Passive transfer of *Mycoplasma bovis*-specific antibodies in calves born to vaccinated dams

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

Passive transfer of *Mycoplasma bovis*-specific antibodies in calves born to vaccinated dams

presented by Christopher Douglas Calloway,

a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

Mycoplasma bovis is a bacterial pathogen that has been shown to cause respiratory disease, mastitis, polyarthritis, keratoconjunctivitis, and other diseases in cattle worldwide. High costs associated with these diseases are attributed to treatment, culling, deaths, and purchase of replacement animals. Commercially available vaccinations are also available to producers and veterinarians to be used as an added preventative measure.

This research, consisting of two studies, evaluated antibody responses in late gestation dairy cattle given a commercially available *M. bovis* vaccination. Serum, colostrum, and milk antibodies were compared between cows receiving the vaccine and those not receiving the vaccine. The second study evaluated serum antibody response in female calves born to cows from the first study. These calves received colostrum from the cows to which they were born. Serum antibody responses were measured before ingestion of colostrum, after ingestion of colostrum, and 30 days of age.

Serum antibody responses against *M. bovis* in vaccinated cows were significantly greater than those of non-vaccinated cows at 3 weeks after first vaccination ($P < 0.0001$) and at calving ($P = 0.047$). Colostrum antibody response was also significantly higher in vaccinated cows than in non-vaccinated cows ($P = 0.0106$). However, there was no difference noted in the serum antibody responses between female calves born to vaccinated cows and those born to non-vaccinated cows ($P = 0.7864$).

CHAPTER 1

MYCOPLASMA BOVIS: CHARACTERISTICS, EPIDEMIOLOGY, CLINICAL DISEASE, TREATMENT, AND PREVENTION

Introduction

Mycoplasma bovis is an important pathogen recognized in veterinary medicine. *Mycoplasma bovis* has been known to cause mastitis, pneumonia, arthritis, and a host of other diseases in cattle. Economical losses as a result of *Mycoplasma bovis* infections include treatment costs, premature culling of infected animals, and herd replacement costs.

Etiology and Characteristics

Taxonomically, *Mycoplasma bovis* is a bacteria belonging to the phylum Firmicutes, class Mollicutes, order Mycoplasmatales, and family Mycoplasmataceae (Boone et al., 2001). A noted feature of all *Mycoplasma* spp. is the apparent lack of a typical cell wall. *Mycoplasma bovis* was once considered to be a subspecies of *Mycoplasma agalactaie*. However, *Mycoplasma bovis* was elevated to species rank and given its name in 1976 (Askaa and Erno, 1976). It is important to note that these two *Mycoplasma* species are biochemically similar. Neither ferment glucose nor hydrolyse arginine; they do utilize the organic acids lactate and pyruvate as energy sources (Miles et al., 1988). *Mycoplasma bovis* is small and pleomorphic with a genome size of 1,080 kilo-basepairs (Boone et al., 2001).

Mycoplasmas exhibit a notable fragility within the environment; however, it is documented that *Mycoplasma bovis* can survive for nearly 2 months at 4°C within milk and for over 2 weeks in water. *Mycoplasma bovis*, when exposed to higher environmental temperatures, survives at considerably lower rates (Pfutzner and Sachse, 1996). Additionally, strains of *M. bovis* can survive up to 126 days at 4°C on dry paper discs (Nagatomo et al., 2001). Disinfection of *M. bovis* prevalent areas can therefore be problematic. Traditional practices of removal of organic material from areas followed by a period of animal absence may not be sufficient to disinfect contaminated areas.

M. bovis is unique in that the organism has the ability to express a variety of membrane surface proteins, known as variable surface proteins [vsp]. Vsp's act as virulence factors specific to this mycoplasma. To date, thirteen vsp genes have been identified. The antigenic character of *M. bovis* is thought to be altered by vsp's; there is an enhancement of colonization and/or adherence of the organism within the host. Additionally, there is thought to be an increased evasion of immune defense mechanism brought about by alterations in vsp's (Lysnyansky et al., 2001, Pfutzner and Sachse, 1996). Vsp genes operate by various mechanisms including DNA transposition and intrachromosomal recombination within the vsp locus (Nussbaum et al., 2002).

