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Ten holstein calves were challenged to induce pneumonia using transtracheal inoculation of rumen contents; transtracheal inoculation of Pasteurella hemolytica; cold stress followed immediately by aerosol inoculation of infectious rhinotracheitis virus (IBR) or adenovirus type 3 (ADV-3) followed by transtracheal inoculation with P. hemolytica 6 hours following IBR inoculation and 36 hours following ADV-3 inoculation; and cold stress immediately followed by aerosol inoculation with IBR and ADV-3. Pre- and post-inoculation body temperatures and clinical responses were recorded and blood samples were collected, starting 7 to 10 days pre-inoculation and continuing 10 to 12 days post-inoculation. Two of the calves receiving stress, IBR and P. hemolytica died and the remaining calves in this group including those inoculated with ADV-3 developed severe signs of respiratory disease. Lesions in the respiratory tract of these calves closely resembled shipping fever pneumonia. Calves inoculated by other methods developed mild to moderate signs of

pneumonia. Complete post-mortem examinations were conducted on all calves. The severity of pneumonia was rated by two methods, a subjective score ranging from 1 to 5 in severity and an objective score based on the calculated percentage of total lung volume that was consolidated.

The blood samples from the calves were tested for 23 serum and plasma constituents and the results were analyzed to determine correlations with severity of pneumonia. Relative to the mean pre-inoculation values, there were significant ($p \leq .05$) increases in the mean post-inoculation values of lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), bilirubin, blood urea nitrogen (BUN), body temperature, plasma fibrinogen, and alpha 1-antitrypsinase (A_1AT). Significant ($p \leq .05$) decreases were observed in the mean post-inoculation values of alkaline phosphatase, phosphorous (P), calcium, total protein, albumin, cholesterol, glucose, chloride, packed cell volume (PCV), lysozyme, and angiotensin-converting enzyme (ACE).

There were significant direct correlations ($p \leq .05$) between severity of pneumonia and mean post-inoculation values of plasma fibrinogen, body temperature, SGOT, LDH, bilirubin, P, BUN, and potassium (K), while ACE, albumin, and glucose exhibited inverse correlations. The degree of change between mean pre- and post-inoculation values of these factors, excluding K, glucose, and albumin also had the strongest association with pathology ratings of the calves, i.e., the changes were milder in the calves with mild pneumonia and greater in the calves with severe pulmonary damage.

Evaluation of Serum Enzymatic and Biochemical Changes as Indicators of Severity of Pneumonia in Calves

by

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EVALUATION OF SERUM ENZYMATIC AND BIOCHEMICAL CHANGES AS INDICATORS OF SEVERITY OF PNEUMONIA IN CALVES

LITERATURE REVIEW

Introduction

Inflammation of the lungs which can occur from a variety of causes is conventionally termed pneumonia. Pneumonitis, a more correct term for pulmonary inflammation, is generally restricted to lesions characterized by cellular infiltration and proliferation within the walls of the alveoli while pneumonia is used to describe those cases in which the lumina of alveoli exhibit the most marked change (49). There is often not a clear distinction between the interstitial and exudative types of pneumonic lesions however, because there is considerable etiological and anatomical overlap between the two.

The lungs are susceptible to infection by two routes, externally via the upper alimentary and respiratory tracts and internally via the circulatory system. The inflammatory process can be initiated by inhaled irritant gases, aspirated foreign matter, by aerogenous and hematogenous viruses, bacteria, fungi, and metazoa (49). Because of the wide range of agents that can induce damage an etiological definition of the lesions is often impossible.

Pneumonia is a major cause of illness and death in the cattle industry yearly. The most important type of pneumonia based on economic losses, especially to feedlot enterprises, is shipping fever pneumonia.

History of Shipping Fever Pneumonia (Bovine Respiratory Disease Complex)

Shipping fever pneumonia is an acute respiratory infection of cattle characterized by fever, dyspnea, anorexia, depression, and fibrinous bronchopneumonia (19, 47). Because of the great variety of infectious and noninfectious causes as well as conditions that can cause or lead to the development of pneumonia in cattle, the term bovine respiratory disease complex is preferred by many veterinary practitioners and researchers. Nevertheless, shipping fever pneumonia is a commonly used and widely accepted terminology. The disease has been extensively studied for many years. In addition to shipping fever pneumonia the malady has been termed "haemorrhagic septicemia", "pasteurellosis", "pneumonic pasteurellosis", "stockyards' pneumonia", and "transit fever". The disorder produces extensive losses to the cattle industry annually, especially in feedlot operations where economic losses are caused as a result of death, expensive treatments, and inefficient feed conversions. It is believed that many stress conditions, or factors that lower natural resistance, are necessary for the development of clinical infection (45,47). This stress can take many forms including cold or rapidly-changing weather conditions, feed changes, shipment of animals, and exposure to "strange" animals. Stress in combination with infectious agents is believed responsible for producing shipping fever pneumonia.

Etiological Agents

Pasteurella spp. were reported to be recovered from the respiratory tracts of cattle as early as 1895 (59) and have been associated with

the respiratory disease complex for over fifty years. In 1925 Pasteurella boviseptica was found in cattle with shipping fever pneumonia (48). Scott and Farley did a field study of 125 herds of cattle affected with shipping fever pneumonia and were able to isolate Pasteurella boviseptica from over 80% of the infected animals (78). The nomenclature for the Pasteurellae has been changed and Pasteurella boviseptica has been replaced by the term Pasteurella multocida. In 1954 Carter (13) examined 26 cases of shipping fever and was able to isolate either Pasteurella multocida or Pasteurella hemolytica from all but two of the animals. Carter and McSherry in a later study of 33 affected animals isolated Pasteurella hemolytica from 27 of the cattle (14). The results of these studies and others led Collier to put forth the hypothesis that the Pasteurellae (Pasteurella multocida and Pasteurella hemolytica) are essential components in the etiology of shipping fever and are principally responsible for the advanced stages of the disease (20, 21); however, both P. multocida and P. hemolytica have been identified in the nasopharyngeal membranes of healthy cattle and are considered by some investigators to be part of the normal microbial flora (12, 17, 18). Attempts to produce shipping fever pneumonia experimentally using only Pasteurella spp. have for the most part been unsuccessful. However Carter in 1956 (15) and in several more recent trials (16, 20, 34, 37) using P. hemolytica has been able to produce lesions similar morphologically to field cases of shipping fever pneumonia. The facts that Pasteurella spp. are commonly found in healthy animals and the difficulty encountered in readily reproducing the disease experimentally with Pasteurellae alone suggest that other factors are involved.

Research in the late 1950's and early 1960's revealed that the malady probably resulted from the interaction of environmental stress, virus, and bacteria (35, 41, 44). Several viruses have since been incriminated including myxovirus parainfluenza-3 (3, 4, 43, 91), infectious bovine rhinotracheitis (15, 17, 47), bovine viral diarrhea virus (16), and adenovirus (15, 16). Hamby et al., in 1964, using several combinations of stress, different viruses, and Pasteurella in groups of cattle showed that only those combinations that included all three of these factors produced lesions similar to those seen in clinical cases of shipping fever pneumonia (41).

Pathogenesis

Extensive examination of field cases and experimental data strongly indicate that shipping fever pneumonia is caused by the sequential interaction of stress, viruses, and bacteria. A hypothesis for pathogenesis put forth by Jensen et al., in 1976 (47), which is essentially supported by others (15, 20) is summarized as follows. First chilling of the mucous membranes allows infection of the nasopharynx by viruses causing tissue changes that allow the colonization of Pasteurella spp. and the development of rhinitis. Next, pathogens especially Pasteurella spp. and possibly Mycoplasma spp. transfer from the nasopharynx to the lungs by gravitational drainage and/or inhalation of infective droplets. These pathogens then colonize the ventral parts of the lungs and produce pneumonia. Pasteurella endotoxin causes clotting and thrombosis of lymphatics and blood vessels resulting in ischemia and necrosis. Death is a result of hypoxemia, endotoxemia, pulmonary necrosis, shock, and heart failure.

Rationale for Study

Current methods of diagnosing pneumonia, especially severity of pneumonia in cattle are of a subjective rather than an objective nature and are not very precise. At present pulmonary damage in cattle is assessed largely on the basis of such clinical parameters as depression, body temperature, and auscultation of the lungs. Radiological examinations and the evaluations of blood PCO_2 and PO_2 content, often used in human medicine to quantitate pulmonary damage, are not practical for routine use in large animal medicine.

Methods of precisely diagnosing pneumonia and its severity would be of value to both veterinary practitioners and researchers. Such information could provide practioners with prognostic information on the animal as well as aid in determining the efficacy of treatment regimes and monitoring the progress of individual animals. Reliable indicators of severity of lung damage could be of value to researchers in evaluating pathogenicity of various potential pulmonary disease-causing organisms or agents, evaluating the efficacy of therapeutic agents or combinations of therapeutic agents, and evaluating the efficacy of various prophylactic measures such as immunization, management, prophylactic drugs, etc.

Presently the best means of measuring the severity of pulmonary lesions in cattle is by killing the animal and conducting a post mortem examination. This has several obvious disadvantages. First it provides a determination of severity of pneumonia at only one point in time, i.e., the day the animal is killed. Secondly, it again is largely a subjective evaluation. A nonfatal method of quantitating or evaluating

the severity of pneumonia throughout the experimental period without necessitating sacrificing of the animal would be extremely valuable. This study was therefore carried out to test various serum and plasma constituents to see if they might be of value as indicators of pulmonary damage. The serum enzymes that were evaluated are discussed below.

I. Alpha 1-Antitrypsin

Alpha 1-antitrypsin (A_1AT) is a low molecular weight glycoprotein synthesized in the microsomal fraction of the liver and accounts for over 90 per cent of the protein content of the $alpha_1$-globulin fraction demonstrated on serum protein electrophoresis (56, 89). This enzyme is an acute phase reactant, showing rapid and reversible changes indicating an inflammatory response to tissue damage or infectious organisms (46, 54, 62, 93). Alpha 1-antitrypsin has the ability to inhibit a variety of enzymes including trypsin (92), chymotrypsin (92), pancreatic elastase (89), plasmin (89), and thrombin (89).

The exact biological role of $alpha_1$-antitrypsin has not been determined. It has been postulated to have a protective role in the lung by inhibiting excessive protease-induced damage to lung tissue. Such proteases are released from macrophages and granulocytes during lysis of inflammatory exudate (54, 92). The ability of A_1AT to inhibit plasmin has lead to the suggestion that it may play an important role in the control of the fibrinolytic system (36, 73). Also, the ability of A_1AT to inhibit thrombin indicates that it may act to control coagulation (36).

People deficient in A_1AT have been shown to be especially susceptible to several lung disorders including pulmonary emphysema (27, 28, 54), chronic obstructive pulmonary disease (28, 50, 53, 74), and respiratory-distress syndrome (29, 56). The trait for ability to produce A_1AT in humans is heritable as an autosomal codominant characteristic and 21 different phenotypes have been identified (30, 91). The ZZ phenotype is deficient in A_1AT. In addition to its association with the above disorders, it has recently been suggested that A_1AT may have value as a prognostic indicator in lung damage due to Klebsiella pneumoniae (8).

II. Angiotensin-Converting Enzyme

The conversion of the decapeptide angiotensin I to the potent pressor substance angiotensin II, important in blood pressure regulation, is carried out by the angiotensin converting enzyme (ACE) (64, 65, 87). The enzyme is presently thought to be produced in the pulmonary vascular endothelium, directly accessible to the pulmonary circulation where it carries out the conversion of angiotensin I to angiotensin II (65, 75, 86). This process is considered to be one of the major metabolic functions of the lung (65, 75). Angiotensin-converting enzyme activity has been detected in the blood and several tissues other than lungs; however, its concentration has been found to be thirty times greater in lung tissue (23, 24, 95).

Angiotensin-converting enzyme acquired medical attention when Lieberman (54) examined the activity of this enzyme in patients with a number of different lung diseases and found that patients with

active cases of sarcoidosis had significantly elevated levels, indicating that ACE might be a useful diagnostic aid in confirming this disorder. This correlation was confirmed by Ashutosh and Keighley (6) who further postulated that any lung damage associated with decreased vascularity or abnormal pulmonary blood supply would lead to decreased ACE activity and, conversely, lesions that produced proliferation of pulmonary capillaries would result in a rise in circulating ACE.

III. Lysozyme (Muramidase)

Lysozyme was first observed to be a powerful bacteriolytic agent by Fleming (31) when he observed that certain bacteria, particularly Micrococcus lysodeikticus, were susceptible to the lytic action of this enzyme. Lysozyme has since been detected in tears, saliva, and serum as well as nasal, gastric, and intestinal secretions (31, 90). Several tissues have been reported to contain lysozyme with highest concentrations in bone marrow, lung, intestine, spleen, and kidney (32, 42, 71). Briggs et al., (12) and Asamer et al., (5) observed that the concentration of lysozyme is higher in granulocytes and monocytes than in other leukocyte types.

Lysozyme is a low molecular weight protein that acts as a mucolytic enzyme by disrupting aminopolysaccharides in the cell walls of bacteria (76). It accomplishes the breakdown by catalyzing the hydrolysis of the B-(1-4) glycosidic linkage between N-acetylmuramic acid and N-acetyl-d-glucosamine in the polysaccharide component of bacterial cell walls (77).

Several clinical applications have been put forth for the monitoring

of changing serum lysozyme levels. It has received major medical attention in its use in the diagnosis and prognosis of leukemia, a disease producing elevated levels of muramidase (22, 67, 69, 85, 94, 96). Measurements of urinary muramidase is useful in the diagnosis of renal damage where increased levels have been found in patients with severe renal impairment (63, 66). Increased values have been found in patients with granulomatous diseases such as tuberculosis (70) and sarcoidosis (68, 84). A possible role for lysozyme and ACE in the management of sarcoidosis has been suggested (55, 68, 83). Respiratory bacterial infections have also been reported to produce increased levels of lysozyme (8, 10) with one study suggesting that it could be used as an index of severity of infection (8).

IV. Alpha-fetoprotein

Fetoproteins are globulins that are specific to fetal sera, not being found in detectable amounts in adult sera (7, 9, 38, 40, 51). An alpha-globulin detected in the serum of human fetuses (38) as well as a number of other mammalian species (1, 38, 51) has been termed alpha-fetoprotein. Alpha-fetoprotein (AFP) is believed to be produced in fetal hepatocytes (79, 80), the alimenatary canal (39), and the yolk sac (39).

The specific biological role of AFP is still unknown but a number of suggestions have been put forth. Since this globulin is structurally similar to albumin it has been suggested to function as a fetal albumin (82). It has been suggested that AFP may suppress the immune response and therefore be influential in protecting the fetus from

maternal rejection (25, 60, 61); however, recent evidence does not support this hypothesis (81, 82). Alpha-fetoprotein has received considerable interest in the last decade because of its reappearance in the sera of patients with primary hepatomas (2). Serum AFP measurements are now widely used in the diagnosis and monitoring of treatment of liver cancer (2, 57) and teratomas (11). Alpha-fetoprotein has also been reported to appear in serum during inflammation and infection (72). A recent study by Berendt et al., (8) has indicated that this enzyme may also be of value in determining the severity of tissue damage due to certain microbial agents.

