

Supplemental Materials. Bassel et al. The effect of aerosolized bacterial lysate on experimentally induced *Mannheimia haemolytica* pneumonia in calves.

Supplemental Table S1. Calves used for the study, pre-challenge antibody levels to *Mannheimia* leukotoxin, and dose of *M. haemolytica* used for the aerosol challenge.

Calf ID/ eartag	Treatment	Leukotoxin titre^a	Challenge dose (CFU)
C1/1586	Control	700	8.6x10 ¹⁰
L1/1587	Lysate	1300	8.6x10 ¹⁰
L2/1593	Lysate	300	5.4x10 ¹⁰
C2/1595	Control	900	5.4x10 ¹⁰
C3 /1597	Control	100	5.4x10 ¹⁰
L3/1598	Lysate	100	5.4x10 ¹⁰
L4/1599	Control	400	7.4x10 ⁹
C4	Lysate	200	7.4x10 ⁹
C5 /1615	Control	N/A ^b	2.2x10 ⁹
L5/1624	Lysate	N/A ^b	2.2x10 ⁹

^a Antibody levels to *Mannheimia haemolytica* leukotoxin determined by ELISA at Prairie Diagnostic Services, Saskatoon, Saskatchewan. Reported reference values range from <100 to >100000 and specific positive cut-off values have not been reported.

^b Leukotoxin antibody levels are not available; samples lost by the laboratory.

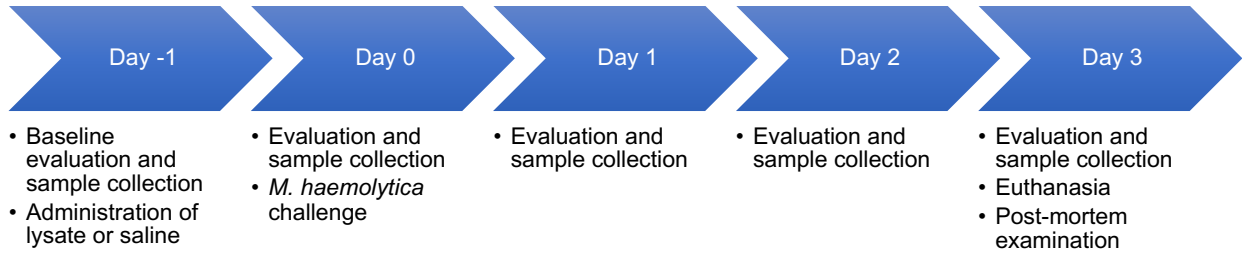
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Supplemental Table S2. Objective clinical scoring system for calves.

