Measuring periciliary liquid depth in newborn pigs with cystic fibrosis disease and in control pigs

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Tuesday

Research Team

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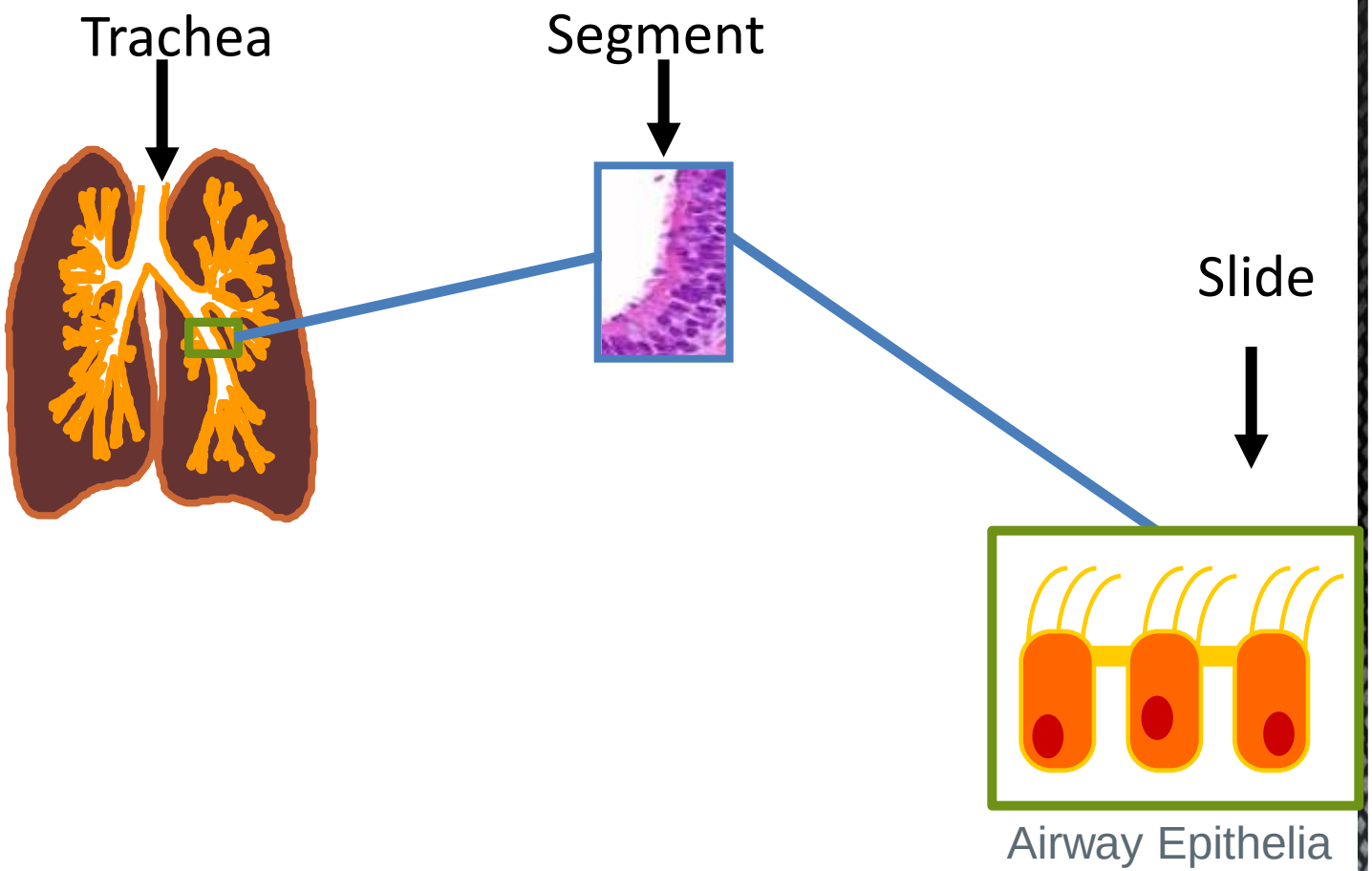
- Michael V. Rector, Research Assistant, Carver College of Medicine, University of Iowa
- Dr. Michael J. Welsh, Professor, Howard Hughes Medical Institute, Carver College of Medicine, University of Iowa

Introduction

- What is cystic fibrosis (CF)
- How is CF caused
  - CFTR protein
- Primary problem: lung disease and mucus in the airways

