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Pasteurella hemolytica : a bacteriological and seriological study of its role in bovine respiratory disease in market stressed feeder steers

Robert H. Wills

To the Graduate Council:

I am submitting herewith a thesis written by Robert H. Wills entitled "Pasteurella hemolytica : a bacteriological and seriological study of its role in bovine respiratory disease in market stressed feeder steers." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

J.B. McLaren, Major Professor

We have read this thesis and recommend its acceptance:

P.C. Smith, R. D. Walker, D.A. Bemis

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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We have read this thesis
and recommend its acceptance:

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PASTEURELLA HEMOLYTICA: A BACTERIOLOGICAL AND SERIOLOGICAL STUDY OF ITS ROLE IN BOVINE RESPIRATORY DISEASE IN MARKET STRESSED FEEDER STEERS

A Thesis

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Robert H. Wills

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ABSTRACT

The role of Pasteurella hemolytica in the bovine respiratory disease (BRD) complex was studied in two trials involving feeder steer calves. In Trial I, 54 calves were purchased from two Tennessee producers. The calves were followed through the market-transit chain which terminated at a feedlot at the Highland Rim Experiment Station (HRES) near Springfield, Tennessee. The calves were subjected to one of three infectious bovine rhinotracheitis (IBR) vaccination regimes, (1) injection with an IBR vaccine 30 days prior to delivery to the auction barn (AB), (2) injection with vaccine 30 days prior to delivery to the AB followed by a cell mediated immune booster upon arrival at the AB, and (3) nonvaccinated control group. On the day prior to departure from the orderbuyer barn (OBB) the calves were challenged by nasal inoculation with IBR virus. In Trial II, 48 calves purchased from auction barns were transported to a feedlot at the University of Tennessee Blount Farms at Knoxville, Tennessee.

Body weights of the calves in both trials were taken at various sampling dates. The weights were used to calculate gains as a measure of feedlot performance. Serum harvested from blood samples obtained at various points during the two trials were used to demonstrate the presence of and changes in antibody titers against Pasteurella hemolytica. An Enzyme-Linked Immunosorbant Assay (ELISA) technique was used to measure antibody titers in serum of calves from both trials. The presence of antibodies in the serum of the Blount Farm calves was also determined by an agglutination technique.

A scoring system involving the evaluation of various health parameters was used to monitor clinical signs of disease in the HRES calves. The number of days the calves were judged by the herdsman to require treatment for disease was used as a measure of disease severity in the Blount Farm calves.

Chi-square analysis of the frequencies of positive and negative antibody titers indicated that the ELISA and agglutination techniques gave comparable results when ELISA titers greater than or equal to 128 were considered positive and titers less than 128 were considered negative.

Pasteurella hemolytica was isolated from 2% of the HRES calves and 47% of the Blount Farm calves which were sampled. Pasteurella hemolytica antibody titers in the HRES calves remained low and consistent throughout the market-transit and feedlot phases. In the Blount Farm calves, a large increase in antibody titers occurred in sera taken upon arrival at the feedlot and sera obtained 38 or 43 days later. These results indicate that Pasteurella hemolytica infection occurred in the Blount Farm calves but not in the HRES calves.

Antibody titers of the second serum samples were positively correlated with the number of days sick and negatively correlated with the average daily gains of Blount Farm calves. This suggests that Pasteurella hemolytica infection had an adverse affect on feedlot performance.

Clinical signs of disease evident in the HRES calves suggested that the calves were infected with IBR virus. The nonvaccinated calves were more severely affected by the IBR infection than the vaccinated

calves. This was evident in greater degrees of respiratory distress, necrotic rhinitis, higher rectal temperatures and higher daily health indexes. No differences in degrees of mucopurulent nasal discharge and percent packed cell volumes were observed. Calves receiving the IBR vaccination followed by the cell mediated immune booster gained more weight from feedlot day 1 to feedlot day 10 than nonvaccinated calves. Weight gain differences due to IBR vaccination had disappeared by feedlot day 22.

In conclusion, the results indicate that Pasteurella hemolytica is an important etiological agent of the BRD complex in market stressed calves. Due to the apparent lack of Pasteurella hemolytica infection, a relationship between Pasteurella hemolytica and IBR virus in the BRD complex could not be demonstrated in the HRES calves.

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CHAPTER I

INTRODUCTION

The bovine respiratory disease (BRD) complex also known as shipping fever pneumonia is an acute respiratory infection of young cattle. This disease complex has been estimated to cost the cattle industry 500 million dollars a year (Flemming, 1975). BRD complex characteristically affects feeder-calves within 45 days after entering the market-transit chain which terminates in the feedlot (Jensen et al., 1976b). Economic losses result from death, excessive shrinkage, treatment expenses, decreased feed efficiency and marketing delays (Jensen et al., 1976b). Therefore, the disease results in immediate economic losses to the feedlot operator. The price of feeder-calves suspected to be susceptible to BRD are discounted by the feedlot operator and the loss of gain due to morbidity and mortality is passed onto the consumer.

The etiology of the BRD complex is comprised of several different agents. Carter (1973) outlined the principle organisms and other etiological agents associated with BRD; these included Pasteurella multocida, Pasteurella hemolytica, Corynebacterium pyogenes, Chlamydia, Mycoplasma species, infectious bovine rhinotracheitis (IBR) virus, parainfluenza-3 (PI-3) virus, bovine viral diarrhea (BVD) virus, reovirus, rhinovirus, adenovirus and environmental stresses. Collier (1968a), in reviewing the literature, concluded that Pasteurella hemolytica and Pasteurella multocida are very important, if not the most important, etiological agents of the disease. For this reason, one rather distinct clinical entity of the disease is often referred to by the more descriptive name of bovine pneumonic pasteurellosis.

Hoerlein and Marsh (1957) described shipping fever as a respiratory disease exhibiting clinical signs of depression, gauntness due to anorexia, high fever, either a dry nose or mucopurulent nasal exudate, soft cough and increased respiration rate.

Attempts to prevent the bovine respiratory disease complex by vaccination have met with varying degrees of success although evidence suggests that protective antibody titers are produced in calves when live Pasteurella species (Gilka et al., 1974, and Friend et al., 1977) or a viral component of the disease complex are used (Jericho et al., 1976; Stockdale et al., 1979a; and Stockdale et al., 1979b).

Although there have been numerous attempts to define the etiology of the bovine respiratory disease complex, the resulting evidence is inconclusive. The complex nature of the disease involving both microbiological and environmental factors makes it difficult to adequately reproduce field conditions in the laboratory. For the same reasons, it is difficult to adequately monitor all the important parameters under field conditions. McLaren (1978) purposed to study many of these parameters by a cooperative effort involving the University of Tennessee, Texas A&M, Oklahoma State University, the National Animal Disease Laboratory and other USDA-SEA research groups. The study reported herein is a part of that cooperative effort designed to identify as many nutritional, physiological, environmental and microbiological parameters as possible under field conditions in an effort to further define the etiology and epidemiology of the BRD complex. In support of this goal the objectives of this study were:

(1) To define in greater detail the role of Pasteurella hemolytica in the etiology and epidemiology of bovine respiratory disease complex.

(2) To study the relationship of Pasteurella hemolytica and infectious bovine rhinotracheitis virus with respect to bovine respiratory disease.

(3) To demonstrate the usefulness of enzyme linked immunosorbant assay (ELISA) as a serological technique used in the measurement of antibody titers against Pasteurella hemolytica.

(4) To compare the relative values of serological and isolation techniques as indicators of bovine pneumonic Pasteurellosis.

CHAPTER II

REVIEW OF LITERATURE

Bovine pneumonic pasteurellosis is an acute respiratory disease of young cattle. This disease is part of the bovine respiratory disease (BRD) complex termed shipping fever pneumonia and is of great economic importance to the cattle industry. Shipping fever pneumonia characteristically affects young cattle within 45 days after entering the market-transit chain which terminates in the feedlot (Jensen et al., 1976b). Economic importance arises from losses due to death, excessive shrinkage, treatment expense, decreased feed efficiency, and marketing delays (Jensen et al., 1976b). In one study in which 407,000 feedlot cattle were surveyed over a one-year period, it was reported that respiratory diseases were the most prevalent disease affecting the feedlot cattle (Jensen et al., 1976a).

The clinical signs characteristic of shipping fever pneumonia include depression, gauntness, high fever, dry muzzle, mucopurulent nasal discharge, a soft cough, and increased respiration rate (Hoerlein and Marsh, 1957).

A. Stress

Cattle are confronted with many severe forms of stress during transportation. These stresses include hunger, thirst, overcrowding, exposure to aberrant weather conditions, temperature extremes, fear, fatigue, and anxiety (King et al., 1958; McKercher, 1978; and Jensen et al., 1979). Stress is apparently an important etiological factor predisposing cattle to respiratory disease (Sinha and Abinanti, 1962; and McKercher,

1978). Sinha and Abinanti (1962) suggested that stress may participate in respiratory disease by influencing the development of viral infections. Phillip (1972) suggested that stress interferes with the ciliated and mucus secreting cells of the respiratory epithelium thereby interfering with the removal of pathogens from the respiratory tract.

B. Pasteurella

Pasteurellosis is a manifestation of a bacterial infection. The two species most often reported to be the causative agents of bovine pasteurellosis are Pasteurella hemolytica and Pasteurella multocida. In a bacteriological study of 33 cattle with shipping fever, Pasteurella hemolytica was isolated from the nasal secretions of 27 of the animals (Carter and McSherry, 1955). Two hundred calves were followed from the salebarn to the feedlot in another bacteriological study. Pasteurella species were isolated from only 3% of the nasal swabs taken from the calves at the salebarn. Pasteurella species were isolated from 59.6% of the nasal swabs of 52 calves with clinical shipping fever. Pasteurella species were isolated from the nasal secretions of 39.4% of the calves convalescent from shipping fever. Pasteurella species were isolated from 14% of the nasal swabs taken from calves displaying clinical shipping fever and receiving intensive antibiotic treatment. From these results the authors concluded that although it could not be shown that Pasteurella was totally responsible for the clinical signs of disease, control of bacteria is necessary for treatment of the disease (Hoerlein et al., 1961).

Researchers isolated Pasteurella hemolytica in pure culture from the pneumonic lungs of cattle in 12 of 13 outbreaks of shipping

fever and Pasteurella multocida in pure culture from the lungs of cattle of one outbreak (Collier et al., 1962). Bacteriological study of the nasal washings of the 13 outbreaks revealed exclusively Pasteurella hemolytica isolates in nine outbreaks, Pasteurella multocida in one outbreak and both species in three outbreaks (Collier et al., 1962). Pasteurella hemolytica and/or Pasteurella multocida were isolated from 48% of calves exhibiting shipping fever while the Pasteurella species were isolated from 20% of normal calves in a study conducted by Saunders et al. (1964). Pasteurella hemolytica was isolated more often from nasal swabs from calves with shipping fever than healthy calves in another study (Thomson et al., 1969). In this same study, (Thomson et al., 1969), found that Pasteurella hemolytica and Pasteurella multocida were isolated with nearly equal frequency but Pasteurella hemolytica was generally present in greater numbers. The high frequency of isolation as well as high numbers of organisms cultivated indicated that Pasteurella hemolytica is the more important bacterial agent in shipping fever.