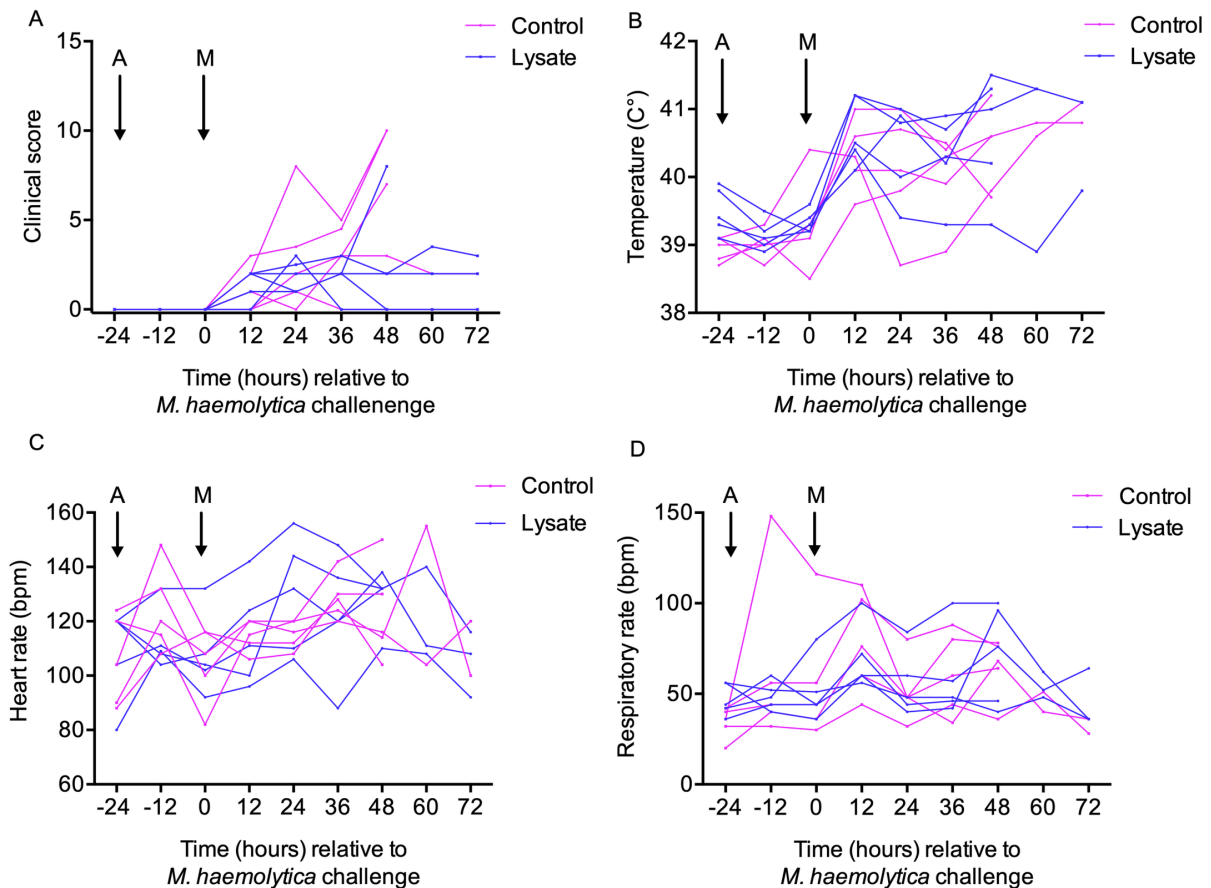
Feature	Score	Description
Demeanor	0	Not depressed.
	1	Rarely stands alone, ears droop slightly, moves away when people approach.
	2	Walks slowly, lethargic, sometimes stands with head low, easy to corner but difficult to catch.
	3	Uninterested in environment, little response when people enter pen, easy to catch, lies in sternal recumbency frequently.
	4	Lies down most of the time, stands only occasionally, doesn't respond when people enter pen.
Appetite (only assessed at feedings)	0	Animal is seen eating with normal vigour.
	1	Nibbles food but little is consumed.
	2	Doesn't eat.
Strength	0	Normal and difficult to catch.
	1	Walks slowly, mildly unsteady gait, easier to catch than normal.
	2	Staggers or knuckles occasionally, recumbent less than 50% of the time, becomes recumbent from standing when people are in the pen, obviously unsteady gait.
	3	Recumbent most of the time but will rise when stimulated.
	4	Recumbent and will not rise when stimulated.
Effort of breathing	0	Normal breathing pattern. Mild but detectable increase in respiratory effort when stressed.
	1	Obvious increase in respiratory effort when stressed, subtle increase when not stressed.
	2	Obvious increase in respiratory effort when not stressed (observed from a distance).
	3	Open mouth breathing or marked increase in respiratory effort.

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Supplemental Figure S1. Experimental timeline with sampling points.