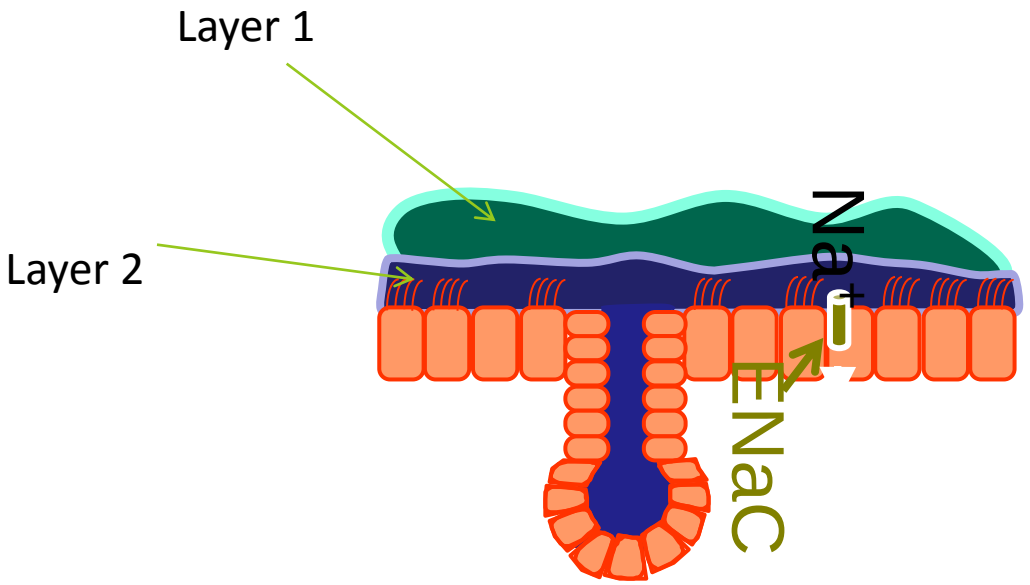
The Lung



Picture by Dr. David Stoltz

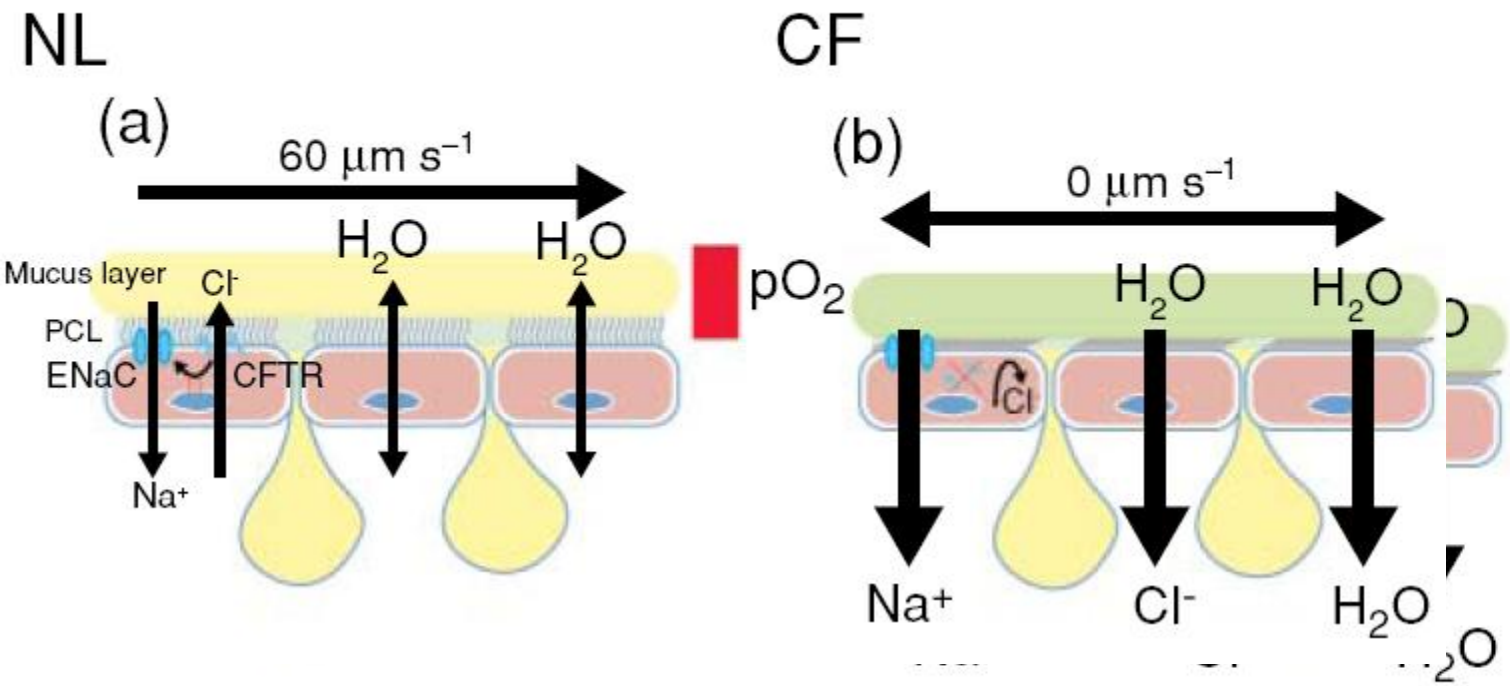
Mucociliary Clearance

- Mucociliary clearance is by airway surface liquid and cilia.
- When CFTR is absent, water is transported out of the epithelium



Picture by Dr. David Stoltz

PCL depth



Boucher, R. C. (2007). Airway Surface Dehydration in Cystic Fibrosis: Pathogenesis and Therapy. *The Annual Review of Medicine*, 157-170.

Hypothesis

- In CF pigs, PCL depth is reduced through the sodium ion transport channel in the epithelial cells at the airway surface.

Objectives

- To model the data from the experiment measuring PCL in newborn pigs
- To estimate of the variance components
- To examine if there is a difference in the Periciliary Liquid (PCL) depth between CF and Non-CF newborn pigs

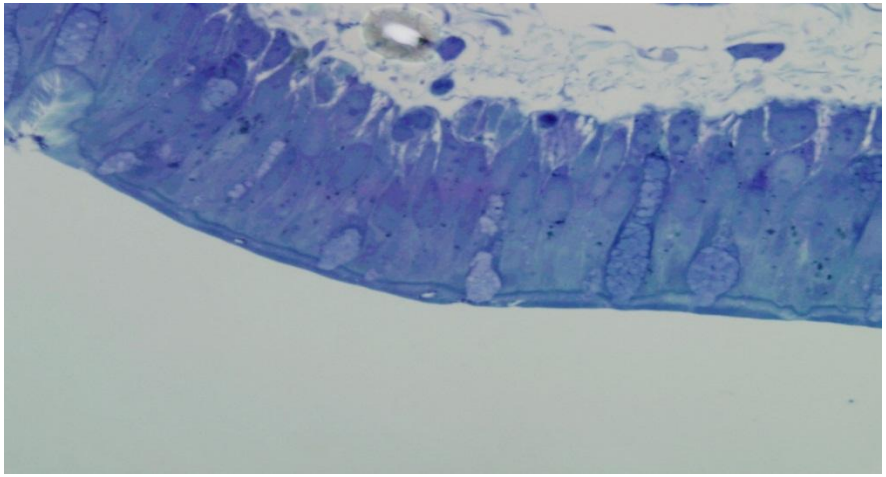
Justification

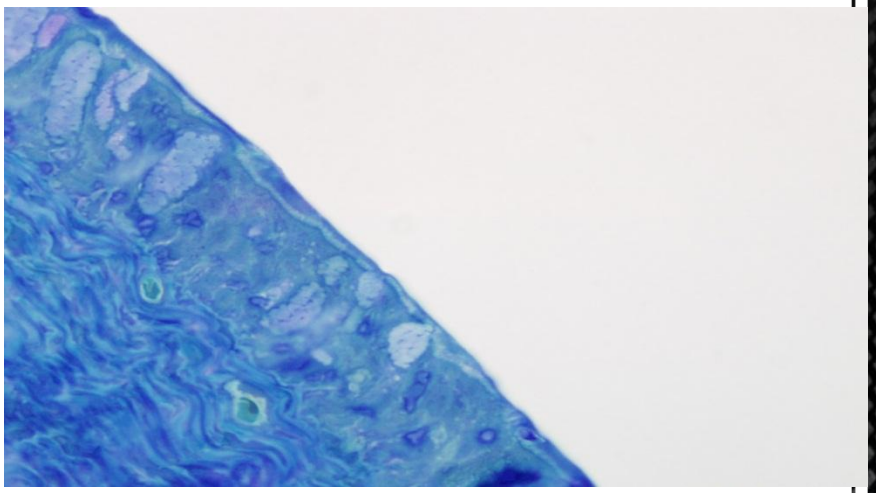
- PCL depth is hard to measure in humans
- PCL depth was measured in pigs
- The pig model was generated by researchers at the University of Iowa (mice with CF genotype do not get sick)

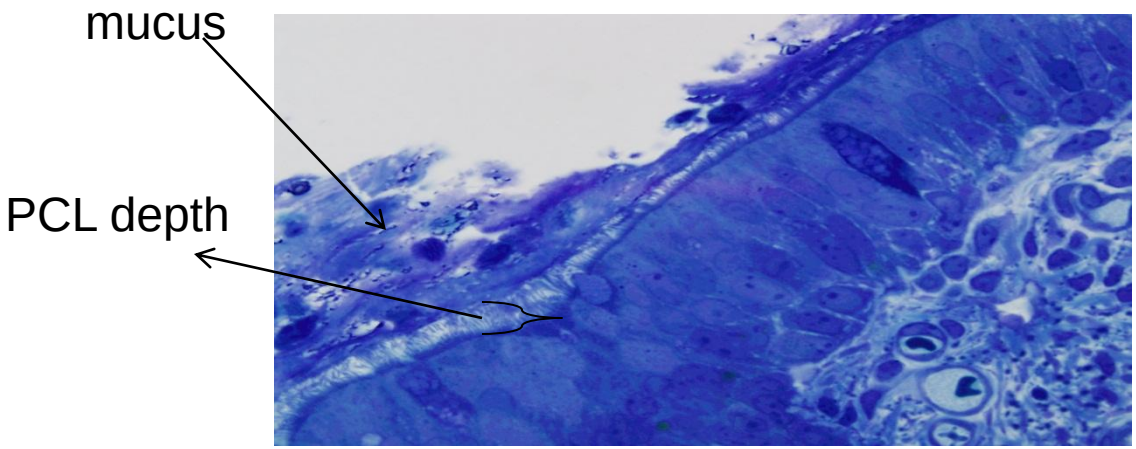
Pigs litter (Pictures taken from Dr. Welsh laboratory)

PCL depth

(Pictures taken from Dr. Welsh laboratory)