MATERIALS AND METHODS

Animals

Eight holstein calves were acquired from the Oregon State University dairy facilities at one to two weeks of age. These animals were placed in individual calf stalls in a maximum isolation room at the Oregon State University Veterinary Medical Animal Isolation Laboratory (VMAIL) and raised until four to five months of age. Two other holstein calves, numbers 9 and 11, ages seven and five months of age, respectively, were obtained from Dr. Mattson, School of Veterinary Medicine. Both of the latter two animals were colostrum deprived at birth and raised in maximum isolation at VMAIL.

Throughout the rearing of these calves every effort was made to insure that they remained in good health. All calves except numbers 9 and 11 were raised in individual stalls that were constructed to prevent physical contact between animals. These calves were fed using separate suckling bottles and the food and water for each calf was also kept separate. The calves were checked daily and if one appeared sick it was removed from the other calves, monitored and treated until clinical signs returned to normal. At eight to ten weeks of age the calves were placed together in groups of three to five in maximum isolation rooms. At the time of exposure the calf to be inoculated was moved to a separate room before inoculation.

Preparation of Bacterial Inoculum

Pasteurella hemolytica type I was the bacterial agent used throughout. Lypholized cultures of this bacteria were obtained from

Dr. Robert Ellis, Department of Microbiology, College of Veterinary Medicine and Biomedical Science, Colorado State University. Cultures of the bacteria had been isolated from feedlot calves with typical shipping fever pneumonia and had been used to induce the disease experimentally. The lypholized cultures of P. hemolytica were reconstituted with sterile brain heart infusion (BHI) broth and streaked onto blood agar plates to check for purity. Isolated colonies were then picked, inoculated into flasks containing 250 ml of BHI broth and then incubated at 37° C in a shaker incubator. Following incubation for 10 to 12 hours, 45 ml aliquots were removed and centrifuged at 6000 rpm for 20 minutes. The supernatant was then discarded and the pellet reconstituted with 5 ml of .15 M KPO_4 buffer at pH 7.2.

Viral Titration and Serology

The viruses used in this study were infectious bovine rhinotracheitis (IBR)[a] and adenovirus type 3 (ADV-3)[a]. The viruses were replicated in secondary bovine fetal kidney cell cultures and suspended in Earles balanced salt solution which contained 10% bovine serum. The IBR virus inoculum contained 5.7×10^4 infective particles/ml and ADV-3 had 9.0×10^5 infective particles/ml. The inocula were frozen at -20° C until the day of inoculation when they were thawed at room temperature.

Serum neutralization tests for both IBR and ADV-3 viruses were carried out on serum from all calves prior to experimental inoculation. This was done to insure that the calves did not have a protective

[a] IBR strain Cooper, ADV-3 strain 5C. Both viral pools were prepared by Dr. D. Mattson, School of Veterinary Medicine, Oregon State University.

titer against the virus before infection. These tests were also conducted by Dr. Mattson.

Animal Inoculation

Several inoculation procedures were used during the course of the study in an attempt to induce pneumonia cases of varying severity. Inoculation Method I. Calf number 172 received 100 ml of a fluid suspension of rumen contents introduced transtracheally. The transtracheal inoculation technique was as follows. A small incision was made through the skin of the neck over the trachea approximately 10 cm distal to the larynx and a 12 gauge needle was inserted between the tracheal rings into the tracheal lumen. Sterile polyethylene tubing[b] (internal diameter .062 inches) was then inserted through the 12 gauge needle and passed posteriorly, approximately to the bifarcation of the trachea (Fig. 1). A syringe containing the inoculum and fitted with a 16 gauge needle was inserted into the polyethylene tubing and the contents discharged. The inoculum was followed with 50 ml of air to clear the tubing.

Inoculation Method II. Calves 173 and 174 were anaesthetized by an intramuscular injection of Rompun[c] at .5 mg/lb body weight and inoculated transtracheally as above with suspensions containing P. hemolytica. Calf number 173 received 25 ml of an inoculum containing 2.97×10^6 colony-forming-units (CFU)/ml while calf number 174 received 36 ml of an inoculum containing 1.71×10^6 CFU/ml.

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[b] Intramedic, Clay Adams, Parsippany, New Jersey

[c] Chemagro, Division of Baychem Corporation, Kansas City, Missouri

Figure 1. Transtracheal inoculation technique showing inoculum being discharged into sterile polythylene tubing which has been surgically inserted into the tracheal lumen.

Inoculation Method III. This procedure utilized the combined effects of stress, virus, and bacteria on calves 9, 11, 82, 171, 175, and 176. The day before inoculation each calf was completely clipped using a Oster clippers[d] fitted with a number 10 blade. The morning of inoculation each calf was thoroughly soaked with cold water and placed for one and a half hours in a refrigerated room, set at 37 to 40° F, with a fan blowing on them. Following this the calves were returned to a maximum isolation pen, at a temperature of approximately 72° F, for inoculation. Calves 82, 175, 176, and 171 were aerosally inoculated with 5 ml of IBR virus inoculum. Calf number 82 received 5 ml of a 1:2 diluted suspension while calves numbers 175, 176, and 171 received an undiluted suspension of virus. Aerosal inoculation of the virus was accomplished by placing a muzzle, consisting of a box approximately 10 cm square by 15 cm long, over the calf's nose and mouth (Fig. 2). A model 40 DeVilbiss nebulizer[e] containing the viral suspension was then tightly secured in a hole at (Figure 3) the closed end of the box and the suspension discharged into the muzzle over a 20 to 25 minute period. A plastic sack was placed over the muzzle and the entire head of the calf to keep contained the aerolized inoculum. Particle size for this nebulizer is .3 to 2 microns in diameter. Six to eight hours later these calves received a suspension of P. hemolytica transtracheally as previously described. Two other calves, numbers 9 and 11, were aerosally inoculated as described above with 10 ml of BAV-3 virus suspension followed by a transtracheal inoculation of P. hemolytica

[d] Oster Corporation, Milwaukee, Wisconsin

[e] DeVilbiss Company, Somerset, Philadelphia

Figure 2. Aerosol inoculation of a calf with virus illustrating box (muzzle) and nebulizer. The muzzle and the calf's head are enclosed in a plastic sack.

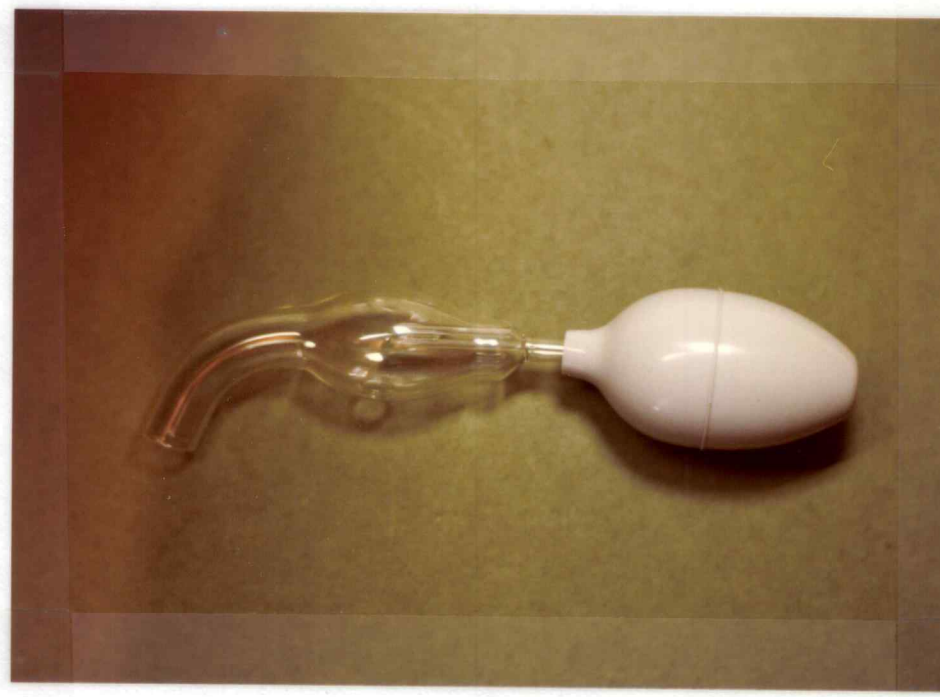

Figure 3. DeVilbiss model 40 nebulizer used for aerosol inoculation of viruses.

36 hours later.

Inoculation Method IV. The final inoculation method, on calf number 170, consisted of stressing as described followed immediately by aerosolized inoculation with 5 ml undiluted IBR virus suspension and a subsequent aerosal inoculation six hours later with 10 ml of BAV-3 virus suspension.

Specimen Collection, Preparation, and Storage

Blood specimens for serum assays were drawn by jugular venipuncture into 15 ml sterile, evacuated tubes[f] and allowed to clot at room temperature for two hours then refrigerated at 5^{o} C overnight. The samples were then centrifuged and the serum pipetted off in 5 ml aliquots in plastic-capped glass vials and stored at -20^{o} C. Separate vials were removed from the freezer for each analysis. Serum samples were also sent to the clinical pathology laboratory of Good Samaritan Hospital, Corvallis, Oregon, for a chemistry analysis that measured 17 different components of serum utilizing a Hycel, Model 17. For listing of components measured see Table II of Appendix.

Blood samples for complete blood counts (CBC) and plasma fibrinogen determinations were collected in 3 ml vacutainer tubes containing ethylene diamine-tetraacetic acid (EDTA). These samples were assayed the day blood was drawn.

Staging

Each trial lasted 15 to 20 days. Each calf was subjected to a

[f] Vacutainer, Becton, Dickinson, and Company, Rutherford, New Jersey

seven to ten day pre-inoculation period during which three blood samples were drawn to establish control values for CBC and plasma fibrinogen as well as serum levels of ACE, A_1AT, lysozyme, and alpha-fetoprotein. Base levels for the chemistry profile were determined on blood samples drawn on two separate days during the pre-inoculation period. The animals were inoculated on day zero except calf numbers 9 and 11 which were inoculated with ADV-3 one day prior to inoculation and P. hemolytica on day zero. Between day one and day ten post-inoculation at least five blood samples were taken from each calf at one to three day intervals. Each day post-inoculation the clinical appearance of the animals was observed, the lungs auscultated, and the rectal temperature recorded.

Assay of EDTA-Treated Blood Samples

The EDTA blood tubes were analyzed the day of collection to determine total leukocyte count (TLC), hematocrit, total erythrocyte count, and plasma protein to fibrinogen ratio. Plasma fibrinogen was evaluated by the method of Millar, et al., (58). A Coulter Counter, model ZBI, was used for the total leukocyte, hematocrit and erythrocyte counts.

Necropsy and Assessment of Lung Pathology

At the end of each trial the calves were euthanized using sodium pentabarbital and necropsied. All organs including the brain were examined. The lungs were removed at necropsy and the lateral and medial aspect of both lungs were photographed using kodachrome slide film. Pathology findings were recorded.

The degree of pulmonary pathology was determined by two methods, a "subjective" and an "objective" method. At necropsy each lobe of the lungs were examined and the number and character of lesions was described. These observations were used to score the severity of lung damage "subjectively" on a scale from zero (no abnormal tissue to five (severe involvement of one or both lungs). For the "objective" method the proportion of inflamed or consolidated lung area was determined for the lateral and medial aspect of both lungs by projecting the slides on a 200 square grid and counting the number of grids containing consolidated lung tissue as well as counting the total number of grids having lung tissue.

Viral and Bacterial Isolation

Lung and associated lymph nodes from all calves inoculated with viruses were collected at necropsy, stored at -70^{o} C and subsequently cultured for viruses by Dr. Mattson. Nasal swabs were taken from all calves except calf 172 prior to inoculation. Bacteriological cultures were carried out on these samples by the Veterinary Diagnostic Laboratory at Oregon State University to check for the presence of *Pasteurella sp*. Samples of lung, associated lymph nodes, and other selected tissues were collected at necropsy and bacterial isolation studies were performed as above on all calves except calves 170 and 172.

Serum Assay Procedures

I. Serum Lysozyme A fluorimetric assay was used employing fluorescamine

labeled cell walls[g], prepared from killed Micrococcus lysodeikticus[g] as explained in the procedure by H. J. Klass, et al. (55).

II. Angiotensin-Converting Enzyme (ACE). This assay was done fluorimetrically by quantifying the conversion of the substrate analog, hippuryl-L-histidyl-L-leucine[h] to hippurate and L-histidyl-L-leucine[g] by formation of a fluorescent adduct with o-phthaldi-aldenyde[g]. The technique used was the same as that described by J. Friedland and E. Silverstein (35).

III. Alpha 1-Antitypsinase. The amount of alpha 1-antitrypsinase was estimated spectrophotometrically by the method of A. A. Dietz, et al. (27). This method is designed to quantitate the amount of antitryptic proteins present in serum based on their ability to inhibit the hydrolysis of alpha-N-benzoyl-DL-arginine-para-nitroanilide (BAPNA)[g] by trypsin in tris buffer.

IV. Alpha-Fetoprotein. The content of serum alpha-fetoprotein was estimated via radioimmunoassay at the Metropolitan Hospitals Laboratory in Portland. In this procedure samples are incubated with 125-I-labeled $alpha_1$-fetoprotein and anti-$alpha_1$-fetoprotein. Antibody bound complexes of $alpha_1$-fetoprotein, labeled and unlabeled, are separated from the free fraction by means of a polyethylene glycol accelerated second antibody precipitation and centrifugation. Samples are counted in an automatic gamma spectrometer and log of counts bound are plotted against the log of standards treated in an identical manner.

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[g] Sigma Chemical Company, St. Louis, Missouri (hereafter referred to as Sigma)

[h] Bachem Fine Chemicals, Torrance, California

Statistical Analysis

Two nonparametric statistical procedures, performed by the Statistics Department, Oregon State University, were employed to evaluate the data collected from the calves. The pre- and post-inoculation mean values for each variable for each calf were calculated and these values were used for all statistical analyses. The Wilcoxon matched-pairs signed-ranks test was used to determine if there was a significant difference ($p \leq .05$) between pre- and post-inoculation mean values for each of the measured variables and the Spearman rank correlation coefficient test was performed on all data to see if there was a significant correlation ($p \leq .05$) between the severity of pneumonia of the calves, as denoted by the subjective and objective scoring schemes, and the post-inoculation mean values for each variable.

RESULTS

Calf Clinical Signs and Hematologic Responses to Inoculation

Daily pre- and post-inoculation values for body temperature, PCV, TLC, and fibrinogen are listed in Appendix Table I. The clinical and hematologic responses of the calves to challenge varied significantly depending upon the inoculation method used. Calf 172, challenged by method I was recumbent and dyspneic immediatley following inoculation but had returned to normal within four hours post-inoculation. No coughing or abnormal lung sounds were noted. Total white blood cell counts increased slightly with a post-inoculation average 16.1% higher than pre-inoculation levels. The highest count was observed on post-inoculation day 9 (Appendix Table IA). Plasma fibrinogen levels showed a steady increase through post-inoculation day (PID) 12 and the post-inoculation mean was 60.9% greater than the pre-inoculation mean (Table I). There was a slight body temperature increase following challenge that peaked on PID 9 at 103.1^{o} F, 1.6^{o} above the pre-inoculation mean (Appendix Table IA). Packed cell volume decreased slightly post-inoculation.