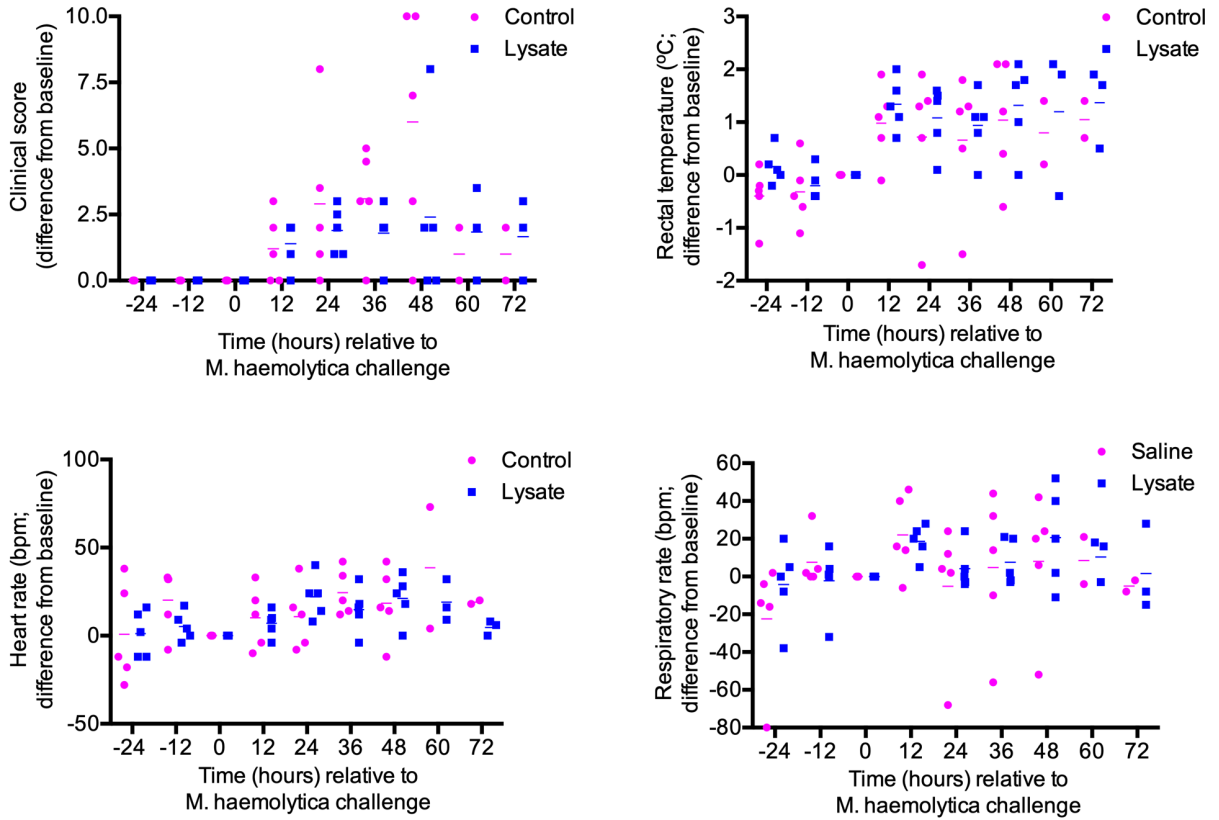


Supplemental Figure S2. Clinical parameters in individual calves over time. Five pairs of calves received aerosolized bacterial lysate or saline at time -24h (arrow A) (n=5 per group). All calves received aerosolized *M. haemolytica* at time 0h (arrow M). Calves were examined every 12 hours to evaluate: A) clinical scores, B) body temperature, C) heart rate and D) respiratory rate. Clinical scores were assigned based on the sum of individual assigned scores for demeanor (0-4), strength (0-4), appetite (0-3), respiratory effort (0-3) for a maximum score of 14.