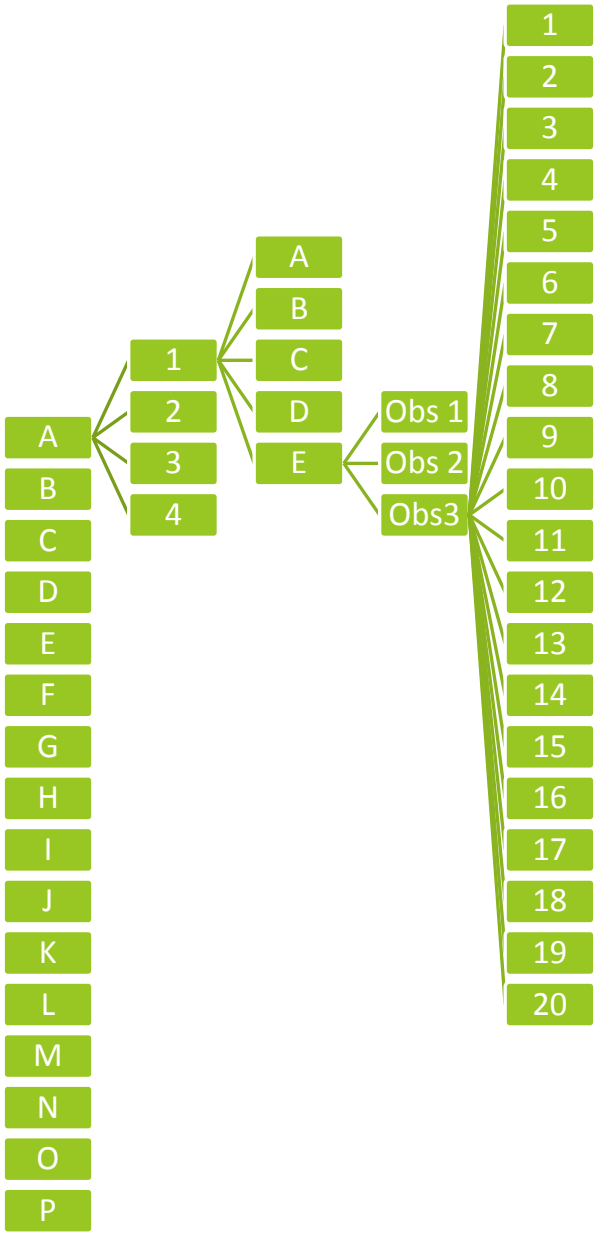




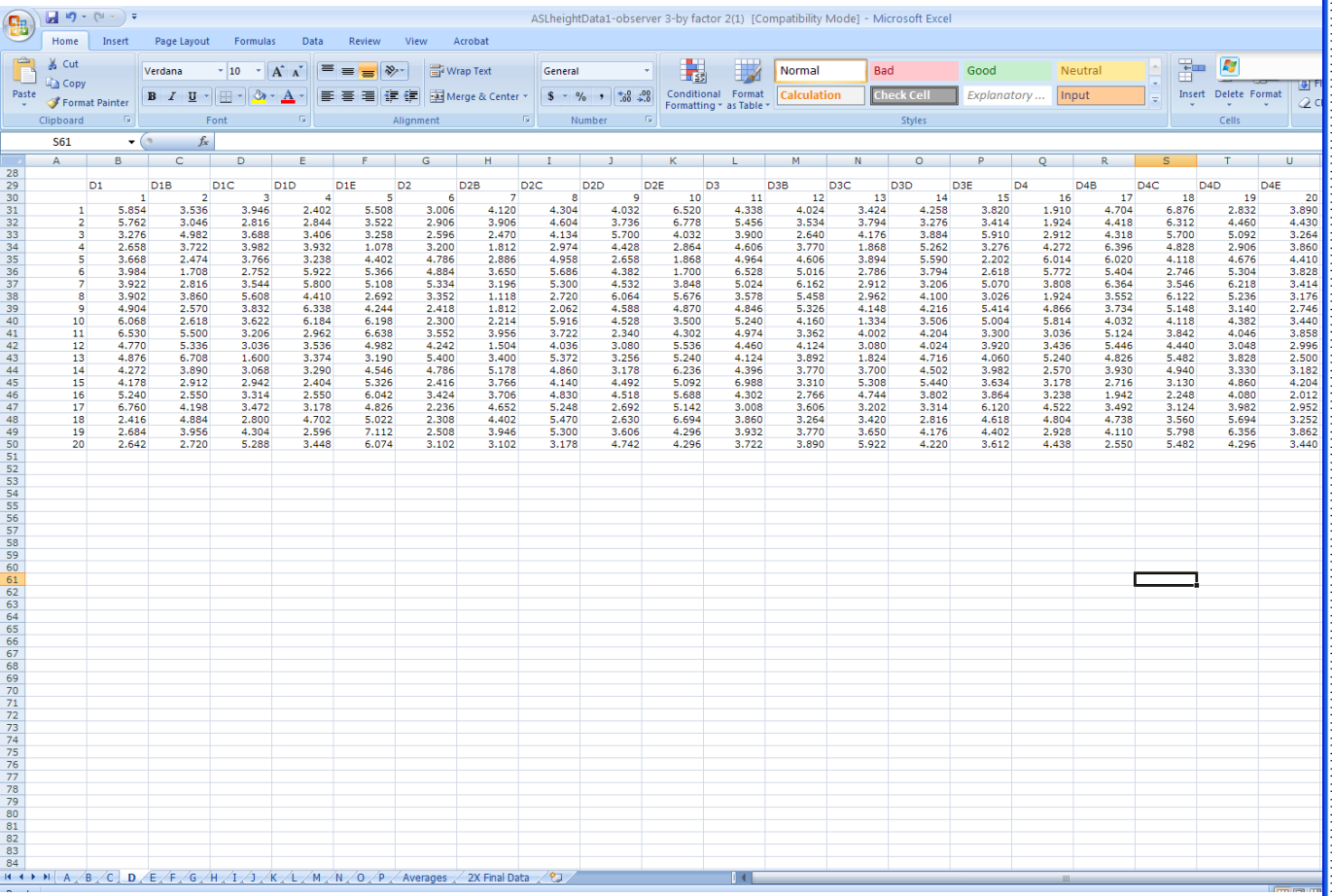
Experimental Methods

- Pigs with mutated *CFTR* gene
- Airway surface was analyzed in 16 pigs; 5 of them newborns with CF and 8 newborn controls, as well as 3 additional pigs.
- Each trachea was divided into 4 segments, and 5 slides were made from each segment.
- 20 measurement of PCL depth (µm)were made by each of 3 independent observers on each slide.
- Over 18,000 measurements to analyze.