Other work has shown that large numbers of Pasteurella hemolytica in the nasal passages is not necessarily associated with pneumonia. Pasteurella hemolytica was isolated from the nasal secretions of 11 out of 14 bulls suffering from pneumonia at a test station. In another herd surveyed, the same bacterium was isolated from the nasal secretions of 9 out of 12 animals, none of which exhibited clinical signs of pneumonia (Magwood et al., 1969). This would suggest that Pasteurella hemolytica may be endemic while not being pathogenic. It is important to note that the 43 bulls came from 28 herds and had been transported to a common facility while the survey herd had not. During transportation the bulls were probably stressed and exposed to other etiological agents.

Long term studies in which the nasal bacterial flora of six normal calves were monitored for 7 months indicated that Pasteurella hemolytica colonization of nasal passages was rhythmic in nature, in that periodic shedding of bacteria occurred (Magwood et al., 1969). Pasteurella hemolytica was frequently isolated for a period of a week or more followed by several weeks in which the bacterium was not isolated. Pasteurella multocida on the other hand was isolated from every calf during each week (Magwood et al., 1969). They suggested such a rhythmic shedding of bacteria indicates that colonization by Pasteurella hemolytica induces a protective host response which inhibits the active colonization of the nasal passages by Pasteurella hemolytica for a period of several weeks. If this is true, the host's immune status would be important in the etiology and epidemiology of shipping fever.

Serological studies have also produced evidence suggesting that Pasteurella species play a principle role in shipping fever. It was found in a Canadian study that complement-fixing antibody titers for Pasteurella hemolytica and Pasteurella multocida type C were higher in serum samples taken after shipment than those taken before. Since these two bacterial species were also isolated from the lungs of fatal cases of shipping fever, it was concluded that the serum titer increases were due to infection developed during shipment (Rice et al., 1955). An increase in hemagglutination titers had also been observed following the experimental induction of Pasteurella hemolytica infections (Carter 1956). However, in another study, groups of sick and well calves did not show consistent differences in Pasteurella hemolytica antibody titer determined by indirect hemagglutination. The authors concluded that these inconsistencies may have been due to the manner in which sick

animals were classified (Thomson et al., 1969). Nasal secretion antibodies against Pasteurella hemolytica have been demonstrated in naturally exposed calves (Thomson et al., 1969; and Duncan and Thomson, 1970b). Therefore, nasal antibodies may have a role in providing protection against Pasteurella hemolytica infection although Duncan and Thomson (1970a) have shown that Pasteurella hemolytica is able to colonize the nasal passages in the presence of nasal antibodies.

C. Viral Etiological Agents

The greater frequency of isolation of Pasteurella hemolytica and, to a lesser degree, Pasteurella multocida in calves with shipping fever compared to healthy calves and the increase in antibody titer to these bacteria following shipment indicated that pneumonic pasteurellosis is a principle component of the BRD complex. Although it is generally accepted that the terminal lesions of shipping fever or pneumonic pasteurellosis are due to infection by Pasteurella hemolytica and/or Pasteurella multocida (Thomson, 1974), there is extensive evidence in the literature supporting the contention that several other etiological agents are involved. Parainfluenza-3 (PI-3) virus has been associated with pneumonic pasteurellosis by both isolation and serological changes (Reisinger et al., 1959; Saunders et al., 1964; and Hamdy and Trapp, 1967). In a study conducted by Heddleston et al. (1962), it was concluded that pneumonic pasteurellosis was successfully transmitted to a group of healthy calves by introducing them to calves infected with Pasteurella hemolytica, Pasteurella multocida and PI-3 virus. However, antibody responses monitored in the study suggests that the Pasteurella species and not the virus were responsible for the disease

observed in these calves (Heddleston et al., 1962). This indicates that the PI-3 infection is not necessary for the establishment of the Pasteurella infection. Other research suggests that although an active PI-3 virus infection often occurs during or soon after shipment, it is not an obvious contributor to the disease (Thomson et al., 1969). In experiments attempting to induce shipping fever, PI-3 virus, Pasteurella hemolytica and Pasteurella multocida or stress singly did not produce shipping fever although Pasteurella species and PI-3 virus in combination did induce signs of the disease. Furthermore, the clinical signs and lesions associated with shipping fever were more evident when the calves were subjected to stress as well as the bacteria and virus (Hamdy et al., 1963).

Infectious bovine rhinotracheitis (IBR) virus can produce a respiratory disease distinct from pneumonic pasteurellosis (Collier et al., 1960). Pasteurella hemolytica was found to potentiate the effect of an IBR virus infection when calves were inoculated with bacteria 4 or 5 days after inoculation with IBR virus (Jericho and Langford, 1978). In another experiment, the severity of infection due to Pasteurella hemolytica was increased when bacterial inoculation was preceded 3 days earlier by IBR virus inoculation (Collier et al., 1960). However, they did not find that IBR virus greatly potentiated the pneumonic pasteurellosis. The potentiating effect of the IBR virus apparently depends on the time period separating IBR virus and Pasteurella hemolytica inoculation (Jericho and Langford, 1978).

In addition to IBR virus and PI-3 virus, Potgieter (1977) reviewed other viral agents associated with bovine respiratory disease, these viruses included bovine viral diarrhea virus, adendovirus, bovine

respiratory syncytial virus, bovine herpesvirus 5, bovine rhinovirus, bovine reoviruses, influenza virus and enteroviruses.

D. Vaccination

Since the etiology of the shipping fever complex is comprised of several different agents, the development of immunization procedures effective in the control of the disease has had varying degrees of success. Immunologic responses to viruses such as IBR virus, PI-3 and BVD virus have been induced by immunization procedures in order to provide protection against primary infection by these etiological agents (Kahrs, 1976). Another approach that has been studied is the use of Pasteurella multocida and Pasteurella hemolytica bacterins in a effort to prevent secondary infections by these bacteria (Collier, 1968b and Engelbrecht, 1968). A third approach has been the use of combination vaccines which provide protection against several of the etiological agents of shipping fever (Bittle and York, 1968 and Kahrs, 1976).

Pasteurella bacterins have not been successful in preventing shipping fever (Pierson, 1968 and Kahrs, 1976). Friend et al., (1977) found that calves vaccinated with killed Pasteurella hemolytica via subcutaneous and aerosol routes developed more severe pneumonia than that seen in nonvaccinated calves. Serological results in the same study, however, indicated that protective antibody titers may be produced in calves when live organisms are used to invoke an immune response. The serotypes of the Pasteurella hemolytica and Pasteurella multocida strains used to produce bacterins are important considerations to be made in the development of future vaccines (Frank, 1968).

After reviewing the literature, Gale (1968) noted that although killed PI-3 vaccines appear to be capable of providing protection against PI-3 virus infection, incomplete protection has also been observed with killed vaccines. Gale (1968) also suggested that attenuated live PI-3 vaccines have the advantages of more rapidly developing immunity and the production of secretory antibodies. He also stated that live vaccines have several inherent disadvantages including possible reversion to virulence, transmission of the disease to nonvaccinated animals and possible abortion in pregnant animals. Potgieter (1977) indicated that the live PI-3 vaccines may be safely used with pregnant animals.

In a review of the literature, Potgieter (1977) concluded that modified live IBR vaccines more effectively prevent IBR disease than do killed vaccines. He also pointed out that live vaccines may interfere with diagnosis of IBR viral infection because of the difficulty involved in descriminating between the vaccine and "wild" strains of the virus. Intranasal live IBR virus vaccines have been found in some cases to be more effective than parenteral live vaccines in providing protection against IBR disease (Potgieter, 1977). In addition to prevention of disease induced by the primary agent, IBR virus, research has also shown that the prevalence of disease caused by secondary infection with Pasteurella hemolytica may be somewhat reduced by vaccination with IBR virus prior to challenging with IBR virus and Pasteurella hemolytica (Jericho et al., 1976; Stockdale et al., 1979a; and Stockdale et al., 1979b).

Bovine viral diarrhea virus vaccines have been associated with the occurrence of post vaccinal mucosal disease (Fuller, 1965). This complication is apparently due to failure of the immune mechanism occurring in some animals after BVD vaccination (Peter et al., 1967).

CHAPTER III

EXPERIMENTAL PROCEDURE

A. General Procedures Used in the Overall Shipping Fever Studies

Various groups cooperating in a multi-region shipping fever research project conducted intensive surveys to determine the prevailing marketing procedures of the feeder-calf industry in the Southeast in preparation for a series of research studies including the two trials reported herein. They found that most Tennessee cow herds which produced feeder-calves were small with approximately 28 cows per herd. The producers seldom shipped their calves directly to the feedlot; instead, the calves were weaned and immediately transported to an auction barn. At the auction, orderbuyers purchased the calves which they combined with calves purchased from other farms. The calves usually remained at the auction barn for a time period of about 18-36 hr (mean = 24 hr) before assembly at the orderbuyer's barn. During their stay at the auction barn the calves were supplied with 0.65 to 0.95 m^2/steer of pen space but not water or hay. At the orderbuyer barn, calves purchased at various auction sales were combined until enough calves of a specific weight and grade were assembled for a specific feedlot order. The time period required to assemble a truckload (20,000 kg) of calves varied with the season. One to 4 days were required during the intensive marketing season (Fall) while it required up to 10 days during other seasons. Generally, calves spent a mean time of 72 hr in the orderbuyer barn where they were provided with hay, water and 1.42 to 2.80 m^2/steer of pen space.

A commercial marketing facility at Algood, Tennessee, which was used as a weekly auction market and an intermediate assembly point by several orderbuyers, was provided by the owners to be used for the overall shipping fever study. This facility was used for one of the trials in this study. In order to duplicate normal industry conditions as closely as possible, the means for times and pen space obtained from the surveys were used to determine pen space allotment and time spent by calves in the auction and orderbuyer barn.