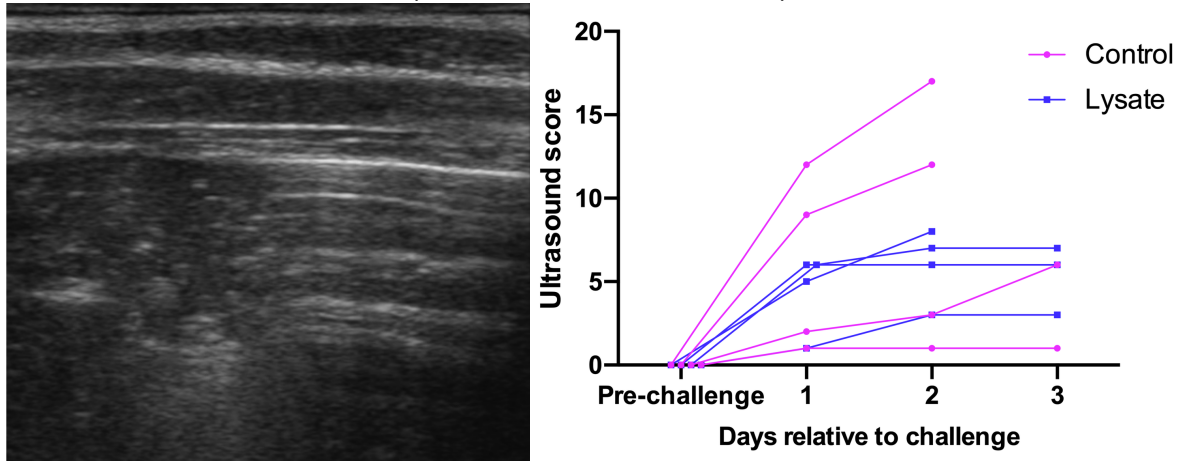
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Supplemental Figure S3. Clinical parameters in individual calves over time. The data show differences from time 0 for individual calves. The horizontal lines show the mean.

