

DR. SIMMONS: Thank you, Dr. Campbell.

Our next presenter this morning on the use of animal models for dose determination will be Dr. Robert Furrow. Dr. Furrow received his D.V.M. degree from The Ohio State University in 1960. He is a diplomate with the American College of Veterinary Pathology. Dr. Furrow served 17 years with the USDA and is currently the Acting Deputy Director of the Division of Veterinary Research at the Bureau of Veterinary Medicine. Dr. Furrow...

DR. FURROW: Thank you. I have one distinction this morning in that I graduated one ahead of Don Gable at The Ohio State University!

Pasteurella Multocida and Stress Induced Bovine Pneumonia for Experimental Purposes II. Evaluation of Adrenocorticotropic Hormone in Calves to Aid the Induction of Pneumonic Pasteurellosis

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Bovine pneumonic pasteurellosis is a devastating disease complex in calves. It includes shipping fever and bacterial pneumonia of calves and is usually accompanied by stress and/or viral infection. More than 50% of the New Animal Drug applications for antimicrobials in cattle submitted to the Bureau of Veterinary Medicine claim efficacy against bovine pneumonic pasteurellosis or shipping fever pneumonia.

The Bureau of Veterinary Medicine currently accepts antimicrobial drug dosage regimens based upon clinical response in diseased animals. Claims for efficacy are supported by data generated from a variety of field trials which are costly to perform and at best are difficult to interpret.

Even though bovine respiratory disease complex is a severe problem in the cattle industry, it has been difficult to consistently produce pneumonia in cattle for experimental purposes. The bovine respiratory system is protected by an efficient immunologic, macrophage and ciliary system that must be compromised before bacterial infections can become established.

A number of investigators have been able to produce pneumonia in cattle by superimposing pasteurella infections upon induced parainfluenza III or infectious bovine rhinotracheitis virus infections, but these techniques require sophisticated virology laboratory support. Consistent production of bovine pneumonic pasteurellosis in weaned calves using hot and cold water stress followed by intratracheal infusion of Pasteurella multocida, serotype B was reported in 1979 by Breeze and Magonigle. We have previously reproduced pneumonic pasteurellosis using this procedure and documented the changes of the clinical, hematologic and biochemical parameters associated with the disease. In this current experiment we replaced this physical stress with multiple injections of adrenocorticotropic hormone (ACTH) and evaluated the clinical and pathologic development of the disease. We chose ACTH as the chemical stress agent because we suspect that the Breeze and Magonigle model is successful because of the general adaptation syndrome which is probably mediated via the pituitary adrenal axis.

Materials and Methods:

Colostrum fed, Holstein male calves were reared in isolation, weaned at 6 weeks of age, and maintained on a diet of hay and

grain for 1 to 3 weeks until the start of the experiment.

To determine the effect of stress on plasma cortisol concentrations five male calves were monitored for 2 days to establish background concentrations. These animals were stressed with the same hot and cold water applications that were used by Breeze and Magonigle and in our previous work. Plasma cortisol concentrations were monitored prior to, during, and following the period of stress.

To determine the effect of ACTH on selected hematologic and clinical chemistry parameters and mean basal cortisol concentrations, blood was collected from 8 healthy 12-week-old calves for 2 days prior to the start of ACTH injections. The animals were given a series of 5 intramuscular injections of 20, 50, or 200 international units (IU) of adrenocorticotrophic hormone (porcine grade II, Sigma) at 8 hour intervals. Blood was collected at 4 hour intervals and the plasma was analyzed for cortisol by solid phase radioimmunoassay (Travenol).

A total of eighteen calves were stressed with intramuscular injections of 20 IU ACTH at 29, 21, 13, and 4 hours, and intratracheal injections of 1 ml 8% acetic acid at 21 and 4 hours before inoculation. The calves were inoculated with broth cultures of Pasteurella multocida MSU 7, ATCC 12946, serotype B and then observed for development of clinical signs. Stock cultures were grown up in tryptose phosphate broth, enriched with 5% heat inactivated serum and then washed 3 times to remove any toxins. The final inoculum contained approximately 100 million colony forming units and was administered using a #22 FRENCH rubber rectal tube passed through the nares into the trachea.

An initial group of six animals was stressed, inoculated with Pasteurella multocida, and observed for 93 hours. Observations included rectal temperature, depression and respiratory indices, and mortality. The experiment was terminated 93 hours after inoculation and the surviving animals killed.

A second group of 1 control and 12 ACTH-stressed infected calves were used to evaluate the pathogenesis of the pneumonia over time. The calves were divided into 4 groups scheduled to be terminated at 8, 30, 42, and 54 hours after inoculation. Due to the severity of the pneumonia that developed, and animal welfare considerations, some of the animals were killed prior to scheduled termination time.

Postmortem examinations were done immediately on animals that were moribund or died prior to end of the experiment. Surviving animals were killed at the end of the experiment and complete postmortem examinations were performed. Lung lesions of each animal were graded according to severity and extent. Lung,

respiratory lymph nodes, liver, kidney, spleen, and tissues from any lesion detected at postmortem were fixed and examined microscopically.

Clinical, Hematologic and Biochemical Observations:

Calves stressed with hot and cold water showering showed an increase in plasma cortisol levels from a pre-stress average of approximately 5 ng/ml to 8 ng/ml with the peak cortisol concentration occurring at 32 hours after the beginning of stress.

Calves treated with either 20, 50, or 200 IU of ACTH demonstrated a classic dose response to the hormone. The effect of 20 IU gave a more pronounced plasma cortisol response than the hot and cold water showering. Therefore, a dose of 20 IU units of ACTH was chosen to replace the hot and cold water stress in our Pasteurella multocida and stress induced bovine pneumonia model.