B. Overall Procedures Used in Trial I

Feeder calves weighing 234 to 3.4 kg were purchased from two Tennessee producers. Thirty days prior to delivery to the auction barn, the calves at each of the farms were randomly assigned to treatment groups in a factorial arrangement as the calves entered the working chute to be weighed. The treatment groups included combinations of infectious bovine rhinotracheitis (IBR) vaccination and growth stimulant implantation[a]. The IBR virus vaccination treatment groups were: (1) injection 30 days prior to delivery to the auction barn with a commercial IBR vaccine[b], (2) injection with vaccine 30 days prior to delivery to the auction barn followed by a cell mediated immune booster upon arrival at the auction barn and (3) a non-vaccinated control group. The growth stimulant implantation groups were: (1) 30 days prior to delivery to the auction barn calves were implanted at the base of the ear with 36 mg of Ralgro, (2) calves were implanted with 36 mg of Ralgro at the farm of origin 30 days prior to transporting to the auction barn and reimplanted with 36 mg upon arrival at the

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[a] Ralgro® [b] TSV2, Intranasol, Nordon's

feedlot and (3) a non-implanted control group. On the second day at the orderbuyer barn, the calves were challenged by nasal inoculation with IBR virus. At the end of the auction and orderbuyer barn phase, the calves were transported 483 km to a feedlot at the Highland Rim Experiment Station (HRES) at Springfield, Tennessee.

From feedlot day 0 to feedlot day 140 the steers received corn silage ad libitum plus 1.82 kg cracked-shelled corn and 0.45 kg cotton-seed meal per steer per day. In addition grass-alfalfa hay was fed during the feedlot adaptation period. From feedlot day 140 to feedlot day 149 the corn silage in the diet was gradually decreased and the proportion of corn was increased until the diet consisted of whole-shelled corn ad libitum and 0.5 kg of a commercial protein supplement[a] designed to be fed with whole shelled corn.

The data collected included body weights, packed cell volume, rectal temperature and clinical evaluation. In addition serological, pathological, immunological, microbiological and rumen fluid samples were collected. Serum was used to determine antibody titers against Pasteurella hemolytica. Bacteriological samples (nasal swabs) were used to isolate Pasteurella hemolytica and Pasteurella multocida.

Data and samples were taken from the calves at the following points: (1) at the farm of origin 30 days prior to transport to the auction barn (FO), (2) upon arrival at the auction barn (AAB), (3) 24 hr after arrival at the auction barn or the beginning of the orderbuyer barn phase (AOBB), (4) departure from the orderbuyer barn (DOBB), (5) arrival at the feedlot (AFL) and (6) at various subsequent intervals at the feedlot (FL).

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[a]Tend-R-Leen[R]

C. Overall Procedure Used in Trial II

The second trial used 48 calves weighing from 223 ± 2.4 kg which were part of a larger group of cattle purchased at various auction sales and transported to a feedlot at the University of Tennessee Blount Farm at Knoxville, Tennessee. Serum samples were obtained soon after transporting the calves from the auction barns and 38 or 43 days after arrival at Blount Farm. These samples were used to determine antibody titers against *Pasteurella hemolytica*. Body weights and nasal swabs were taken at various sampling dates after arrival at the feedlot. The nasal swabs were used in isolation studies of *Pasteurella* species. The cattle were fed either corn silage or a concentrate ration described by Cole *et al.*, (1979) during their stay at the feedlot.

CHAPTER IV

A BACTERIOLOGICAL AND SEROLOGICAL STUDY OF PASTEURELLA HEMOLYTICA AND ITS ROLE IN BOVINE RESPIRATORY DISEASE IN MARKET STRESSED FEEDER STEERS

A. Summary

During the Fall of 1979, 48 feeder steer calves, weighing 223 $\pm$ 2.4 kg, were purchased at two auction sales and transported to a feedlot at the University of Tennessee Blount Farms at Knoxville, Tennessee. Body weights were taken upon arrival at the feedlot (AFL) and at various sampling dates after arrival. Nasal swabs used for isolation of Pasteurella species were taken soon after AFL. Serum samples were obtained soon after transportation and 38 or 43 days after arrival at the feedlot. Serum antibody titers against Pasteurella hemolytica were determined by an Enzyme-Linked Immunosorbant Assay (ELISA) technique and an agglutination method.

The frequencies of positive and negative titers determined by the two serological techniques were compared by Chi-square analysis to assess the homogeneity of results by the two techniques. When the ELISA titers equal to or greater than 128 were considered positive, the two techniques gave equivilent results. ELISA antibody titer increases between the paired sera indicated that infection of the calves by Pasteurella hemolytica occurred.

Pasteurella hemolytica was isolated in 47% of the calves which were sampled. The presence of the bacteria in the nasal passages of calves did not predispose them to disease. The mean preshipment antibody

titers of calves from which Pasteurella hemolytica was isolated were less than titers of calves from which Pasteurella hemolytica was not isolated. Mean postshipment antibody titers were not different.

Postshipment antibody titers were positively correlated with the number of days the calves were sick. ADG was negatively correlated with number of days sick. Postshipment Pasteurella hemolytica antibody titers were related to ADG. Calves with greater ADG from AFL to feedlot day 56 had lower postshipment antibody titers.

B. Introduction

The bovine respiratory disease (BRD) complex known as shipping fever pneumonia is an acute respiratory infection of feedlot cattle and is of great economic importance to the entire cattle industry. The etiology of the disease appears to be complex. Pasteurella hemolytica and/or Pasteurella multocida have been isolated more often from calves with the disease than from normal calves (Hoerlein et al., 1961; Saunders et al., 1964; and Thomson et al., 1969). Serum antibody titers against Pasteurella multocida type C and especially Pasteurella hemolytica were found to be higher in calves after shipment than before (Rice et al., 1955). However, in another study, sick and well calves did not consistently demonstrate significant differences in Pasteurella hemolytica antibody titer (Thomson et al., 1969).

Other etiological agents including parainfluenza-3 virus (Reisinger et al., 1959; Saunders et al., 1964; Hamdy et al., 1963; and Hamdy and Trapp, 1967), infectious bovine rhinotracheitis virus (Collier et al., 1960 and Jericho and Langford, 1978) and stress (Hamdy et al., 1963) have been found to contribute to the disease complex.

Although considerable effort has been expended in order to define the etiology and epidemiology of bovine respiratory disease complex, much of the resulting evidence has been inconclusive. The purposes of this study were to determine by isolation and serological methods the importance of Pasteurella hemolytica in the bovine respiratory disease complex and to compare the Enzyme-Linked Immunosorbant Assay (ELISA) and agglutination techniques as methods of measuring serum antibody titers against Pasteurella hemolytica.

C. Experimental Procedure

Forty-eight feeder calves weighing 223 $\pm$ 2.4 kg upon arrival at the auction barn were used. The calves were a part of a larger group of calves purchased at various auction sales, assembled and transported to a feedlot at the University of Tennessee Blount Farms at Knoxville, Tennessee. Thirty-six of the 48 calves were purchased at an auction barn in Knoxville, Tennessee. The remaining 12 calves were purchased the following day at an auction barn in Morristown, Tennessee. The calves purchased in Knoxville and Morristown were processed at the feedlot 1 and 5 days, respectively, after arrival at the feedlot. Processing included assigning to treatment groups and collecting data and samples as the calves were worked in a chute. The data collected included body weights and clinical evaluations. Blood samples from all and nasal swabs from 18 of the calves were taken. Thirty-eight days following the arrival of the Morristown calves, blood samples were taken from all of the calves. At this time 36 of the calves were weighed and placed on a feeding trial. Twenty-two of the 36 calves were fed corn silage and the remaining 14 animals were fed a concentrate ration described

by Cole et al., (1979). These calves were again weighed on feed trial days 28 (FT 28), 56 (FT 56) and 77 (FT 77).

Isolation and Identification of Pasteurella

At each sampling time two nasal swabs were taken from each calf by inserting a 6 inch cotton-tipped wooden applicator into the nostrils of the calf after thorough cleaning of the external nares. The swabs were then placed into a sterile plastic tube. Within 4 hr after sampling, the two swabs were used to streak Brain Heart Infusion agar[a] plates enriched with 5% bovine blood, 1% inactivated horse serum and 1% yeast extract. After overnight incubation at 37°C in a carbon dioxide (CO_2) candlejar, the plates were inspected for growth. Small to medium off-white colonies which were visually assessed as possible Pasteurella colonies were removed with an inoculating loop and restreaked on Trypticase Soy Agar[a] enriched with 5% bovine blood. The plates were again incubated at 37°C in a candlejar. The resulting colonies were observed for signs of hemolysis. Bacteria from the colonies were Gram stained and tested for oxidase activity[b]. Colonies of Gram negative rods which were oxidase postive were then used to inoculate Triple-sugar-iron agar[a] slants (TSI), Indole Nitrate Broth[a], and MacConkey agar[a]. A weak acid over acid (A/A) TSI reaction was indicative of Pasteurella species. The criteria used in determining positive identification of Pasteurella hemolytica and Pasteurella multocida are summarized in Table 1.

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[a]BBL, Becton Dickenson and Company

[b]Oxidase Test Reagent Droppers, Marion Scientific Corporation

Table 1. Criteria Used for the Identification of *Pasteurella* Species*

Species	Morphology	Gram Stain	TSI	Oxidase	Hemolysis	Growth on MacConkey	Indole	Nitrate
Pasteurella hemolytica	rod	-	A/A	+	+	+	-	+
Pasteurella multocida	coccibacillus	-	A/A	+	-	-	+	+

*Eisenberg and Cavanaugh (1974)

Serology

Serum antibody titers against Pasteurella hemolytica were determined by the Enzyme-Linked Immunosorbant Assay (ELISA) and by agglutination assay. The reference positive serum used in both the ELISA and agglutination assay was taken from a calf infected with Pasteurella hemolytica type 1 and stored at -20°C. Fetal calf serum (FCS) was used as the reference negative control serum in both techniques. The sera of calves in the experimental trial were harvested from blood samples obtained by venapuncture of the external jugular vein. The blood samples were collected in vacutainers, allowed to clot and centrifuged. The serum was then aliquoted and stored at -20°C. Sera from blood samples drawn during processing soon after arrival at the feedlot was designated as preshipment sera. Postshipment sera was harvested from blood samples drawn 43 days after the second group of calves were processed.

Antigen

The antigen used in both the ELISA and agglutination assays was prepared from Pasteurella hemolytica type 1. Bacteria used for the ELISA assays were grown on dextrose starch agar[b]. Antigen suspensions for the ELISA test were prepared by collecting the bacteria and suspending them in PBS with 0.05% Tween 20 (PBS/Tween). The final concentration of the bacterial cells was determined by visual comparison to a 0.5 M $BaSO_4$ standard. Bacteria used for the agglutination assay were grown on enriched blood agar. A very concentrated suspension of bacterial cells and PBS/Tween was prepared for the agglutination assay.