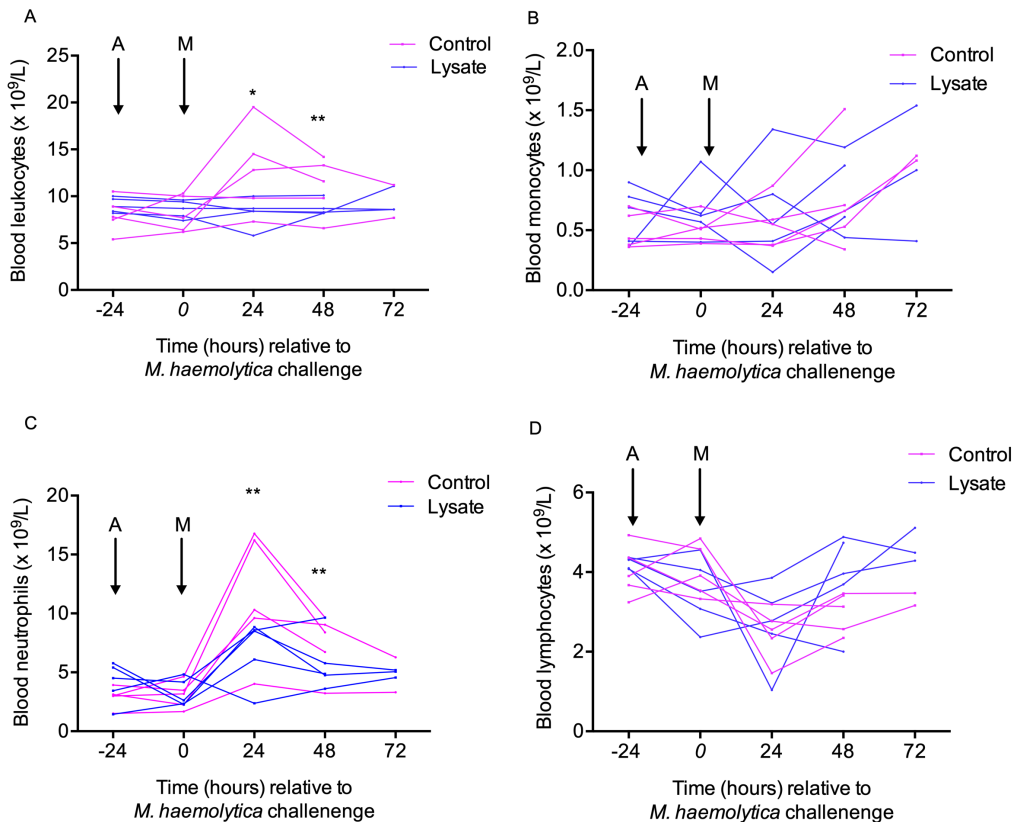


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Supplemental Figure S4. Thoracic ultrasound findings of individual calves after challenge with *Mannheimia haemolytica*. A) Ultrasound evidence of consolidation (left of image) at 2 days after challenge with *M. haemolytica*. B) Individual-animal ultrasound scores over time (maximum score 34; n = 8).

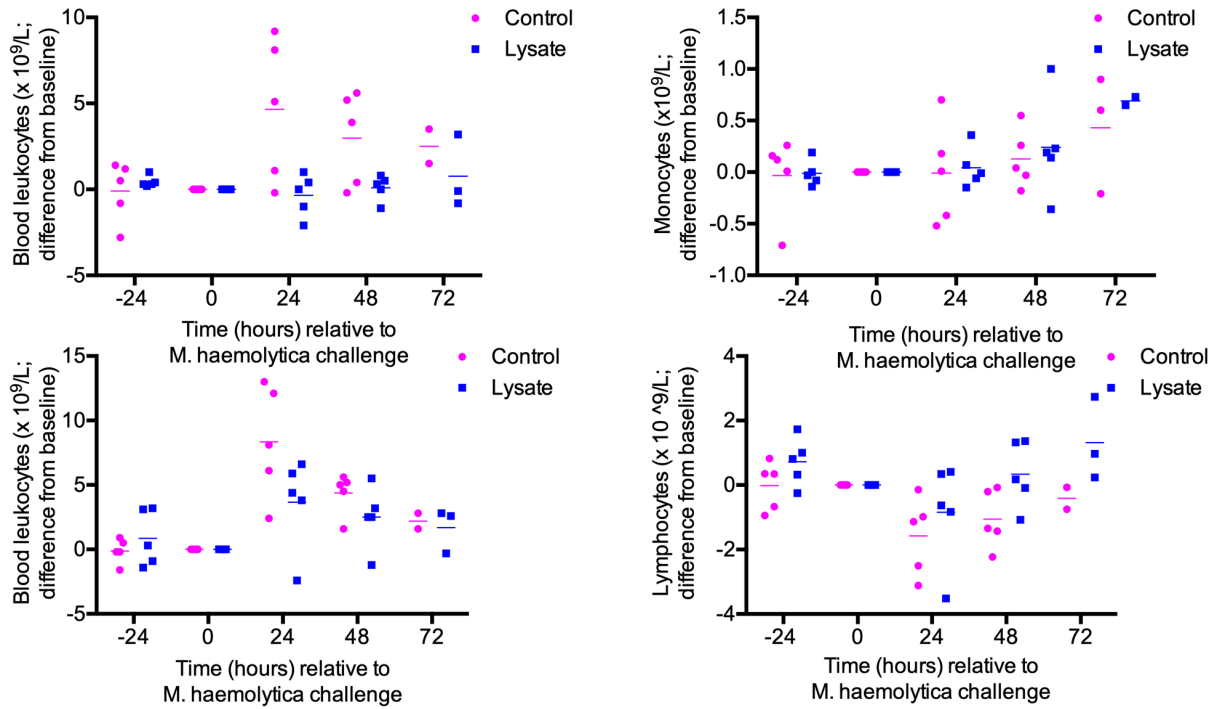


Supplemental Figure S5. Changes in blood leukocytes of individual calves over time. Five pairs of calves received aerosolized bacterial lysate or saline at day -1 (arrow A) (n=5 per group). All calves received aerosolized *M. haemolytica* at time 0 (arrow M). Calves had daily blood collection for evaluation of: A) total leukocytes, B) monocytes, C) neutrophils and D) lymphocytes.



