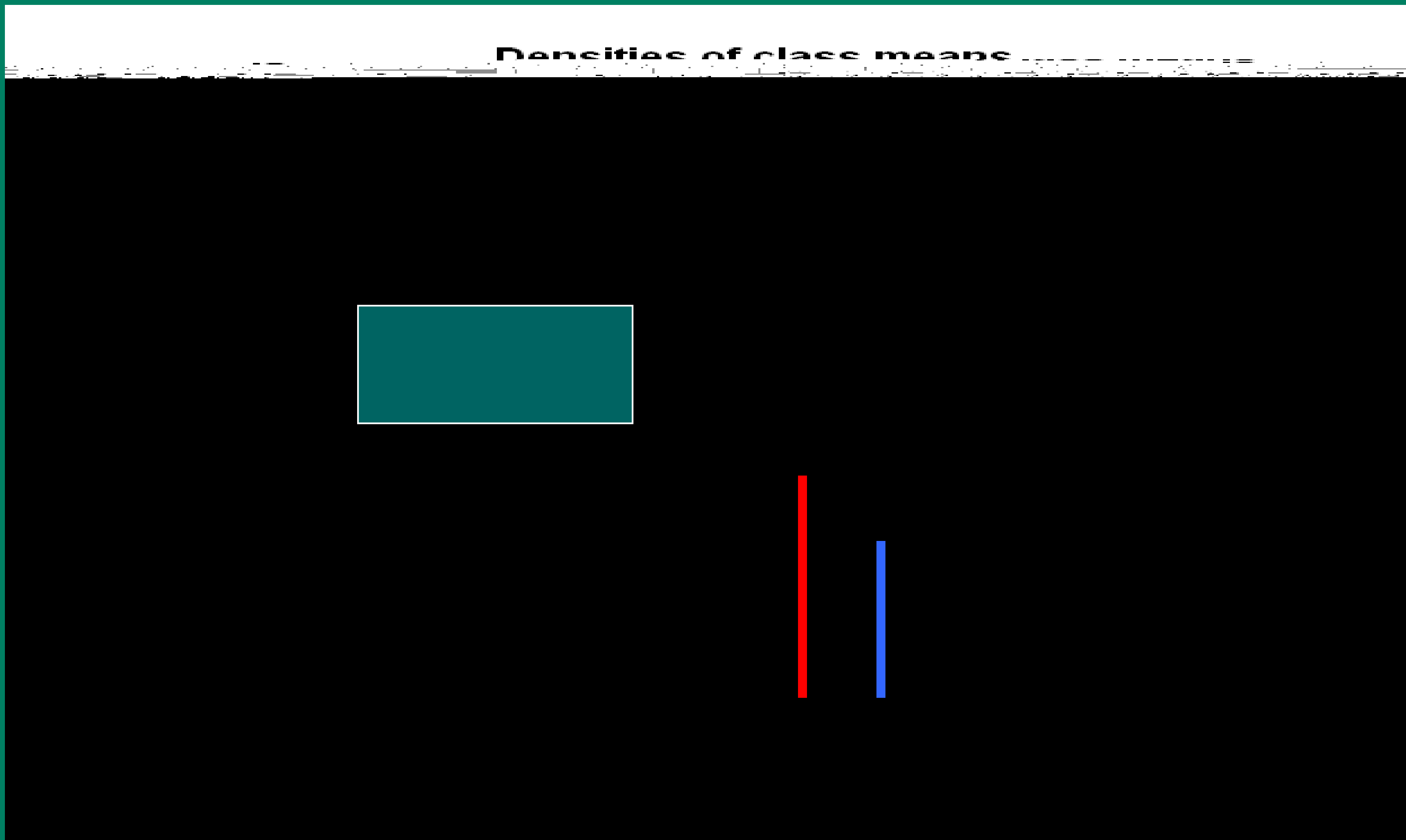
T-test (1)

- Suppose we have 2 groups (for example, the class of Mr. First and the class of Ms. Second)
- We want to compare mean response between groups (what is our response? suppose - the mean number of tardy slips)
- We assume that our means are approximately normally distributed