Calves 173 and 174 challenged by inoculation method II displayed rather mild overall clinical changes. There were no coughing or abnormal lung sounds noted in calf 173 following inoculation. This calf responded with a slight initial increase in body temperature that returned to normal by PID 5 (Appendix Table IB). Packed cell volume and white blood cell counts were also depressed slightly. Plasma fibrinogen levels had doubled in calf 173 by PID 3 after which time they started decreasing toward control levels. Calf 174 was

TABLE I Pre- and Post-inoculation Mean Values for Packed Cell Volume, Total Leukocyte Count, Fibrinogen, and Body Temperature

Calf Number	Pre-inoculation				Post-inoculation			
	PCV	TLC	Fib	Temp	PCV	TLC	Fib	Temp
173	34.2	8901	483	101.9	32.1	7991	777	102.3
170	39	14,178	656	101.6	36	8744	700	103
172	40	8367	504	101.5	34.8	9714	937	102.4
174	39.8	6779	512	101.6	38.7	11,448	966	102.7
175	39.5	9817	610	101.3	33.3	8479	968	105
11	39.3	8732	506	101.3	32.4	7662	1045	104.3
9	38.8	10,054	556	101.3	32.5	6283	932	105.7
176	37.8	8017	508	101.6	39	2743	607	103.5
171	39	8619	816	101.4	32.5	6450	1044	104.5
82	36.5	10,285	494	101.6	33.5	8400	736	105.3

depressed for the first 42 hours after inoculation, had a temperature of 1051° F (4.5° above normal) on PID 1 and was off feed. Moist rales and harsh bronchial tones could be detected through PID 5 in the left lung. By day 7 post-inoculation the animal appeared alert, the lung sounds had returned to normal, and the body temperature was back to normal. Post-inoculation white blood cell counts and plasma fibrinogen levels were significantly elevated (Appendix Table IC).

All six calves subjected to inoculation method III had a much more severe clinical response. Four calves, numbers 82, 171, 175 and 176, received IBR virus and two calves, numbers 82 and 176, died from the infection. Calf 82 was off feed by PID 1. Anorexia and depression increased until the animal died on PID 7. By day 4 post-inoculation, abnormal lung sounds could be detected including moist rales in the left lung and harsh bronchial sounds in both lungs. Pronounced dysnea was evident by PID 5 as well as mucopurulent nasal and ocular discharges. On PID 6 the animal was suffering from severe dysnea and was frothing from the mouth. Body temperature was elevated to 105.2° F by PID 2 and increased to over 106° F by death on PID 7 (Appendix Table ID). Fibrinogen levels were significantly increased with a post-inoculation mean 49% higher than pre-inoculation values and a last day level of 858 mg/100 ml, 74% above pre-inoculation mean, (Table I and Appendix Table ID). White blood cell counts and packed cell volume after initial reductions were both elevated on the last day of sampling.

Calves 175 and 171 showed a similar response. After inoculation both animals became depressed and anorexic. Abnormal lung sounds including moist rales and harsh bronchial tones were detected. Frequent

coughing was observed. Body temperatures were elevated throughout the trial period in both calves with temperatures in excess of 105^{0} F recorded for both. Plasma fibrinogen levels were rapidly and significantly elevated within 48-72 hours after challenge and remained elevated until necropsy. Both calves had depressed total white blood cell counts during the first 8 to 10 days after inoculation with normal or slightly increased levels observed just prior to necropsy (Appendix Table IE and IF).

Calf 176 became severely ill immediately after inoculation and its condition deteriorated until death on PID 3. Twenty-four hours after challenge it was severely depressed. On PID 2 it was too weak to stand and dysnea was evident. Rough bronchial tones could be heard in both lungs on this day and there were mucopurulent ocular and nasal discharges noted. Depression and dysnea increased and by PID 3 the animal was frothing from its mouth. Lung sounds had worsened and moist rales could be heard on both sides of the lung prior to death. Body temperature had elevated to 104.8 on PID 2 (Appendix Table IG). There was no significant change in packed cell volume or plasma fibrinogen levels, however the white blood cell counts had decreased to 43% of pre-inoculation levels by PID 3 (Table I).

Calves 9 and 11 were also challenged by method III using ADV-3 in place of IBR as the viral agent. Both calves were depressed and anorexic for several days post-inoculation. Moist coughing was occasionally observed and both had abnormal lung sounds including rough bronchial sounds that persisted until euthanasia. Calf 9 had a temperature of 106.5 by PID 1 and it remained above 105.8 throughout the remainder of the trial (Appendix Table IH). Calf 11 had a body temperature of

106.0 on PID 1 but it gradually decreased from this peak until it was 103.6 on the last day (Appendix Table IJ). Both animals showed marked increases in their plasma fibrinogen levels following challenge. Packed cell volume and total white blood cell counts were decreased after inoculation also (Appendix Table IH and IJ).

Calf 170 subjected to inoculation method IV exhibited a relatively minor clinical response. There was no noticeable change at the time of challenge with the animal remaining alert and displaying no anorexia. No coughing was observed and the only abnormal lung sounds detected was a slight harshness in bronchial tones on PID 7 and PID 9. No nasal or ocular discharges were observed. There was a slight increase in packed cell volume and white blood cell counts after inoculation (Appendix Table IK). There was not a noticeable change in plasma fibrinogen levels until PID 7. From PID 7 to PID 10 a fibrinogen increase was observed with PID 11 showing a 24% increase over the pre-inoculation mean (Appendix Table IJ and Table I).

Serum Enzyme Responses to Inoculation

Alpha 1-Antitrypsinase

The daily serum A_1AT activities, measured as a function of trypsin inhibitory capacity (umol/min/ml), for each calf are listed in Appendix Table III and the mean pre- and post-inoculation values are listed in Table II. Calf 172, inoculated by method I, displayed an initial decrease in A_1AT activity and then a gradual increase PID 3 through PID 7. Enzyme activity remained relatively constant PID 7 through PID 12 and a decrease in activity was noted on PID 13, the necropsy date.

Calves 173 and 174 challenged by inoculation method II both showed rapid increases in A_1AT activity following challenge. Calf 173 had a post-inoculation mean for A_1AT 61% higher than its pre-inoculation mean and the mean post-inoculation A_1AT activity of calf 174 was 49% greater than the pre-inoculation mean.

The changes in A_1AT activity were more erratic in Calves 82, 171, 175, and 176 inoculated by method III. Calf 82 inoculated by aerosol with IBR virus had increased A_1AT activity on PID 1 and enzyme activity continued to increase through PID 6, the last day serum samples were taken before death. The enzyme activity was 93% greater than the pre-inoculation mean on PID 6. Calf 176 was exhibiting a slight increase in A_1AT activity on PID 3 when it died. Increased enzyme levels were noted between PID 1 and PID 7 in calf 175. The A_1AT activity remained elevated through the last day of sampling. Calf 171 showed only minor changes in A_1AT after challenge. The post-inoculation mean of 3.54 umol/min/ml represented only a 17% increase over the pre-inoculation mean.

Calves 9 and 11 challenged with ADV-3 virus and P. hemolytica both had post-inoculation increases in A_1AT activity. Calf 11 had the greater response with peak activity on PID 5 at 6.06 umol/min/ml, 109% above pre-inoculation mean. Enzyme activity remained markedly elevated until necropsy. The changes in activity for Calf 9 were more gradual with peak activity on PID 9. Activity remained elevated through the last day of sampling.

Calf 170 had a pre-inoculation A_1AT mean of 3.72 umol/min/ml (Table II). There was a gradual decreasing trend in activity following inoculation (Appendix Table IIIK).

TABLE II Pre- and Post-inoculation Mean Values for Alpha 1-Antitrypsinase, Lysozyme, and Angiotensin-Converting Enzyme for all Calves

	A_1AT (umol/min/ml)		Lysozyme (ug/ml)		ACE (L-His-L-Leu,nM)	
Calf No.	Pre-inoc	Post-inoc	Pre-inoc	Post-inoc	Pre-inoc	Post-inoc
173	3.27	5.27	3.23	2.43	14.2	10.4
170	3.72	3.20	3.04	3.23	8.31	9.95
172	3.54	4.23	4.06	3.13	12.27	12.37
174	3.34	4.97	3.82	2.52	11.23	8.92
175	3.12	5.79	2.82	2.62	7.58	7.73
11	2.90	4.95	2.38	2.28	12.84	9.33
9	2.72	4.22	2.65	2.15	11.42	8.63
176	1.93	2.22	2.66	1.95	13.1	7.97
171	3.02	3.54	3.56	2.51	11.8	7.47
82	2.97	4.97	3.60	2.25	12.6	12.07

Serum Lactate Dehydrogenase

The daily levels of LDH for each calf are provided in Appendix Table II. Marked increases in LDH activity were observed in all calves except 170 and 172. Calf 172 had a pre-inoculation mean activity for LDH of 532 U/L which dropped to 392 U/L on PID 1 and slowly continued to decrease to a level of 339 U/L on PID 11. The mean pre-inoculation LDH activity was 525 U/L for Calf 170. By PID 3 serum LDH activity had decreased to a level of 240 U/L. Activity returned to a level of 333 U/L by PID 11.

The two calves, 82 and 176, that died spontaneously during the post-inoculation period, had serum LDH activities that were markedly elevated and appeared to be increasing at the time of death. Calf 82 with a pre-inoculation mean for LDH of 320 U/L was showing an increase on day 0, the day inoculated with Pasteurella, this increase continued until death on PID 7. This calf had a post-inoculation mean increased 165% over control levels and a PID 7 LDH activity of 1058 U/L, 230% greater than the pre-inoculation mean. Serum lactate dehydrogenase activity for Calf 176 was markedly increased on PID 2 and PID 3. The activity on PID 3 was 1364 U/L, an 89% increase over pre-inoculation mean.

Calves 171 and 175 inoculated by method III also exhibited post-inoculation increases in serum LDH activity. Calf 171 had a LDH activity that was increased 82% on PID 1 and an average post-inoculation increase of 50%. There was a gradual increase of serum LDH in calf 175 through day 11. The LDH level of 585 U/L recorded on day 11 represented an increase of 117% over pre-inoculation mean.

In Calves 173 and 174 there was a peak of serum LDH activity observed on PID 1. Enzyme activity had returned to normal levels in both calves by PID 11.

The LDH activities of the two animals challenged with ADV-3 and P. hemolytica differed somewhat from each other. Calf 9 had a LDH level of 1284 U/L on PID 10, 172% greater than the activity level noted before inoculation, and a post-inoculation mean 83% greater than pre-inoculation mean. In Calf 11 the serum LDH activity peaked on PID 1 at 768 U/L, 71% higher than control levels, and this calf had an overall increase in LDH of 42%.

Serum Transaminases

The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) values for all calves are recorded in Appendix Table II. All calves except 170 showed an increase in transaminase levels after inoculation. Calf 170 had a slight decrease in both SGOT and SGPT after challenge. Calves 171 and 175 had increased transaminase levels on PID 1 with enzyme activity back to normal by the end of the experimental period. There was a 10.5 fold increase in SGOT and a 5.6 fold increase in SGPT activity noted by PID 4 in Calf 82. These enzyme levels remained elevated until the death of the animal on PID 7. Calf 176 also exhibited marked increases in transaminase levels with SGOT increased greater than six fold by PID 2 and SGPT increased 3.6 fold before death on PID 3. Calves 9 and 11 the animals challenged with ADV-3 and P. hemolytica had increases in both enzymes with Calf 11 having the greater activity changes. This calf had SGPT levels four times that of control values on PID 3 and SGOT levels five times

control values on PID 1. Calf 173 after challenge with Pasteurella responded with over a 2.5 fold increase in SGPT levels by PID 5 but the SGOT activity was reduced post-inoculation. The other calf challenged with Pasteurella only, Calf 174, had a moderate increase in SGPT with peak activity on PID 5 and a pronounced increase in SGOT on PID 1. The transaminases in the serum of Calf 172 exhibited less increase than other calves but appeared to be increasing at the time the calf was euthanized.

Alkaline Phosphatase

Daily pre- and post-inoculation values for alkaline phosphatase are listed in Appendix Table II. Calves 172 and 176 had overall post-inoculation decreases in alkaline phosphatase activity of 20%. Enzyme activity was also decreased in Calf 173 with the post-inoculation mean only 54% of the pre-inoculation mean. There was no marked change in alkaline phosphatase levels for Calf 174 following inoculation. There were post-inoculation decreases of 39%, 35% and 32.5% observed in Calves 82, 11, and 170 respectively. Calf numbers 175 and 171 exhibited steady declines in enzyme activity following challenge. Calf number 175 had a last day level of 22 IU/L, representing a 70% decrease in activity from the pre-inoculation mean, while calf number 171 had a last day level of 19 IU/L, representing a 71.4% decrease. In calf number 9 alkaline phosphatase activity was reduced to 17 IU/L by PID 1, only 31% of pre-inoculation mean, and remained at this low level throughout trial.

Serum Alpha-Fetoprotein

Preliminary tests with bovine serum indicated that enough cross reactivity occurred between the human and bovine alpha-fetoprotein to allow the tests to be run in a human medical laboratory; however, when the assays were done on the serum from the inoculated calves, the results were always below detectable levels of the test. Serum from an adult cow was used in the preliminary trial and perhaps this cow had an unusually high alpha fetoprotein level which was detectable or perhaps nonspecific or false readings were obtained on the preliminary tests.

Serum Lysozyme

Individual pre- and post-inoculation serum lysozyme levels for all calves are listed in Appendix Table III and mean pre- and post-inoculation values are listed in Table II. Decreases in lysozyme activity were observed in all of the calves within 48 hours post-inoculation. With the exception of Calf 170 all of the animals had post-inoculation means lower than control levels (Table II). Calf numbers 82 and 176, the animals that died as a result of infection, showed enzyme activities that decreased until death while the lysozyme activity in all other calves was returning toward control levels by the end of trial.

Serum Angiotensin-Converting Enzyme

The mean pre- and post-inoculation values of ACE for each calf are recorded in Table II and the individual measurements for each calf are listed in Appendix Table III. Calf numbers 170, 172, and 175 had slight

increases in ACE activity post-inoculation, all other calves had decreased post-inoculation means. Calf numbers 9, 11, 171, 173 and 176 showed the most marked decreases in ACE activity after inoculation with post-inoculation means 25% to 36% lower than pre-inoculation means.

Chemistry Profile Results

The daily values for the serum chemistry profile results for each calf are listed in Appendix Table II. A descriptive account of the post-inoculation changes in each of these factors, other than the enzymes already discussed, will not be covered here. Rather these factors will be analyzed in the statistical results section and discussion.

Viral Isolation

Infectious bovine rhinotracheitis virus was recovered from tissue samples of the left lung as well as the bronchial and mediastinal lymph nodes of calf 82. Tissue samples from both lungs as well as the bronchial and mediastinal lymph nodes of calf 176 were found to contain IBR virus. The IBR virus was isolated from the left lung of calf 171 and from the right lung of calf 175. Calf 170 was negative for IBR virus in all tissue samples examined.