Hierarchical Model: Fixed Effect with Nested Random Effects



How the data looks



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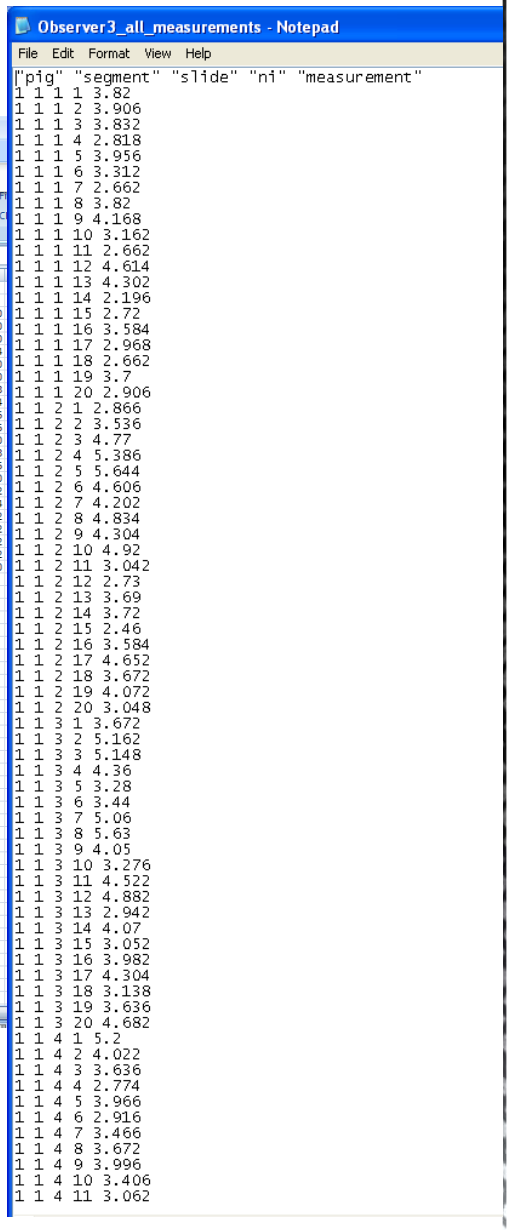
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	5.854	3.536	3.946	2.402	5.508	3.006	4.120	4.304	4.032	6.520	4.338	4.024	3.424	4.258	3.820	1.910	4.704	6.876	2.832	3.890
2	5.762	3.046	2.816	2.844	3.522	2.906	3.906	4.604	3.736	6.778	5.456	3.534	3.794	3.276	3.414	1.924	4.418	6.312	4.460	4.430
3	3.276	4.982	3.688	3.406	3.258	2.596	2.470	4.134	5.700	4.032	3.900	2.640	4.176	3.884	5.910	2.912	4.318	5.700	5.092	3.264
4	2.658	3.722	3.982	3.932	1.078	3.200	1.812	2.974	4.428	2.864	4.606	3.770	1.868	5.262	3.276	4.272	6.396	4.828	2.906	3.860
5	3.668	2.474	3.766	3.238	4.402	4.786	2.886	4.958	2.658	1.868	4.964	4.606	3.894	5.590	2.202	6.014	6.020	4.118	4.676	4.410
6	3.984	1.708	2.752	5.922	5.366	4.884	3.650	5.686	4.382	1.700	6.528	5.016	2.786	3.794	2.618	5.772	5.404	2.746	5.304	3.828
7	3.922	2.816	3.544	5.800	5.108	5.334	3.196	5.300	4.532	3.848	5.024	6.162	2.912	3.206	5.070	3.808	6.364	3.546	6.218	3.414
8	3.902	3.860	5.608	4.410	2.692	3.352	1.118	2.720	6.064	5.676	3.578	5.458	2.962	4.100	3.026	1.924	3.552	6.122	5.236	3.176
9	4.904	2.570	3.832	6.338	4.244	2.418	1.812	2.062	4.588	4.870	4.846	5.326	4.148	4.216	5.414	4.866	3.734	5.148	3.140	2.746
10	6.068	2.618	3.622	6.184	6.198	2.300	2.214	5.916	4.528	3.500	5.240	4.160	1.334	3.506	5.004	5.814	4.032	4.118	4.382	3.440
11	6.530	5.500	3.206	2.962	6.638	3.552	3.956	3.722	2.340	4.302	4.974	3.362	4.002	4.204	3.300	3.036	5.124	3.842	4.046	3.858
12	4.770	5.336	3.036	3.536	4.982	4.242	1.504	4.036	3.080	5.536	4.460	4.124	3.080	4.024	3.920	3.436	5.446	4.440	3.048	2.996
13	4.876	6.708	1.600	3.374	3.190	5.400	3.400	5.372	3.256	5.240	4.124	3.892	1.824	4.716	4.060	5.240	4.826	5.482	3.828	2.500
14	4.272	3.890	3.068	3.290	4.546	4.786	5.178	4.860	3.178	6.236	4.396	3.770	3.700	4.502	3.982	2.570	3.930	4.940	3.330	3.182
15	4.178	2.912	2.942	2.404	5.326	2.416	3.766	4.140	4.492	5.092	6.988	3.310	5.308	5.440	3.634	3.178	2.716	3.130	4.860	4.204
16	5.240	2.550	3.314	2.550	6.042	3.424	3.706	4.830	4.518	5.688	4.302	2.766	4.744	3.802	3.864	3.238	1.942	2.248	4.080	2.012
17	6.760	4.198	3.472	3.178	4.826	2.236	4.652	5.248	2.692	5.142	3.008	3.606	3.202	3.314	6.120	4.522	3.492	3.124	3.982	2.952
18	2.416	4.884	2.800	4.702	5.022	2.308	4.402	5.470	2.630	6.694	3.860	3.264	3.420	2.816	4.618	4.804	4.738	3.560	5.694	3.252
19	2.684	3.956	4.304	2.596	7.112	2.508	3.946	5.300	3.606	4.296	3.932	3.770	3.650	4.176	4.402	2.928	4.110	5.798	6.356	3.862
20	2.642	2.720	5.288	3.448	6.074	3.102	3.102	3.178	4.742	4.296	3.722	3.890	5.922	4.220	3.612	4.438	2.550	5.482	4.296	3.440




```
"pig" "segment" "slide" "ni" "measurement"
1 1 1 1 3.82
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1 1 1 3 3.832
1 1 1 4 2.818
1 1 1 5 3.956
1 1 1 6 3.312
1 1 1 7 2.662
1 1 1 8 3.82
1 1 1 9 4.168
1 1 1 10 3.162
1 1 1 11 2.662
1 1 1 12 4.614
1 1 1 13 4.302
1 1 1 14 2.196
1 1 1 15 2.72
1 1 1 16 3.584
1 1 1 17 2.968
1 1 1 18 2.662
1 1 1 19 3.7
1 1 1 20 2.906
1 1 2 1 2.866
1 1 2 2 3.536
1 1 2 3 4.77
1 1 2 4 5.386
1 1 2 5 5.644
1 1 2 6 4.606
1 1 2 7 4.202
1 1 2 8 4.834
1 1 2 9 4.304
1 1 2 10 4.92
1 1 2 11 3.042
1 1 2 12 2.73
1 1 2 13 3.69
1 1 2 14 3.72
1 1 2 15 2.46
1 1 2 16 3.584
1 1 2 17 4.652
1 1 2 18 3.672
1 1 2 19 4.072
1 1 2 20 3.048
1 1 3 1 3.672
1 1 3 2 5.162
1 1 3 3 5.148
1 1 3 4 4.36
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1 1 3 14 4.07
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1 1 4 2 4.022
1 1 4 3 3.636
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1 1 4 6 2.916
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1 1 4 8 3.672
1 1 4 9 3.996
1 1 4 10 3.406
1 1 4 11 3.062
```

Statistical Methods

- No cf effect: $y_{ijklm} = \mu + \delta_l + e_i + e_{i(j)} + e_{ij(k)} + e_{ijklm}$

  $l=1,\ldots,3;\ i=1,\ldots,16;\ j=1,\ldots,4;\ k=1,\ldots,5;\ m=1,\ldots,20$

- cf effect: $y_{ijklm} = \mu + \theta\, cf_i + \delta_l + e_i + e_{i(j)} + e_{ij(k)} + e_{ijklm}$

  where cf_i is 1 for pigs with CF and 0 for non-CF

- Plot all the data (19,199 measurements)
- Plot means and medians of y_{ijklm} (the 20 measurements by each observer on each slide)

Analysis

- Analyze mean of y_{ijklm} over 20 measurements
- Analyze medians, similarly
- Alternative analysis uses all 19,199 measurements
- Some measurements missing, the data is unbalanced.
- We used lmer method of R, with REML
- We import the data into R, from the Excel files

R code

- library(lme4)

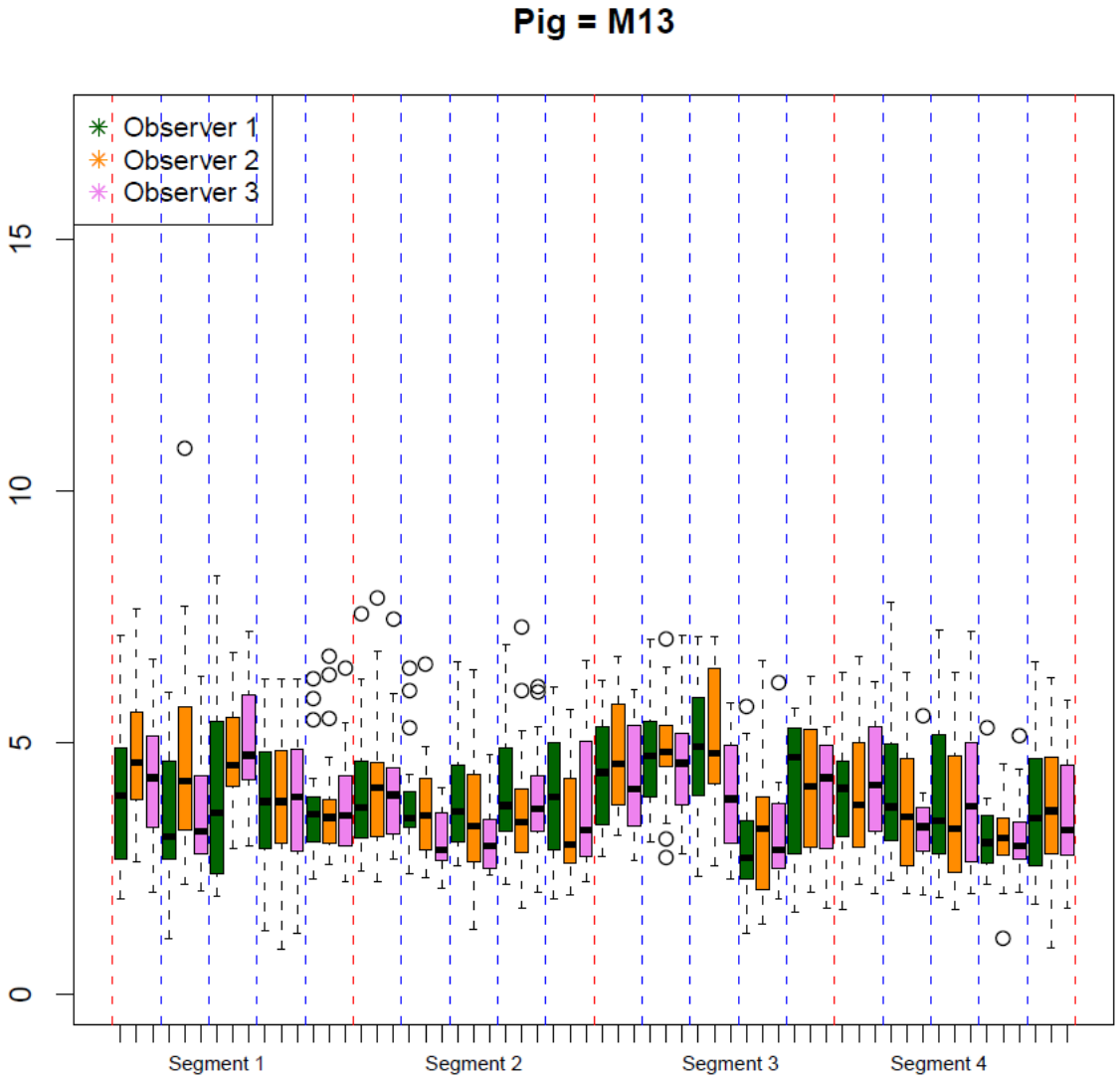
- means <- read.table("H:\\Observers_means_Long_pigs.txt",header=T)