Epidemiology

The distribution and severity of *M. bovis* infection differ for each clinical disease; however, there is an overall recognizable pattern similar for all *M. bovis*

associated diseases. Transmission and intermittent shedding of the organism by clinically and sub-clinically infected animals tends to complicate proper diagnostic testing strategies and preventative veterinary medical practices.

Within the United States, *Mycoplasma bovis* was first diagnosed as a cause of clinical bovine mastitis in 1961 (Hale et al., 1962). Within Canada, *Mycoplasma bovis* was also diagnosed as a cause of clinical bovine mastitis in 1972 (Ruhnke et al., 1976). *Mycoplasma bovis* detection in Israel occurred in 1964, Spain in 1967, Australia in 1970, France in 1974, Great Britain in 1975, Czechoslovakia in 1975, Germany in 1977, Denmark in 1981, Switzerland in 1983, Morocco in 1988, South Korea in 1989, Brazil in 1989, Northern Ireland in 1993, Republic of Ireland in 1994, and Chile in 2000 (Nicholas and Ayling, 2003). However, one can debate whether *M. bovis* spread truly occurred via animal movements or improved diagnostic testing techniques allowed for positive identification of infected animals and herds. Currently, *Mycoplasma bovis* is widely spread within the world's bovine population by means of enzootic transmission (Nicholas and Ayling, 2003). It can be interpreted that *M. bovis* has been or could potentially be reported worldwide, especially amongst the major cattle export markets.

Mycoplasma bovis is usually introduced to naïve herds by animals shedding this bacterium via numerous routes. Infection via the respiratory tract, mammary system, or genital tract is common (Pfutzner and Sachse, 1996). Once established within the male genital tract, an ascending infection resulting in

orchitis and/or vesiculitis as well as decreased seminal quality (Kreusel et al., 1989).

Economic Losses

M. bovis and *M. bovis*-related disease has been responsible for major economic loss amongst major cattle producing nations. Cost within the USA has been estimated at $32 million per year. Sources of loss include decreased rate of gain and decreased carcass value (Rosengarten and Citti, 1999). Cost of *M. bovis* mastitis has been estimated at $108 million per year with a herd prevalence rate of 70% (Rosengarten and Citti, 1999). Within the United Kingdom, a loss of £54 million to the cattle industry has been attributed to an estimated 1.9 million cattle being infected with respiratory disease (Reeve-Johnson, 1999). European losses due to respiratory disease total 576 million euros. It is hypothesized that approximately 25-33% of these total losses are attributed to *M. bovis* solely (Nicholas et al., 2000)

Clinical Disease

Mycoplasma bovis is implicated as either a sufficient or necessary cause or play ancillary roles in bovine respiratory disease [BRD], intramammary infection [IMI], polyarthritis and tenosynovitis, infectious bovine keratoconjunctivitis [IBK], otitis media and interna, and a host of other disease processes of minor incidence.

Bovine Respiratory Disease Complex

INTRODUCTION

Mycoplasma bovis is an integral part of the bovine respiratory disease (BRD) complex in some nations. A cross-sectional study with a study population from Canadian feedlot cattle identified a *M. bovis* prevalence of 80% (Haines et al., 2001).

Other bacterial pathogens, such as *Mannheimia hemolytica* or *Pasteurella multocida*, are usually identified in the lungs of calves infected with *M. bovis*. However, when *M. bovis* alone is implicated, the results are usually subclinical BRD cases within cattle populations. In clinical studies involving dairy calves within *Mycoplasma bovis* and *Pasterella multocida* prevalent herds, nearly 50% of subjects were shedding mycoplasma by 5 days of age; 90% of these clinically normal calves were shedding mycoplasma by 4 weeks of age (Stipkovits et al., 2001). Calves with BRD with an associated mortality of up to 10% resulting in severe serofibrinous pneumonia were highest at 10-15 days post-exposure. Those surviving calves demonstrated poor weight gain rates.

