Bovine respiratory disease research (1983–2009)

Robert W. Fulton

Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA

Abstract

Bovine respiratory disease (BRD) research has provided significant understanding of the disease over the past 26 years. Modern research tools that have been used include monoclonal antibodies, genomics, polymerase chain reaction, immunohistochemistry (IHC), DNA vaccines and viral vectors coding for immunogens. Emerging/reemerging viruses and new antigenic strains of viruses and bacteria have been identified. Methods of detection and the role for cattle persistently infected bovine viral diarrhea virus (BVDV) were identified; viral subunits, cellular components and bacterial products have been characterized. Product advances have included vaccines for bovine respiratory syncytial virus, *Mannheimia haemolytica* and *Pasteurella multocida*; the addition of BVDV2 to the existing vaccines and new antibiotics. The role of *Mycoplasma* spp., particularly *Mycoplasma bovis* in BRD, has been more extensively studied. Bovine immunology research has provided more specific information on immune responses, T cell subsets and cytokines. The molecular and genetic basis for viral–bacterial synergy in BRD has been described. Attempts have been made to document how prevention of BRD by proper vaccination and management prior to exposure to infectious agents can minimize disease and serve as economic incentives for certified health programs.

Keywords: infectious agents, prevention, control, vaccines, bovine respiratory disease, cattle, shipping fever, immunity

Overview of bovine respiratory disease (BRD) and status of research

The impact of BRD is extensive with economic losses occurring due to morbidity, mortality, treatment and prevention costs, loss of production and reduced carcass value (Griffin, 1997; Smith, 2000; Irsik *et al.*, 2006). Research on BRD has been a longstanding priority for the United States Department of Agriculture (USDA) and the veterinary profession. Various types of research are in place ranging from basic discovery to clinical studies in the field applying the knowledge gained from the basic studies. There has been a multistate research project, initially supported in the North Central (NC) region of the United States by agricultural experiment stations (AES), with projects involving veterinary medical colleges and veterinary science departments. That regional distinction

(North Central US) does not reflect the national participation within the group, including California, Georgia and many states in between. This research group sponsored a BRD research symposium in 1983 in Amarillo, TX and the proceedings book was published in 1984 as *Bovine Respiratory Disease: A Symposium* (1984). That symposium was designed for researchers to interact with the industry and veterinarians involved with BRD, and to obtain guidance for future research.

This national research involvement reflects the fact that the cattle industry is represented by cowherds in most regions with the movement of cattle across many states from forage post-weaning to feedlots now predominating in the southwest and central regions where the feeds are grown for the feedlot rations. In addition to the multistate project, other funding sources are from the USDA, Hatch projects and commercial firms, as well as limited funds from producer groups and private foundations. To a small extent, some basic research with cattle as a model for human disease has been supported by the National

E-mail: robert.fulton@okstate.edu

Institutes of Health (NIH). Accountability for the BRD research positively impacting the industry will be critical for funding. Outcomes indicating the benefits of BRD research include but are not limited to: (1) reduction of disease incidence; (2) better understanding of the pathogenesis of BRD, especially viral–bacterial inter-actions; (3) detection of new infectious agents and new strains of the current agents; (4) understanding and application of knowledge of the acquired and innate immune systems; (5) development of new vaccines and therapeutic agents that complete the approval process, and are used in the industry and recommended by veterinarians; (6) industry and veterinarians successfully applying research findings to the prevention and control of BRD and (7) recognition by medical and basic science researchers of the scientific discoveries in veterinary medical research. On the latter point the US Library of Medicine website (PubMed) shows that from 1982 through 29 April 2009 there were 1952 publications on various aspects of 'bovine respiratory diseases' in that database.