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[a]Vacutainer Serum Seperation Tube, B·D

[b]Gibco Diagnostics

ELISA

The ELISA technique was a modification of the method of Walker (personal communication). In order to sensitize polystyrene microtiter plates[a] 50 ul of antigen suspension were pipetted into each well of the plates. The plates were then placed in a 37°C incubator until all of the wells were dry, approximately 7 hr. The sensitized plates were then stored at -70 C until time for the test at which time they were thawed and washed with PBS/Tween. New, nonsensitized microtiter plates[b], were used to make 2-fold serial dilutions of the sera. One positive control, one negative control and 10 test sera samples were used per dilution plate. The first dilution was made by adding 50 ul of serum to 150 ul of PBS/Tween; subsequent dilutions were made by removing 100 ul of diluted serum from the previous well and adding to 100 ul of PBS/Tween in the succeeding well within a column of wells on the plate. After all dilutions had been made, the plates were incubated at 37°C for 30 minutes. Fifty ul from each well of the dilution plate were then transferred to a corresponding well in a sensitized plate. After incubation at 37°C for 30 minutes, the sensitized plates were rinsed once with PBS/Tween, emptied, refilled with PBS/Tween, and allowed to sit for 3 minutes. This wash procedure was repeated three times, the PBS/Tween was shaken out after each wash. Next, 25 ul of anti-bovine Ig G horseradish peroxidase conjugate[c] were added to each well in the plate and incubated for 30 minutes at 37°C. The proper conjugate concentration was previously

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[a]Microelisa[R] Plates, Dynatech Microelisa[R] Systems.

[b]MicroTest II[T.M.], Tissue Culture Plates, Falcon[T.M.]

[c]Miles-Yeda Ltd.

determined by a checkerboard titration of dilutions of positive control serum and dilution of the conjugate.

During the incubation with the conjugate, substrate for the conjugate was prepared. Sixteen mg of 5 aminosalycylic acid (5-ASCA) added to 20 ml of hot distilled H_2O was stirred, cooled and adjusted to pH 6.0 by addition of sodium hydroxide or hydrochloric acid. Two ml of hydrogen peroxide (H_2O_2) diluted to 0.05% in distilled H_2O were then added to the 5-ASCA to form the final substrate solution.

Following incubation with the conjugate, the plates were washed with PBS/Tween as described above. Fifty ul of the substrate solution were then added to each well and allowed to react for 12 minutes. The reaction was then stopped by adding 50 ul of sodium hydroxide per well. Fifteen minutes later the color intensity in each of the wells was measured as percent transmission (%T) by a Microelisa^R Reader[a] coupled to an Artek Compu-Print 700[b] and TI-59 calculator[c]. The Microelisa^R Reader instrument was calibrated to 99.9%T for each plate on the negative control dilution which had the lowest color intensity.

The endpoint titration was found by determining the highest dilution of an individual sample which had the %T value closest to 50 but less than 55. The reciprocal of this dilution value was designated as the antibody titer for that serum sample.

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[a]Dynatech Laboratories, Inc.

[b]Artek System Corporation

[c]Texas Instruments

Agglutination Assay

The agglutination assay used was the procedure of Walker (personal communication). A 50 ul drop of serum was placed on a clean glass plate. Ten ul of antigen suspension was then added to the serum and mixed with a wooden applicator stick. If clumping and clearing occurred within two minutes, the serum was designated positive. If the serum remained turbid, the reaction was considered to be negative.

D. Results and Discussion

Comparison of ELISA and Agglutination Techniques

Antibody titers against Pasteurella hemolytica ranging from 0 to 512 were determined by the ELISA technique. The agglutination technique was used to determine if antibodies against Pasteurella hemolytica were present or absent in undiluted serum. In order to compare the ELISA and agglutination technique, the ELISA titers were classified as positive or negative according to their magnitudes. Different minimum values were assessed to determine at which level the ELISA titers were positively correlated with the agglutination results (Table 2). The frequencies of positive and negative results as determined by the two techniques were compared by Chi-square analysis of means to to determine if the techniques produced equivalent results. When a minimum ELISA titer of 128 was considered positive and any titers less than 128 were classified as negative, the two techniques gave equivalent results (P .2). At minimum ELISA titer values less than 128, the ELISA technique gave more positives than did the agglutination technique. This indicates that the agglutination technique is less sensitive than the ELISA technique.

Table 2. Comparison of ELISA and Agglutination Techniques as Measures of Serum *Pasteurella hemolytica* Titers

ELISA Titer[a]	ELISA		Agglutination		X^2	Probability[b]
	-	+	-	+		
16	12	84	54	42	40.727	.0001
32	26	70	54	42	16.800	.0001
64	35	61	54	42	7.561	.0060
128	46	50	54	42	1.336	.2478
256	60	36	54	42	.777	.3780

[a]ELISA titers greater than or equal to this value are classified positive.

[b]Probability that a larger X^2 will occur due to chance.

ELISA Results

The mean preshipment and postshipment antibody titers against Pasteurella hemolytica as determined by the ELISA technique were 38 ± 6.5 and 320 ± 24.0 respectively. These results indicate that an immune response to a Pasteurella hemolytica infection occurred in most of the calves. The distribution of the calves considered to be serologically positive within each antibody titer level are given in Table 3. There was considerably more variation with respect to titer magnitude within the preshipment titers than in the postshipment titers as would be expected if the calves were infected with Pasteurella hemolytica during the time between the sample dates. Antibody titers against Pasteurella hemolytica were also higher in serum samples taken after shipment than those taken before in a study conducted by Rice et al., (1955). An increase in hemagglutination titers has also been observed following the experimental induction of Pasteurella hemolytica infections (Carter, 1956).

Isolation of Pasteurella hemolytica

Isolation of Pasteurella hemolytica was attempted from nineteen of the calves. In some cases Pasteurella multocida was isolated. In others, the specific Pasteurella species could not be determined. Calves exhibiting either of these isolation results were designated as positive for Pasteurella species. Calves from which Pasteurella hemolytica was isolated were designated positive for Pasteurella hemolytica.

Table 3. Frequency Distribution of Calves by *Pasteurella hemolytica* Antibody Titer Levels[a]

Titer Level	Frequency of Observation of Each Titer Level[b]	
	Preshipment	Postshipment
0	1	0
4	1	0
8	10	0
16	14	0
32	9	0
64	9	2
128	3	11
256	1	16
512	0	19

[a]Antibody titers against *Pasteurella hemolytica* were determined by the ELISA technique.

[b]Total of 48 observations.

Determination of Disease

The number of days the herdsman subjectively judged treatment for shipping fever to be necessary was used as a measure of the extent of sickness. Ninty-two percent of the calves exhibited signs of shipping fever. Forty-eight percent of the calves were considered sick for 5 to 10 days.

Pasteurella Isolation and Disease

The mean number of days sick, mean preshipment antibody titer and mean postshipment antibody titer against Pasteurella hemolytica are outlined in Table 4. No significant differences ($P<.05$) were demonstrated between the means of the number of days sick among the Pasteurella groups. However, calves from which Pasteurella hemolytica was isolated were sick 1.2 days or more than the other calves. The frequency of isolation of Pasteurella hemolytica and Pasteurella species are also given in Table 4. Although these results do not demonstrate a significant relationship between the isolation of Pasteurella hemolytica and the number of days sick, they do show that the organism was endemic and could be a possible pathogen. Pasteurella hemolytica and/or other Pasteurella species have been indicated as an etiological agent of BRD in several studies (Carter and McSherry, 1955; Hoerlein et al., 1961; Collier et al., 1962; Saunders et al., 1964; and Thomson et al., 1969).

Pasteurella Isolation and Antibody Titer

Preshipment titer. Calves from which Pasteurella hemolytica was not isolated had a significantly greater ($P<.05$) mean preshipment antibody titer against the organism than did calves from which Pasteurella hemolytica was isolated (Table 4). The mean antibody titer against

Table 4. Days Sick, Preshipment Antibody Titer[a], and Postshipment Antibody Titer[a] by Nasal Isolation Classification

Isolation Group	No. of Animals	Days Sick	Titer	
			Preshipment	Postshipment
Pasteurella hemolytica	9	4.7[b]	13.3[b]	320.0[b]
Pasteurella spp.	5	3.4[b]	20.8[b,c]	243.2[b]
No *Pasteurella* spp.	5	3.6[b]	36.8[c]	256.0[b]

[a]Antibody titers were determined by the ELISA technique.

[b,c]Means within the same column superscripted with different letters are significantly different ($P<.05$).

Pasteurella hemolytica of calves from which Pasteurella species were isolated was intermediate between the other two isolation groups (Table 4). This would indicate that Pasteurella hemolytica titers between 1:21 and 1:37 afford the animal some protection against colonization of the nasal passages by that bacteria.

Postshipment titers. Mean postshipment antibody titers were not different ($P<.05$). This suggests that although preshipment titers may have partially protected calves from infections of Pasteurella hemolytica which were demonstratable by sampling with nasal swabs, they did not prevent infections which evoked an antigenic response. The reason for this may be the degree of infection. A serious or widespread infection of the nasal passages might be required in order for Pasteurella hemolytica to be isolated while a milder infection might still produce an antigenic response measurable by the ELISA technique even though the bacteria was not isolated.

Antibody Titer and Extent of Disease

The number of days sick was negatively correlated ($P<.2$) with preshipment antibody titer (Figure 1). Although a great deal of confidence cannot be placed on this relationship, it does support the contention that Pasteurella hemolytica is responsible in part for BRD and that higher preshipment antibody titers against Pasteurella hemolytica may be one of the mechanisms that protect the animal from disease. Postshipment antibody titers were found to increase as the number of days sick increased ($P<.05$) (Figure 1). A possible explanation of this is that calves which were sick for several days were suffering from pasteurellosis which resulted in the production of elevated postshipment antibody titers against Pasteurella hemolytica.