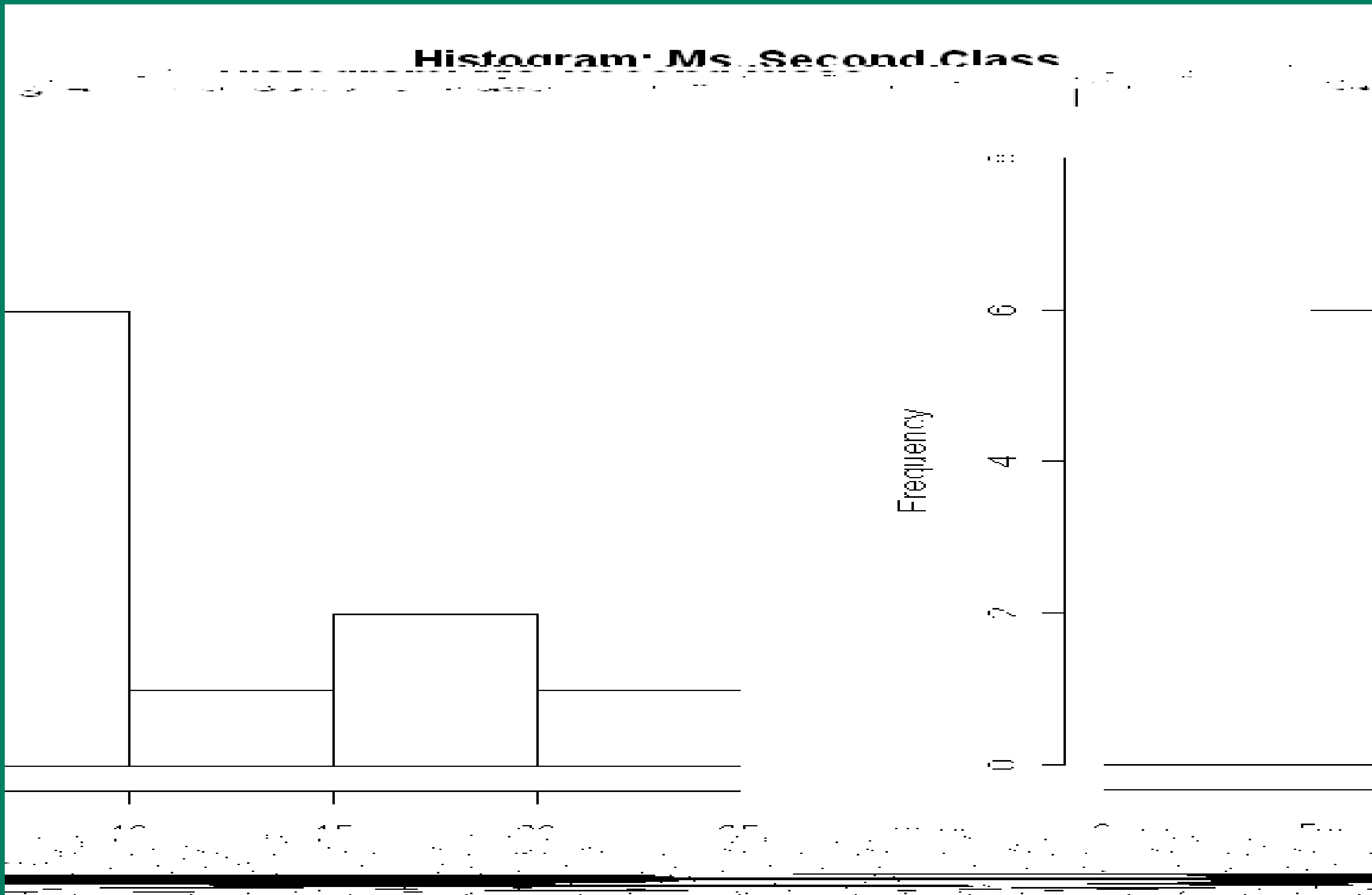
Recall what t-test does (2)

- What does it mean normally distributed? … bell-shaped curve ☺.
- Suppose Mr. First provided the following data: 1,10,12,0,0,5,6,2,3,7,8,9,12,12,10,0.
- Ms. Second reported: 10,20,3,5,6,2,13,19,8,2,5,2,5,7,9,22,1,10
- We can see that there were 16 students in Mr. First class and 18 in Ms. Second.