- attach(means)
- Seg <- factor(means$segment)
- Slide <- factor(means$slide)
- Pig <- factor(means$pig)
- Obs <- factor(means$v)

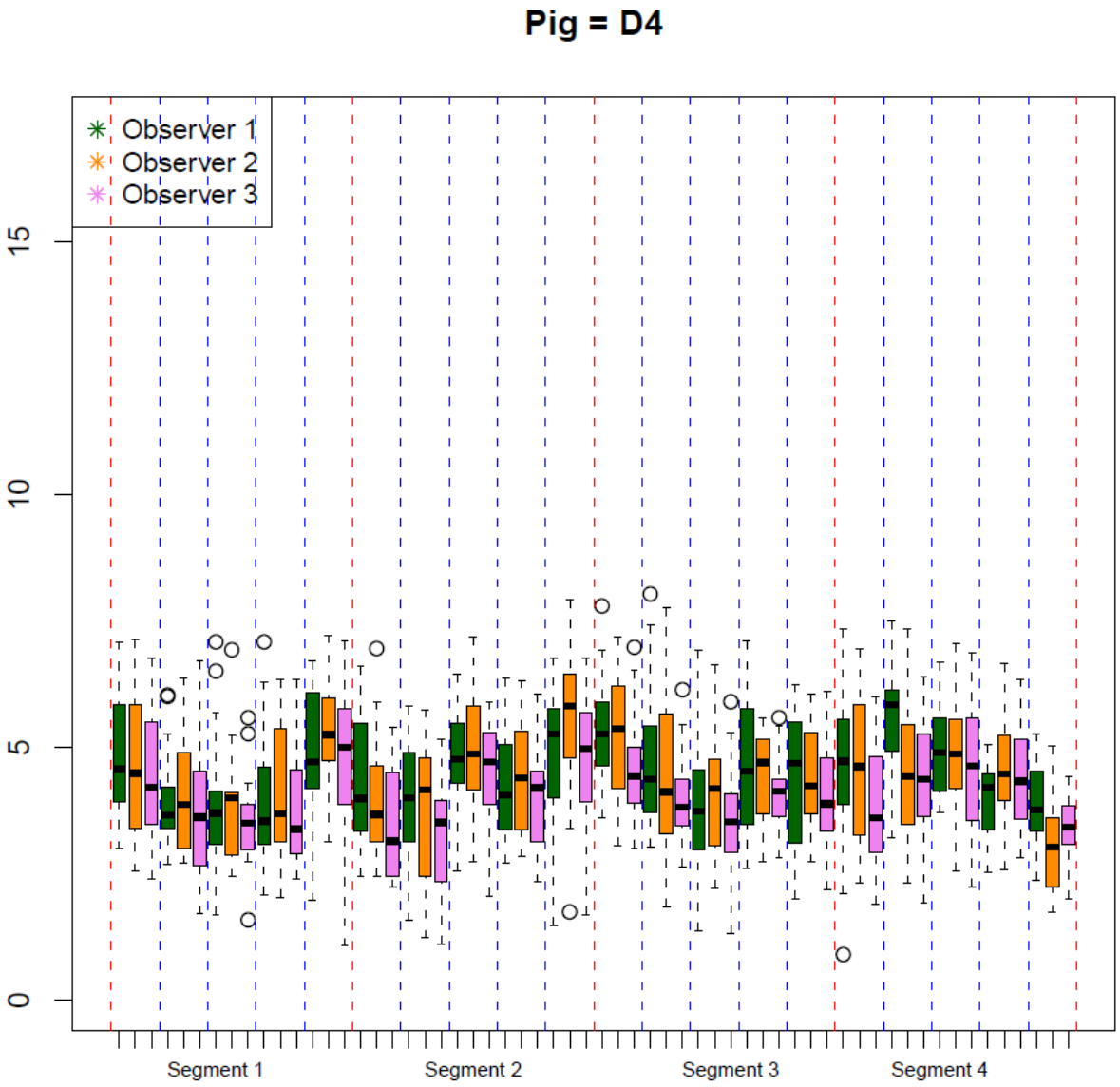
- cf <- rep(1:length(measurement))
- cf[Pig==1] <- 0
- cf[Pig==4] <- 0
- cf[Pig==5] <- 0
- cf[Pig==6] <- 0
- cf[Pig==11] <- 0
- cf[Pig==12] <- 0
- cf[Pig==14] <- 0
- cf[Pig==16] <- 0
- cf
- meanspigs <-
- lmer(measurement ~ Obs + (1|Pig/Seg/Slide))
- summary(meanspigs)