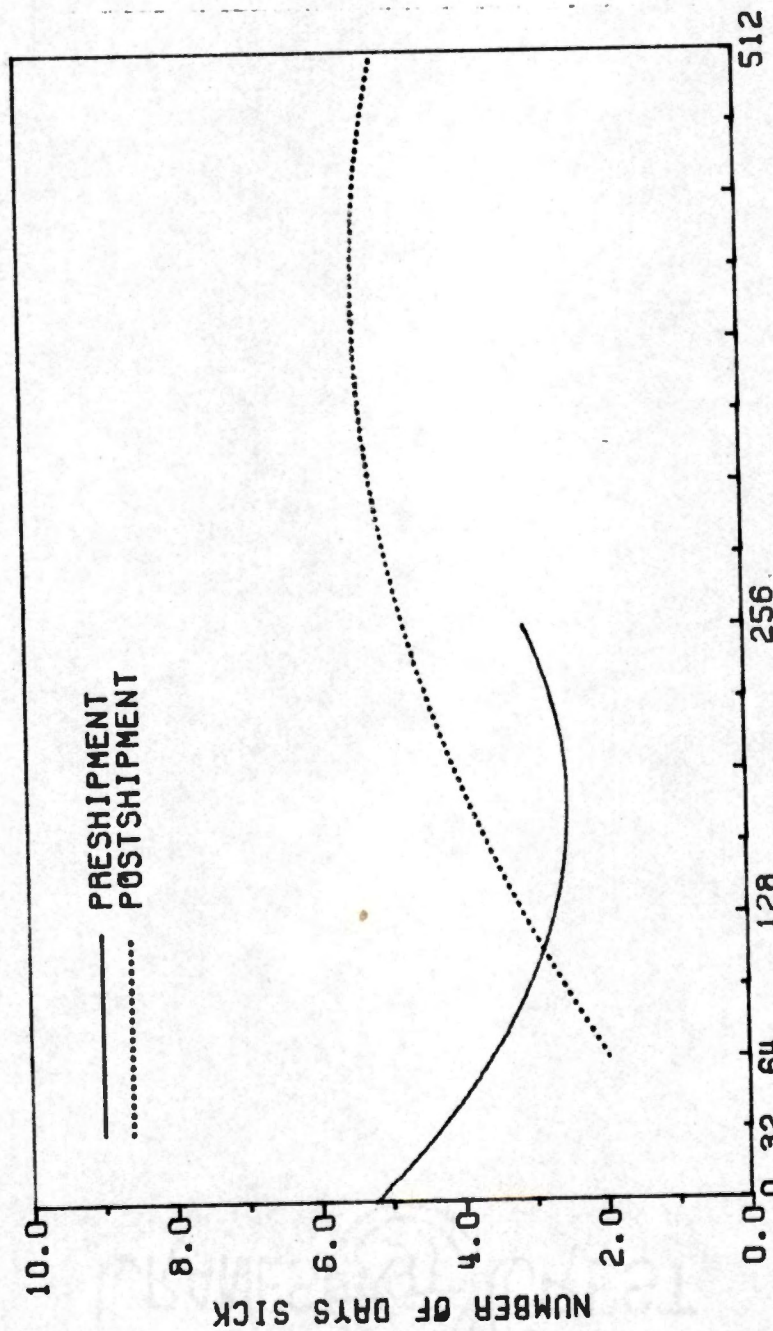

Figure 1. Relationship of pre- and post-shipment Pasteurella hemolytica serum titers to number of days animals were sick.

Calves which had a preshipment antibody titer against Pasteurella hemolytica greater than or equal to 128 were sick 3.1 days less ($P<.05$) than calves with titers less than 128. A preshipment antibody titer of 128 or greater may have given the calves some immunity to Pasteurella hemolytica infection and thereby decreased the severity of illness. Lack of complete immunity against Pasteurella hemolytica, other pathogens or the fact that some calves were sick when the preshipment serum samples were taken would probably explain the clinical signs of disease which were seen. Conversely, calves with postshipment antibody titers of at least 128 were sick 3.0 days more ($P<.05$) than calves with titers less than 128. Although a cause and effect relationship between Pasteurella hemolytica infection and days sick cannot be proven from these results, such a relationship would partially explain them. In other words, a severe infection of Pasteurella hemolytica would induce high antibody titers; concurrently, animals with severe infection would be expected to be sick more days. Therefore, calves which had been sick more would also be expected to have elevated antibody titers against Pasteurella hemolytica.

Average Daily Gains

Weight gains were converted to average daily gains (ADG) to remove variation in dates of purchase and other variation. Daily gains were then used as a measure of feedlot performance. For the purposes of this study, the day which the calves were processed will be considered the date of arrival at the feedlot (AFL). ADG were also calculated from weights taken from the 36 calves which were placed on the feeding trial. The calves were weighed on the first day of the trial (FT 0) and FT 28, FT 56 and Ft 77.

ADG and Disease

Average daily gains from AFL to FT 77 were negatively correlated ($P<.001$) with the number of days the calves were sick (Table 5). No relationships ($P<.1$) were shown to exist between ADG and number of days sick for the time periods of FT 28 to FT 56 and FT 56 to FT 77 probably because the calves had recovered from the shipping fever by this time (Table 5). The negative correlations ($P<.05$) between ADG and days sick for the time periods of AFL to FT 0 and FT 0 to FT 28 (Table 5) were probably due to a detrimental effect the shipping fever had on the calves resulting in lower ADG. This detrimental effect apparently influenced accumulative weight gains until FT 77.

ADG and Antibody Titer

The relationship of ADG which preshipment and postshipment antibody titers against Pasteurella hemolytica was also considered. Preshipment antibody titer was not apparently ($P<.1$) related to ADG. Although preshipment titer was found to be inversely related to mean days sick, the protective immune status which might be inferred from that relationship was not expressed as increased ADG. High preshipment antibody titers did not appear to have any beneficial affect on feedlot performance.

Postshipment Pasteurella hemolytica antibody titers were related to ADG. Calves with greater ADG for the time period of AFL to FL 56 had lower postshipment titers ($P<.05$). Once again this might be explained by the contention that higher postshipment antibody titers are the result of more severe infections of Pasteurella hemolytica which resulted in more severe disease.

Table 5. Correlation of ADG with Number of Days Sick

	Mean ADG (kg)	Correlation Coefficients
AFL to FT 0	0.8	-0.43421[a]
AFL to FT 28	0.9	-0.58554[a]
AFL to FT 56	0.9	-0.49117[a]
AFL to FT 77	0.9	-0.44207[a]
FT 0 to FT 28	1.0	-0.36095[a]
FT 28 to FT 56	1.0	-0.07188
FT 56 to FT 77	0.7	0.15081

[a]Correlations are significant ($P<.05$).

The results of this study indicate that the ELISA technique used to determine antibody titers against Pasteurella hemolytica is a useful serological technique. Due to its ability to measure antibody at higher dilutions, it is a more sensitive test than the agglutination technique employed in this study. Due to its greater sensitivity, the ELISA technique has the potential to more accurately assess the immune status of calves to Pasteurella hemolytica.

Isolation of Pasteurella hemolytica from the nasal passages of feeder calves showed that the organism was present in the population. However, the presence of Pasteurella hemolytica in the nasal passages was not shown to predispose the calves to shipping fever. Preshipment Pasteurella hemolytica antibody titers greater than or equal to 128, as determined by the ELISA technique, appeared to give calves some protection against infection. This was made evident by the lower frequency of isolation of Pasteurella hemolytica and by the lower mean days sick seen in calves with preshipment titers of at least 128. Greater postshipment antibody titers against Pasteurella hemolytica following more severe illness suggests that Pasteurella hemolytica is an important etiological agent in the bovine respiratory disease complex.

CHAPTER V

A BACTERIOLOGICAL AND SEROLOGICAL STUDY OF PASTEURELLA HEMOLYTICA AND ITS RELATIONSHIP WITH INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN THE BOVINE RESPIRATORY DISEASE COMPLEX

A. Summary

Fifty-four feeder steer calves weighing 234 $\pm$ 3.4 kg upon arrival at the auction barn were purchased from two Tennessee producers. The calves were subjected to one of the three following infectious bovine rhinotracheitis (IBR) virus vaccination treatment groups: (1) injection with an IBR vaccine 30 days prior to delivery to the auction barn (AB), (2) injection with vaccine 30 days prior to delivery to the AB followed by a cell mediated immune booster upon arrival at the AB, and (3) nonvaccinated control group. On the day prior to departure from the orderbuyer barn (OBB) the calves were challenged by intranasal inoculation with virulent IBR virus. The calves were transported 483 km to a feedlot at the Highland Rim Experiment Station.

Body weights and packed cell volumes were determined at various sampling times during the market-transit and feedlot phases. The animals were clinically evaluated daily for different health parameters which were used to develop a daily health index. Nasal swabs used for the isolation of Pasteurella hemolytica and Pasteurella multocida were collected during the market-transit and feedlot phases. Serum harvested from blood samples collected at several points during the experiment were analyzed for antibody titers against Pasteurella hemolytica by an ELISA technique.

Nonvaccinated calves were found to be more severely affected by the IBR infection than the vaccinated calves. This was evident in greater degrees of respiratory distress, necrotic rhinitis, higher rectal temperatures and higher daily health indexes. No difference in degree of mucopurulent nasal discharge and percent packed cell volumes were observed. Calves receiving IBR vaccination and the cell mediated immune booster gained more weight from feedlot (FL) day 1 to FL day 10 than nonvaccinated calves. Weight gain differences due to IBR vaccination treatment had disappeared by FL day 22.

Pasteurella hemolytica and Pasteurella multocida were isolated from one calf each. Pasteurella hemolytica antibody titers did not change over time nor were any differences in titer demonstrated between IBR treatment groups. Infection of the calves by Pasteurella hemolytica did not apparently occur. Therefore no relationship between it and the IBR virus could be demonstrated.

B. Introduction

The bovine respiratory disease (BRD) complex known as shipping fever pneumonia is an acute respiratory infection of feedlot cattle and is of great economic importance to the entire cattle industry. The etiology of the disease appears to be complex. Pasteurella hemolytica and/or Pasteurella multocida have been isolated more often from calves with the disease than from normal calves (Hoerlein et al., 1961; Saunders et al., 1964; and Thomson et al., 1969). Serum antibody titers against Pasteurella hemolytica, especially, and Pasteurella multocida type C were found to be higher in calves after shipment than before shipment (Rice et al., 1955). However, in another study, sick and well calves

did not consistently demonstrate significant differences in Pasteurella hemolytica antibody titers (Thomson et al., 1969).

Other etiological agents including infectious bovine rhinotracheitis (IBR) virus (Collier et al., 1960 and Jericho and Langford, 1978), parainfluenza-3 (PI-3) virus (Reisinger et al., 1959; Saunders et al., 1964; Hamdy et al., 1963; and Hamdy and Trapp, 1967) and stress (Hamdy et al., 1963) have been found to contribute to the disease complex.

Although considerable effort has been expended in order to define the etiology and epidemiology of BRD complex, much of the resulting evidence has been inconclusive. The purposes of this study were to determine by isolation and serological methods the importance of Pasteurella hemolytica in the BRD complex and to study its relationship with IBR virus in the BRD complex.