Normal Densities of the two classes



Histograms (2)



Normal data?

- Our data do NOT look normal – it is obvious from the histograms
- This means that our data cannot be fully described only through means and standard deviations
- However, for large sample sizes averages approximately follow a normal distribution
- Thus, t-test allows comparing means between two groups for large sample sizes (some claim that 30 per group is enough)

T-test (3)

- Our data are likely not normally distributed… but we will proceed with our illustrative example, as if they were
- We estimate the mean for Mr. First group as (1+10+…+12+10)/16=6.0625. Standard deviation as 4.567549.
- The estimated mean and standard deviation are 8.3 and 6.5

ANOVA (R output)

the output is

Note, P-value is the same

```
Analysis of Variance Table
Response: data[, 1]
          Df  Sum Sq Mean Sq F value Pr(>F)
data[, 2]  1   41.57   41.57  1.3034 0.2621
Residuals 32 1020.55   31.89
```

So, ANOVA applied to two groups is the same as a two sample t-test

Three classes

Multiple comparisons

- Now we have 3 groups.
- We can use t-test to compare whether the number of “tardy slips” is different between Mr. First and Ms. Second classes.
- Similarly we can compare Mr. First and Mrs. Third classes, and Ms. Second and Mrs. Third classes.
- What would be our type I error?

Three t-tests

Comparing 1st vs 3rd classes P-value = 29.6%

Comparing 2nd vs 3rd classes P-value = 1.9%

Comparing 1st vs 2nd classes P-value = 26.2%

What can we say if we see these results?
…the mean number of "tardy slips" is significantly higher in Ms. Second than in Mrs. Third class!
but this is true only if we are looking at
5% significance level
and testing only ONE hypothesis (2nd vs 3rd)

Beware: Type I Error (1)

- One test: 5% significance level means if P-value is below 5% we reject the null of no difference, otherwise "fail to reject"
- In other words: 5 times out of 100 we falsely reject the null when it is true !!!
- Two (independent) tests: reject the null if P-value < 5%. No error in 95 out of 100 cases in each of the tests. So, the probability that we fail to reject the null is 0.95*0.95 = 0.91 !!! (91 out of 100)

ANOVA (1)

- The null hypothesis: The mean number of "tardy slips" is the same across all three groups!!!
- This hypothesis can be tested using ANOVA test and the type I error will stay at a priory defined significance level (say 5%).
- ANOVA has a mathematical justification via sum of squares partitioning… not in this lecture

ANOVA (2) ("tardy slips" example, now 3 groups)