Results

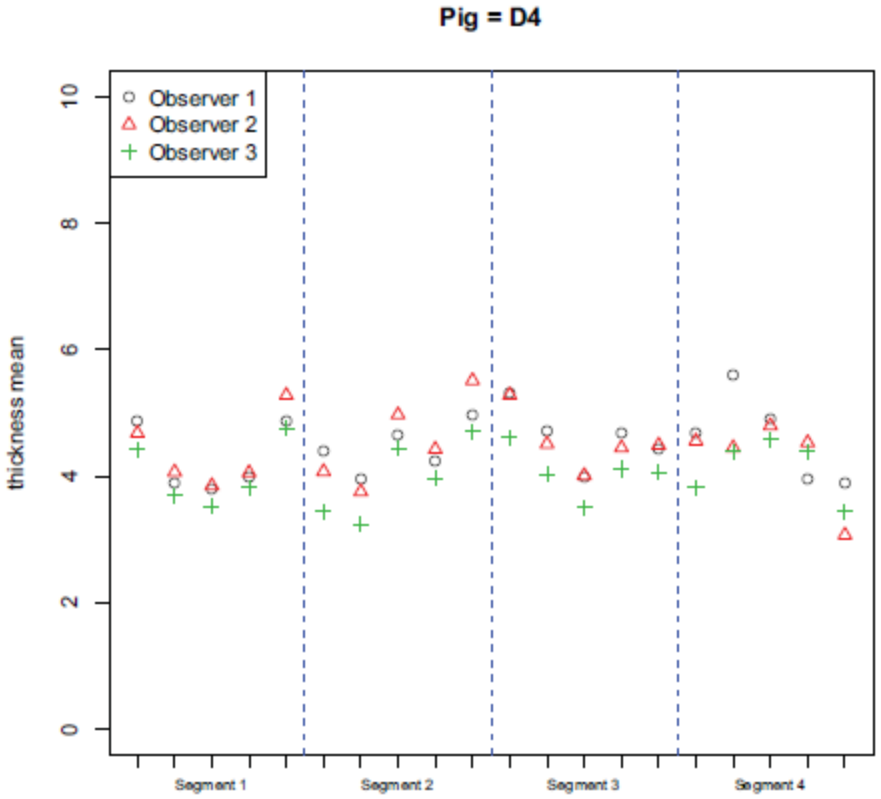
Example of data on one CF pig

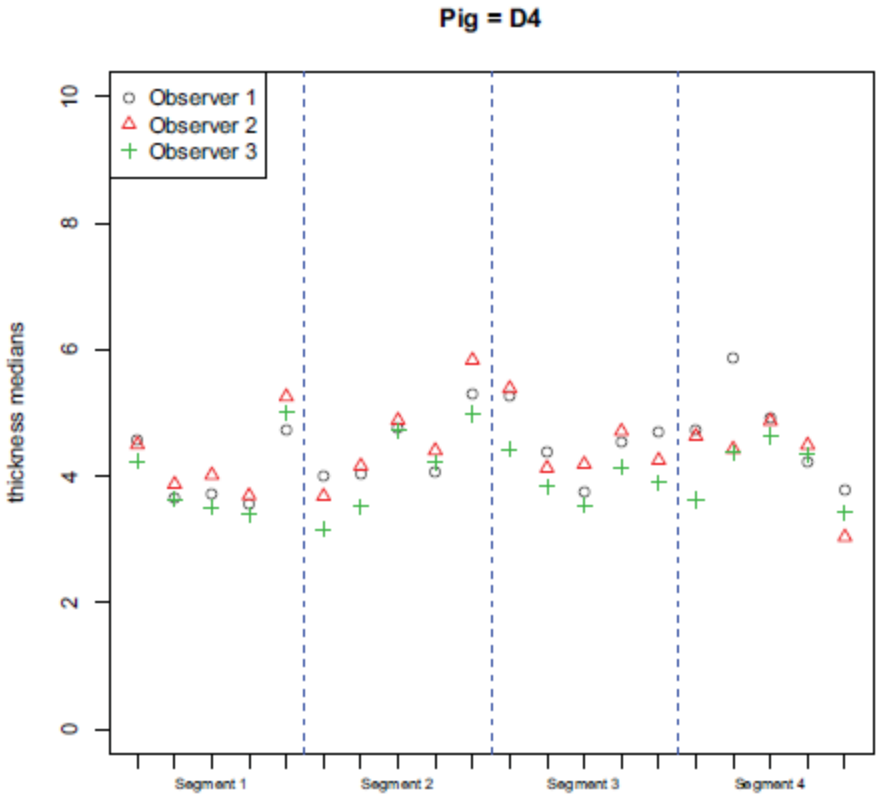


Example of data for Non-CF pig

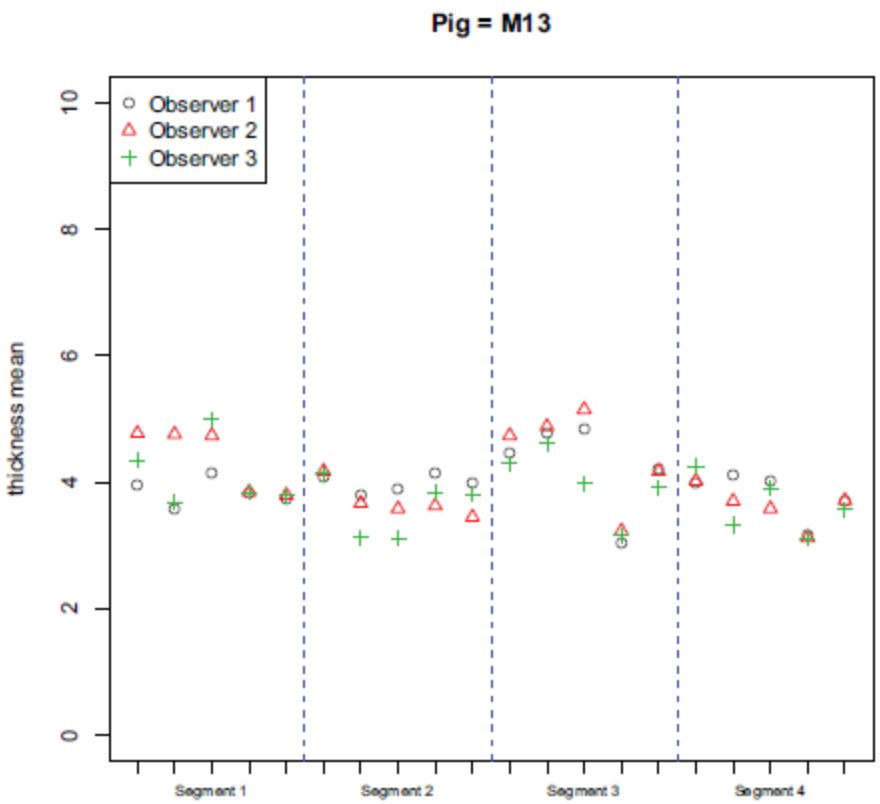


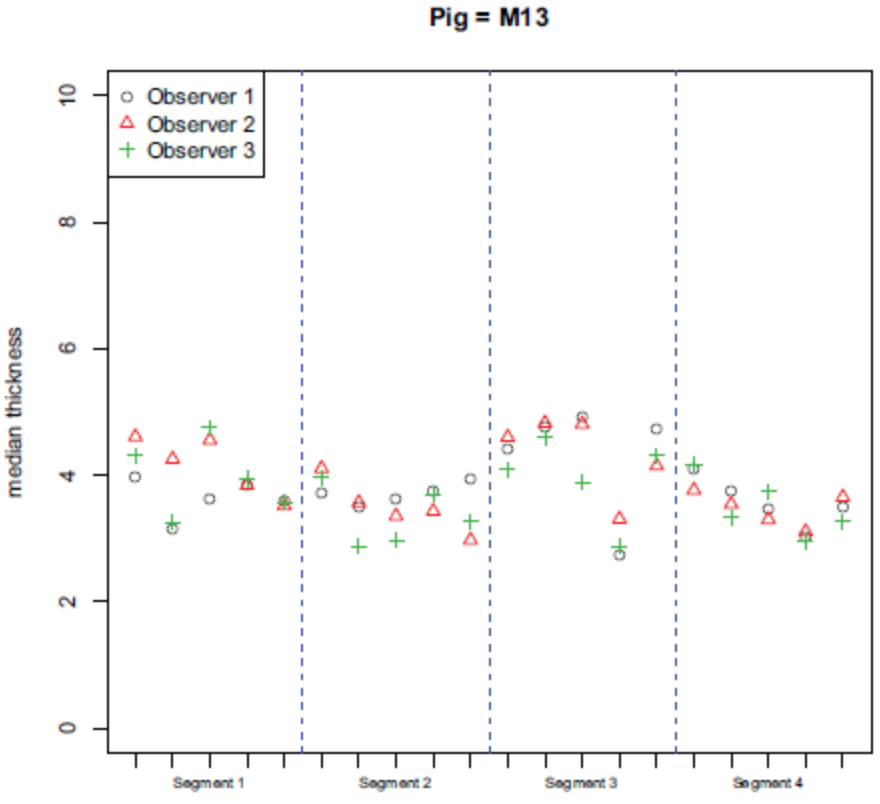
CF pig: means (left) and medians (right)





Non-CF pig: means (left) and medians (right)





Analysis results using library lme4

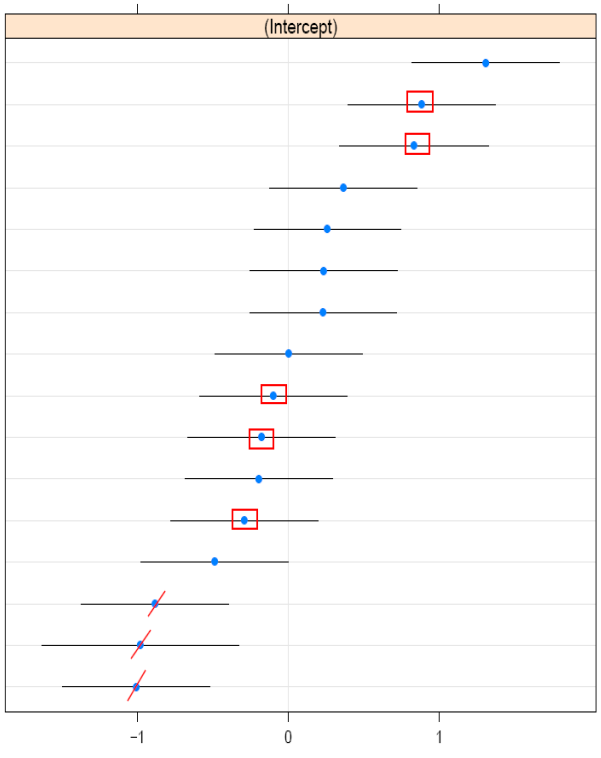
Source of Random variability	σ estimate with means	σ estimate with medians	σ estimate with all the data
Between pigs $(\sigma_A)^2$	0.5383	0.5523	0.7138
Between Segments $(\sigma_B)^2$	0.4181	0.4152	0.4554
Between Slides $(\sigma_C)^2$	0.5852	0.6229	0.5940
Residual between Observers on the same slide (σ^2)	0.3843	0.4209	
Between and within observers on the same slide			1.2503