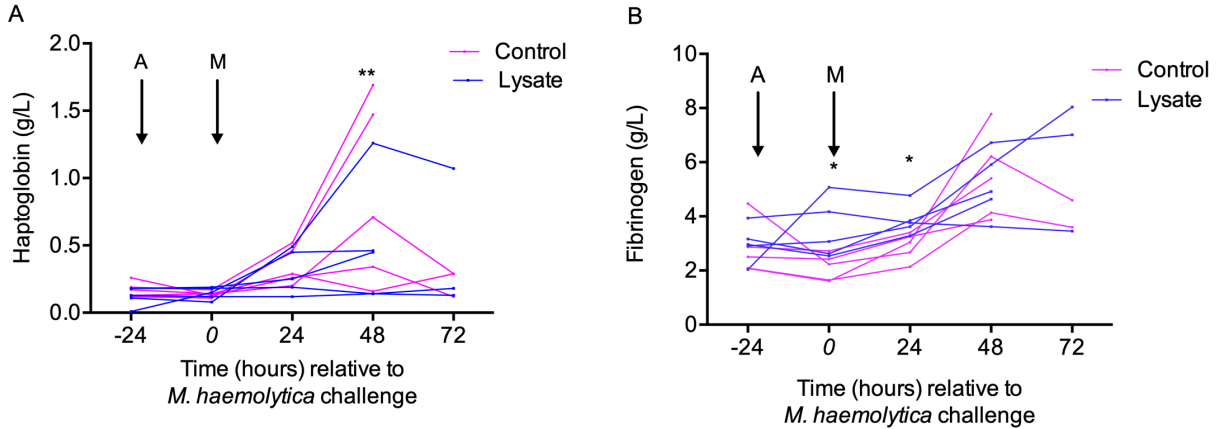
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Supplemental Figure S6. Changes in blood leukocytes of individual calves over time. The data show differences from time 0 for individual calves. The horizontal lines show the mean.

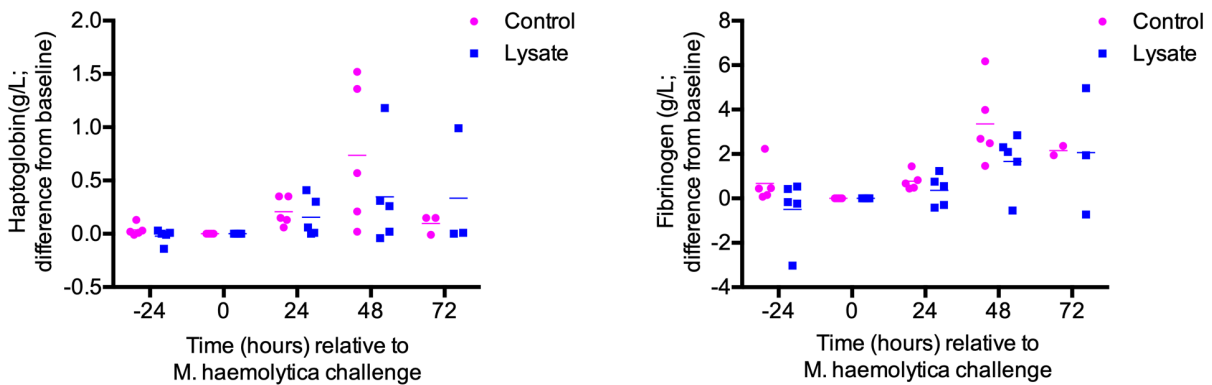


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Supplemental Figure S7. Changes in acute phase protein concentrations in individual calves over time. Five pairs of calves received aerosolized bacterial lysate or saline at day -1 (arrow A). All calves received aerosolized *M. haemolytica* at time 0 (arrow M). Calves had daily blood collection for evaluation of: A) haptoglobin and B) fibrinogen. * $P < 0.05$, ** $P < 0.01$. (n=5 per group).