Several publications have addressed the current state of BRD. Representative publications have surveyed the industry for BRD in feedlots. In a survey using a feedlot monitoring program through feedlot veterinary con-sultants overseeing 21.8 million cattle in 121 US feed-lots, there was a trend for increased mortality ratio for respiratory tract disorders from 1994 to 1999 (10.3 deaths/1000 in 1994 to 14.2 deaths/1000 in 1999) (Loneragan et al., 2001). Also, a 2005 survey (Woolums et al., 2005) of feedlots indicated that the BRD complex was the leading cause of morbidity and mortality: 12.8% of the cattle were treated for BRD and 0.8% died of BRD. Thus even with current prevention and control measures, the clinical impact of BRD continues. In addition to research publications and clinical reports, knowledge related to BRD has been summarized with reference to diseases and their control using modern terminology (Bovine Respira-tory Disease Handbook, 2007).

Interestingly, when one examines the vaccines and therapeutic agents available in 1983 compared to 2009, it becomes clear that there have been considerable advances. This represents an impressive measure of the research over the past 26 years. The list of vaccines and therapeutic agents in the Veterinary Pharmaceuticals and Biologicals 1982/1983 revealed several modified live viral (MLV) vaccines containing bovine herpesvirus-1 (BHV-1), also known as infectious bovine rhinotracheitis virus; bovine viral diarrhea virus (BVDV) with no genotype noted, and parainfluenza-3 (PI-3V) for parenteral use. There were a limited number of MLV BHV-1 vaccines for intranasal use. One killed virus (KV) vaccine with BHV-1, PI-3V and BVDV, and a second KV vaccine with BHV-1 and PI-3V were noted. There were no licensed bovine respiratory syncytial virus (BRSV) vaccines available. Mannheimia haemolytica (formerly Pasteurella haemo-lytica) and Pasteurella multocida bacterins were available,

but it is likely they have been replaced by the current products. Also many viral products were marketed with a leptospiral bacterin component. The antibiotics listed in 1982–1983 will be unknown to recent graduates. Those for BRD treatment included erythromycin, penicillin–dihydrostreptomycin, tylosin injectable and oral oxyte-tracycline, and injectable sulfamethazine. Also many of the animal health companies in 1983 have been merged, sold, or renamed. Twenty six years later in 2009 there is an extensive list of vaccines and therapeutics in the Compendium of Veterinary Products, 11th edition (2008). A new list of companies is noted in the reference above and the list will be entirely renamed as potential mergers are completed.

The available vaccines in 2009 reflect the research in the identification of selected viruses, and the addition of selected strains of viruses. There are MLV vaccines for injection or intranasal use for BHV-1, BVDV1a and BVDV2a, PI-3V, and BRSV as well as KV vaccines for these same viruses for injection. In some cases the vaccine strains of 1983 are now described more fully, such as the BVDV1a and BVDV2a identified in many current vaccines, which was simply listed as BVDV in 1983. Although updates have occurred, many of the vaccinal strains of BHV-1, BVDV, PI-3V are isolates from 35 to over 50 years ago. A case in point is the BHV-1 Colorado strain reported as an MLV vaccine in 1957. An almost-complete new list of therapeutic agents in 2009 is listed in the Compendium of Veterinary Products, 11th edition (2008).

Many of the antimicrobials available in 1983 are no longer available, often due to residue and safety issues. Antibiotics in use today include the principal marketed injectables: ceftiofur, oxytetracycline, enrofloxicin, florfe-nicol, danofloxacin, tilmicosin and tulathromycin. Also listed in the 2008 publication are injectable tylosin, erythromycin and penicillin. A new therapeutic agent available in 2009 is the non-steroidal anti-inflammatory product flunixin meglumine. There are also numerous oral medications such as sulfas, tetracyclines and tylosin.

Methods of study

Since 1983 the development and application of many molecular tests has had a remarkable impact on infectious disease and immunological research. Techniques and tools now available include: restriction fragment poly-morphism (REFP) for DNA analysis, ELISA tests for antibodies, monoclonal antibodies (MoAb), recombinant DNA expressed proteins, deletion mutants (with selected genes deleted), knock outs, poxvirus or adenovirus vectored vaccines, microarrays (gene chips), DNA vaccines, immunohistochemistry (IHC), antigen-capture ELISA tests, polymerase chain reaction (PCR) using gel-based and real time PCR, and proteomics (determination of proteins expressed by the genome and their role in the pathophysiologic response). Currently considerable

emphasis is given to the sequencing of selected regions of the viral and bacterial genomes. Not only are these tests commonly used for research; many of them have been a significant benefit for veterinary diagnostic laboratories. It is routine that IHC, antigen capture ELISA and PCR are utilized for more accurate and efficient testing, and they have often replaced many longstanding tests. The following discussion of research on infectious agents and the immune system in BRD will include the many advances made using these techniques and tools.