Calves 9, 11, and 170 were all inoculated with ADV-3, however isolation attempts to recover this virus from tissue samples proved negative.

Bacterial Isolation

Pasteurella hemolytica was isolated from tissue samples taken from the

right lung of calf 173. This bacteria was also isolated from both lungs of calves 82 and 174. In addition to P. hemolytica, P. multocida was isolated from tissue samples of the left lung and bronchial lymph nodes of calf 82. Pasteurella hemolytica was recovered from the right lung of calf 175 and the left lungs of calves 9, 11, and 171. Pasteurella multocida was isolated from both lungs of calf 176.

Macroscopic Pathology Findings

In general the severity of pulmonary pathology determined at necropsy correlated to the method of inoculation of the calves. Calf 172 inoculated with rumen contents (method I) had relatively mild pneumonia which was scored 1 by the subjective scoring method at the time of necropsy and which had consolidation or inflammation of 8.8% of the total lung area as determined by the objective scoring method. The pneumonia was of a purulent nature with multiple well-encapsulated abscesses.

Calves 173 and 174, inoculated only with P. hemolytica (method II) had subjective pathology scores of 1 and 4 respectively. Calf 173 had 3.7% lung area consolidation and calf 174 had 20% lung area consolidated by the objective scoring system. The pneumonia was of a purulent nature with abscessation especially in Calf 174 (Figure 4).

Calves 82, 171, 175, and 176 inoculated following stress with IBR virus and P. hemolytica (method III) had severe pneumonia. Calves 82 and 176 died spontaneously during the experimental period with subjective pulmonary scores of 5 and 4.5 respectively and objective pulmonary pathology scores of 67.3% and 53.4% respectively. Calves 171 and 175 had subjective pathology scores of 5 and 4

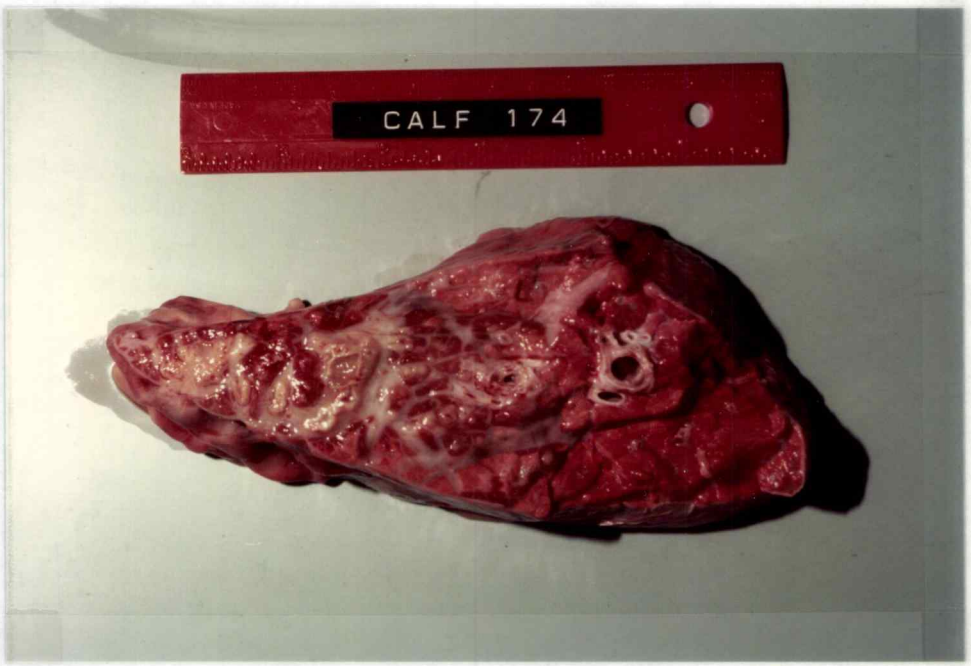


Figure 4. Cross section of left diaphragmatic lobe of calf 174 (inoculation: stress + IBR virus + P. hemolytica) showing fibrinopurulent pneumonia similar to naturally-occurring shipping fever pneumonia.

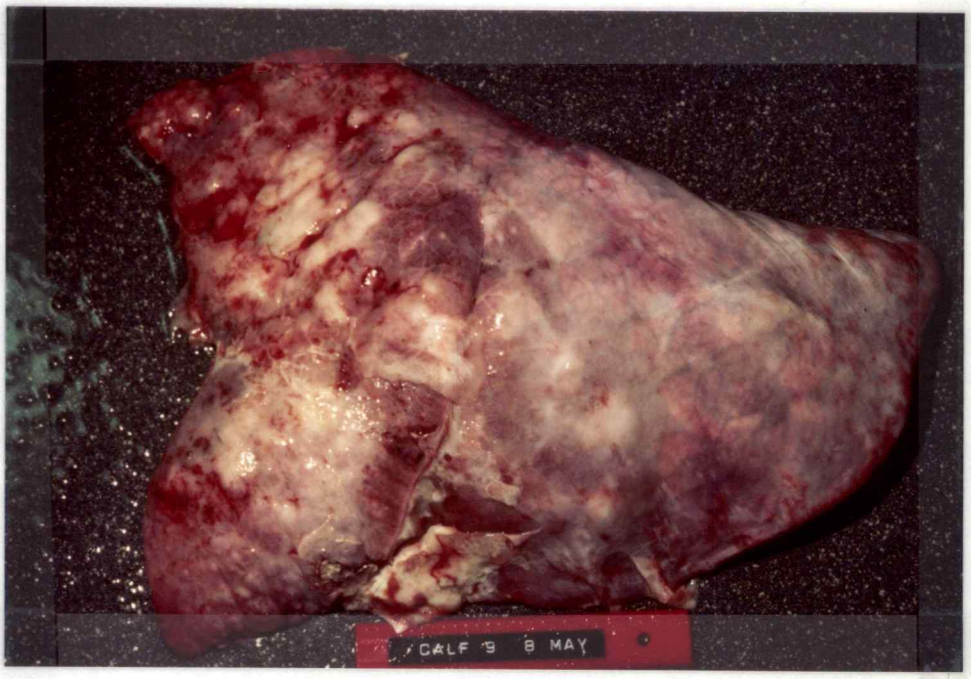
respectively and objective pathology scores of 27.6% and 67.2% respectively. The nature of the pneumonia was a fibrinopurulent exudation in some cases with marked pleuritis. Necropurulent tracheitis was present also in calves 82 and 176. Mesenteric lymph nodes and ileal Peyers' Patches were enlarged in calves 82 and 176.

Calves 9 and 11 inoculated with ADV-3 and P. hemolytica following stress (method III) also had severe pneumonia (Figures 5a and b). Both calves had a subjective pathology score of 4. Calf 11 had 44.6% consolidated lung area by the objective scoring system and Calf 9 had 50.1% consolidated area. The pneumonia in these calves was also of a fibrinopurulent exudative nature. There were ulcerations of the intestinal mucosa and inflammation of intestinal lymph nodes in Calf 11.

Calf 170 inoculated with ADV-3 and IBR virus following stress (method IV) had a subjective pathology rating of 1. By the objective scoring system this calf had 6.3% consolidated lung tissue. The inflammation in this calf could be classified as a suppurative bronchopneumonia (Figures 6a and b). Following are detailed descriptions of the pathology findings of each calf.

Calf 172 inoculated intratracheally with rumen contents (method I) had several enlarged bronchial and mediastinal lymph nodes. The cardiac lobe of the left lung had two consolidated, depressed, plum-colored foci located at tip and hilar region of lobe. The anterior one half of the left diaphragmatic lobe contained seven consolidated foci scattered throughout the parenchyma ranging from .5 to 1 cm in diameter. There were two consolidated foci (.5 and 1 cm in diameter) in the anterior part of the right cardiac lobe. The posterior part

a



b

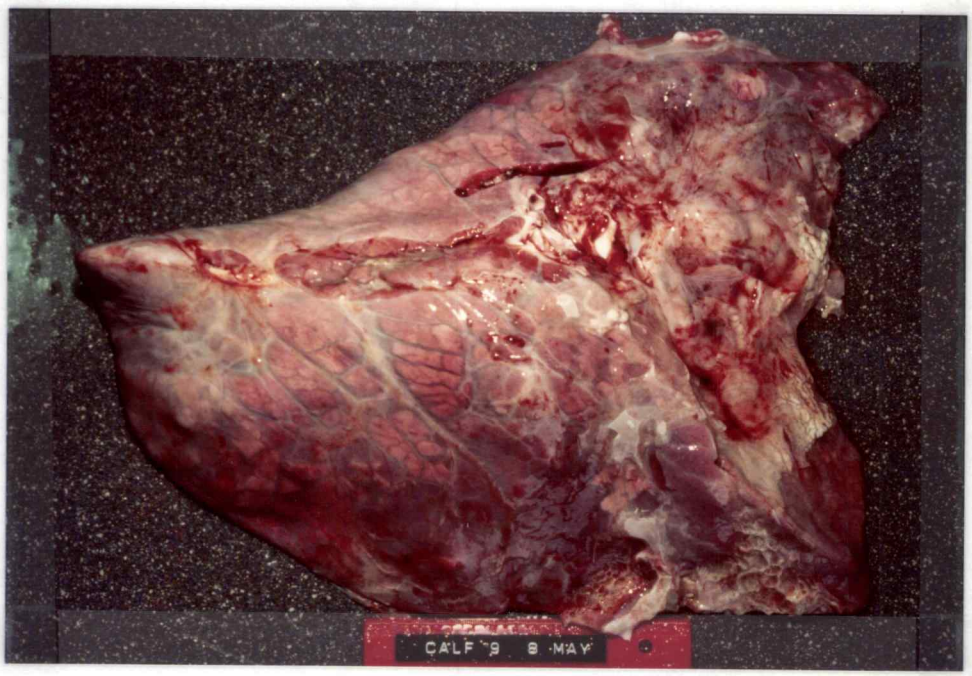
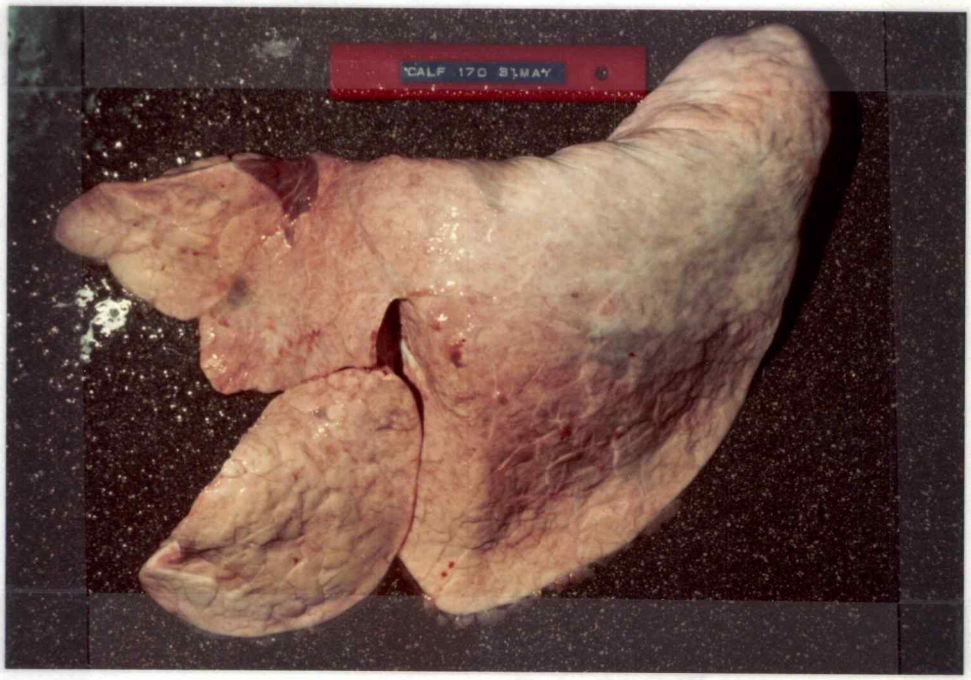


Figure 5. Lateral (a) and medial (b) views of completely consolidated left lung of calf 9 (inoculation: stress + ADV-3 virus + P. hemolytica) pneumonia and multiple collapsing abscesses,

a



b

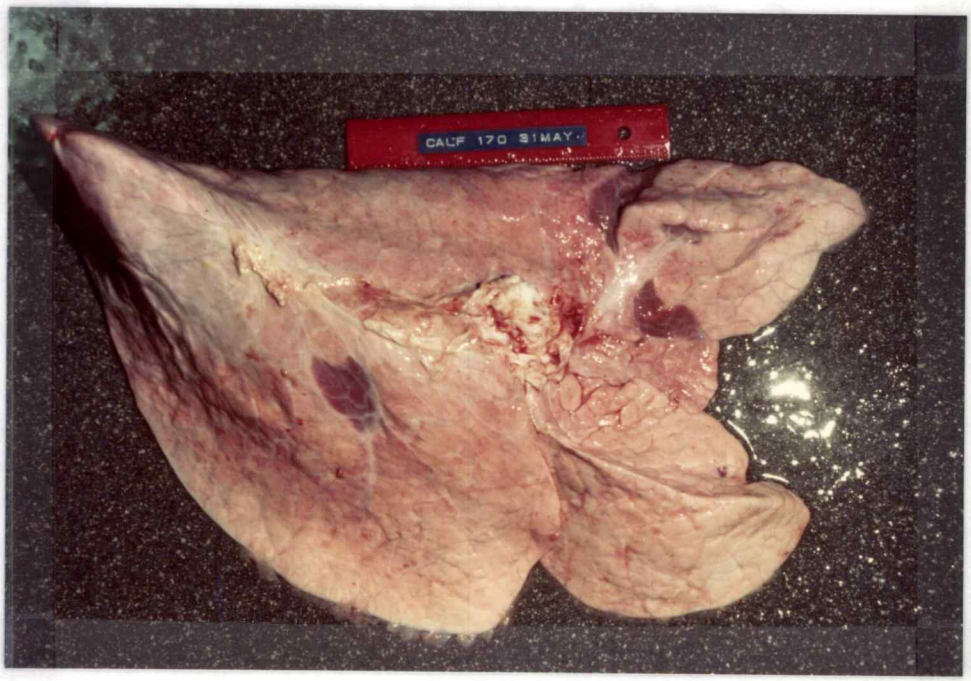


Figure 6. a. Lateral aspect of left lung of calf 170 (inoculation: stress + IBR and ADV-3 viruses) with two collapsed consolidated plum-colored foci in apical lobe and two similar foci in diaphragmatic lobe.

b. Medial aspect of left lung of calf 170 showing one collapsed consolidated plum-colored focus in apical lobe.

of this same lobe contained four collapsed plum-colored foci, 1 to 3 cm in diameter, and two of these had associated emphysematous bullae. The anterior one half of the diaphragmatic lobe contained six consolidated foci of a fibrous nature up to 1.5 cm in diameter. There were no visible lesions in the intermediate lobe.

