

Comparison of Two Non Human Primate Pneumonic Plague Models

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Abstract

Background

Animal models must be developed because vaccines and treatments of primary pneumonic plague can not be ethically evaluated in humans. Differences among nonhuman primate species in their response to challenge with other select agent pathogens led us to compare our experiences with the African green monkey (AGM) and cynomolgus macaque (CM) after aerosol exposure to *Yersinia pestis* CO92.

Methods

Ten AGM, *Chlorocebus aethiops*, and 18 CM of Indonesian origin, *Macaca fascicularis* implanted with telemeters were exposed to *Y. pestis* aerosolized by a Collison nebulizer in a head only exposure chamber controlled by real time whole body plethysmography. The LD₅₀ was previously established at 75 colony forming units (CFU) for CM and 350 CFU for AGM. Blood was collected for bacterial load (BL), clinical chemistry, hematology, and blood gas parameters. Continuous radio-telemetry collected body temperature, electrocardiogram, and heart rate (HR), and respiration rate (RR) in unanesthetized animals.

Results

Two days post exposure (PE) 3 AGM and 1 of 15 CM had detectable BL. Three days PE 8 AGM had a higher mean BL, 2.9 X10⁵ CFU/ml, than 5 of 12 CM, 4.7 X 10² CFU/ml. Fever in the CM was not as clear as in the AGM whose temperatures consistently elevated above 39°C. Coinciding with fever, HR and RR increases in both models. Average time from exposure until death in the AGM was 93 ± 9 hours exposed to 44 – 255 LD₅₀ and in the CM 91 ± 15 challenge with 97 – 185 LD₅₀. Both models showed pneumonia on x-ray, necropsy, and histopathology.

Conclusions

Both AGM and CM, regardless of exposure dose, have a rapid disease course of approximately four days. Compared to CM, AGM may be less resistant to disease progression as suggested by higher BL. However, difference in resistance does not influence the time until death for either model. In contrast to variable fever in CM, the consistently higher fever in AGM provides a clear milestone to mark the initiation of interventions, and therefore, may be better suited for treatment studies.

Methods

Exposure

Anesthetized (Telazol) animals are exposed in a head only unit contained in a Class III Biosafety cabinet. Bacteria are nebulized using a Collison nebulizer (MRE-3 jet, BGI, Inc.). The aerosol is sampled downstream of the primate's nares in an impinger (AGI, Ace Glass, Inc.). Dose is determined by combining culture results from impinger with the data collected during exposure using real-time plethysmography.

Telemetry

Temperature was recorded continuously using telemetry (Integrated Telemetry Services (ITS)). Baseline data curves were generated from the acclimation period, seven days prior to challenge.

Blood draw

Blood was drawn each morning during study and prior to moribund euthanasia. Tissue was collected from euthanized animals. All African greens were euthanized by day four, thus, do not have a sample of blood on day five. Blood was drawn from unanesthetized chair-restrained nonhuman primates.

Arterial blood

Arterial blood drawn from the tail was analyzed using an ISTAT. Blood was drawn into a syringe containing powdered heparin. Only samples filling cylinder, coming into contact with the heparin were analyzed. Animals are housed at the LRRRI facility at approximately 5,000 feet above sea level.

Euthanasia

Animals exhibiting euthanasia criteria were anesthetized with ketamine. Radiographs were taken using the ventral-dorsal aspect. Tissue was collected at necropsy from scheduled (cynomolgus macaque only) and moribund euthanasia

Exposure and Duration

No Correlation Between Aerosol Exposure Dose and Time Until Death

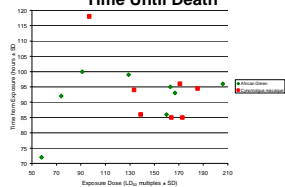


Figure 1. Exposure dose and time until death comparison. The mean exposure dose and time until death for each model are plotted with the associated standard deviations. The blue closed-diamond represents the cynomolgus macaque model and the closed-green circle represents the African green model. Results: Wide variation above exposure between 50 and 200 LD₅₀ result in consistent time until death. Unprotected nonhuman primate will become moribund within one day of four days after challenge. There is no difference between the models.

Bacterial Load, Temperature, and Death Relation

Cynomolgus Macaques Become Febrile Sooner Than African Green Monkeys and Bacteremic After Fever

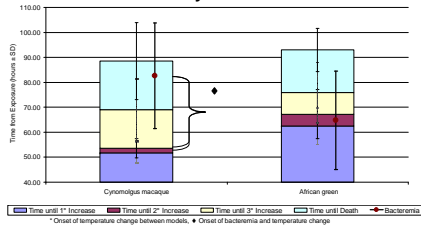


Figure 2. Temperature and Bacteremia Comparison. Figure legend indicates the time prior to the determined temperature change in degrees Celsius. The asterisks indicate the statistical difference between the models for time until 1 °C and 2 °C temperature increase. A closed-diamond illustrates that the cynomolgus macaque has a fever prior to bacteremia. Results: Temperature in the cynomolgus macaque model left the baseline (one degree change) and increased two degrees from normal (fever) prior to bacteremia. African green monkeys had a change in body temperature and became febrile later than the macaque.