Infectious agents

Research progress on infectious BRDs ranges from additional information on their epidemiology, role in clinical disease, characterization based on genomes and proteins of the agents, and demonstration of pathogenicity in challenge studies. The types of studies include reports of a single case; summaries of diagnostic lab reports (usually the end stage pneumonia and nasal swab isolates from sick calves); and studies over time, such as feedlot seroepidemiologic studies, with or without periodic virus isolation. Examples of these types of studies with the identification of agents including viruses and bacteria are reported (Martin and Bohac, 1986; Fulton et al., 2000, 2002b). Also reported are a wide variety of experimental challenge studies of susceptible calves with one or more of most of the agents involved in BRD. In addition studies have been performed where calves dying in a feedlot over time are necropsied, the pathologic lesions characterized, and the agents isolated or detected. Although such studies may emphasize end stage pneumonia, and initiating viruses and/or bacteria may not be detected, they do provide information regarding agents involved and lesions associated with fatal pneumonic cases.

A very significant study was reported in the late 1970s, which identified the pneumonic lesions and infectious agents found in a long-term survey of fatal pneumonia cases in a Colorado feedlot. This study was widely referenced and formed the basis for description of fatal feedlot pneumonias (Jensen et al., 1976a, b). In 2006 and later there were additional studies of fatal feedlot deaths with lesions described and infectious agent reported. Many similarities were found between the 1976 study and the more recent studies, although some technologies for agent identification used in more recent studies (such as IHC and PCR) were not available in the 1970s. These 2006–2009 reports included an Ontario, Canada study of 99 calves dying in 72 Ontario feedlots in the first 60 days of the feeding period (Gagea et al., 2006b); and a study by Booker et al. (2008) of 99 calves dying in the first 60 days after entry into 17 feedlots. A 2009 study (Fulton et al., 2009) was from one feedlot with 237 samples from animals dying in the feed yard regardless of time after arrival, with a range of 1–241 days after arrival.

Research on the viral infectious agents in BRD since 1983 has largely focused on BHV-1, BVDV and BRSV. Few publications dealing with PI-3V singly were reported, with most reports including PI-3V as one of multiple viruses and bacteria described. The bovine coronavirus (BCV) also received attention with its isolation from calves with BRD after shipment and commingling (detailed below). Beginning in the late 1970s and the early 1980s a major emphasis was placed on M. haemolytica as the stand alone disease potential for M. haemolytica was substantiated by challenge studies. Also P. multocida has received increased research focus. Histophilus somni (formerly Haemophilus somnus) remains a significant pathogen as reported by diagnostic laboratories; however, considerably more reports from Canada have been published on the prevalence of disease and specific lesions associated with H. somni, as compared to the US Mycoplasma spp. in cattle have long been recognized as a cause of respiratory disease, but the involvement of Mycoplasma bovis in respiratory disease and its relative role in BRD has received considerable attention since 1983, both from the diagnostic laboratory and in regard to the potential role for vaccination. The research on BHV-1, BVDV, BRSV, M. haemolytica and P. multocida has led to advances in the various vaccines for these agents, and their use in the management of cattle. Any discussion of the agents will include involvement of the immune system. The coverage of the research findings on each agent is extensive and the reader is referred to excellent review articles for each infectious agent. Selected publications will be cited to illustrate the basis for certain research directions.