C. Experimental Procedure

Fifty-four Angus or Hereford feeder calves weighing 234 $\pm$ 3.4 kg upon arrival at the auction barn were purchased from two Tennessee producers. Thirty days prior to shipment to the auction barn, the calves were randomly assigned to treatment groups as they entered the working chute to be weighed. The treatment groups consisted of various combinations of infectious bovine rhinotracheitis (IBR) modified live intranasal vaccination and growth stimulant implantation in a factorial arrangement. The IBR virus vaccination treatment consisted of 3 levels: (1) nonvaccinated control group (Group I), (2) injection with an IBR vaccine 30 days prior to delivery to the auction barn (Group II), (3) injection with vaccine 30 days prior to delivery to the auction barn followed by an intradermal cell mediated immune booster upon arrival at the

auction barn (Group III). There were also three growth stimulant implantation groups: (1) 30 days prior to delivery to the auction barn calves were implanted at the base of the ear with 36 mg of Ralgro, (2) calves were implanted with 36 mg of Ralgro at the farm of origin 30 days prior to shipment to the auction barn and were reimplanted with 36 mg upon arrival at the feedlot and (3) a non-implanted control group. On the day prior to departure from the orderbuyer barn the calves were challenged by nasal inoculation with virulent IBR virus[a]. The calves were transported 80 to 120 km from the farm of origin (FO) to a commercial auction barn (AB) at Algood, Tennessee where they remained for the auction barn and orderbuyer phase. While in the auction barn phase the calves were provided 0.65 to 0.95 m^2/steer of pen space but no water or feed for 18 hr. During the orderbuyer barn (OBB) phase the pen space was increased to 1.42 to 2.80 m^2/steer. Hay and water were also provided during this phase for 72 hr. The calves were then shipped 483 km by truck to a feedlot facility at the Highland Rim Experiment Station (HRES) at Springfield, Tennessee. Here the calves were allotted to pens and introduced to a corn silage, cracked-shelled corn and hay diet. The calves remained here until slaughter.

Data Collection

Body weights were taken at the farm of origin (FO), arrival at the AB, arrival at the OBB, departure from OBB and feedlot (FL) days 1, 3, 10, 22, 37, 87, 149 and 240. Packed cell volumes were determined[b]

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[a]Challenge Strain (Colorado Strain), National Animal Disease Center, 1 x $10^{7.5}$ PFU/ml

[b]Winthrobe Macro Hematicrit Tubes, Fischerbrand

on arrival AB, arrival OBB, departure OBB, arrival FL and FL day 3, 10, and 37.

Clinical Evaluations

The calves were clinically evaluated daily before feeding from arrival at the AB to FL day 9. A scoring system involving several different health parameters was used in an attempt to derive an objective health index. Each health parameter was scored from 0-4 with a score of 4 representing the most severe condition. Rectal temperatures were taken at the FO, arrival AB, departure OBB, arrival FL and FL days 2, 4, 6 and 9. The different health parameters evaluated are outlined in Table 6. Accumulative health scores were determined by adding individual values of the different health parameters. This cumulative score was then used as a daily health index.

Sample Collection

Pasteurella isolation. Nasal swabs used for the isolation of *Pasteurella hemolytica* and *Pasteurella multocida* were taken upon arrival AB (AAB), departure OBB (DOBB), arrival FL (AFL) and FL days 2, 4, 6 and 9. The procedure used for the isolation of the two bacterial species was described in Chapter IV, page 19.

Serology. Serum was harvested from blood samples obtained by vein puncture of the external jugular vein. The blood samples were collected in tubes[a] and centrifuged. The serum was then aliquoted and stored at -20°C. The blood samples were obtained at the FO, AAB, DOBB, FL 37 and 149. Antibody titers against *Pasteurella hemolytica* were determined by the ELISA technique procedure described in Chapter IV, page 22.

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[a]Serum Separation Tubes, Vaccutainer.

Table 6. Outline of the Parameters Used to Evaluate Clinical Signs of Disease

I. Respiration
   A. Frequency
   B. Depth
   C. Labored
   D. Coughing

II. Attitude
   A. Anorexia
   B. Depression

III. Eyes
   A. Photophobia
   B. Lacrimation
   C. Purulent discharge
   D. Conjunctivitis
   E. Keratitis

IV. Nasal Passages
   A. Dryness
   B. Mucopurulent discharge
   C. Rhinitis
      1. Redness
      2. Papular
      3. Necrotic

V. Locomotion
   A. Antimation
   B. Weaving
   C. Wandering
   D. Circling

VI. Other
   A. Fill
   B. Diarrhea
      1. Blood
      2. Mucus
      3. Volume
      4. Frequency

VII. Temperature Scoring Classification
   A. 39.4 to 39.9 = 1
   B. 40.0 to 40.5 = 2
   C. 40.6 to 41.0 = 3
   D. $\geq$ 41.0 = 4

D. Results and Discussion

Clinical Evaluation

Although 18 of the 54 calves were treated for respiratory disease, for the most part, the cases of respiratory disease observed were mild. The daily health index was used to determine which calves were to be treated. If the health index was equal to or greater than 10, treatment with antibiotics was initiated.

Health index. From FL 2 to the end of the tests, the IBR treatment control calves had health indexes greater ($P<.05$) than those of the calves in the other two groups (Figure 2). The mean of the health index of the control calves was greatest on FL 3 and then began to decline on subsequent days. IBR treatment Groups II and III had equivalent health indexes ($P<.05$) throughout the test.

Respiratory index. The respiratory parameter scores for frequency of respiration, depth of respiration, labored respiration and coughing were combined to form a respiratory index. The relatively low values indicated in Figure 3 are consistent with the earlier observation of only mild cases of respiratory disease. The IBR control calves had mean respiratory indexes greater ($P<.01$) than Group II calves at AAB, FL 1 and FL 6. The mean respiratory index of Group III was intermediate to the other two groups on these days. On FL 7 and FL 9, the control calves had greater ($P<.05$) respiratory indexes than either of the other groups. The mean respiratory index of Group II was intermediate to Group I and III with Group I having the greatest mean ($P<.05$). Although the mean respiratory indexes of Groups II and III fluctuated, the mean of Group I was consistently greater than or equal to the other groups thus indicating that the control calves were more severely affected.

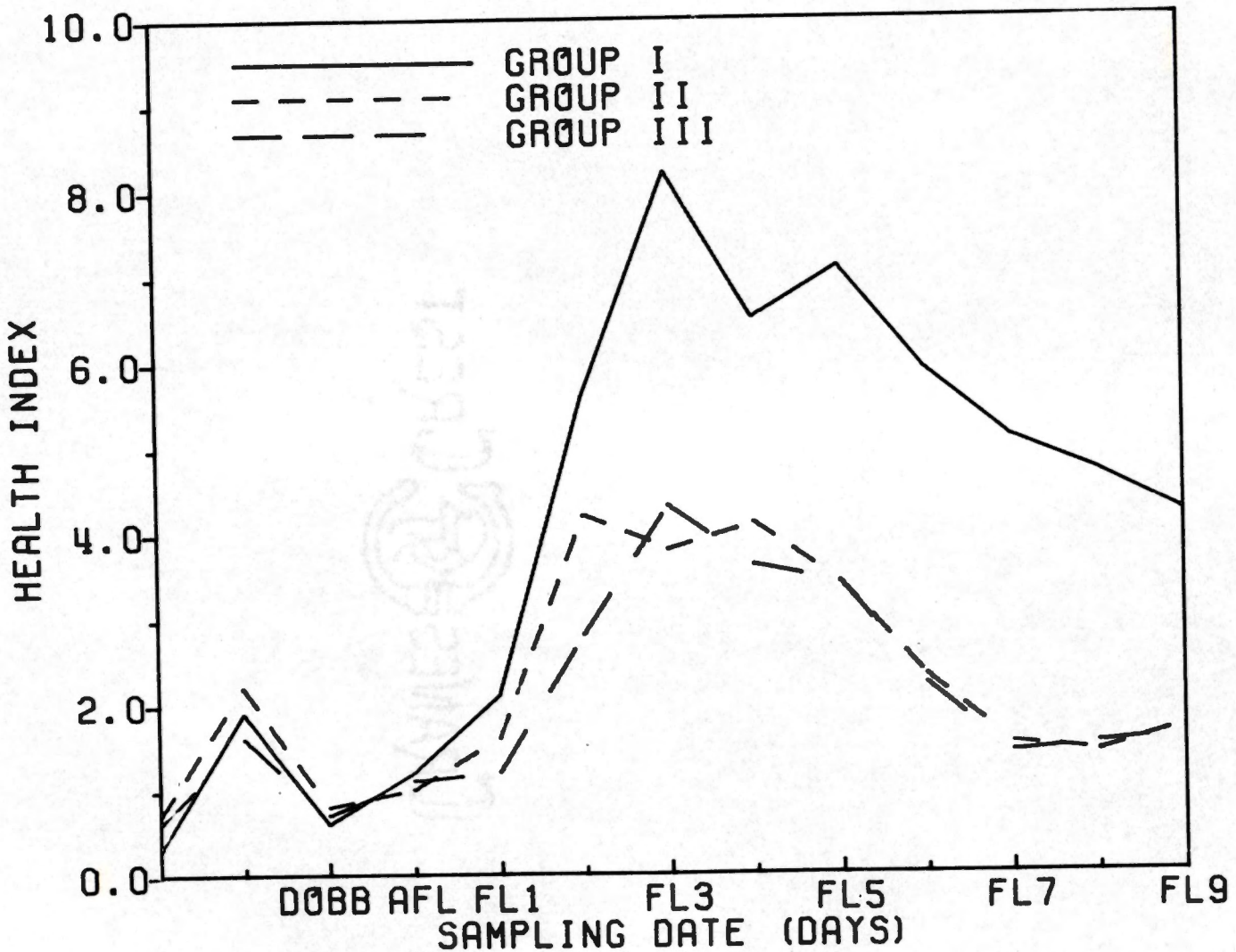

Figure 2. Effect of IBR vaccination on health index during the market, transit and feedlot phases.