Examination of Calf 173, inoculated intratracheally with P. hemolytica only (method II) revealed two areas in the left cardiac lobe, one dorsal and one ventral, with multiple fibrinopurulent foci, both foci were approximately 3 x 3 x 6 cm in diameter. There were also two adhesions between the cardiac and diaphragmatic lobes. The diaphragmatic lobe of the left lung contained multiple abscesses, .5 to 2 cm in diameter, along the anterioventral border extending up to the cardiac notch. There were no visible lesions in the intermediate lobe, the left apical lobe, or the lobes of the right lung.

The bronchial lymph nodes of Calf 174, also inoculated with Pasteurella alone, were enlarged four times normal size. There were adhesions 5 to 25 cm in diameter between the posterior ventral margin of dorsal surface of left lung and chest wall. The dorsal surface of the left cardiac lobe contained multiple small adhesions. There were several atelectatic foci in both the cardiac and apical lobes of the left lung that ranged in size from .5 to 1.5 cm wide by 3 to 5 cm long. In addition to these there was one consolidated focus, 3 x 5 cm, in the apical lobe and one consolidated focus 10 x 1.5 cm in the cardiac lobe. The entire ventral portion of the left diaphragmatic lobe was consolidated, measuring 16 x 11 x 5 cm. There was considerable fibrous proliferation throughout the consolidated area. Two abscesses 1.5 and 3.0 cm in diameter, surrounded by a thick fibrous capsule

were found in the left diaphragmatic lobe. The bronchi and abscesses contained a yellowish grey exudate. There were no visible lesions in the right lung.

Calf 82, inoculated following cold stress with both IBR virus and P. hemolytica (method III) died during the experiment. The right thoracic cavity of this calf contained approximately three liters of turbid yellow fluid. The visceral and parietal pleura in this area was uniformly coated with a layer of yellow fibrinous exudate which covered the pericardial sac as well. The entire trachea was involved with a necropurulent inflammation and much of the mucosa was covered with a cheesy exudate. The bronchial and mediastinal lymph nodes were enlarged 10 times normal size. The entire left apical lobe of the lung was collapsed and plum-colored with foci of purulent inflammation. The left cardiac lobe was consolidated and plum-colored with foci of necropurulent inflammation. This lobe had a solid meaty texture and the major bronchi contained a cheesy exudate. The anterior ventral one-fourth of the left diaphragmatic lobe was atelectatic, collapsed, and plum-colored and contained one purulent focus. The entire right apical and cardiac lobes were consolidated with necropurulent inflammation. These lobes were covered with a yellow fibrinous layer and there was no recognizable normal tissue present. The entire surface of the right diaphragmatic lobe was covered with a fibrinous exudate and the anteroventral two-thirds of the lobe was consolidated with necropurulent inflammation. The whole lobe had a swollen meaty texture and the airways were filled with a cheesy exudate. The intermediate lobe was also covered with a fibrinopurulent exudate and

the entire lobe was consolidated. Examination of viscera and intestinal tract revealed that the colonic lymph nodes were slightly enlarged and there was a slight edema in folds of large intestinal mesentery, possibly due to serous atrophy. There was also a 1 cm diameter focus in mucosa of the ileocaecocolic valve. Mucosal hyperemia was evident in the small intestine and Peyer's Patches were raised and white. In the stomach the abomasal leaves were edematous and the mucosa was hyperemic. The liver appeared swollen and turbid and the gall bladder was distended with bile. There was edema around the hepatic lymph nodes and the bile duct.

Examination of thoracic cavity of calf 175, inoculated by method III with IBR virus and P. hemolytica, showed fibrinopurulent adhesions between the lung and rib cage. There was a fibrinopurulent layer covering the ventral two-thirds of the right thoracic wall. The retropharyngeal lymph nodes were enlarged 10 times normal size. The epiglottis and trachea were swollen and roughened but intact. A thin layer of serous exudate covered the lumen of trachea. There was a fibrinopurulent thickening of the entire pericardial sac with adhesions to the entire right apical and cardiac lobes of the lung. A fibrinous exudate was present in the pericardial sac and there were adhesions to the epicardial sac. In the left side of the lung there was one 2 cm diameter plum-colored atelectatic focus in the anterioventral portion of the apical lobe. The left cardiac lobe contained one plum-colored atelectatic focus on the anterior border, 6 cm x 2 cm, and two similar one cm diameter foci in the dorsal aspect. In the ventral aspect of the left diaphragmatic lobe there were 8 foci of

atelectasis, .5 cm in diameter. In the right lung the anterior half of the apical lobe was covered with a fibrinopurulent exudate and there were adhesions to heart and thoracic wall. The entire right cardiac lobe was consolidated with necropurulent inflammation and there was adhesions to the thoracic wall and anterioventral aspect of the diaphragmatic lobe. There was also a 2 x 5 cm atelectatic focus in dorsal aspect of the lobe. The right diaphragmatic lobe had four foci of atelectasis 5 cm in diameter and the entire intermediate lobe was collapsed and congested or consolidated. In the small intestine the Peyer's patches were raised and thickened but there was no definite inflammation.

In calf number 9, inoculated by method III with ADV-3 virus and P. hemolytica, the bronchial and mediastinal lymph nodes were 10 times enlarged. There was a 2.5 cm diameter foci of proliferative fibrous tissue associated with the septum between apical and cardiac lobes of the right lung. The right apical lobe contained three collapsed plum-colored foci ranging from 1 to 2 cm in diameter. In the right cardiac lobe there was one collapsed plum-colored focus, .5 x 2 cm in size, in the anterior portion and six similar foci of irregular conformation in the posterior portion of the lobe. The right diaphragmatic lobe contained two collapsed plum-colored foci, 1.0 and 1.5 cm in diameter. There were no visable lesions in the intermediate lobe. Adhesions between the rib cage and total outer surface of left lung were observed. There were also adhesions between pericardial sac and anterior lobes on medial surface of left side as well as to the diaphragm on the posterior medial surface. Adhesions

were also noted between the apical, cardiac, and diaphragmatic lobes forming a solid unit. The entire left lung was consolidated with multiple collapsing abscesses, .5 to 8 cm in diameter. These abcesses were filled with semifluid, greenish exudate or caseated yellowish pus.

In calf number 11, inoculated by method III with ADV-3 virus and P. hemolytica, the airways of the right lung had a slight amount of watery yellowish exudate, however there were no significant lesions in any of the lobes. The left thoracic cavity was covered by a fibrinopurulent exudate with adhesions to rib cage. In the left lung the apical, cardiac, and anterior third of the diaphragmatic lobes were consolidated with fibrinopurulent inflammation. The consolidated portions were covered with a thick layer of yellowish-white fibrinous exudate. The consolidated area in the apical lobe contained multiple cyst-like cavities lined by fibrous tissue and filled with a watery yellowish fluid. Normal lung tissue was virtually completely replaced by inflammatory tissue. In the intestinal tract mesenteric lymph nodes were enlarged three times normal size and several healing ulcers, 2 mm in diameter, were found in the posterior abomasal mucosa. Peyer's Patches were hyperplastic and enlarged with raised centers.

Calf number 176 was inoculated by method III with IBR virus and P. hemolytica and died during the experiment. This calf had multiple foci of erosion and early ulceration in trachea up to 3 cm in diameter. These foci were scattered in clusters and located in mid and distal trachea. In the thoracic cavity there was a fibrinopurulent pericarditis with adhesions to medial side of left anterior lobe and right intermediate lobe of the lung. There was a fibrinopurulent consolidation of left

cardiac, left apical, and anterior-ventral half of left diaphragmatic lobes. Adhesions between the left cardiac and left diaphragmatic lobes were also evident. The pleural surface of the left lung was covered with a yellowish-white fibrinous exudate approximately 2 to 3 mm in diameter. A fibrinous exudate layer covered the medial side of left lung. The intermediate lobe was entirely consolidated and covered with a similar fibrinous exudate. The right apical lobe had a tag of fibrinous exudate 1 cm in diameter on the lateral surface. There were areas of atelectasis in the apical and ventral portions of the right cardiac lobe. There was also purulent consolidation of the anterioventral portion of the posterior part of the cardiac lobe. Similar consolidation was noted in the right diaphragmatic lobe extending from the anterior portion to the junction of the cardiac lobe. The bronchi of the right lung contained large amounts of yellowish watery fluid. In the intestinal tract approximately 20 ulcers, .5 to 1 cm in diameter, were noted in the mucosa of the distal abomasum. The ileocaecocolic lymph node was enlarged three times normal. Catarrhal enteritis was present in the lower half of the small intestine.

In Calf 171, inoculated by method III with IBR virus and P. hemolytica, there was a focus of pus in the trachea at the sight where the needle was inserted. The airways contained a watery purulent exudate. In the lung the right apical lobe was completely consolidated with a fibrinopurulent inflammation throughout. There were also fibrinous adhesions to the lateral surface of thoracic wall. The ventral half of the anterior portion of the right cardiac lobe was consolidated with multiple purulent foci surrounded by areas of

atelectasis and inflammation. The posterior portion of this lobe had one large area of atelectasis and purulent inflammation approximately 8 cm in diameter containing multiple foci of pus. Two small foci of atelectasis and inflammation were found in the ventral portion of the right cardiac lobe. The right diaphragmatic lobe contained approximately 10 areas of consolidation, atelectasis, and inflammation. These areas were depressed and plum-colored, scattered throughout the lobe and ranged from .5 to 2 cm in diameter. The intermediate lobe contained approximately four foci of consolidation and atelectasis up to 1 cm in diameter. The entire left apical lobe was consolidated with multiple foci of pus up to 5 cm in diameter. Several of these foci surrounded areas undergoing frank necrosis. There were also adhesions between left apical and left cardiac lobes. The dorsal two-thirds of the cardiac lobe contained inflammation similar to the apical lobe with several large necropurulent foci up to 6 cm in diameter. The ventral tip of this lobe contained several atelectatic foci. There were adhesions between the cardiac and diaphragmatic lobes and the thoracic wall. The left diaphragmatic lobe contained inflammation similar to previous lobes involving the central three-fourths of the lobe with only a band of normal tissue along the ventral margin and posterior dorsal surface.

Calf number 170, inoculated by method IV with IBR and ADV-3 viruses, had one 2.5 x 3.5 cm consolidated focus in the mid-portion of the right apical lobe that was slightly atelectatic and plum-colored. The right cardiac lobe contained one focus 5 x 2 cm in the posterior part of the lobe. The focus was consolidated, plum-colored and

slightly raised. There was also one 1 cm diameter focus along the posterior margin of this lobe. The right diaphragmatic lobe contained a 1 cm focus similar in character to those in previous lobes and it was located along the anterior margin of the lobe. The intermediate lobe also had a similar focus 4 x 2 cm in size. In the left lung the apical lobe contained two consolidated plum-colored foci, one 5.5 x 3 cm extending through the lobe and the other 4 x 2.5 cm. In the left cardiac lobe there was one focus .75 cm in diameter located in the upper portion of lobe. The left diaphragmatic lobe contained three foci, similar in character to previous foci; one 3.5 x 2.5 cm focus on the medial surface, one 3 x 2.5 cm focus on the anteriodorsal margin, and one .5 cm focus on the anterioventral margin.

Histopathology

Inoculation method I (Rumen contents) Calf 172. There was severe consolidation of a lobular pattern characterized by infusion of much proteinaceous fluid (inflammatory fluid or plasma) and large numbers of leukocytes predominately neutrophils. Multiple abscesses with well-developed fibrous capsules were present. Multinucleated foreign body-type giant cells were present in some areas and plant material from the rumen fluid was present in a few bronchi or bronchioles. Many bronchioles contained purulent exudate. The mediastinal lymph nodes had moderate lymphoid hyperplasia and edema.

Overall, the characteristics of this pneumonia are that of an acute, purulent or fibrinopurulent nature with some early indication of a granulomatous response as would be expected of a foreign body pneumonia.

Inoculation method II (Pasteurella hemolytica alone). Calves 173 and 174. The histologic changes of these two calves were similar in nature and consisted of a moderate multifocal pneumonitis with atelectasis and infiltration of a mixed leukocyte population. Several areas of purulent inflammation characterized by infiltration of neutrophils and abscessation were present. Mild to moderate purulent bronchitis and bronchiolitis were evidenced by disorganization and cuboidal metaplasia of epithelium occassionally covered with small bits of purulent exudate. Thickening of interlobular septa due to fluid accumulation was present in some lobes. In Calf 174 some pulmonary blood vessels contained thrombi.

Overall the primary changes in the lungs represented a moderate pneumonitis involving rather large areas of the lung with multiple lobules exhibiting atelectasis. Multiple purulent foci were present as well as mild purulent bronchopneumonia. There was marked lymphocytic hyperplasia and edema of the bronchial and mediastinal lymph nodes.

Inoculation method III (stress followed by virus and P. hemolytica). Calves 82, 171, 175 and 176 received IBR virus. Calves 82 and 176 died from the experimental inoculation. The histologic nature of the pulmonary response was basically the same in all the calves and was of a fibrino-purulent nature with multiple necrotic foci. Massive infusion of proteinaceous fluid with intense leukocytic, predominately neutrophils, infiltrates were present. Areas of interstitial pneumonitis were also present. Interlobular septae were distended and there were many thrombotic blood vessels and lymphatics. Fibrinopurulent pleuritis was present in all calves to some degree. Suppurative,

ulcerative tracheitis and rhinitis was present to some extent in all calves but was especially evident in the calves that died. Lymph nodes of the respiratory tract as well as the intestinal tract displayed evidence of lymphoid hyperplasia followed by lymphoid depletion and necrosis with marked edema. Similar changes were present in the lymphoid nodules of the Peyer's Patches in the ileum especially in the calves that died. Calf 176 had edema and several ulcers in the mucosa of the abomasum.

Calves 9 and 11 were inoculated with ADV-3 virus. These calves developed fibrinopurulent bronchopneumonia and pleuritis with necrotic foci, similar histologically to the calves inoculated with IBR virus however there was no tracheitis and rhinitis. There was marked reaction of the respiratory as well as the intestinal lymph nodes as seen in the IBR virus infected calves. Focal ileitis with necrosis of Peyer's Patches was a feature in both calves.

Overall the calves inoculated by method III developed similar pulmonary lesions described as acute, severe, extensive fibrinopurulent bronchopneumonia and pleuritis.

Inoculation method IV (ADV-3 and IBR viruses). Calf 170 developed pneumonia that would be classified as a suppurative bronchopneumonia. However it was almost more of a suppurative bronchitis since the neutrophilic infiltrate was primarily confined to the bronchioles. In several of the lobules there were necropurulent foci involving the pulmonary parenchyma. In these cases it appeared that the necropurulent reaction had originated in the bronchioles and expanded into the adjacent parenchyma. There were large areas of

interstitial pneumonitis with areas of atelectasis. There was hyperplasia and edema of the lymph nodes. Necrotic foci were present in Peyer's Patches of the ileum.