CLINICAL SIGNS

Typical clinical signs associated with *M. bovis* BRD follows suit of those of generalized BRD and associated BRD agents. Clinical signs include fever, depression, hyperpnea, dyspnea, nasal discharge, mild and continuous cough, and appetite loss (Nicholas and Ayling, 2003). In animals with severe lower respiratory signs, a severe protracted fever has been noted (Thomas et al.,

1986). Animals with *M. bovis* BRD have a chronic bronchopneumonia coupled with necrosis and bronchiectasis. Often, this pneumonia is associated with fibrinous and/or fibrous pleuritis (Adegboye et al., 1996). These calves tend to be chronically ill and fail to thrive (Shahriar et al., 2002) and are usually eliminated from the herd due to a lack of appropriate weight gain and failure to respond to antimicrobial therapies.

Cattle that are coinfected with *M. bovis* and bovine viral diarrhea virus (BVDV) develop lesions beyond that expected with *M. bovis* BRD cases. Notably, these calves develop vasculitis of the heart and lungs (Shahriar et al., 2002).

Pulmonic lesions associated with *M. bovis*-BRD include an exudative bronchopneumonia with extensive foci of coagulative necrosis surrounded by inflammatory cells. Up to 30% of the lung surfaces were affected in gnotobiotic calves experimentally infected with *M. bovis* (Thomas et al., 1986). Chronic pneumonia cases associated with *M. bovis* yield pulmonic lesions involving lymphocytic cuffing with marked hyperplastic peribronchial lymphoid tissue causing stenotic airway lumina and compression and subsequent collapse of adjacent pulmonary parenchyma. *M. bovis* antigen was detected in the periphery of the necrotic areas, necrotic exudates, and in close association with infiltrating macrophages and neutrophils (Rodriguez et al., 1996).

TREATMENT, PREVENTION, AND CONTROL

In vitro susceptabilities to danofloxacin, florfenicol, oxytetracycline, spectinomycin, and tilmicosin were evaluated using 62 British field isolates. Isolates were most susceptible to danofloxacin; oxytetracycline and spectinomycin exhibited little effect against the majority of isolates. Tilmicosin was found to be ineffective (Ayling et al., 2000). Antibiotic susceptibility to enrofloxacin (Ball et al., 1995) and tylosin (Adegboye et al., 1996, ter Laak et al., 1993) has been demonstrated.

Bovine mastitis

INTRODUCTION

Eleven *Mycoplasma* species have been identified as causative agents of intramammary infection, or IMI with the most common of these being *Mycoplasma alkalescens*, *M. bovigenitalium*, *M. bovis*, *M. californicum* and *M. canadense* (Gonzalez and Wilson, 2003). It is accepted within veterinary medical literature that introduction of *Mycoplasma bovis* to a dairy herd population is facilitated through introduction of new animals, namely animals deemed as herd replacements (Bushnell, 1984; Jasper, 1981; Gonzalez and Wilson, 2003). Replacement animals purchased and introduced to a dairy production facility have been the implicated cause of *Mycoplasma bovis* IMI in several cases (Ruhnke et al., 1976; Bicknell et al., 1978, 1983; Gunning and

Shepherd, 1996). Greater risk for transmission of and infection with *Mycoplasma bovis* seems to be evident within larger dairy herds where the mere animal population numbers and turn-over of the animal population through culling and introduction of replacement heifers and cows are present (Fox et al., 2003, Thomas et al., 1981). Surveying and monitoring for *Mycoplasma bovis* within dairy herd is accomplished through bulk tank sampling and subsequent culturing (Bray et al., 1997, Farnsworth, 1993, Gonzalez and Wilson, 2003). The assumption is made that if *Mycoplasma bovis* is found within a bulk tank sample at least one cow from the associated herd is infected (Bray et al., 1997, Brown et al., 1990).

Mechanical transfer of respiratory and/or urogenital tract *Mycoplasma bovis* infection(s) to the mammary system is possible (Bushnell, 1984). Supported by more evidence is the notion that internal transfer of *Mycoplasma bovis* from extramammary sites to the mammary gland occurs. Following intramammary inoculation of *Mycoplasma* sp. and subsequent *Mycoplasma* sp. IMI development, the same *Mycoplasma* sp. was identified via blood cultures and within other organ systems (Bennett and Jasper, 1978, Jain et al., 1969). Therefore, it can be concluded that there exists a hemotogenous spread of *Mycoplasma* sp. from the mammary gland to other organ systems.