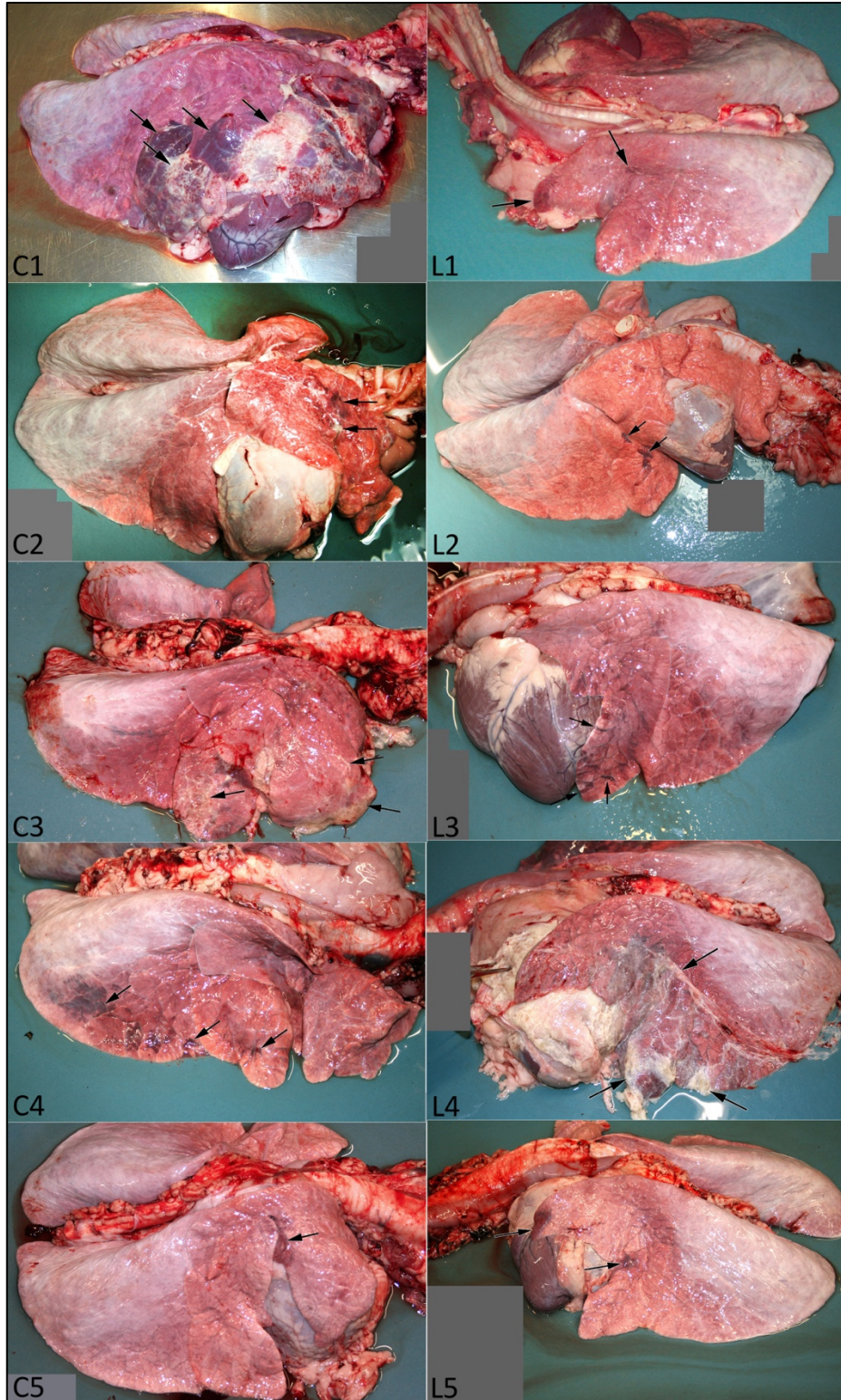


Supplemental Figure S8. Changes in acute phase protein concentrations in individual calves over time. The data show differences from time 0 for individual calves. The horizontal lines show the mean.