Statistical Analysis

Using the objective and subjective pulmonary pathology rating schemes, the calves were arranged in groups (Table III). Based on the subjective scoring system, there were two groups - calves with mild pulmonary pathology (numbers 170, 172 and 173) and calves with severe pulmonary pathology (numbers 9, 11, 171, 174 and 175). By the objective scoring system there were three groupings - mild pulmonary pathology (calves 171, 172 and 173), moderate (calves 174 and 175), and severe (calves 9, 11 and 171). Calves that died during the experiment were placed in a separate group (calves 82 and 176). The mean values of the pre- and post-inoculation measurements of the calves in each of these groups are listed in Tables IV and V.

The Wilcoxon matched-pairs signs-test was used for statistical analysis of the mean pre- and post-inoculation values to determine which variables developed significant ($P \leq .05$) changes. When all ten calves were examined as a composite a significant difference ($p \leq .05$) in the pre- and post-inoculation values was observed in all the variables except TLC, triglyceride, uric acid, Na, and K. A second Wilcoxon matched-pairs signs-test was performed to examine the pre- and post-inoculation mean values from all calves except numbers 82 and 176, which died spontaneously as a result of infection. This procedure demonstrated significant differences ($p \leq .05$) between pre-

TABLE III Calf Lung Pathology Scoring Results

Calf No.	Agents Inoculated	Method 1 Objective Score[a]	Method 2 Subjective Score[b]
173	*P. hemolytica*	3.7	1 (slight)
170	IBR and ADV-3	6.3	1
172	Rumen Contents	8.8	2 (mild)
174	*P. hemolytica*	20.0	4 (severe)
175	IBR and *P. hemolytica*	27.6	4
11	BAV-3 and *P. hemolytica*	44.6	4
9	BAV-3 and *P. hemolytica*	50.1	4
176	IBR and *P. hemolytica*	53.4 (died)	4.5
171	IBR and *P. hemolytica*	67.2	5 (severe)
82	IBR and *P. hemolytica*	67.3 (died)	5

[a] Percentage of total lung area with macroscopic pathologic change (consolidation or inflammation). Calculated by projection of photographs of lungs onto a 200 square grid and counting the squares having consolidated tissue and the total number of grids having lung tissue.

[b] A score of 1 to 5 was assigned at the time of necropsy with a "1" designated slight pulmonary damage and "5" designated extremely severe pneumonia based on the amount of tissue involved and the character of the pathology.

TABLE IV. Pre- and Post-inoculation Mean Blood Chemistry, Leukocyte, Hematocrit, and Temperature Measurements for Calves with Mild, Moderate, or Severe Pulmonary Pathology Based on the "Objective" Scoring System[a]

	Pulmonary Pathology Rating					
	Mild[b]		Moderate[c]		Severe[d]	
	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.
Lactate Dehydrogenase (IU/L)	467	467.3	353.5	502	459.3	729
Serum Glutamate Oxaloacetate Transaminase (IU/L)	40.8	35.0	58.0	94.0	47.0	98.0
Serum Glutamate Pyruvate Transaminase (IU/L)	40.7	60.9	31.3	41.2	27.8	58.3
Alkaline Phosphatase (IU/L)	64.2	42.6	65.8	44.2	58.5	28.8
Alpha 1-Antitrypsinase (umol/mn/ml)	3.51	4.23	3.23	5.38	2.9	4.24
Lysozyme (ug/ml)	3.44	2.93	3.32	2.57	2.86	2.45
Angiotensin-Converting Enzyme (L-Histidine-L-Leucine, nM)	11.59	10.91	9.4	8.33	12.0	8.5
Triglyceride (mg/dl)	60.2	52.2	42.0	48.5	56.7	49.7
Phosphorus (mg/dl)	7.72	7.03	6.75	6.60	6.82	4.93
Calcium (mg/dl)	12.22	11.03	11.98	9.80	11.25	10.33
Bilirubin (mg/dl)	.32	.20	.15	.55	.13	.50
Uric Acid (mg/dl)	1.23	1.12	1.0	1.14	1.1	1.1

Continued on next page

TABLE IV Continued

	Pulmonary Pathology Rating					
	Mild[b]		Moderate[c]		Severe[d]	
	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.
Total Protein (mg/dl)	7.08	6.47	5.93	5.85	5.98	5.9
Albumin (mg/dl)	4.3	3.73	3.7	3.34	3.9	3.53
Cholesterol (mg/dl)	107.7	84.6	65.3	49.5	90.5	69.0
Blood Urea Nitrogen (mg/dl)	4.5	4.43	3.0	6.7	5.2	8.9
Glucose (mg/dl)	101.8	97.6	94.5	69.5	92.7	83.2
Sodium (meq/l)	136.3	141.4	137.0	136.5	142.5	140.3
Potassium (meq/l)	4.7	4.6	4.65	4.85	5.4	5.43
Chloride (meq/l)	103.5	98.6	96.8	88.4	98.3	97.6
Packed Cell Volume (%)	37.7	34.0	39.7	36.0	39.0	32.5
Total Leukocyte Count/cmm	10,482	8816	8298	9964	9135	6798
Fibrinogen (mg/dl)	548	805	561	967	626	1007
Body Temperature (^{o} F)	101.7	102.6	101.5	103.9	101.3	104.9

[a]See text for description of this method

[b]Calf numbers 170, 172, and 173

[c]Calf numbers 174 and 175

[d]Calf numbers 9, 11, and 171

TABLE V. Pre- and Post-inoculation Mean Blood Chemistry, Leukocyte, Hematocrit, and Temperature Measurements for Calves with Mild or Severe Pulmonary Pathology Based on the "Subjective" Scoring System[a]. Mean Values for the Calves that Died are also Listed

	Pulmonary Pathology Rating					
	Mild[b]		Severe[c]		Died During Experiment	
	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.
Lactate Dehydrogenase (IU/L)	467	467.3	417	638.2	521.5	1063.5
Serum Glutamate Oxaloacetate Transaminase (IU/L)	40.8	35.0	51.4	96.4	33	226.5
Serum Glutamate Pyruvate Transaminase (IU/L)	40.7	60.9	29.2	51.5	36.3	133.5
Alkaline Phosphatase (IU/L)	64.2	24.5	61.4	34.9	59.5	40.5
Alpha 1-Antitrypsinase (umol/min/ml)	3.51	4.23	3.02	4.69	2.45	4.26
Lysozyme (ug/ml)	3.44	2.93	3.05	2.50	3.13	2.08
Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)	11.6	10.9	10.97	8.42	12.85	10.0
Triglyceride (mg/dl)	60.2	52.2	50.8	49.2	51.8	55.4
Phosphorus (mg/dl)	7.72	7.03	6.79	5.6	6.75	4.55
Calcium (mg/dl)	12.2	11.0	11.54	10.12	10.58	8.75
Bilirubin (mg/dl)	.32	.20	.14	.52	.15	2.8
Uric Acid (mg/dl)	1.23	1.12	1.06	1.11	1.15	1.34

Continued on next page

TABLE V. Continued

	Pulmonary Pathology Rating					
	Mild[b]		Severe[c]		Died During Experiment	
	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.	Pre-inoc.	Post-inoc.
Total Protein (mg/dl)	7.08	6.47	5.96	5.88	5.85	4.75
Albumin (mg/dl)	4.3	3.73	3.8	3.45	3.65	3.0
Cholesterol (mg/dl)	107.7	84.6	80.4	61.2	65.3	55.5
Blood Urea Nitrogen (mg/dl)	4.5	4.43	4.3	8.0	3.5	14.7
Glucose (mg/dl)	101.8	97.6	93.4	77.7	82.5	57.2
Sodium (meq/l)	136.3	141.4	140.3	138.8	140.8	142.5
Potassium (meq/l)	4.7	4.6	5.1	5.2	4.6	3.8
Chloride (meq/l)	103.5	98.6	97.7	93.9	94.3	90.0
Packed Cell Volume (%)	37.7	34.0	39.3	33.9	37.2	36.3
Total Leukocyte Count/cmm Blood	10,482	8816	8800	8064	9151	5572
Fibrinogen (mg/dl)	548	805	600	991	501	672
Temperature (^{o} F)	101.7	102.6	101.4	104.2	101.6	104.4

[a]See text for description of this method

[b]Calf numbers 170, 172, and 173

[c]Calf numbers 9, 11, 171, 174, and 175

[d]Calf numbers 82 and 176

and post-inoculation mean values in PCV, fibrinogen, body temperature, lysozyme, SGPT, A_1AT, P, Ca, alkaline phosphatase, albumin, cholesterol, BUN, and glucose. Finally, a third Wilcoxon test was carried out to examine the pre- and post-inoculation mean values of the calves excluding those that died during the experiment and those that were rated as having moderate pneumonia based on the objective scoring system. The results of this test indicated that there were significant differences in PCV, fibrinogen, body temperature, SGPT, A_1AT, P, alkaline phosphatase, albumin, and cholesterol.

The Spearman correlation coefficients test was used to evaluate the post-inoculation values of the calves. Initially a test was performed to see which variables or measurements correlated with the pathology rating schemes when all ten calves were included. For the subjective rating system significant correlations existed between pulmonary pathology rating and the following variables: body temperature, LDH, lysozyme, SGOT, P, Ca, bilirubin, total protein, albumin, cholesterol, BUN, and glucose. The objective rating scheme showed the same correlations except cholesterol was replaced by TLC. A second Spearman correlation coefficients test was performed to examine the data for all calves except numbers 82 and 176, the animals that died during the experiment. The results of this test revealed that in the subjective rating scheme there was a significant correlation between animal rank and post-inoculation mean values for fibrinogen, body temperature, SGOT, ACE, bilirubin, albumin, cholesterol, BUN, and glucose. For the objective rating scheme, a significant correlation between body temperature, LDH, ACE, P, bilirubin, BUN, and K values and severity of

pneumonia was demonstrated. When a third Spearman correlation coefficients test was carried out on the data of only the mild and severe groups of the objective rating scheme (6 calves) a significant correlation ($p \leq .05$) was demonstrated for TLC, body temperature, LDH, ACE, P, bilirubin, and BUN.

DISCUSSION

The clinical and pathology results indicate that the efforts to produce pulmonary damage of varying degrees of severity through the use of different inoculation methods were successful. Five of the six calves inoculated by method III (stress followed by virus and P. hemolytica) developed severe pneumonia and two of these calves died from the infection. The sixth calf developed pneumonia that was rated moderate by the objective scoring system and severe by the subjective scoring system. The two calves inoculated with P. hemolytica alone (method II) developed less severe pneumonia. One calf developed mild pneumonia, while the second calf developed pneumonia classified as moderate by the objective scoring system and severe by the subjective scoring system. The calf inoculated with viruses only (method IV) developed mild pneumonia. Somewhat surprisingly to us, the calf inoculated with rumen fluid (method I) developed only mild pneumonia. These results add support to the hypothesis of the pathogenosis of shipping fever pneumonia put forth by Jensen et al., (47).

Because of the success of method III in experimentally inducing disease that parralleled shipping fever pneumonia, this would appear to be the method of choice for future studies on this disease problem. Interestingly, severe pneumonia was induced when both IBR and ADV-3 viruses were used although spontaneous deaths occurred only in the calves inoculated with IBR virus. In future studies it would be interesting to study the influence of different viruses more extensively as well as different viral concentrations. Different methods

of viral and bacterial inoculation, different strains of bacteria and different types of stress situations might also influence severity of pneumonia.

Microscopic examination of the lungs revealed basically similar changes in all the calves. Purulent inflammation was present to some extent in the lungs of all calves, even in the calf inoculated only with viruses where bacteria normally present in the respiratory tract apparently proliferated following the action of viruses on the respiratory tissues. There was a severe infusion of plasma or inflammatory fluid in most of the calves inoculated by method III which was absent or present only to a mild degree in calves inoculated by the other methods. Transtracheal inoculation of a calf with rumen fluid would be expected to produce pneumonia of a different character than inoculation with viruses or bacteria only; however, at the early stage at which the calves were euthanized, the character of the pneumonia in the calf inoculated with rumen fluid was very similar to the purulent nature induced by the other inocula. Thus, overall the character of the experimentally-induced pneumonia was similar enough to allow comparison between calves in terms of the blood constituent parameters.

As noted in the results, P. multocida was isolated from tissue samples taken from four of the calves at necropsy. All of these calves were negative for P. multocida as well as P. hemolytica on pre-inoculation culture of nasal swabs. The isolation of P. multocida at necropsy indicates that nasal cultures are not reliable in detecting P. multocida and that P. multocida is a normal or common inhabitant of the bovine respiratory tract as has been previously reported.

The role of the P. multocida in the pathogenesis of pneumonia in the calves from which it was isolated cannot be determined. Certainly P. multocida is an important microorganism in naturally-occurring shipping fever pneumonia.

Prior to bacterial inoculation calves of inoculation method III were aerosally challenged with virus. Infectious bovine rhinotracheitis virus and adenovirus type 3 were used because they have both been incriminated in the disease complex, are widespread and common pathogens, and primarily restrict their pathological effect to the respiratory tract. The 30 hour incubation period was used for adenovirus type 3 because it is a slower replicating virus than IBR. The failure to isolate ADV-3 from the tissue samples taken at necropsy of those calves inoculated with ADV-3 may have been due to the effects of host antibody production. Adenovirus is a non-enveloped virus and therefore more susceptible to action by antibodies than herpes viruses such as IBR. For this reason Dr. Mattson, School of Veterinary Medicine, does not routinely attempt isolation of ADV-3 if the infection has lasted longer than five days.

In a recent study by Berendt et al., (8) in which rats were subjected to respiratory infection by Klebsiella pneumoniae it was found that changes in serum lysozyme activity were a good index of severity of infection. He showed that rats dying from infection had progressively increasing lysozyme levels which were significantly higher than infected survivors. This was not observed in the calves in our experiment. On the contrary lysozyme activity decreased in all calves except Calf 170 following inoculation.

Serum glutamate oxaloacetic transaminase activity increased after inoculation in the moderate and severely involved calves as well as those that died during the experiment. Enzyme activity spiked early in infection with peak activity detected on the first sampling day post-inoculation in six of the ten calves. Because only three samplings were taken for post-inoculation chemistry analysis with up to five days between each, it cannot be known if there were higher levels of SGOT on those days not sampled; however, examination of the change in post-inoculation mean values for SGOT reveals that the greatest change occurred in the more severely infected calves. By the objective rating scheme the mild group had an overall 5.8 IU/L decrease in SGOT post-inoculation while the moderate and severe groups exhibited 36.5 U/L and 51 IU/L increases respectively.

Using the subjective pulmonary pathology rating the mild group again had a 5.8 IU/L decrease in SGOT whereas the severe group showed a 45 IU/L increase (Tables IV and V). Those calves that died spontaneously during infection revealed a 193.5 IU/L increase post-inoculation, representing nearly a seven fold increase.

Lactate dehydrogenase levels increased after inoculation and showed a similar positive correlation between extent of post-inoculation change and degree of pulmonary pathology. The mild, moderate, and severe groups of the objective scheme had a .3 IU/L, 148.5 IU/L, and 269.7 IU/L increases respectively. For the subjective rating scheme the mild group had a .3 IU/L increase and the severe group a 221.2 IU/L increase. The calves that died during the experiment exhibited a post-inoculation mean 542 IU/L greater than pre-inoculation levels.