R OUTPUT:

```
Analysis of Variance Table
Response: data[, 1]
                     Df  Sum Sq Mean Sq F value  Pr(>F)
as.factor(data[, 2])  2  145.97   72.98  3.0632 0.05376 .
Residuals            63 1501.02   23.83
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

P-value

ANOVA model (5)

y_{ij} is what we observe (actual numbers)

ε_{ij} is a random noise or error

$$y_{ij} \qquad\qquad\qquad\qquad {}_{i} \qquad\qquad {}_{ij}$$

μ and α_i are not observed (this is what we are estimating)

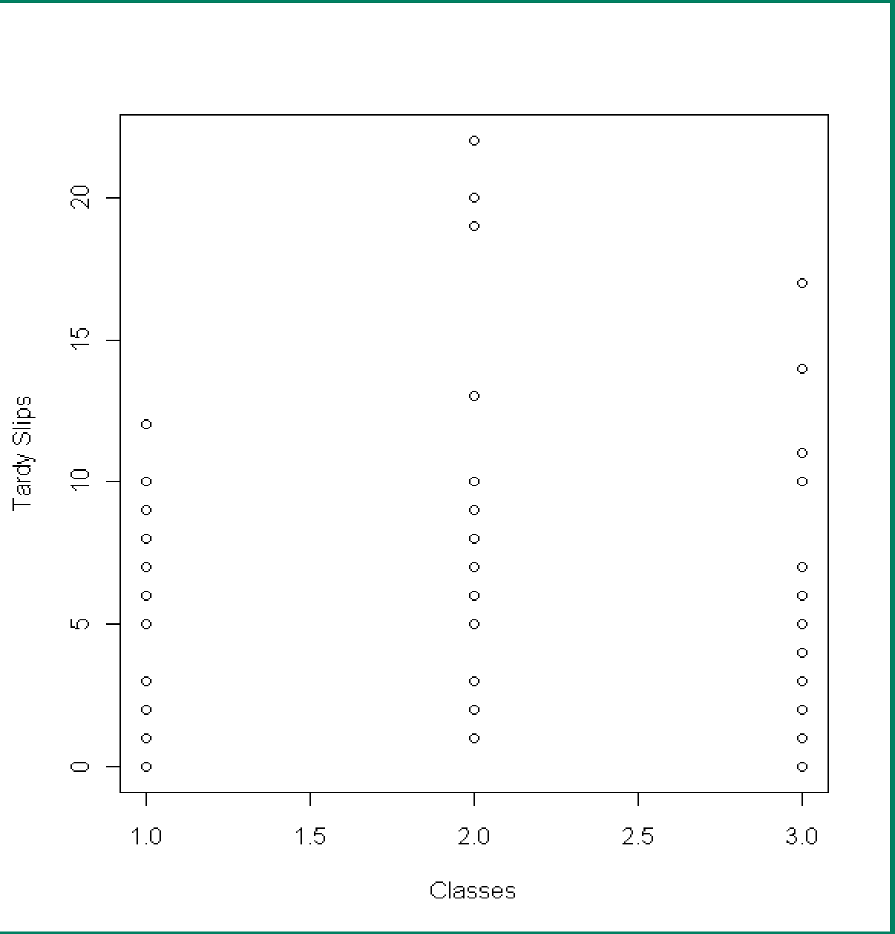
ANOVA Model (8) "tardy slips" example

- We will use reference parameterization. So, we set α_1=0 and we will need to estimate α_2, α_3, and μ.
- In this case
  (1) μ represents the mean number of "tardy slips" in Mr. First class,
  (2) $\mu + \alpha_2$ is the mean for Ms. Second, and