BHV-1

BHV-1 is an alpha herpesvirus subfamily member that causes diseases of the respiratory tract, fetal infections including abortions, reproductive tract disease in the female and male (vulvo-vaginitis and balanoposthitis), conjunctivitis and severe neonatal disease (Jones and Chowdhury, 2007; Muylkens et al., 2007). There are three BHV-1 subtypes based on antigenic and genomic differences: BHV1.1, BHV-1.2a and BHV-1.2b. In addition, since the 1983 symposium another BHV was identified in cattle with central nervous system (CNS) signs and lesions. Although these CNS BHV-1 strains share antigens with the above BHV-1, there are genomic differences with the virus referred to as BHV-1.3. The BHV-1, as a herpesvirus, is noted for the latency or sequestration of the virus in neural tissues in animals recovering from acute infections. Stress or steroid treatment may cause shedding of the virus after reverse migration from the ganglia via nerves. The immune response to BHV-1 includes both B-cell (humoral or antibody) and T-cell responses of the acquired immune response. Numerous assays have been used since 1983 to

investigate the T-cell response to both the field strains and the vaccinal strains given to cattle. From a clinical standpoint, immunity to BHV-1 vaccines may not be long-lived. For example in a feedlot environment calves administered MLV vaccine at entry/processing may break with an infectious bovine rhinotracheitis (IBR)-like disease 70–100 days after vaccination with infection confirmed by fluorescent antibody test (Bryant *et al.*, 2008). This led to studies evaluating the duration of immunity induced by MLV BHV-1 vaccines (Ellis *et al.*, 2005). While antigenically relevant mutation of field isolates of BHV-1 was hypothesized to contribute to the short duration of vaccine-induced immunity, this was not confirmed on analysis of a strain of virus isolated from vaccinated cattle with disease late in the feeding period (van Drunen Little-van den Hurk *et al.*, 2001). The reason that cattle vaccinated on feedlot entry sometimes succumb to BHV-1 infection later in the feeding period is still unknown, and this is an area where research of 'new' BHV-1 strains could still be useful. Some consider that clinically important strain variation is still a possible contributing factor in feedlot outbreaks of BHV-1 in vaccinated cattle, since the Colorado 1 strain has been used in the current MLV vaccines since 1957.

Current research on BHV-1 has utilized modern technologies. For example, the entire BHV-1 genome (Cooper strain) has been sequenced, and the essential and non-essential genes of BHV-1 have been determined with a functional map of the open reading frames encoded by the viral genome (Robinson *et al.*, 2008). Sequencing these various regions may identify unique strains. In addition to studies of pathogenesis, research has utilized genetic modification of BHV-1 as applied to vaccination. A limited number of studies have been reported wherein BHV-1 was used as a vaccine vector expressing proteins for different agents. To date these vaccines have not been commercialized.

BVDV

Probably no virus involved with BRD has received as much research attention since 1983 as BVDV (Ridpath and Fulton, 2009). The use of MoAb and the sequencing of the viral genome have facilitated BVDV research and diagnostic testing. In 1993, a severe acute outbreak of BVDV disease occurred in Ontario, Canada (Carman *et al.*, 1998). The virus was found to be a BVDV2 strain, while vaccines in use from the 1970s contained only BVDV1 strains. In 1994, it was reported that BVDV could be grouped in subgenotypes of BVDV1 and BVDV2 (Ridpath *et al.*, 1994). With the serious disease caused by a BVDV2, both MLV and KV vaccines were developed and marketed that contained both BVDV1a and BVDV2a. Evaluation of the diversity of BVDV strains indicates there are 12 BVDV1 subgenotypes (BVDV1a to BVDV1l) and two BVDV2 subgenotypes (BVDV2a and BVDV2b)

(Ridpath and Fulton, 2009). The relevance of these differences as they apply to protection afforded by heterologous vaccine strains, and the potential for discordant results in diagnostic testing, remains unclear; this is an important area for future research.

Improved understanding of the impact of BVDV infection of a susceptible dam at a critical stage of pregnancy, with the resultant delivery of a calf persistently infected (PI) for its lifetime, has greatly increased knowledge of the epidemiology and source/transmission of the virus. While acute/transient BVDV infections that may be inapparent or that may cause respiratory and digestive tract disease can lead to BVDV transmission among a group of cattle, many believe that the PI calf is the key to sustaining the virus in a population of cattle. Thus identifying PI cattle is crucial to control programs. A landmark test was developed and validated to detect PI cattle using IHC on fixed skin biopsy samples. This test is now widely used in diagnostic laboratories for PI detection. A few years later an antigen capture ELISA test (ACE) was developed for the detection of BVDV antigen in fluids of skin samples collected in phosphate buffered saline (PBS). The IHC and ACE tests are both examples of the value of MoAb, which are key components of each of these tests. Gel-based and real time PCR tests have also been applied to detect BVDV genomic material in samples of fluids from PI cattle.