There was a significant correlation ($p \leq .05$) between the pulmonary pathology rating and post-inoculation means for ACE. This trend was also noted when the difference in post-inoculation values were examined for the different groups of the two rating schemes. By the objective pathology scheme decreases of .7 nM, 1.07 nM, and 3.5 nM were observed for the mild, moderate, and severe groups respectively (Table IV). In the mild group of the subjective scheme there again was a .7 nM decrease while the severe group had a 2.55 nM decrease. The animals that died spontaneously during infection showed a 2.55 nM decrease (Table V). Because of the high concentration of ACE in the lungs, relative to other tissues, it could be very useful if this trend proved true in further studies.

Blood urea nitrogen and bilirubin activity increased in the more severely infected samples after inoculation. Post-inoculation activity of BUN in the mildly involved group decreased .7 mg/dl whereas increases of 3.7 mg/dl and 3.9 mg/dl post-inoculation increases were noted in the calves of the moderate and severely involved groups based on the objective rating scheme. The severe group of the subjective scheme had a 3.7 mg/dl increase in BUN activity post-inoculation. The mildly involved group of both pathology rating schemes also showed a slight post-inoculation decrease of .12 mg/dl in bilirubin. The severe group of the subjective scheme showed a .38 mg/dl increase while the moderate and severe group of the objective pathology rating scheme had .4 mg/dl and .37 mg/dl increases respectively. The animals succumbing to infection had very marked post-inoculation increases with BUN and bilirubin

increases of four fold and 19 fold respectively.

The results for glucose were somewhat confusing in that even though there was a significant decrease observed post-inoculation by the subjective rating scheme, in the objective scheme the moderate group showed a greater decrease post-inoculation than the severe group, 25 mg/dl vs 9.5 mg/dl respectively (Table IV and V). The two calves (174 and 175) comprising the moderate pathology group of the objective scoring scheme were included in the severe pathology group of the subjective scheme and thus help to account for the statistically significant differences in glucose demonstrated in the subjective pathology scheme. A similar change was noted for P in that the mild group of the objective scheme had a greater decrease post-inoculation than the calves of the moderate pulmonary pathology group, .69 mg/dl and .15 mg/dl respectively. The reason for this trend for glucose and P are not know.

Fibrinogen levels and body temperature increased after inoculation. Both of these variables showed a positive correlation between degree of lung damage and mean post-inoculation value.

As stated earlier the primary aim of this study was to examine the changes in blood constituents and body temperature during the course of shipping fever pneumonia in these calves in the hope that certain parameters might correlate with severity of lung damage and therefore be of prognostic and/or diagnostic value as well as serve as a research tool. Post mortem examinations were conducted when each calf was euthanized or died to determine particularly the extent of pulmonary pathology, but also to determine if lesions other than

those of the respiratory system might have contributed significantly to the blood constituent changes. Since rating of pulmonary pathology is primarily done on a subjective scoring system by the prosector, an attempt was made to evaluate the quantity or relative proportion of lung tissue involved to determine if an objective method of scoring might be better than the subjective system.

The pre- and post-inoculation means for the 24 measurements taken were evaluated using nonparametric statistical procedures. Non-parametric procedures were employed because the sampling size (number of calves) was not large enough to meet the criterion of the more precise parametric procedures such as the paired T test. The data was first subjected to the Wilcoxon matched-pairs test to determine which of the 24 measurements exhibited significant differences ($p \leq .05$) between the pre- and post-inoculation means. All but five of the variables including total leukocyte count, triglyceride, uric acid, Na, and K, exhibited a significant post-inoculation change. Two further Wilcoxon tests were carried out, one including eight calves in which the two calves that died were removed from consideration and the other with six calves excluding values from the two calves that died and the two calves ranked as having moderate pneumonia by the objective rating scheme. These tests indicated that there were statistically significant differences between pre- and post-inoculation mean values of PCV, fibrinogen, body temperature, lysozyme, SGPT, A_1AT, P, Ca, alkaline phosphatase, albumin, cholesterol, BUN, and glucose, but they provided little information relating the magnitude of changes to the degree of pulmonary pathology. For this

type of analysis, the Spearman correlation coefficients tests were of more value.

The results from the first Spearman test including the data from all lung pathology ranking classes revealed both ranking schemes to be in agreement for all parameters except that in the objective scheme there was a correlation between lung pathology score and TLC level but not with cholesterol. The reverse occurred with the subjective scheme, i.e., cholesterol level was significantly correlated with pulmonary pathology score and TLC was not. This was largely due to the fact that calf numbers 174 and 175 of the moderate group had a much lower overall pre-inoculation cholesterol activity than the mild or severely involved groups, 65.3 mg/dl vs 107.7 mg/dl and 90.5 mg/dl respectively. Because the Spearman correlation test only evaluated the post-inoculation means it appeared as if the moderately involved calves of the objective scheme illicited the most drastic response and therefore failed to fit into the ranking scheme. When the difference between pre- and post-inoculation means for each group is calculated however, it is seen that the decrease in cholesterol activity is very similar for all groups: mild - 23.1 mg/dl; moderate - 16.1 mg/dl; severe - 21.4 mg/dl. Because of this similar response after inoculation by all groups, cholesterol activity would appear to provide no information about severity of infection. Similarly, since the pre-inoculation values for TLC also show a wide range of activities (Table II and III) and both the mild and severe groups exhibited significant decreases post-inoculation, this variable appears from these results to be of limited significance.

The final two Spearman correlation coefficient tests carried out, using data from eight calves and six calves as explained earlier, revealed the following parameters to have significant correlations with lung pathology: total leukocyte count, fibrinogen, body temperature, SGOT, LDH, ACE, bilirubin, P, albumin, cholesterol, BUN, K, and glucose.

Evaluation of the data without statistical tests or analysis may be as valuable as statistical tests in arriving at practical conclusions. The greatest changes between pre- and post-inoculation levels occurred in fibrinogen, body temperature, SGOT, LDH, ACE, bilirubin, P, and BUN. These parameters also had the strongest association with the pathology ratings of the calves, i.e., the changes were least in the calves with mild pneumonia and greater in the calves with severe pulmonary pathology. Furthermore, SGOT, BUN, and bilirubin all exhibited marked increases in activity in those calves that died spontaneously as a result of infection. Serum glutamate oxaloacetate showed nearly a seven fold increase, BUN over a four fold increase, and bilirubin a 19 fold increase in these calves.

Although the statistical tests showed statistically significant changes in pre- and post-inoculation levels and correlations between mean post-inoculation values and pulmonary pathology for K and albumin, the changes in these parameters were quite minimal. Considering the objective ranking system, K exhibited increasing values from mild to severe when only the post-inoculation means were examined, however the total change between pre- and post-inoculation values for the mild and severe groups was a .07 meq/l decrease. Post-inoculation mean albumin values were significantly depressed relative to the mild group of the

subjective ranking scheme; however, when the pre- and post-inoculation means of each group are compared, the mildly involved calves show a greater overall decrease.

The inverse correlation between serum ACE activity and severity of pulmonary pathology that was demonstrated in this study is very interesting. These results support the theory of Ashutosh and Keighley (6) that any lung damage associated with decreased vascularity or abnormal pulmonary blood supply would lead to decreased ACE activity. Considering its high concentration in lung tissue relative to other organs in the body, ACE might be expected to show better correlation with pulmonary pathology than other enzymes. Further studies, perhaps using more frequent sampling periods, will help to substantiate the results of this experiment.

It would be desirable if more enzymes such as ACE or other serum factors, specific for lung function, could be found to monitor pulmonary pathology; however, because the lungs play such a central role in the health of the individual, changes in the lungs during pneumonia will effect other organ systems. Therefore alterations in serum enzymes such as SGOT and LDH as well as blood components such as bilirubin, P, BUN, and plasma fibrinogen may provide additional information in evaluating the severity of pulmonary pathology or pneumonia in calves.

When the various factors were examined using the objective and subjective pulmonary pathology scoring systems very similar results were obtained. It appears at least from these results that subjectively scoring macroscopic lung pathology by character and extent of lesions

at the time of necropsy is as effective as quantitating damage on the basis of percentage of consolidated to non-consolidated lung tissue (objective scoring system).

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APPENDIX

TABLE I. PRE- AND POST-INOCULATION VALUES OF PACKED CELL VOLUME, TOTAL LEUKOCYTE COUNT, PLASMA FIBRINOGEN AND TEMPERATURE FOR EACH CALF

Appendix Table IA Calf No. 172

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-5	41	8250	493	101.6
-2	40	8700	516	101.4
0	39	8150	504	101.7
Post-inoculation				
2	40	8700	526	101.8
3	40	9600	676	102.6
7	34	10,100	934	102.2
9	31	10,900	1123	103.1
12	30.5	10,350	1352	102.4
13	33.5	8631	1010	102.0

Appendix Table IB Calf No. 173

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-9	35	9126	491	101.9
-7	34	8861	480	101.9
-2	33.5	8734	479	101.8
Post-inoculation				
1	33	8692	844	102.9
3	31.5	6496	975	102.9
5	30	6381	892	101.5
7	30	8923	579	102.2
9	36	8005	707	101.5
11	32	9470	662	101.3

TABLE I. Continued

Appendix Table IC Calf No. 174

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-11	40.5	6598	----	101.9
-8	39.5	7538	521	101.3
-5	41	7279	460	101.6
-1	38	5701	555	101.4
Post-inoculation				
1	45	4510	447	105.1
3	32.5	13,514	938	103.7
5	39	14,232	1266	102.6
7	35.5	12,428	1322	101.6
9	33	11,459	1077	101.4
11	38	11,762	995	101.7
13	40	12,232	716	-----

Appendix Table ID Calf No. 82

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-6	35.5	12,236	399	-----
-4	38	9257	498	101.7
-2	35.5	9044	564	101.4
-1	37	10,602	515	101.6
Post-inoculation				
1	----	------	655	105.0
2	34	3247	784	105.2
4	29	5643	647	106.0
6	37.5	16,310	858	106.2

TABLE I. Continued

Appendix Table IE Calf No. 175

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-7	38.5	9686	661	-----
-5	41	9998	538	101.5
-3	39	9768	632	101.0
Post-inoculation				
1	42.5	10,324	661	104.6
3	28.5	4384	1006	106.2
5	31	7666	933	106.3
7	31.5	8272	1107	104.1
9	33	10,100	1092	103.6
11	34	10,160	1008	-----

Appendix Table IF Calf No. 171

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-7	--	9266	919	101.7
-4	41	6814	898	101.5
-2	37	9776	632	101
Post-inoculation				
1	35	6270	791	102.4
3	40	3647	1139	105.0
5	30	4504	1258	105.2
7	28	5552	1043	105.6
9	28	6889	1005	105.0
12	34	11,840	1026	103.5

TABLE I. Continued

Appendix Table IG Calf No. 176

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-7	40	7950	433	101.3
-5	36	7749	526	101.5
-2	37.5	8338	564	102.0
Post-inoculation				
1	---	------	----	103.6
2	41	1923	601	104.8
3	37	3563	611	102.0

Appendix Table IH Calf No. 9

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-10	39.5	10,500	461	101.5
-6	40	10,200	607	101.6
-1	37	9493	600	100.7
Post-inoculation				
1	41.5	8738	750	106.5
2	39	8738	1035	103.2
4	30	6749	1156	106.0
6	27	5943	1088	106.0
9	29	2504	806	106.4
10	28.5	5023	755	105.8

TABLE I. Continued

Appendix Table IJ Calf No. 11

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-12	40	10,366	453	101.5
-10	39.5	9012	532	101.4
-7	39	7815	549	101.5
-5	40	8079	524	101.5
-2	38	8387	472	100.4
Post-inoculation				
1	42	8772	856	106.0
3	32	7018	916	104.6
5	26.5	7773	1239	104.0
7	28	7477	1198	103.5
9	33.5	7268	1017	103.6

Appendix Table IK Calf No. 170

Experimental Day	Packed Cell Volume (%)	Total Leukocyte Count/cmm	Plasma Fibrinogen (mg/dl)	Body Temperature (°F)
Pre-inoculation				
-10	41	16,400	731	101.5
-7	38.5	11,733	637	101.3
-3	38	14,400	599	102.0
Post-inoculation				
1	38	11,800	561	100.9
3	42	14,360	690	101.4
5	40.5	14,833	606	102.4
7	32.5	8966	751	104.6
9	33	8605	778	104.8
11	30	8730	813	103.7

TABLE II. Pre- and Post-inoculation Serum Chemistry Values for Each Calf

Appendix Table IIA Calf No. 172

	Pre-inoculation		Post-inoculation		
Experimental day	-5	-2	2	7	12
Lactate dehydrogenase (IU/L)[a]	561	502	392	383	339
Serum glutamate oxaloacetate transaminase (IU/L)	20	6	38	3	32
Serum glutamate pyruvate transaminase (IU/L)	26	31	43	23	80
Alkaline phosphatase (IU/L)	48	44	42	26	42
Triglyceride (mg/dl)	56	56	59	42	86
Phosphorus (mg/dl)	5.9	6.7	6.7	5.8	8.5
Calcium (mg/dl)	11.1	11.2	11.9	11.3	13.2
Bilirubin (mg/dl)	.1	.3	.4	.2	.4
Uric acid (mg/dl)	1.0	1.3	1.7	1.1	1.3
Total protein (mg/dl)	6.2	6.2	6.5	6.6	8.5
Albumin (mg/dl)	3.8	3.7	3.9	3.4	4.2
Cholesterol (mg/dl)	104	81	84	87	107
Blood urea nitrogen (mg/dl)	3	4	6	2	4
Glucose (mg/dl)	112	83	78	99	122
Sodium (meq/l)[b]	152	150	149	146	116
Potassium (meq/l)	4.7	5.4	4.8	4.2	3.4
Chloride (meq/l)	98	97	99	99	109

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIB Calf No. 173

	Pre-inoculation		Post-inoculation		
Experimental day	-8	-2	1	5	10
Lactate dehydrogenase (IU/L)[a]	350	337	1008	538	348
Serum glutamate oxaloacetate transaminase (IU/L)	---	226	58	191	16
Serum glutamate pyruvate transaminase (IU/L)	52	55	93	145	71
Alkaline phosphatase (IU/L)	54	64	27	33	36
Triglyceride (mg/dl)	64	65	52	42	41
Phosphorus (mg/dl)	8.1	8.8	7.2	5.9	6.4
Calcium (mg/dl)	12.0	13.4	9.7	10.7	11.3
Bilirubin (mg/dl)	.3	.6	.1	.2	.2
Uric acid (mg/dl)	1.7	1.2	1.2	.9	.9
Total protein (mg/dl)	6.7	8.1	5.7	6.2	6.4
Albumin (mg/dl)	4.2	5.1	3.5	3.6	3.8
Cholesterol (mg/dl)	99	114	68	68	75
Blood urea nitrogen (mg/dl)	5	3	4	3	3
Glucose (mg/dl)	95	113	89	117	114
Sodium (meq/l)[b]	156	96	148	149	144
Potassium (meq/l)	4.8	3.2	4.2	4.4	4.3
Chloride (meq/l)	101	115	98	97	99