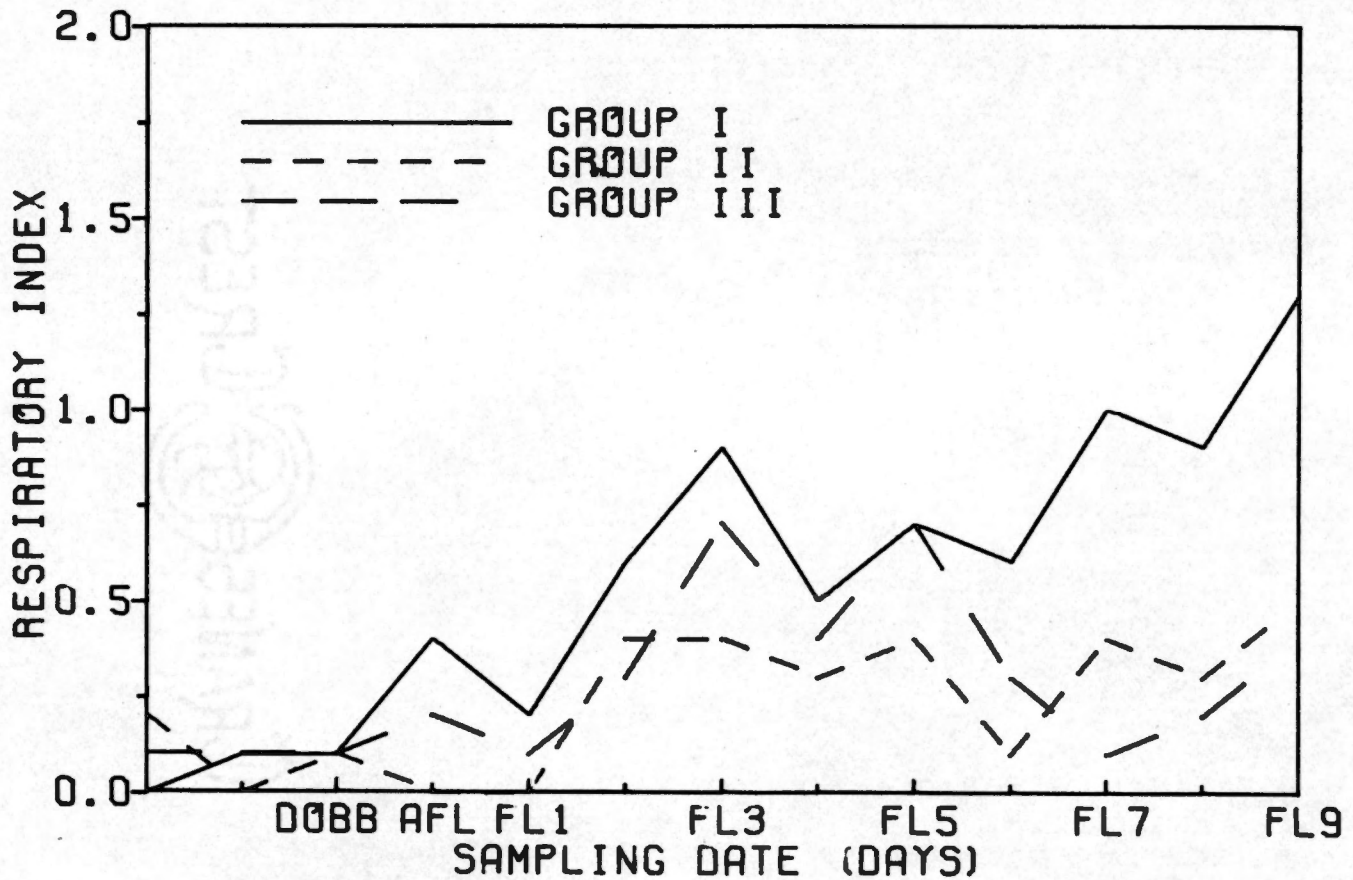

Figure 3. Effect of IBR vaccination on respiratory index during market, transit and feedlot phases.

Necrotic rhinitis. Control calves exhibited more severe necrotic rhinitis than calves in the other groups ($P<.05$) from FL 3 to FL 9. The mean necrotic rhinitis reached a maximum point on FL 3 and 6 in the control calves (Figure 4). The means of the other two groups were not different ($P<.05$). Groups II and III had maximum values on FL 4 (Figure 4). The mean necrotic rhinitis for both groups decreased rapidly from this point.

Mucopurulent exudate. No differences in the severity of mucopurulent discharge were seen among the three IBR treatment groups ($P<.05$). The mean score for all three groups increased until about FL day 5 and then began to drop.

Percent Packed Cell Volume. Differences in mean percent packed volume were not observed among the three groups ($P<.05$). A maximum percent packed cell volume was reached in all groups on the day of arrival at the feedlot. The observed changes were probably due to dehydration incurred during the auction barn, orderbuyer barn and transit phases.

Rectal temperatures. The mean rectal temperatures of Groups II and III were consistently lower than those of Group I from FL 2 to FL 6 ($P<.05$) (Figure 5). A maximum mean temperature of 40.4^{o}C was reached in the control group on FL 3 (Figure 5). The mean rectal temperature of Groups II and III were not different ($P<.05$) throughout the experiment.

Feedlot Performance. Weight gains over time were calculated from body weights. The calves in Group III, gained more ($P<.05$) from FL 1 to FL 10 than did calves in Group I (Figure 6). The weight gains in Group II were intermediate between the other two groups during this time period. Differences in weight gains were not significant ($P<.05$) after FL 10 (Figure 6).

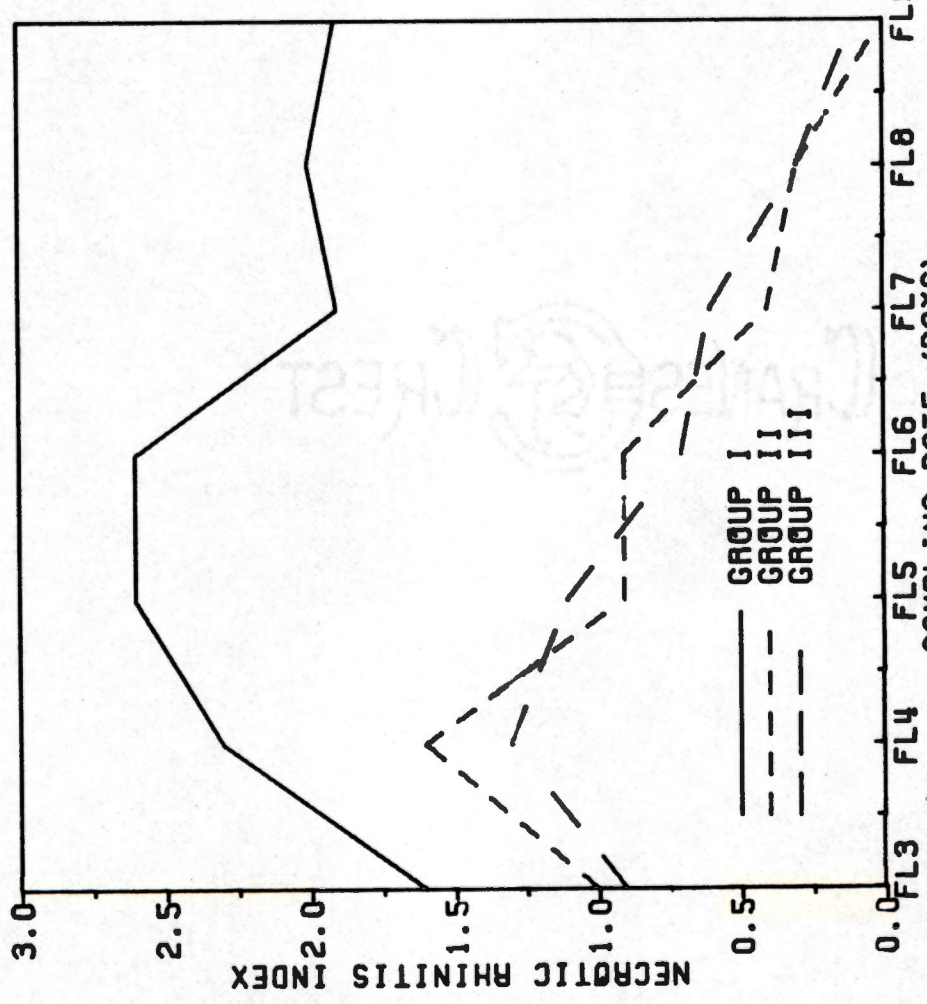

Figure 4. Effect of IBR vaccination on necrotic rhinitis during the feedlot phases.

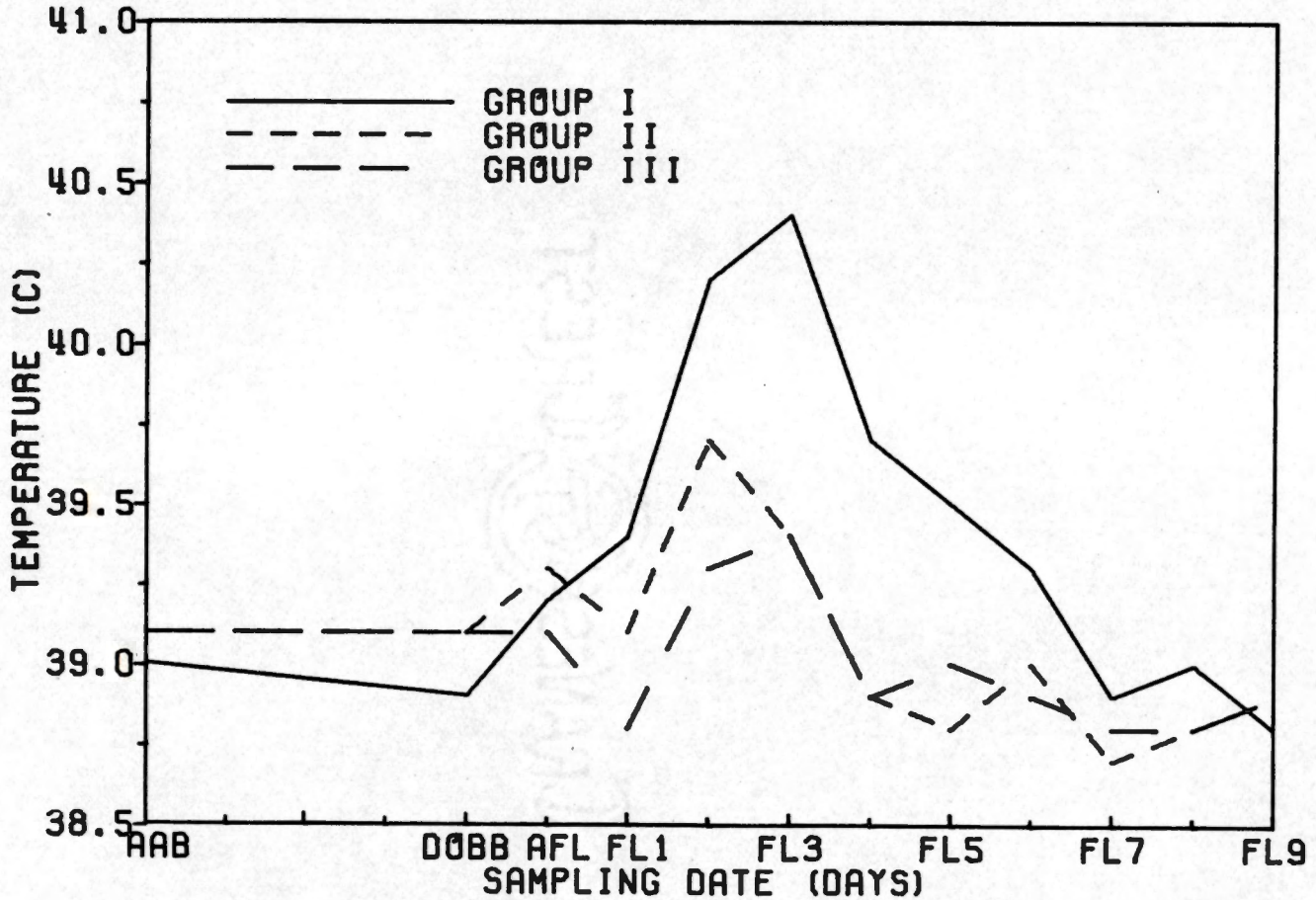

Figure 5. Effect of IBR vaccination on rectal temperature during the market, transit and feedlot phases.