The immunosuppressive nature of BVDV is a critical factor in the interaction of BVDV with other viruses and bacteria, especially in BRD. For example, BVDV and *M. bovis* were found by IHC in tissues of feedlot cattle with chronic respiratory disease and arthritis (Haines *et al.*, 2001). Since 1983 the research on immunosuppression by BVDV, the knowledge of how PI cattle occur, the use of the MoAb in tests such as IHC and ACE, and the role of PI cattle as a source of the virus are excellent examples of the application of research findings to the development of diagnostic tests and control programs.

BRSV

BRSV was initially described in the US in the 1970s, and its role in BRD was established over the following years. Shortly after the 1983 symposium an MLV BRSV vaccine was licensed and incorporated into MLV vaccines with BHV-1, BVDV and PI-3V. The term 'big four' has been coined for these four viruses due to their typical inclusion in vaccines and their important role in BRD. The addition of BVDV2 to BRD vaccines has necessitated a change in that terminology. BRSV research in particular has focused on the importance of the host's immune response to BRSV in the pathogenesis of disease due to the virus. Extensive reviews on BRSV and the host response have been published by Gershwin (2007) and Valarcher and Taylor (2007). Important research has focused on the viral surface proteins and their role in the host immune

response. Different types of T helper cell response (Th1 or Th2) have been linked to immune responses that contribute to disease in cattle. Experimentally, an immune-mediated pathogenesis for BRSV has been shown in calves after use of a formalin inactivated BRSV vaccine followed by aerosol with a virulent BRSV strain. However, the licensed MLV BRSV vaccines given by injection are used widely in the industry and appear to be safe. Recently, an MLV vaccine given by the intranasal route with BRSV, BHV-1, BVDV1a and 2a, and PI-3V has been licensed for use in young calves.

BCV

BCV isolates from the respiratory tract have received attention as they have been isolated from cattle with signs of BRD and from lungs of cattle with BRD. Also the BCV has been shown to be transmitted among calves, and animals respond with an immune response which coincides with the reduction in nasal shedding (Storz et al., 1996; Thomas et al., 2006). BCV may occur in conjunction with other viruses and with bacteria such as M. haemolytica. A specialized human rectal tumor cell line is quite sensitive for viral isolations, and its use may improve recovery of BCV by diagnostic laboratories using this cell line. While BCV has repeatedly been found in BRD cases, the case for BCV as a major BRD pathogen would be advanced if lung pathology was demonstrated in calves challenged with the virus. This virus may be the next virus with potential as an immunizing product.

M. haemolytica

This bacterium, M. haemolytica, is the subject of the most published studies since the 1983 symposium (Rice et al., 2007). There were criticisms of the M. haemolytica bacterins in use in the late 1970s and early 1980s. Most of the M. haemolytica vaccines in use today, which are bacterin-toxoid types, have replaced those used in 1983. Today, the literature contains numerous publications describing the bacterial components including virulence factors and antigens. There are multiple serotypes (16) for biotypes A and T, with 12 serotypes based on capsular serotypes for the A serotype. Also with more effective challenge systems described, the pathogenicity of the M. haemolytica isolates can be evaluated. Likewise with characterization of the various cell components and the host's immune response measured, the likelihood of the cellular components and exotoxins inducing protective immunity have been determined. One of these cellular products, the leukotoxin, has received considerable attention. This is a virulence factor as evidenced by its interactions with leukocytes and other blood components. The leukotoxin stimulates the host's immune system to produce serum antibodies to the leukotoxin.