CLINICAL SIGNS

IMI in the bovine can be classified as either subclinical or clinical mastitis. Mastitis is said to be subclinical when there is supporting evidence of inflammation without any visible abnormality of the milk or udder. Clinical mastitis may include findings of abnormalities of the size, consistency and temperature of the mammary glands and frequently a systemic reaction (Radostits, 2000). *M. bovis* mastitis can take both of these forms with subclinical mastitis usually leading to clinical mastitis.

Mycoplasma bovis is regarded as a contagious bovine mastitis pathogen. Herd-wide contagious mastitis outbreaks and/or individual cases are marked by low to medium rates of clinical IMI; however, they exhibit progressive increases in somatic cell counts (Wilson et al., 1997).

Individual cows infected with *M. bovis* mastitis exhibit decreased milk yields followed by clotting of the milk in affected quarters. Flakes will also appear in milk at the beginning of the course of disease. As the disease progresses, the clots and flakes begin to appear larger. The milk itself becomes a pale yellow color. Purulent material is also evident in mammary secretions (Ruhnke et al., 1976). The udder of severely affected cows may become swollen but are not hot or painful when palpated (Ruhnke et al., 1976). In many animals, infection will spread to uninfected quarters within 7-10 days (Hale et al., 1962).

DIAGNOSIS

Microbiological testing is primarily used to diagnose *M. bovis* mastitis within individuals or herds (Gonzalez and Wilson, 2003). Specialized growth media is necessary to culture *M. bovis*. Milk is cultured on modified Hayflicks media and incubated at 37°C under 10% CO_2 for 7-10 days (Hogan and National Mastitis Council (U.S.). 1999). It is suggested that further testing, such as ELISA, SDS-PAGE, or immunoblot techniques, are used to assure the *Mycoplasma* sp cultured is in fact *M. bovis* (Fox et al., 2005).

PCR techniques are used as well (Sachse et al., 1993). Nested PCR techniques have been developed for detection of *M. bovis* in preservative-treated field milk samples (Pinnow et al., 2001). Such PCR techniques are practical since herds enrolled in DHIA programs have the added benefit of having monthly collected milk samples tested for *M. bovis*.

Cows can become intermittent shedders of *M. bovis*. It has been shown that as many as 40% of cows can shed <100 CFU/ml *M. bovis* in the milk (Biddle et al., 2003). Therefore, the risk of false negative test results to a herd can be problematic. Conversely, the risk of false positive test results is reduced in view of the fact that non-pathogenic *Mycoplasma* sp. rarely cause mastitis (Kirk and Lauerman, 1994).

Surveying and monitoring for *Mycoplasma bovis* within dairy herd is accomplished through bulk tank sampling and subsequent culturing (Bray et al., 1997, Farnsworth, 1993, Gonzalez and Wilson, 2003). The assumption is made

that if *Mycoplasma bovis* is found within a bulk tank sample at least one cow from the associated herd is infected (Bray et al., 1997, Brown et al., 1990).

TREATMENT, PREVENTION, AND CONTROL

Previous work has indicated that *M. bovis* sensitivities to aminoglycoside and macrolide antibiotics warrant their usage as therapies (Jasper, 1981). With a voluntary ban against aminoglycoside usage in food-producing animals being enforced, such therapy is not defensible. Others have suggested that therapy is not economically feasible and only protracts the time before the infected animal is culled (Bushnell, 1984).

M. bovis is best controlled by stringent milking time hygiene, including post-milking teat asepsis, and detection and culling of infected animals (Gonzalez and Wilson, 2003).

Polyarthritis and tenosynovitis

INTRODUCTION

Arthritis and tenosynovitis associated with *M. bovis* infection is most notably seen within the feedlot and backgrounding phases of U.S. beef cattle operations. Joint inflammation, and subsequent lameness, occurs via hemotogenous dissemination of M. bovis (Pfutzner and Sachse, 1996, Stalheim and Page, 1975). Arthritis tends to develop approximately 2-4 weeks after entry

into backgrounding or feedlot operations (Adegboye et al., 1996). Lameness is usually preceded by a substantial prevalence of BRD in the cattle population. Exact morbidity rates attributable to mycoplasma-associated arthritis have not been well-documented since many researchers report morbidity rates for pneumonia-arthritis syndrome rather than lameness rates singly (Stokka et al., 2001).