  (3) $\mu + \alpha_3$ is the mean for Mrs. Third.

ANOVA Model (10) ("tardy slips" example)

It is good to explore our data first:

SCATTERPLOT



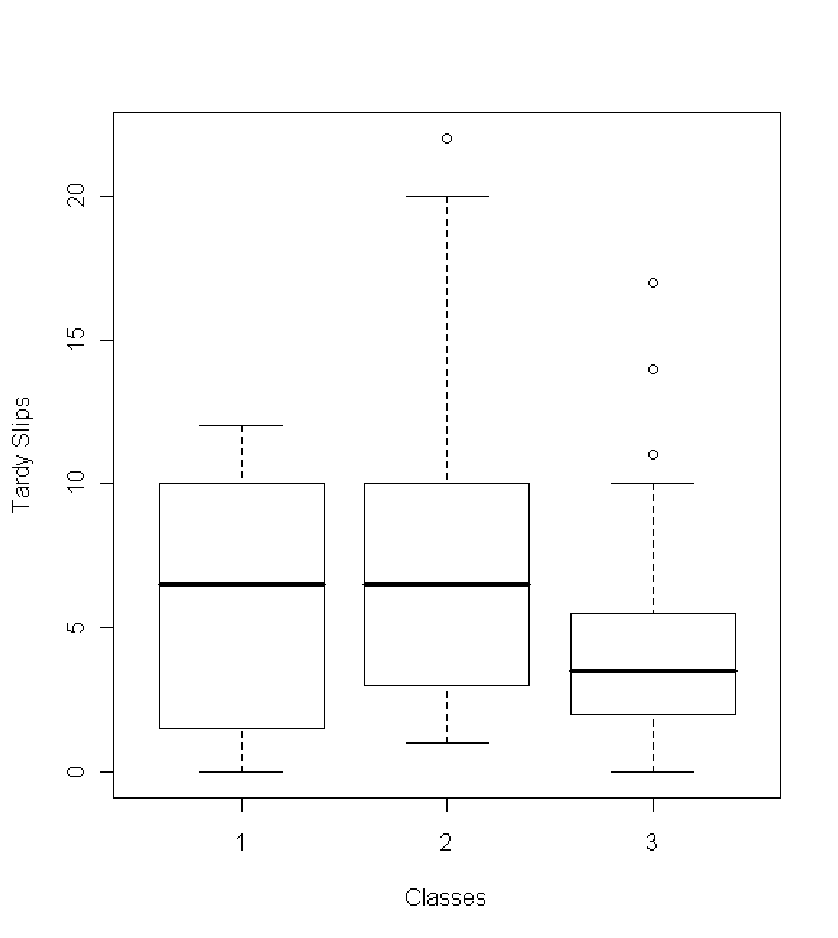
Note, some multiple observations are plotted as a single point

ANOVA Model (11) ("tardy slips" example - R)

Another exploration tool:

BOX PLOTS

Some observations may be outliers and if excluded we may see a smaller variance in the 3rd group



ANOVA model (14)

If the data are not well described by ANOVA model the following can be considered:

- outcome transformation (for example, the natural logarithm of the number of “tardy slips”)

- outlier detection; outliers can be excluded if justified (suppose somebody lives in a different school attendance area)

ANOVA Model (15)
(“tardy slips” example)