Animals with leukotoxin antibodies have been shown to be immune to respiratory challenge with virulent M. haemolytica; thus many current vaccines contain this immunogen. The leukotoxin has been produced by recombinant DNA technology and formulated into a vaccine. Other antigens being investigated include outer membrane proteins (OMP). One such OMP is the PlpE, which has been shown to be immunogenic; moreover, calves with OMP antibodies are resistant to M. haemolytica challenge. There is potential for the recombinant derived leukotoxin and OMP to be used in vaccines. There are also experimental modified live M. haemolytica vaccines.

P. multocida

P. multocida has received great attention over the past several years (Dabo et al., 2007). Often thought of as a secondary invader, there is evidence to reveal its role as a primary lung pathogen in cattle. Thus there are P. multocida immunizing products licensed and marketed in the US (Compendium of Veterinary Products 2008). These are primarily new products compared to those available in 1983. As for M. haemolytica, attention has been given to identifying virulence factors and cellular components that are potential immunogens. An example for potential as a P. multocida vaccine candidate is an OMP which has been described. While experimental studies have shown protection in challenge studies, these vaccines have not consistently shown benefit in commercial feedlot programs.

H. somni

H. somni continues to be a common isolate from BRD cases in cattle as reported by diagnostic laboratories, although the number of isolates is often less than M. haemolytica and P. multocida. As with M. haemolytica and P. multocida, the characterization of cellular components as virulence factors and antigens, including an immunodominant OMP, has been a significant focus of much research (Corbeil, 2007). A model with synergy was demonstrated in H. somni and BRSV challenged calves. There are H. somni vaccines available, however there have been no recently developed and marketed vaccines compared to those from several years ago. It is likely that more effective challenge methods to demonstrate protection by H. somni bacterins in vaccinated cattle would stimulate more interest in the use of these vaccines.

M. bovis

M. bovis has been the subject of many investigations, including laboratory studies characterizing the agent

and measuring the host immune response, as well as field studies to identify infected cattle (Caswell and Archambault, 2007). The *M. bovis* agent has been studied to characterize its antigens and virulence factors as for the bacteria described above. There are tests to detect the immune response to the agent. Diagnostic laboratories are now better able to detect *M. bovis* in pneumonia cases with specific sera and use of PCR. The challenge in establishing *M. bovis* as a significant pathogen in BRD is to determine whether it causes primary infection leading to disease, or whether it is more often a secondary invader in the compromised lung. Often diagnostic laboratories will recover *M. bovis* in the end stage chronic pneumonia, suggesting it is most likely a secondary invader. In fairness, there are studies demonstrating lung disease in animals challenged with *M. bovis*. Two significant current aspects of pathogenesis related to *M. bovis* are: (1) its recovery from cases of chronic pneumonia and poly-arthritis in feedlots (Haines *et al.*, 2001; Gagea *et al.*, 2006a) and the frequency of concurrent infections with BVDV in these cases (Haines *et al.*, 2001).

The development of vaccines to control *M. bovis* has had its challenges. There are vaccines available, but their use has led to mixed results. A recent study in dairy calves indicated that a licensed *M. bovis* bacterin was not efficacious for the prevention of *M. bovis*-associated disease in dairy calves (Maunsell *et al.*, 2009). There are no published reports of efficacy for *M. bovis* vaccines in field studies evaluating their use for preventing pneumonia in feedlot cattle (Caswell and Archambault, 2007).

Immune response and pathogenesis

The review of the literature since the 1983 symposium on BRD indicates a major emphasis on the host response/ immune response to the various infectious agents. These reports often reflect advances made in the understanding of the immune system components and in the technology available to evaluate them, as research advances in lab animal models and human immunology have been applied to the bovine immune system. With advances in the characterization of the various components of bacteria and subunits of viruses, studies of the immune response now focus on the specific immune response to the subcomponents rather than the whole organism or virus. Often these specific immune responses can guide the selection of the most likely epitope for selection as immunogens. A review article by Ellis (2001) summarized the immune responses to various viral and bacterial agents of BRD.