CLINICAL SIGNS

M. bovis-associated lameness is a result of arthritis, or more commonly polyarthritis, and tenosynovitis. *M. bovis* has been documented to be found in tendon sheaths and joint capsules of affected cattle leading to a pyogranulomatous-type lesion (Stokka et al., 2001). Abscess formation within joint capsules, tendon sheaths, and bursae may occur resulting in prolonged clinical course of disease (Adegboye et al., 1996).

Radiocarpal, tibiotarsal, and femorotibial joints are most frequently affected. Experimentally, arthritis of the cervical spine has been induced (Thomas et al., 1986). Joint capsules may become distended with abnormally excessive amounts of synovial fluid. Affected joints will also feel warm upon palpation. Acute lameness appears as a stiff gait with the calf limping when bearing weight on the affected limb(s). As the arthritis becomes more chronic, calves tend to remain recumbent. Decubital ulcers may develop over pressure points (Stokka et al., 2001). Calves with less severe arthritis may spontaneously recover within 4 to 6 weeks (Hjerpe and Knight, 1972).

DIAGNOSIS

Diagnosis of *M. bovis*-associated arthritis is usually based on the temporal association of respiratory disease and arthritis in affected calves. Routine bacteriological testing usually does not detect *Mycoplasma* spp. (Pfutzner et al., 1980). Antemortem synovial fluid analysis reveals fluid that is usually clear to cloudy with the cloudiness being a result of fibrin deposition within the joint space. Samples should be chilled and transported rapidly to a diagnostic laboratory for most reliable results (Whitford et al., 1994). Swabs should be submitted in Amies' or Stuart's transport media (Pfutzner et al., 1980). A multiplex polymerase chain reaction (PCR) is available to detect remnants of bacteria that may not be detected with traditional or special culture techniques (Hotzel et al., 1993). PCR testing may be appropriate after extensive antimicrobial usage in affected calves. Gross examination of affected joints reveals massive fibrinosuppurative synovitis and tenosynovitis, erosion of the joint cartiledge, and polypoid granulation tissues in place of eroded cartiledge. Histologic lesions include ulceration of synovial membranes, leukocytic infiltration of subsynovium, congestion, hyperemia, and thrombosis of subsynovial vessels. Chronic osteomyelitis and pannus tissue may also be present as a result of infection (Ryan et al., 1983).

TREATMENT, PREVENTION, AND CONTROL

M. bovis-associated arthritis and tenosynovitis cases exhibit poor response to antimicrobial therapy (Pfutzner and Sachse, 1996, Romvary et al., 1977a, Romvary et al., 1977b, Stalheim, 1976). In vitro susceptabilities to danofloxacin, florfenicol, oxytetracycline, spectinomycin, and tilmicosin were evaluated using 62 British field isolates. Isolates were most susceptible to danofloxacin; oxytetracycline and spectinomycin exhibited little effect against the majority of isolates. Tilmicosin was found to be ineffective (Ayling et al., 2000). Antibiotic susceptibility to enrofloxacin (Ball et al., 1995) and tylosin (Adegboye et al., 1996, ter Laak et al., 1993) has been demonstrated.

Preventative measures focus on reducing incidence of respiratory disease within cattle populations. Vaccination against respiratory pathogens such as bovine respiratory syncitial virus, parainfluenza type 3 virus, and *M. bovis* has shown to reduce the severity of pneumonia due to *M. bovis* (Howard et al., 1987). A reduction in frequency of *M. bovis*-associated arthritis was seen in calves vaccinated with a *M. bovis* bacterin (Chima et al., 1981).

Control measures against *M. bovis* infections within cattle populations have proven to be problematic. *M. bovis* is highly contagious and can persist within the environment for extended periods of time. Intuitively, standard biosecurity measures should be effective at minimizing spread of *M. bovis* within susceptible cattle populations. However, establishing and maintaining such programs can prove to be difficult in some circumstances.