Analysis results using library lme4 for CF and Non-CF comparison with means

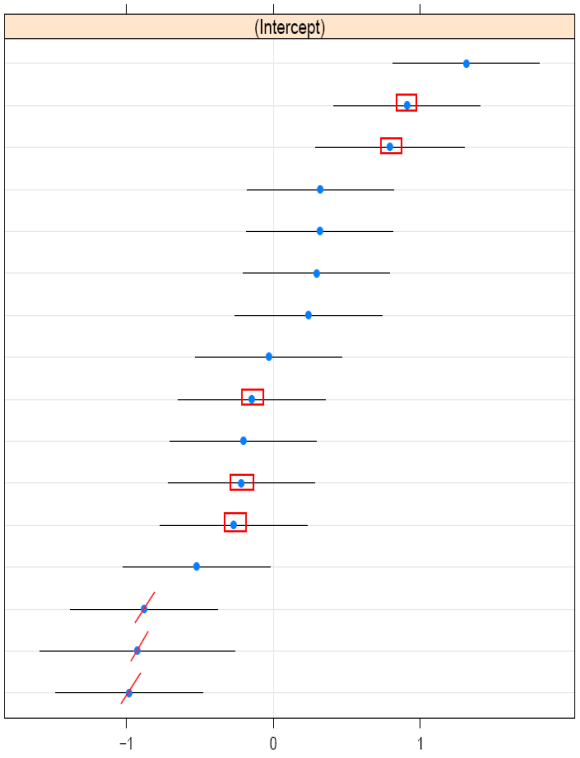
Source of Random variability	σ estimate with means	σ estimate with medians	σ estimate with all the data
Between pigs $(\sigma_A)^2$	0.5673	0.5820	0.5673
Between Segments $(\sigma_B)^2$	0.4182	0.4153	0.4182
Between Slides $(\sigma_C)^2$	0.5851	0.6229	0.6033
Residual between Observers on the same slide (σ^2)	0.3843	0.4209	1.2891
Between and within observers on the same slide			

Discussion of random effects confidence intervals

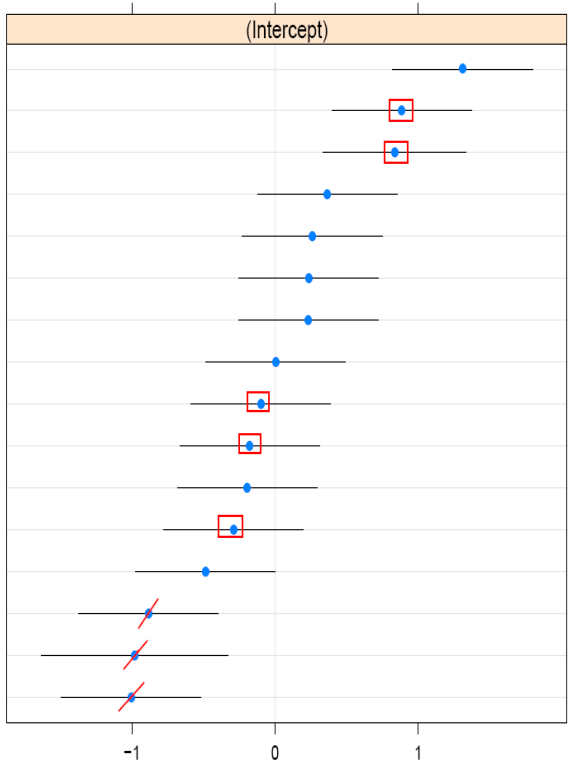
Means



Medians



All the data



Discussion

Confidence Interval	All data	Data medians	Data means
	(-0.6909, 0.7240)	(-0.7282, 0.7222)	(-0.6912, 0.7244)

Same answer!

Conclusion

- There is not enough evidence to reject the null hypothesis of no difference p-value is 0.96.
- There is no statistically significant difference in the reduction of the PCL depth through the sodium ion transport channel in the epithelial cells at the airway surface.
- If there is a difference it is less than 0.7 µm.

What the study suggests

This study suggests that the PCL depth is no different in pigs with CF than non-CF newborn pigs, or, if there is a difference it is less than 0.7 mm with 95% confidence.

Future work

- To measure sodium concentrations of the epithelial cells at the airway surface to determine if there is significant difference between the CF and Non-CF pigs.
- To find a better way, to measure PCL
- Possibly image processing

Acknowledgements

- Dr. David Stoltz, Assistant Professor, Carver College of Medicine, University of Iowa
- Michael V. Rector, Research Assistant, Carver College of Medicine, University of Iowa
- Dr. Michael J. Welsh, Professor, Howard Hughes Medical Institute, Carver College of Medicine, University of Iowa
- Dr. Kathryn Chaloner, Professor, Head of the Department of Biostatistics, University of Iowa
- Jingyang Zhang, Graduate Student Mentor, Department of Biostatistics, University of Iowa
- Ming Yang, Graduate Student, Department of Biostatistics, University of Iowa

Reference

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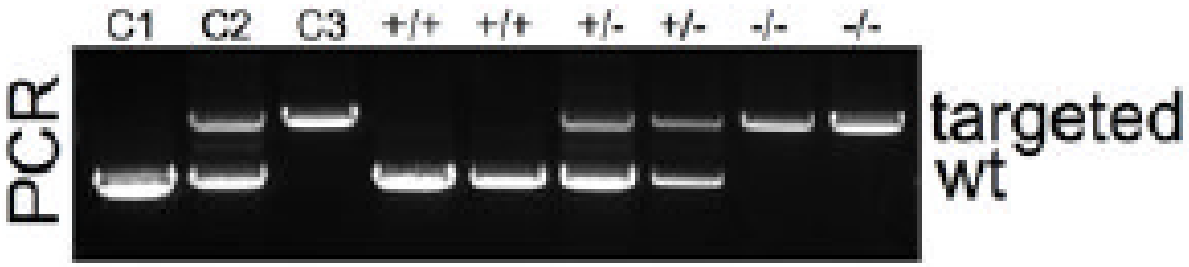
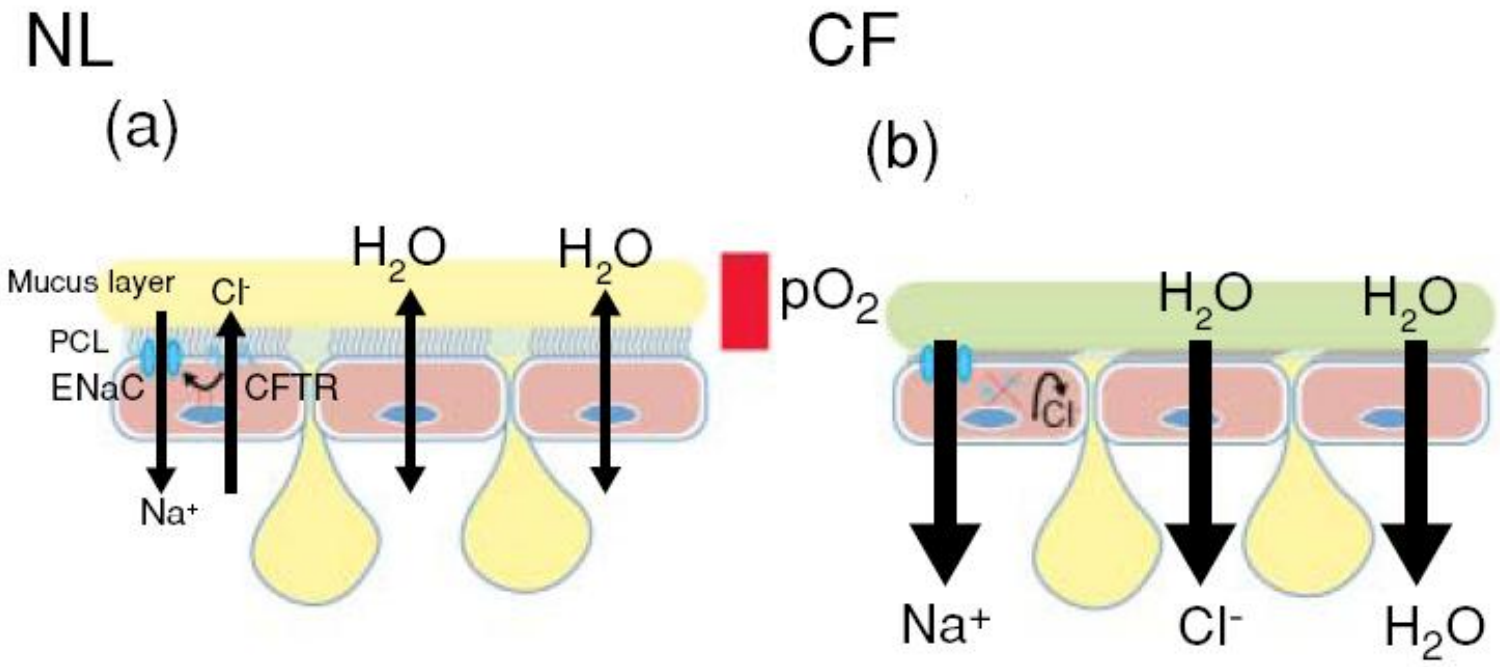
- THANKS!