The immune system is complex with various components often working in concert. Advances in the understanding of acquired immunity have focused on the T-cell (cell-mediated immunity, CMI) and the B-cell (humoral) components of the response (Woodland, 2003). The T-cell system is further divided into classifications such as CD4+ T cells and CD8+ T cells (Esser *et al.*, 2003). The CD8+ T cells are also referred to as cytolytic T cells. The CD4+ T cells can be differentiated into either T helper 1 (Th1) or T helper 2 (Th2) cells that secrete specific sets of cytokines. These cytokines can drive the immune response toward either a strong CMI response, or a strong humoral (antibody) response, and the direction the immune response takes is largely associated with specific cytokines produced by either Th1 or Th2 cells. In general, the Th1 response is associated with a CMI or pro-inflammatory response, while the Th2 response is associated with antibody mediated responses. Cytokine gene expression for CD4+ and CD8+ T cell subsets has now been described for cattle (Tanaka *et al.*, 2007). In addition to the CMI and antibody response related to T cells, the T cell system in cattle includes other components such T regulatory cells (de Almeida *et al.*, 2008). T regulatory cells when activated may limit the Th1 response. In a review article, several studies indicated that numerous viruses, bacteria and parasites induce immunosuppressive T regulatory cells as a normal part of the immune response (Robertson and Hasenkrug, 2006). Also, T cells have a significant role in antigenic memory, especially for protective immunity in animals recovering from initial infection or in the response to vaccines (Esser *et al.*, 2003). Assays of T cell responses to infectious agents in challenged and vaccinated animals have been used in numerous studies. This is illustrated in a study detecting the T-cell and antibody response to BHV-1, BVDV1 and 2, and BRSV in calves following vaccination with an MLV vaccine containing these antigens (Platt *et al.*, 2006). There are several similar studies using T cell function assays as well as tests to measure the mRNA for various cytokines.

Research on the pathogenesis of BRD undertakes examination of the interaction of the agent and the host immune response, including the synergistic effect of viral–bacterial co-infection, which may lead to enhanced disease. For example, increased fatal bacterial respiratory disease following primary viral infection has been observed for several species (Hodgson *et al.*, 2005). An experimental model of this viral–bacterial synergy has been described that utilizes primary BHV-1 respiratory challenge followed by aerosol challenge with *M. haemolytica* (Ohmann and Babiuk, 1985; Ohmann *et al.*, 1991; Babiuk *et al.*, 1996). This model has been used to elucidate mechanisms of viral–bacterial synergy; for example, as found with functional genomic analysis indicating that BHV-1 altered Toll-like receptor (TLR) expression and pro-inflammatory responses, which contributed to the severity of *M. haemolytica* infection (Hodgson *et al.*, 2005). This study was based on advances in immunology that show the importance of pro-inflammatory responses stimulated by interaction of bacterial components with TLR; it is further evidence of the use of molecular techniques to better understand the host response related to viral–bacterial synergy in BRD.

The ability of infectious agents to evade the immune system complicates the pathogenesis of BRD and may render the host unable to develop a protective immune response (Srikumaran *et al.*, 2007). This review summarizes how infections alter the acquired and innate immune systems, and how infectious agents modify themselves to evade the host responses. These points offer explanations for the failure of vaccines to provide complete protection against BRD.

Prevention and control

There are far more effective vaccines and therapeutic agents available today as compared to 1983. The viral and bacterial vaccines have modifications including adjuvants and addition of new strains and serotypes. Vaccine production involves monitoring for contamination by adventitious agents. The *M. haemolytica* vaccines are excellent examples of the upgrading of BRD vaccines, which includes completion of efficacy studies. There have been remarkable changes in available antibiotics since 1983. Despite these new products there continues to be respiratory disease issues among various components of the cattle industry. The BRD losses in the feedlot are noteworthy, as shown by the survey of feedlots by Woolums *et al.* (2005) indicating the continued presence of BRD.