Infectious bovine keratoconjunctivitis

INTRODUCTION

Mycoplasma bovoculi and *Mycoplasma conjunctivae* have previously been identified as causative agents of infectious bovine keratoconjunctivitis, or IBK (Langford, 1977, Naglic et al., 1996, Salih and Rosenbusch, 2001). In addition, *Mycoplasma conjunctivae* is identified as the primary *Mycoplasma* sp. causing infectious keratoconjunctivitis in the ovine (Degiorgis et al., 2000). *Mycoplasma bovis* has previously received little attention as being a primary cause of IBK. The bovine eye was not considered to be an organ site for isolation of *M. bovis* (Jack et al., 1977, Kirby and Nicholas, 1996, Levisohn et al., 2004, Naglic et al., 1996). However, recent work has revealed that *M. bovis* indeed can be a primary cause of IBK in the bovine (Alberti et al., 2006). Advances in diagnostic laboratory procedures along with a better understanding of *M. bovis*'s role in other disease processes such as BRD have aided in making this conclusion.

CLINICAL SIGNS

Moraxella bovis is typically isolated from the majority of IBK cases that present for diagnostic testing. Varying pathogenicity of *Moraxella bovis* strains, environment, host immune status, concurrent pathogens, and the presence of mechanical vectors such as face flies (*Musca autumnalis*), house flies (*Musca domestica*), and barn flies (*Stomoxys calcitrans*) are all various factors involved in the pathogenesis of IBK (Brown et al., 1998). Typical clinical signs of IBK are well described. Unilateral infection initially followed by bilateral disease is

common (Punch and Slatter, 1984). Acutely, conjunctivitis and keratitis are the prominent findings (Bedford, 1976) along with profuse lacrimation, photophobia, blepharospasm. A serous or mucopurulent ocular discharge is also noted as IBK progresses (Brown et al., 1998). Corneal ulceration may present as a subacute finding. As the clinical case of IBK progresses, severe keratoconjunctivitis with descemetocele formation is present. Consequently, ocular rupture may occur. Fulminate forms of IBK include severe corneal ulceration, panophthalmitis, blindness, and possible death due to ascending infection (Bedford, 1976).

Clinical signs indicative of *Mycoplasma bovis* IBK do not vary greatly from those described for other IBK pathogens. In outbreaks of *Mycoplasma bovis* IBK, clinical signs noted are bilateral conjunctivitis and profuse lacrimation followed by keratitis and varying degrees of corneal ulceration (Jack et al., 1977). Some affected animals have become blind for up to 10 days. Remarkable differences noted in these cases versus typical IBK cases include significant swelling of both upper and lower eyelids along with a severe purulent discharge (Kirby and Nicholas, 1996). Additionally, neovascularization of the cornea, diffuse corneal edema, and leukoma have been identified in cases of *Mycoplasma bovis* IBK (Alberti et al., 2006). Insect vectors have not been noted to be necessarily associated with *Mycoplasma bovis* IBK since many of these cases occur during winter months when flies are not active (Kirby and Nicholas, 1996).

DIAGNOSIS

Diagnosis of *Mycoplasma bovis* IBK is based on physical examination and findings concurrent with positive clinical signs. Conjunctival and nasal swabs cultures, identification with PCR, and DNA sequencing have successfully been utilized for identification of *Mycoplasma bovis* in cases of IBK (Alberti et al., 2006, Levisohn et al., 2004). Such laboratory tests have proved advantageous to establish etiological causes. Cattle producers and veterinarians may need to differentiate IBK caused by *Mycoplasma bovis* versus other *Mycoplasma* sp. or other, more traditional IBK agents.

TREATMENT, PREVENTION, AND CONTROL

Typical cases of IBK can be treated by parental administration of antibiotics such as tetracyclines, tylosin, or procaine penicillin with varying results (George et al., 1984). Newer antibiotics such as ceftiofur (Dueger et al., 2004) and florfenicol (Angelos et al., 2000) have been used with promising results. However, these drugs are less efficacious in the face of *Mycoplasma bovis* IBK.

Mycoplasma bovis IBK tends to foreshadow incidence of other *Mycoplasma bovis* associated diseases, namely pneumonia and polyarthritis (Alberti et al., 2006). Therefore, primary treatment of *Mycoplasma bovis* IBK is given little attention when producers are faced with these subsequent diseases.