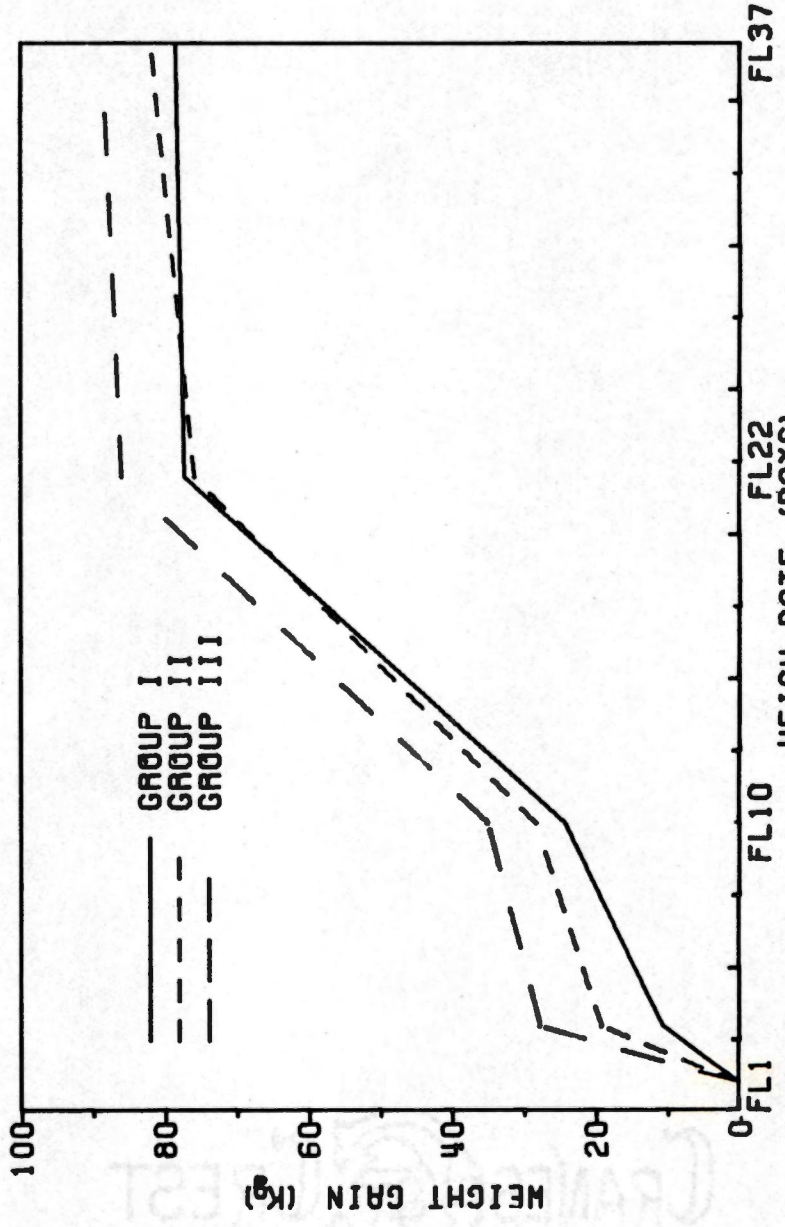

Figure 6. Effect of IBR vaccination on cumulative weight gains at the feedlot.

Pasteurella Isolation Study.

Pasteurella hemolytica was isolated from the nasal passages of only one calf during the experimental period. Only one calf was found to harbour Pasteurella multocida in its nasal passages. The low incidence of isolation of Pasteurella is consistent with the work of Hoerlein et al., (1961) in which Pasteurella was isolated from 3% of 200 calves in the salebarn. As these calves progressed through the market-transit system and contracted shipping fever, the number of calves harbouring Pasteurella increased (Hoerlein et al., 1961). However, in the present study Pasteurella hemolytica and Pasteurella multocida were isolated in one calf each only one time indicating that widespread infection by Pasteurella did not occur.

ELISA Antibody Titers Against Pasteurella Hemolytica

The mean antibody titers against Pasteurella hemolytica for the three treatment groups and the overall mean of all the calves at FO, AAB, DOBB, FL 37 and FL 149 are presented in Table 7. No differences in titer level among the three IBR treatment groups were observed ($P<.05$) nor were significant changes ($P<.05$) in antibody titer over time seen. Carter (1956) demonstrated that an increase in hemagglutination titers occurred following the experimental induction of Pasteurella hemolytica infections. The ELISA technique was successfully used to demonstrate Pasteurella hemolytica antibody titers in the trial discussed in Chapter IV. Burnells et al., (1978) following a different procedure, were able to quantitate antibody titers against Pasteurella hemolytica in ovine sera with the ELISA technique. The calves in this trial did not have serum antibody titer changes indicating infections of Pasteurella hemolytica did not occur. These serological findings correlate with the negative results observed in the isolation study.

Table 7. Serological Response to Pasteurella hemolytica (Trial 1)

Sampling Date	IBR Treatment Group Means			Overall Mean
	I	II	III	
FO	26.0 ± 4.76[a]	25.1 ± 4.77	13.1 ± 2.52	21.4 ± 2.49
AAB	18.2 ± 3.80	28.7 ± 6.83	18.7 ± 3.81	21.8 ± 2.92
DOBB	23.8 ± 4.30	28.4 ± 4.53	20.7 ± 4.30	24.3 ± 2.52
FL day 37	29.1 ± 5.03	24.0 ± 4.28	22.7 ± 3.30	25.2 ± 2.44
FL day 149	25.5 ± 7.87	27.1 ± 4.08	20.2 ± 3.30	24.2 ± 3.02

[a] Reciprocal of endpoint dilution as determined by ELISA technique.

[b] Means are based on 54 animals.

IBR virus alone can produce a clinical respiratory disease which is distinct from the classical signs of shipping fever (Collier, 1960). The results from the present study indicate that the calves were infected by IBR virus but did not suffer from the more severe shipping fever which usually involves other etiological agents. The marked differences between the nonvaccinated control calves and the other two groups indicate that the clinical signs observed were due to IBR infection. The elevated temperatures, evidence of necrotic rhinitis and respiratory distress are characteristic of IBR infection. The IBR vaccine apparently protected the calves in Groups II and III from severe infection. The clinical signs of disease which were evident in these two groups could possibly be due to mild cases of IBR. However, other etiological agents might also have been responsible for the mild disease observed in the calves. It is easier to determine what pathogens did not play a role in the disease.

The bacteriological and serological studies conclusively demonstrate that Pasteurella hemolytica was not responsible. Pasteurella hemolytica has been shown to potentiate the effect of IBR infection (Jericho and Langford, 1978), conversely, IBR virus has been shown to increase the seriousness of infection by Pasteurella hemolytica (Collier et al., 1960). Why an interaction between the bacteria and virus was not evident in this study is open to speculation. One reason might be that Pasteurella hemolytica simply was not present in great enough numbers to initiate severe and widespread infection. Another alternative is that the calves were not severely stressed during transit and were able to combat infection by Pasteurella hemolytica and/or IBR virus. Stress is thought to predispose cattle to respiratory disease (Sinha and Abinanti, 1962; and

McKercher, 1978). Sinha and Abinanti (1962) suggested that stress may participate in respiratory disease by influencing the development of viral infections. With this in mind, the failure of the calves to contract severe cases of shipping fever may have been due to the absence of either Pasteurella hemolytica, stress or other etiological agents.

CHAPTER VI

SUMMARY

The role of Pasteurella hemolytica in the bovine respiratory disease (BRD) complex was studied in two trials involving feeder steer calves. In Trial 1, 54 calves were purchased from two Tennessee producers. The calves were followed through the market-transit chain which terminated at the feedlot at the Highland Rim Experiment Station (HRES) near Springfield, Tennessee. The calves were subjected to one of three infectious bovine rhinotracheitis (IBR) vaccination regimes, (1) injection with an IBR vaccine 30 days prior to delivery to the auction barn (AB), (2) injection with vaccine 30 days prior to delivery to the AB followed by a cell mediated immune booster upon arrival at the AB, and (3) nonvaccinated control group. On the day prior to departure from the orderbuyer barn (OBB) the calves were challenged by nasal inoculation with IBR virus. In Trial 2, 48 calves were purchased from auction barns and transported to a feedlot at The University of Tennessee Blount Farms at Knoxville, Tennessee.

Body weights of the calves in both trials were taken at various sampling dates. The weights were used to calculate gains as a measure of feedlot performance. Serum harvested from blood samples obtained at various points during the two trials were used to demonstrate the presence of and changes in antibody titers against Pasteurella hemolytica. An Enzyme-Linked Immunosorbant Assay (ELISA) technique was used to measure

antibody titers in serum of calves from both trials. The presence of antibodies in the serum of the Blount Farm calves was also determined by an agglutination technique.

A scoring system involving the evaluation of various health parameters was used to monitor clinical signs of disease in the HRES calves. The number of days the calves were judged by the herdsman to require treatment for disease was used as a measure of disease severity in the Blount Farm calves.

Chi-square analysis of the frequencies of positive and negative antibody titers indicated that the ELISA and agglutination techniques gave comparable results when ELISA titers greater than or equal to 128 were considered positive and titers less than 128 were considered negative.

Pasteurella hemolytica was isolated from 2% of the HRES calves and 47% of the Blount Farm calves which were sampled. Pasteurella hemolytica antibody titers in the HRES calves remained low and consistent throughout the market-transit and feedlot phases. In the Blount Farm calves, a large increase in antibody titers occurred in sera taken upon arrival at the feedlot and sera obtained 38 or 43 days later. These results indicate that Pasteurella hemolytica infection occurred in the Blount Farm calves but not in the HRES calves.

Antibody titers of the second serum samples were positively correlated with the number of days sick and negatively correlated with the average daily gains of Blount Farm calves. This suggests that Pasteurella hemolytica infection had an adverse affect on feedlot performance.

Clinical signs of disease evident in the HRES calves suggested that the calves were infected with IBR virus. The nonvaccinated calves

were more severely affected by the IBR infection than the vaccinated calves. This was evident in greater degrees of respiratory distress, necrotic rhinitis, higher rectal temperatures and higher daily health indexes. No differences in degrees of mucopurulent nasal discharge and percent packed cell volumes were observed. Calves receiving the IBR vaccination followed by the cell mediated immune booster gained more weight from feedlot day 1 to feedlot day 10 than nonvaccinated calves. Weight gain differences due to IBR vaccination treatment had disappeared by feedlot day 22.

In conclusion, the results indicate that Pasteurella hemolytica is an important etiological agent of the BRD complex in market stressed calves. Due to the apparent lack of Pasteurella hemolytica infection, a relationship between Pasteurella hemolytica and IBR virus in the BRD complex could not be demonstrated in the HRES calves.

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