```
Analysis of Variance Table

Response: data[, 1]
                     Df  Sum Sq Mean Sq F value  Pr(>F)
as.factor(data[, 2])  2  145.97   72.98  3.0632 0.05376 .
Residuals            63 1501.02   23.83
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1
  ‘ ’ 1
```

Significant at 10%

ANOVA Model (17) ("tardy slips" example)

- So, ANOVA test says that the groups are different (at 10%), but when we compared classes 1 and 2 (P-val =19.1%) and classes 1 and 3 (P-val = 37.2%)… not different (note, P-values are the same as in t-tests)
- The hypothesis of no difference between classes 2 and 3 corresponds to H_0: $\alpha_2 = \alpha_3$.

ANOVA Model (18) (multiple comparisons)

- There many procedures developed for multiple comparisons – the simplest and the most conservative is the Bonferroni method.
- According to Bonferroni, we can divide the significance level (say 5%) on the number of tests (say 50). Then each test is tested at 5% / 50=0.1% significance level.

Summary of ANOVA (19)

- We talked about the simplest ANOVA model and test: one-way ANOVA
- ANOVA represents a generalization of a t-test for more than two groups.
- ANOVA assumes that noise follows $N(0,\sigma^2)$.
- ANOVA assumes equal variance within each group.

Two-way ANOVA (1)

- One-way ANOVA considers only one way to classify subjects. In our example, all students were divided into three classes.
- Two-way ANOVA allows us to consider another factor. Suppose “attendance area” is our second factor. Each student is classified as living in the school attendance area or not.

Two-way ANOVA model (2)

y_{ijk} is what we observe (actual numbers)

ε_{ijk} is a random noise or error

y_{ijk} $\quad$ i $\quad$ j $\quad$ ijk

μ, α_i , and β_j are not observed (this is what we are estimating)

Two-way ANOVA model (3)

Two-way ANOVA model (4)

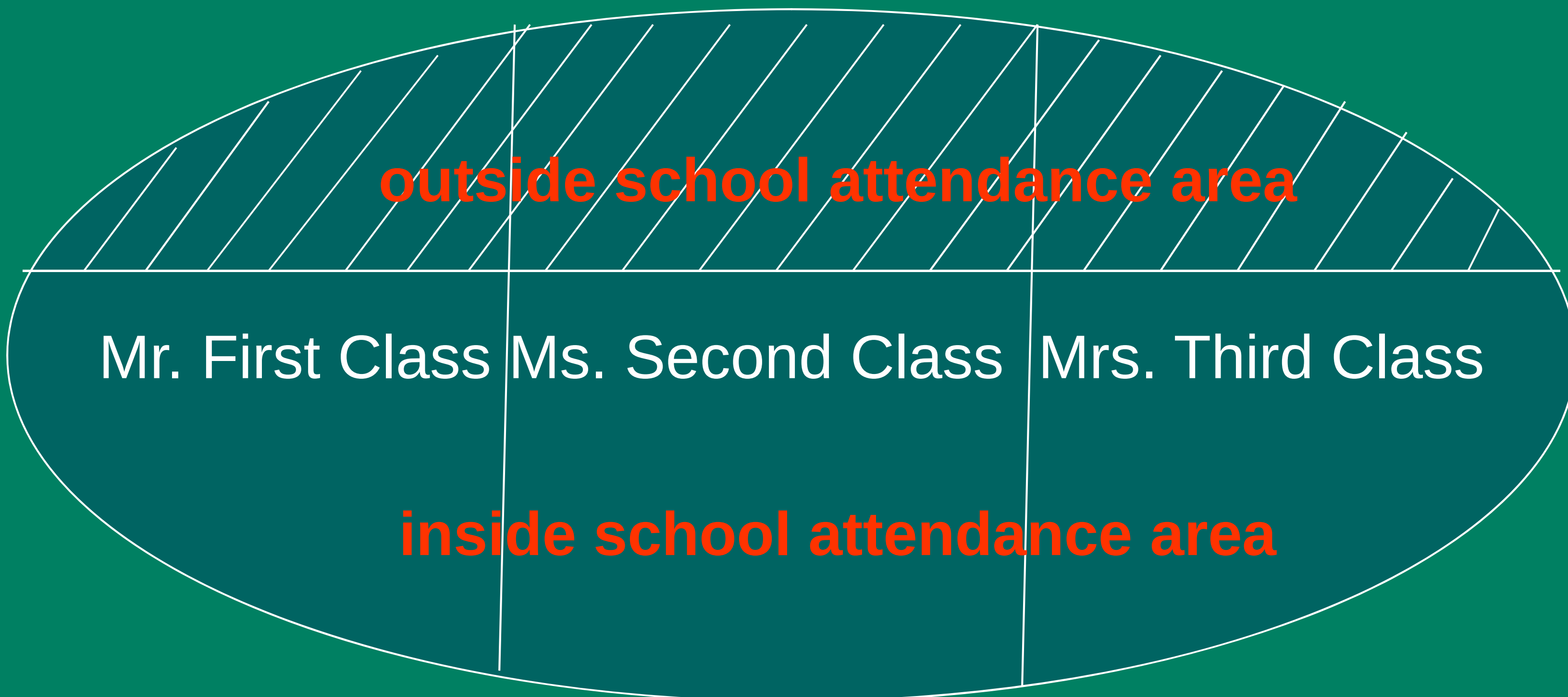
Effect of class continues

```
Analysis of Variance Table

Response: data[,1]
          Df  Sum Sq Mean Sq F value  Pr(>F)
data[,2]   2  145.97   72.98  3.0370 0.05517 .
data[,3]   1   11.05   11.05  0.4596 0.50032
Residuals 62 1489.97   24.03
```

Population

The population we are making inference about is restricted to three groups only, but we also control for "attendance area" factor



Resources

- The Clinical and Translation Science Institute (CTSI) supports education, collaboration, and research in clinical and translational science: www.ctsi.mcw.edu
- The Biostatistics Consulting Service provides comprehensive statistical support

  http://www.mcw.edu/biostatsconsult.htm

Free drop-in consulting

- MCW/Froedtert/CHW:
  - Monday, Wednesday, Friday 1 – 3 PM @ CTSI Administrative offices (LL772A)
  - Tuesday, Thursday 1 – 3 PM @ Health Research Center, H2400
- VA: 1st and 3rd Monday, 8:30-11:30 am
  - VA Medical Center, Building 70, Room D-21
- Marquette: 2nd and 4th Monday, 8:30-11:30 am
  - Olin Engineering Building, Room 338D