Specific Tissue Bacterial Load

Models do not Differ in Bacterial Concentrations Detected from Various Tissues

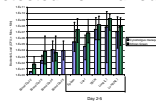


Figure 3. Bacterial Detection in various tissues. Geometric mean titers in colony forming units (CFU) are represented. Error bars denote the highest and lowest detected. Bacterial levels from spleen liver, tracheobronchial lymph node (TBLN), lung with apparent lesion, and lung without gross lesion are an average from those tissues taken at each timed necropsies. Results: Blood drawn one day after exposure was always negative (data not shown). *Yersinia pestis* was only detected in one cynomolgus macaque on day two. Bacterial tissue levels are similar for both models. At two days after exposure one of fifteen cynomolgus macaques are positive versus three of ten African greens. By a nonparametric test of significance ($P = 0.14$) there may be a trend that African greens develop bacteremia sooner than cynomolgus macaques.

Heart Rate, Respiration Rate, and Temperature

Pneumonic Plague Models May Differ in the Onset of Temperature Increase Relative to Heart and Respiration Rate Elevations

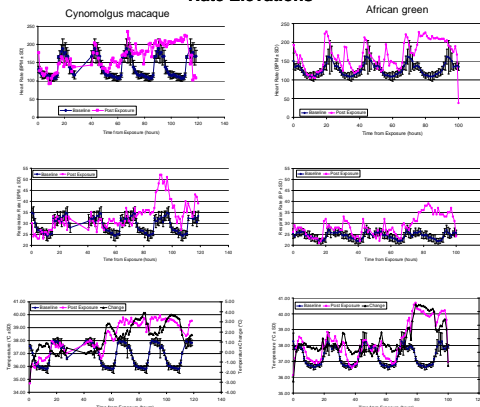


Figure 5. Continuously recorded heart rate, respiration rate, and temperature by telemetry. A representative animal from each model is shown. The blue lines represent that animal's baseline values for that time of day. The purple lines are the post-exposure values. The black lines are the temperature changes from baseline (used to determine fever). Heart rate is beats per minute (BPM). Respiration rate is breaths per minute (BPM). Results: From the represented animals, temperature in the macaque model is increased prior to heart and respiration elevation, whereas, in the African green model each parameter elevates at a similar time post-exposure. Further analyses are needed in order to determine model averages and to fully compare the models.

Arterial Blood Gas

Consistent Arterial Blood Gas Parameters Indicate Appropriate Pneumonic Function

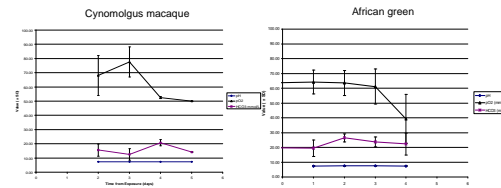


Figure 6. Three arterial blood gas parameters. Closed diamonds denote pH, closed triangles represent the partial pressure of oxygen (pO₂), closed circles signify the bicarbonate levels (HCO₃). Results: Arterial blood oxygen levels only change when measured just prior to moribund euthanasia.

Pathology

Pneumonia is Similar Between Models



Figure 7. Radiographs and photos from euthanized animals. Images have been cropped to highlight thoracic cavity. Results: Pneumonia is evident by radiograph and gross observation.

Model Comparison

Characteristic	Cynomolgus macaque	African green
LD ₅₀ of <i>Y. pestis</i> CO92	75 cfu	350 cfu
Detection of bacteremia	One animal two days post exposure (PE). Similar levels to African green.	Detection three days after challenge
Detection of bacteremia	Occurs after fever for the group.	Is not timed with fever.
Relation to fever		
Fever (2 ° C change)	Happens sooner than African green model at 54 hours PE.	Temperature changes 67 hours PE.
Telemetry parameters	Based on example given, tachycardia and tachypnea occur after fever	All three measurements increase at approximately the same time.
Heart & Respiration rate		
Temperature		
Radiographic pneumonia	Late in disease	Late in disease

Conclusions

Fever is the one consistent and easily measured physiological indicator of disease in primary pneumonic plague.

In African greens the onset of fever and the detection of bacteremia appears to occur simultaneously. In contrast, in the cynomolgus macaques the onset of fever (2 ° C change) precedes detectable bacteremia by approximately 24 hours. Thus, timing the onset of intervention by fever alone in cynomolgus macaque may allow earlier initiation, prior to dissemination of the disease outside the lung.

More data are necessary to confirm the gap between fever and bacteremia in the macaque, and the lack of a gap in the African Greens.

Use of continuous telemetry appears to be critical in defining fever. Fever (2°C change) provides easy initiator of therapeutic intervention. Cynomolgus macaques fever occurs before the African Greens, but all animals display an increase in temperature and a disruption of the diurnal cycle.

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