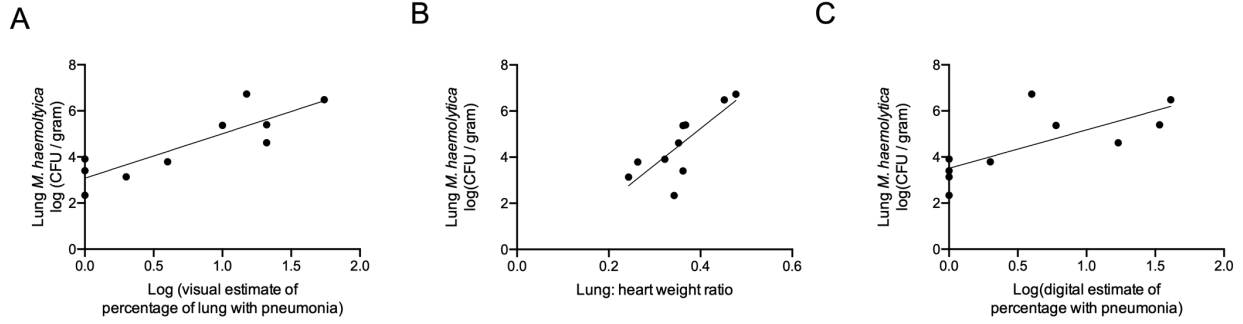
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Supplemental Figure S9. Gross postmortem lung lesions in the calves in the study. Calves were aerosolized with saline (animals C1-C5, controls) or bacterial lysate (animals L1-L5). All 10 calves were challenged by aerosol with *Mannheimia haemolytica*, 24 hours later. Some of the lesions in each lung are indicated by arrows.



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Supplemental Figure S10. Relationship among gross lung lesions and the number of *Mannheimia haemolytica* isolated from lungs. A) Log-transformed mean colony forming units (CFU) of *M. haemolytica* in lungs versus visual estimation of the percentage of lungs with pneumonia ($R^2 = 0.741$, $p = 0.001$). B) Log-transformed mean CFU of *M. haemolytica* in lungs versus the lung:heart weight ratio ($R^2 = 0.8610$, $p = 0.001$). C) Log-transformed mean CFU of *M. haemolytica* in lungs versus estimation of percentage with pneumonia using image analysis ($R^2 = 0.561$, $p < 0.013$).



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Supplemental Figure S11. Spectrum of histologic lung lesions in calves that had been aerosolized with saline or bacterial lysate, and challenged by aerosol with *Mannheimia haemolytica*. Annotations indicate alveoli (A) and bronchioles (B). **A)** Bronchiolitis. Neutrophils and macrophages fill the lumen of bronchioles (inset). **B)** Bronchopneumonia. Fibrin, edema, neutrophils and macrophages fill the lumens of alveoli and bronchioles, and infiltrate the bronchiolar wall (inset). **C)** Bronchopneumonia. Alveoli (lower inset) contain non-lytic neutrophils and leukocytes that have rounded pale nuclei (arrows, necrosis). The upper inset shows an inflamed bronchiole. **D)** Bronchopneumonia with thrombosis. A fibrin thrombus fills a pulmonary vein (arrow, inset). **E)** Lymphatic vessels in interlobular septa are distended and contain fibrin (arrow). Neutrophils and macrophages fill alveoli and bronchioles in adjacent tissue. **F)** A band of numerous inflammatory cells (arrows) surrounds a focal lesion of inflamed lung tissue. The boxed area is shown in figure G. **G)** The inflammatory cells have streaming chromatin typical of “oat cells” (arrows, inset). **H)** Pleuritis. The original surface of the pleura (arrows) is covered by hypertrophied mesothelial cells, and overlain by a pleural exudate of neutrophils and fibrin (P).