There was no observable clinical response to the ACTH administration. With ACTH injections the blood glucose concentration increased by 26% from a mean of 82 mg/dl to 103 mg/dl and the mean neutrophil count increased from about 3000 to 8000/ml of blood.

Following exposure to the organism the calves became depressed and anorectic; the rectal temperature ranged from 104 to 106°F and remained above normal until termination. Plasma glucose concentration dropped precipitously after exposure to the organism and seemed to be correlated with weakness and depression. Plasma fibrinogen concentration increased slightly with ACTH administration then increased significantly as the disease progressed.

The gross lung pathology observed at post mortem was graded according to severity.

Grade I lesions consisted of collapse involving less than half the lobules in one lobe.

This right lung demonstrates small areas of consolidation on the apical and cardiac lung lobes. Notice the multiple fibrin tags on the surface of the rib of this infected calf. Multiple focal areas of red hepatization were seen on the apical and cardiac lobes of this left lung.

Grade II lesions consisted of collapse involving more than half the lobules in one or several lobes.

This right lung shows consolidation and hepatization of approximately half of the right apical and cardiac lung lobes. Red hepatization was often observed. Here it affects more than half of the left cardiac lobe.

Grade III lesions consisted of dark red, very firm consolidated lobules with some collapse and mild fibrinous pleuritis.

In this right lung extensive areas of consolidation and red hepatization with moderate fibrinous pleuritis and interlobular fibrin deposits were observed.

Grade IV lesions consisted of severe and extensive consolidation of cranial, middle, and caudal lobes with fibrinous pleuritis and separation of interlobular septa by fibrin.

Severe fibrinous pleuritis and extensive consolidation in the apical, cardiac and anterior ventral diaphragmatic lobes was seen. A cross section of this grade IV right cardiac lung lobe show extensive consolidation, fibrinous pleuritis, and interlobular fibrin deposition. Extensive hemorrhagic and fibrinous pleuritis of the costal pleura was seen in these grade IV lesions.

Lung Lesions Score Summaries:

Of the 6 initial calves infected after being stressed with ACTH, 2 were found dead 43 hours after infection with lung lesion scores of 3 and 4. Three of the four remaining calves also developed extensive lung consolidation and severe fibrinous pleuritis consistent with pasteurella pneumonia. The remaining calf showed only scattered multiple small areas of consolidation.

LUNG LESION SCORES OF CALVES

43 AND 93 HOURS

AFTER INOCULATION WITH PASTEURELLA MULTOCIDA

<u>Hours After Inoculation</u>	<u>Number Of Calves</u>	<u>Lung Lesions Scores</u>	<u>Average Scores</u>
43	2	3, 4	3.5
93	4	1, 3, 4, 4	3.0

When the second set of 12 calves was stressed, infected, and killed over time an increasing severity of the disease was seen. At seven hours, all animals had lesions of grade 1, at 21 hours they averaged 2.3 and at 31 and 48 hours all were graded as 4.

LUNG LESION SCORES OF CALVES

0, 7, 21, 31, AND 48 HOURS

AFTER INOCULATION WITH PASTEURELLA MULTOCIDA

<u>Hours After Inoculation</u>	<u>Number Of Calves</u>	<u>Lung Lesions Scores</u>	<u>Average Scores</u>
0	1	0	0
7	3	1, 1, 1	1.0
21	3	1, 3, 3	2.3
31	5	4, 4, 4, 4, 4	4.0
48	1	4	4.0

Microscopic Lesions:

Lesions in pneumonic animals included formation of exudate within bronchi, alveoli, and interlobular septa with vascular congestion and extensive fibrinous pleuritis. A mixed cellular inflammatory debris was seen in many of the bronchi. Gram negative bacteria were seen within alveoli, interlobular septa, and within fibrinous inflammatory debris on the pleura.

Discussion:

The clinical observations, hematologic and biochemical findings, and lesions confirm that Pasteurella multocida, serotype B inoculated intratracheally following ACTH stress causes pneumonia that closely mimics the natural disease. Seventeen of the eighteen exposed animals developed acute, severe clinical pneumonia and 14 of 15 animals terminated 21 or more hours after infection developed extensive fibrinous bronchial pneumonia. The success rate of producing the disease was consistent, ranging from 82 - 100% of stressed, infected animals. Clinical findings included neutrophilia, increased plasma fibrinogen concentration, hyperpyrexia, depression, anorexia, cough and respiratory rates consistent with acute pneumonia. The controls did not develop pneumonia.

We have shown that acute severe fibrinous pneumonia can be consistently produced by intratracheal inoculation of broth cultures of Pasteurella multocida following conditioning with multiple intramuscular injections of ACTH and intratracheal injections of acetic acid.

The clinical disease, post mortem lesions and microscopic changes we observed are very similar to bovine pneumonic pasteurellosis seen in field outbreaks. In the natural occurring disease the right anterior lobes are usually most severely affected. In our study both the left and right were affected, although not uniformly.

We recommend this procedure for producing pneumonic pasteurellosis for experimental purposes. We believe this disease model offers potential for use in pharmacokinetic studies and for dosage titration of drugs intended for treatment of bacterial pneumonia.

The next phase of this research now underway is to compare plasma and lung concentration of selected antimicrobials in control calves to calves with induced pneumonia. Preliminary results with sulfa drug administered orally indicate wide variation of drug absorption in animals with pneumonia compared to uninfected ACTH stressed animals. Consolidated lung tissue consistently has a higher concentration of sulfa drug than lung from treated uninfected animals.