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIC Calf No. 174

	Pre-inoculation		Post-inoculation		
Experimental day	-8	-5	1	5	10
Lactate dehydrogenase (IU/L)[a]	480	396	798	299	440
Serum glutamate oxaloacetate transaminase (IU/L)	52	59	182	87	86
Serum glutamate pyruvate transaminase (IU/L)	38	29	55	60	38
Alkaline phosphatase (IU/L)	56	63	57	54	60
Triglyceride (mg/dl)	53	38	45	76	64
Phosphorus (mg/dl)	8.2	6.0	6.7	6.6	8.4
Calcium (mg/dl)	13.1	10.7	8.6	9.9	11.4
Bilirubin (mg/dl)	.2	.2	1.7	.4	.2
Uric acid (mg/dl)	1.0	1.1	1.2	.8	1.9
Total protein (mg/dl)	6.0	5.5	4.8	5.5	6.6
Albumin (mg/dl)	3.7	3.6	3.2	3.2	3.4
Cholesterol (mg/dl)	83	35	46	52	49
Blood urea nitrogen (mg/dl)	3	5	12	4	4
Glucose (mg/dl)	103	86	58	82	66
Sodium (meq/l)[b]	139	136	135	140	138
Potassium (meq/l)	4.4	4.9	3.8	4.9	6.0
Chloride (meq/l)	96	96	88	87	89

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IID Calf No. 175

	Pre-inoculation		Post-inoculation		
Experimental day	-5	-3	1	6	10
Lactate dehydrogenase (IU/L)[a]	324	214	396	570	585
Serum glutamate oxaloacetate transaminase (IU/L)	64	55	77	69	65
Serum glutamate pyruvate transaminase (IU/L)	33	25	46	25	23
Alkaline phosphatase (IU/L)	81	63	53	19	22
Triglyceride (mg/dl)	48	29	46	28	32
Phosphorus (mg/dl)	6.9	5.9	5.8	5.3	7.0
Calcium (mg/dl)	12.3	11.8	9.4	9.5	9.8
Bilirubin (mg/dl)	.1	.1	.4	.4	.2
Uric acid (mg/dl)	.9	1.0	1.2	.7	1.0
Total protein (mg/dl)	6.2	6.0	6.0	5.9	6.5
Albumin (mg/dl)	3.8	3.6	3.6	3.3	3.3
Cholesterol (mg/dl)	74	69	68	44	38
Blood urea nitrogen (mg/dl)	2	2	8	6	6
Glucose (mg/dl)	92	97	57	81	73
Sodium (meq/l)[b]	136	137	136	134	136
Potassium (meq/l)	5.0	4.3	5.3	4.4	4.6
Chloride (meq/l)	97	98	92	90	84

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIE Calf No. 82

	Pre-inoculation		Post-inoculation		
Experimental day	-4	-2	1	4	6
Lactate dehydrogenase (IU/L)[a]	402	237	591	894	1050
Serum glutamate oxaloacetate transaminase (IU/L)	44	44	354	462	288
Serum glutamate pyruvate transaminase (IU/L)	38	28	156	134	142
Alkaline phosphatase (IU/L)	81	69	50	43	44
Triglyceride (mg/dl)	54	40	47	38	52
Phosphorus (mg/dl)	6.2	6.8	4.3	3.4	3.8
Calcium (mg/dl)	10.7	11.0	8.3	8.6	9.2
Bilirubin (mg/dl)	.1	.1	2.1	2.7	3.2
Uric acid (mg/dl)	.9	.9	1.1	1.1	1.5
Total protein (mg/dl)	5.7	5.4	5.3	4.1	4.2
Albumin (mg/dl)	3.7	3.6	3.4	2.9	2.8
Cholesterol (mg/dl)	66	68	74	58	60
Blood urea nitrogen (mg/dl)	3	2	11	14	19
Glucose (mg/dl)	92	97	47	66	74
Sodium (meq/l)[b]	136	142	145	134	141
Potassium (meq/l)	4.9	4.8	2.9	3.7	4.7
Chloride (meq/l)	93	91	93	85	83

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIF Calf No. 11

	Pre-inoculation		Post-inoculation		
Experimental day	-7	-2	1	4	8
Lactate dehydrogenase (IU/L)[a]	446	449	768	554	584
Serum glutamate oxaloacetate transaminase (IU/L)	54	47	254	34	59
Serum glutamate pyruvate transaminase (IU/L)	29	32	114	127	48
Alkaline phosphatase (IU/L)	53	52	37	34	30
Triglyceride (mg/dl)	49	43	53	44	50
Phosphorus (mg/dl)	7.6	5.5	4.5	4.9	6.4
Calcium (mg/dl)	11.2	12.0	10.7	10.2	11.0
Bilirubin (mg/dl)	.1	.1	.8	.9	.2
Uric acid (mg/dl)	1.0	1.0	1.3	1.4	1.1
Total protein (mg/dl)	5.5	6.3	5.4	5.3	6.3
Albumin (mg/dl)	3.7	4.2	4.1	3.4	3.6
Cholesterol (mg/dl)	77	81	68	61	63
Blood urea nitrogen (mg/dl)	4	4	13	8	6
Glucose (mg/dl)	101	107	111	79	90
Sodium (meq/l)[b]	143	146	148	139	144
Potassium (meq/l)	4.3	4.3	4.7	5.3	5.5
Chloride (meq/l)	100	104	104	97	97

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIG Calf No. 9

	Pre-inoculation		Post-inoculation		
Experimental day	-8	-3	1	5	10
Lactate dehydrogenase (IU/L)[a]	487	458	672	644	1284
Serum glutamate oxaloacetate transaminase (IU/L)	59	57	145	112	113
Serum glutamate pyruvate transaminase (IU/L)	39	30	62	50	34
Alkaline phosphatase (IU/L)	53	40	17	17	19
Triglyceride (mg/dl)	70	54	45	43	64
Phosphorus (mg/dl)	7.4	6.3	4.1	4.4	4.1
Calcium (mg/dl)	10.8	10.4	9.6	9.3	10.5
Bilirubin (mg/dl)	.1	.2	.8	.4	.3
Uric acid (mg/dl)	.9	.9	.9	.8	1.1
Total protein (mg/dl)	6.3	6.4	5.7	6.0	6.0
Albumin (mg/dl)	3.9	3.9	4.0	3.5	3.1
Cholesterol (mg/dl)	77	74	66	53	68
Blood urea nitrogen (mg/dl)	4	7	11	7	9
Glucose (mg/dl)	96	92	30	88	78
Sodium (meq/l)[b]	139	141	136	136	137
Potassium (meq/l)	5.8	5.5	5.4	5.6	5.9
Chloride (meq/l)	98	98	97	96	97

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIH Calf No. 170

	Pre-inoculation		Post-inoculation			
Experimental day	-9	-2	1	3	8	11
Lactate dehydrogenase (IU/L)[a]	470	580	550	240	479	333
Serum glutamate oxaloacetate transaminase (IU/L)	70	67	52	55	38	38
Serum glutamate pyruvate transaminase (IU/L)	43	37	34	34	34	26
Alkaline phosphatase (IU/L)	70	105	90	40	61	45
Triglyceride (mg/dl)	64	56	55	55	53	40
Phosphorus (mg/dl)	9.7	7.1	8.2	7.3	7.5	7.4
Calcium (mg/dl)	13.7	11.9	11.0	10.3	10.0	9.9
Bilirubin (mg/dl)	.2	.4	.4	.1	.1	.0
Uric acid (mg/dl)	1.2	1.0	1.1	.9	.9	1.0
Total protein (mg/dl)	8.1	7.2	6.8	6.0	5.9	5.7
Albumin (mg/dl)	4.7	4.3	4.0	3.7	3.6	3.6
Cholesterol (mg/dl)	118	120	111	95	80	77
Blood urea nitrogen (mg/dl)	5	7	5	5	8	6
Glucose (mg/dl)	122	86	82	95	85	83
Sodium (meq/l)[b]	120	144	146	141	140	134
Potassium (meq/l)	4.9	5.0	5.9	5.2	5.4	4.2
Chloride (meq/l)	109	101	100	96	95	92

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIJ Calf No. 176

	Pre-inoculation		Post-inoculation		
Experimental day	-7	-2	1	2	3
Lactate dehydrogenase (IU/L)[a]	664	782	775	1069	1364
Serum glutamate oxaloacetate transaminase (IU/L)	20	24	39	146	69
Serum glutamate pyruvate transaminase (IU/L)	34	43	80	93	142
Alkaline phosphatase (IU/L)	43	45	38	35	33
Triglyceride (mg/dl)	59	53	64	54	77
Phosphorus (mg/dl)	7.0	7.0	6.0	5.3	4.6
Calcium (mg/dl)	10.3	10.3	9.1	8.5	8.8
Bilirubin (mg/dl)	.1	.3	1.1	5.2	2.3
Uric acid (mg/dl)	1.6	1.3	1.4	1.5	1.5
Total protein (mg/dl)	6.0	6.3	5.4	5.0	4.5
Albumin (mg/dl)	3.6	3.7	3.3	3.0	2.8
Cholesterol (mg/dl)	62	65	52	56	33
Blood urea nitrogen (mg/dl)	3	6	13	18	13
Glucose (mg/dl)	86	55	50	47	59
Sodium (meq/l)[b]	144	141	148	143	145
Potassium (meq/l)	4.1	4.5	4.0	3.0	3.0
Chloride (meq/l)	97	96	98	92	90

a. IU = International Units

b. meq = Milliequivalents

TABLE II. Continued

Appendix Table IIK Calf No. 171

	Pre-inoculation		Post-inoculation		
Experimental day	-4	-2	1	4	11
Lactate dehydrogenase (IU/L)[a]	460	454	830	494	732
Serum glutamate oxaloacetate transaminase (IU/L)	30	35	80	51	27
Serum glutamate pyruvate transaminase (IU/L)	21	16	37	32	21
Alkaline phosphatase (IU/L)	67	66	57	30	19
Triglyceride (mg/dl)	65	59	52	46	49
Phosphorus (mg/dl)	7.3	6.8	5.8	4.3	5.9
Calcium (mg/dl)	11.5	11.6	11.0	10.4	10.4
Bilirubin (mg/dl)	.2	.1	.1	.6	.4
Uric acid (mg/dl)	1.4	1.4	1.1	1.0	1.2
Total protein (mg/dl)	5.9	5.5	5.6	5.1	5.8
Albumin (mg/dl)	3.8	3.7	3.7	3.3	3.3
Cholesterol (mg/dl)	120	114	106	80	57
Blood urea nitrogen (mg/dl)	7	5	4	13	9
Glucose (mg/dl)	71	89	82	81	61
Sodium (meq/l)[b]	145	141	145	138	143
Potassium (meq/l)	5.6	5.9	5.3	5.4	5.9
Chloride (meq/l)	94	96	97	98	95

a. IU = International Units

b. meq = Milliequivalents

TABLE III Pre- and Post-inoculation Alpha 1-Antitrypsinase, Lysozyme and Angiotensin-Converting Enzyme Values for Each Calf

Appendix Table IIIA Calf No. 172

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-5	3.54	4.0	11.5
-2	3.64	4.0	11.3
0	3.44	4.2	14.0
Post-inoculation			
2	2.88	2.4	14.2
3	3.74	---	----
5	3.56	3.1	13.1
7	5.03	3.1	13.3
9	5.11	3.2	13.9
12	5.12	4.0	10.3
13	4.17	3.0	10.0

Appendix Table IIIB Calf No. 173

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-9	3.19	3.1	13.5
-7	3.33	3.19	14.0
-2	3.29	3.4	15.0
Post-inoculation			
1	4.23	3.0	10.2
3	5.51	2.6	12.1
5	5.28	2.0	11.8
7	5.45	2.1	9.6
9	5.65	2.4	10.0
11	5.5	2.48	8.8

TABLE III Continued

Appendix Table IIIC Calf No. 174

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-11	3.5	3.7	11.4
-8	3.3	4.0	11.2
-5	3.27	4.2	----
-1	3.27	3.4	11.1
Post-inoculation			
1	3.22	2.2	9.2
3	4.90	2.3	8.6
5	5.53	2.1	8.9
7	5.66	2.6	8.6
9	5.41	2.7	10.1
11	5.14	2.9	8.3
13	4.94	2.9	8.7

Appendix Table IIID Calf No. 82

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-6	3.0	3.2	12
-4	---	---	12
-2	2.99	3.6	13
-1	2.91	4.0	13.4
Post-inoculation			
1	4.01	2.6	13.5
2	4.37	3.0	13.6
3	---	2.2	----
4	5.09	2.2	12.9
5	5.66	2.2	----
6	5.74	1.8	11.0
7	---	1.8	9.35

TABLE III Continued

Appendix Table IIIE Calf No. 175

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-7	3.14	2.7	7.5
-5	3.17	3.16	7.6
-3	3.05	2.6	7.6
Post-inoculation			
1	5.2	2.0	6.2
3	5.85	2.4	7.6
5	5.9	2.6	11.5
7	5.95	2.7	6.5
9	5.89	3.0	7.0
11	5.95	3.02	7.6

Appendix Table IIIF Calf No. 171

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-7	3.08	3.4	11.2
-4	2.78	3.7	12.0
-2	3.19	3.6	12.1
Post-inoculation			
1	3.62	2.8	6.6
3	3.33	2.4	7.0
5	3.49	2.7	8.5
7	3.76	2.4	7.4
9	3.4	2.5	8.3
12	3.64	2.3	7.0

TABLE III Continued

Appendix Table IIIG Calf No. 176

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-7	2.12	2.7	13.0
-5	1.88	2.7	13.3
-2	1.80	2.6	13.0
Post-inoculation			
1	1.93	2.2	9.6
2	1.87	1.8	7.8
3	2.85	1.7	6.5

Appendix Table IIIH Calf No. 9

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-10	2.87	2.5	11.9
-6	2.55	2.6	11.1
-1	2.74	2.9	11.3
Post-inoculation			
1	3.53	2.2	9.75
2	3.75	2.3	8.8
4	4.37	2.1	8.9
6	4.47	1.9	8.0
9	4.87	2.2	7.6
10	4.33	2.2	8.7

TABLE III Continued

Appendix Table III J Calf No. 11

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-12	---	2.3	13.7
-10	2.68	2.2	----
-7	2.89	2.6	11.8
-5	2.86	2.5	12.3
-2	3.15	2.3	13.6
Post-inoculation			
1	3.81	2.0	11.9
3	5.47	1.6	8.1
5	6.06	2.7	8.0
7	4.81	2.7	8.9
9	4.6	2.5	9.75

Appendix Table III K Calf No. 170

Experimental Day	Alpha 1-Antitrypsinase (umol/min/ml)	Lysozyme (ug/ml)	Angiotensin-Converting Enzyme (L-Histidine-L-Leucine,nM)
Pre-inoculation			
-10	3.45	3.1	8.65
-7	3.87	2.9	8.0
-3	3.85	3.1	8.3
Post-inoculation			
1	3.31	2.9	9.1
3	3.31	3.3	11.0
5	4.0	3.4	12.0
7	2.94	3.8	10.4
9	2.9	2.9	9.0
11	2.73	3.1	8.2