Questions…?

Pigs litter (Pictures taken from Dr. Welsh laboratory)





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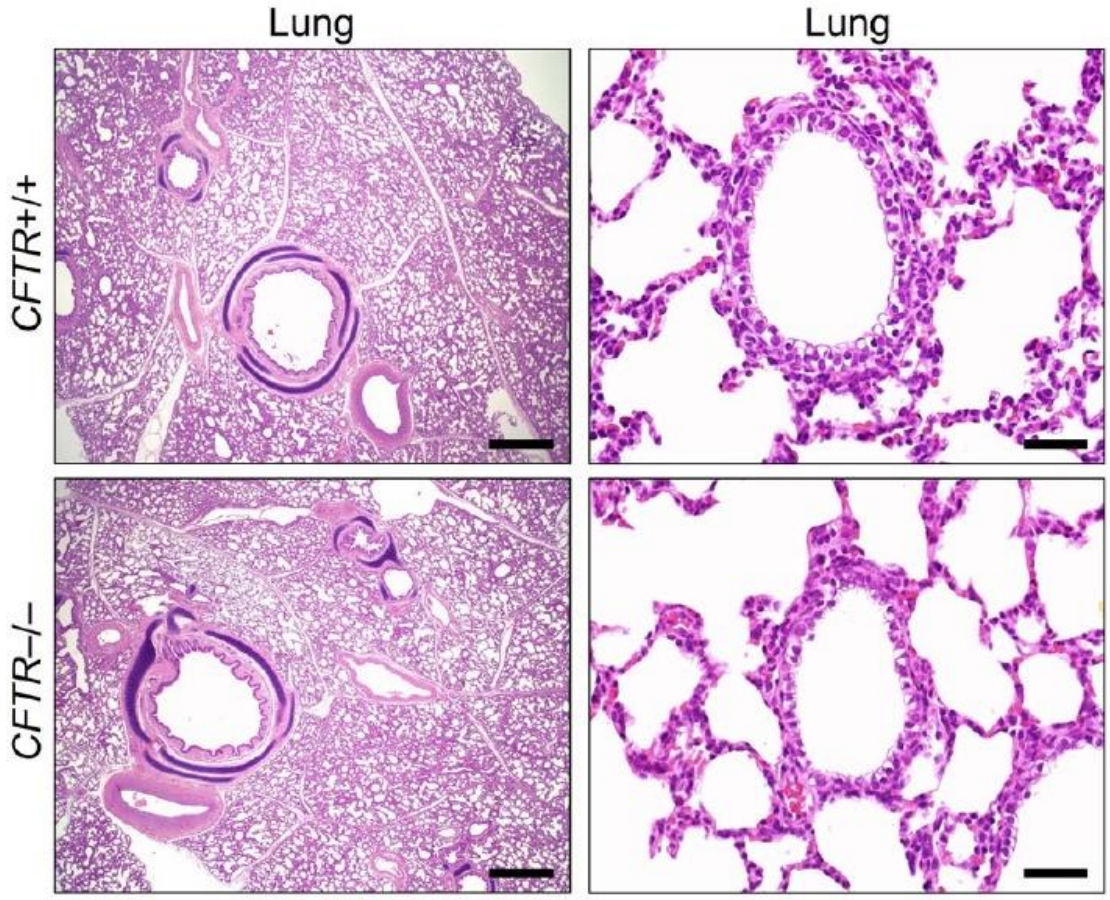
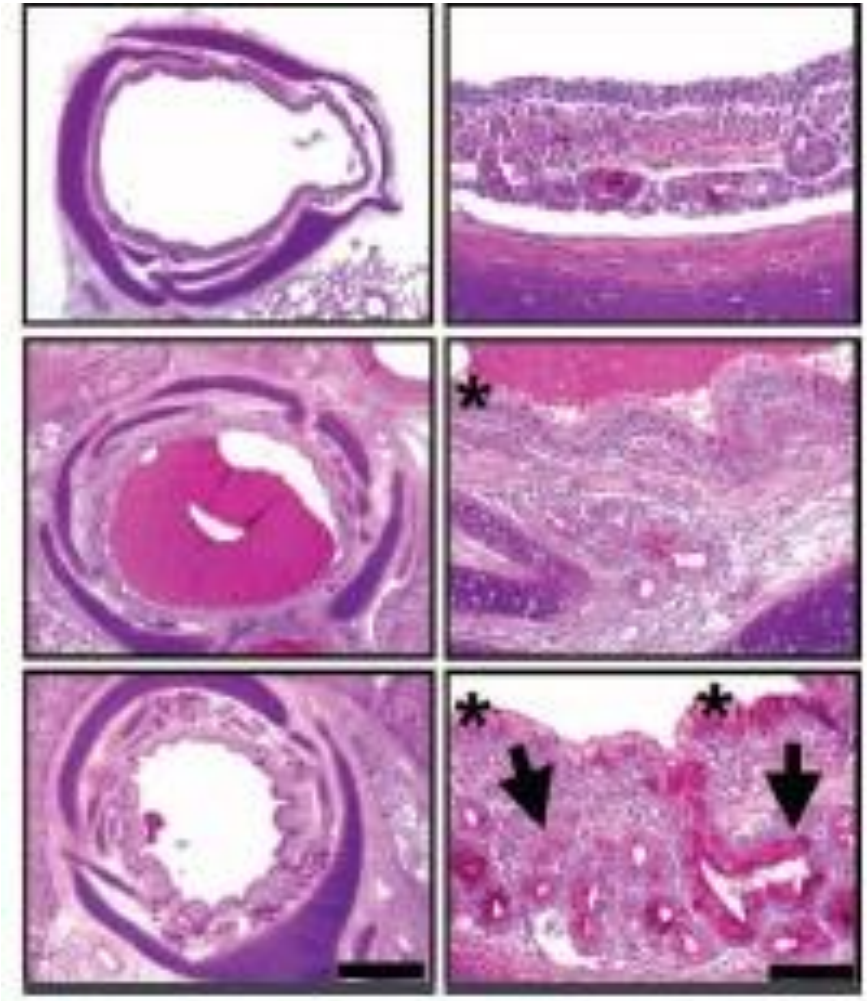
Lung disease

(Pictures taken from Dr. Welsh laboratory)



Airway

(Pictures taken from Dr. Welsh laboratory)



Airway obstruction

(Pictures taken from Dr. Welsh laboratory)