The successful application of prevention and control programs based on reduced disease and economic benefit from these programs is often not realized. Those promoting the use of vaccines and therapeutic agents must look for the critical control points for the intervention, especially, regarding the pathogenesis of the disease. It is difficult to ask a vaccine to alter the pathogenesis of disease if given to an already infected animal with a compromised immune system. Many believe the health status begins in the breeding herd for cattle destined to remain healthy and return a profit. While not a fundamental or basic research assignment, the economics of the beef industry largely drive the marketing and sale of cattle and may impact the effectiveness of these better vaccines and antibiotics. Several studies have shown that the 'preconditioned calf ' that was weaned 30–45 days with proper vaccines and anthelmintic treatment will be more valued and will bring a higher price. However, some believe that there is a potentially greater economic return for the management of disease than can be realized by paying a higher price for healthy calves, thus justifying the lower price for the high risk calves. A relatively new term is 'metaphylaxis' which is the use of antibiotics in calves considered 'high risk' before the clinical signs of bacterial disease are evident (*Bovine Respiratory Disease Handbook*, 2007). Measuring success of new technologies used to manage high risk calves is often difficult. A case in point is the use of vaccines in high risk calves at feedlot arrival and the resulting failure to see benefit for the

vaccine at the feedlot. One such example is a study with *M. haemolytica* vaccine given to calves at entry to the feedlot, with performance compared to non-vaccinates. There was no difference in BRD mortality, morbidity, or average daily gain (MacGregor *et al.*, 2003). However there are reports where cattle with high levels of immunity to selected agents prior to entry to the feedlot had less illness and reduced treatment costs and returned more value to the owner at the end of the feeding period (Fulton *et al.*, 2002a).

The potential for the industry to change offers opportunities for the promotion of health versus managing disease. There have been studies reporting on the economic benefit and disease reduction benefit to 'preconditioned calves'. Calves vaccinated or conditioned and sold through special auctions received a premium compared to producers selling at conventional auctions, and vaccinated and conditioned calves were less likely to receive treatment for BRD in the first 28 days in the feedlot (Macartney *et al.*, 2003a, b). A summary of certified health programs indicated that cattle from the certified health program when sold through livestock videotape auction service consistently yielded improved price and these increased over time (King *et al.*, 2006). Another study from a commercial feedlot where calves from different backgrounds were purchased and fed until processed showed that calves receiving the certified health protocol yielded more net income compared to those with unknown health history (Seeger *et al.*, 2008). In summary there is evidence that under certain conditions cattle with documented health programs may perform better and return more value to the owner than those of unknown history.

Research areas

BRD will continue to be a significant clinical issue for all phases of the cattle industry, affecting value of animals marketed, profitability to producers, and providing challenges to veterinarians and diagnosticians faced with a need to make accurate and timely diagnoses. Research findings validated to improve production will assist veterinarians in making prevention and control recommendations. Animal health companies will be challenged to develop therapeutic agents and vaccines that meet the industry's needs and capabilities, and regulatory agencies' requirements for approval. The industry will potentially have to deal with the disease as an animal welfare issue, and also to attempt to lessen the use of therapeutic agents that have use in human therapy. Public concern over animal welfare and antibiotic use in food animals will likely continue. With numerous technologies available to investigate the agents and the immune system, there is great potential for discoveries. Granting agencies will be expected to assist in the extramural funds required for these projects. Future research areas for investigation

include: (1) monitoring for emerging or re-emerging infectious agents and antigenic variants; (2) evaluation of acquired immunity (T- and B-cell) involved in recovery and vaccine-induced protection; (3) evaluation of innate immunity; (4) development of innovative vaccines using new technologies, and perhaps addition of current strains, as some vaccine strains have been in use for over 50 years ago; (5) development of diagnostic tests for field use; (6) delivery of vaccines given by different routes or based on new technologies; (7) adjuvants that are effective and safe for use in food animals; (8) diagnostic tests that differentiate vaccine-induced immunity versus response to natural infections; (9) new therapeutic agents; (10) studies to determine the economic cost of disease and to confirm enhanced economic return for prevention and control programs; (11) bovine genome mapping with the use of markers for resistance to disease and enhanced immune response to vaccines (The Bovine Mad Consortium [2009]); (12) application of research results to the current marketing system for cattle and (13) animal welfare issues for the cattle industry and producers, including reduction in use of therapeutic agents.

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