Environmental or physical stressors, such as long-distance travel or high stocking density, have been shown to amplify *Mycoplasma bovis* IBK cases (Kirby and Nicholas, 1996). Therefore, prevention strategies should be targeted

to the lessening of such factors. Likewise, concurrent disease processes such as respiratory disease can exacerbate IBK problems within herds (Jack et al., 1977). Infected animals have been shown to transmit *Mycoplasma bovis* IBK to cohorts who are under similar stressful conditions (Kirby and Nicholas, 1996). Consequently, segregation of herd mates may be indicated.

Otitis media and otitis interna

INTRODUCTION

Otitis is defined as an inflammatory process involving the vestibulocochlear organ. Otitis is classified according to location of the primary inflammation, be that external, middle, or inner ear (Bruyette and Lorenz, 1993).

Otitis media is a relatively common finding in calves (Jensen et al., 1983). Populations of dairy and beef calves are both at risk; however, beef calves are more commonly affected (Walz et al., 1997). Dairy calves are mainly affected at one to two months of age (Lamm et al., 2004). Beef calves are usually affected in association with *M. bovis*-associated respiratory disease (Gagea et al., 2006).

Pathogenic bacterial agents associated with otitis in cattle include *Arcanobacterium pyogenes* (Baba et al., 1988, DeChant and Donovan, 1996, Vestweber, 1999, Yeruham et al., 1999), *Corynebacterium pseudotuberculosis* (Duarte and Hamdan, 2004), *Escherichia coli* (Duarte and Hamdan, 2004), *Histophilus somnus* (Nation et al., 1983), *Pasteurella multocida* (Jensen et al., 1983, Rademacher et al., 1991), *Mannheimia haemolytica* (Yeruham et al., 1999), *Pseudomonas* spp. (Duarte and Hamdan, 2004), *Streptococcus* spp.

(Baba et al., 1988), and *Mycoplasma bovis* (DeChant and Donovan, 1996). Of recent importance is the discovery of *Mycoplasma bovirhinis* and *M. alkalescens* as a causative agent of otitis in calves (Lamm et al., 2004).

Exact pathophysiology of otitis media in calves is not fully understood. Extension of infection from the pharynx via the Eustachian tube is believed to be the most common route of entry into the middle ear (Jensen et al., 1983, Yeruham et al., 1999). Additional routes of infection include extension of otitis externa infection or bacteremia (Oliver et al., 1997). Extension of otitis media infection into the inner ear or bacteremia are considered to be the common causes of otitis interna (Vestweber, 1999).

The correlation between *M. bovis* mastitis and otitis media in calves has been reported. After an outbreak of otitis media in calves was reported, subsequent bulk tank milk culture results yielded positive for *M. bovis*. Calves affected with otitis had been discarded milk from cows within the herd (Walz et al., 1997).

CLINICAL SIGNS

Clinical signs of calves affected with *M. bovis*-associated otitis media include unilateral or bilateral ear droop, head tilt, epiphora, and nystagmus (DeChant and Donovan, 1996, Walz et al., 1997). Ear droop and epiphora are consistant with facial nerve paralysis; head tilt and nystagmus are signs of vestibulocochlear nerve dysfunction. Clinical signs of otitis media caused by

other bacterial pathogens commonly do not differ from those of *M. bovis*-associated otitis media. Purulent aural discharge has been noted with *E. coli* and *Pseudomonas* spp. otitis media (Henderson and McCullough, 1993). A severe fibrinopurulent meningitis and supurative otitis media and interna have been noted with otitis caused by *Histophilus somnus* (McEwen and Hulland, 1985).

DIAGNOSIS

Historically, otitis media and/or otitis interna generally follows or is found in conjunction with cases of respiratory disease (Lamm et al., 2004). Aural examination may reveal purulent discharge (Francoz et al., 2004). Tympanic membranes of affected calves may be ruptured or still intact. Radiographic examination of head of calves affected with otitis media and/or otitis interna may be used for diagnostic purposes. Ventrodorsal radiographic views of affected calves reveals osteitis of the tympanic bullae and petrous temporal areas of the skull (Vestweber, 1999). Antemortem diagnostic testing for *M. bovis*-associated otitis includes bacteriological culturing of ruptured tympanic bullae exudate (Lamm et al., 2004, Walz et al., 1997).

TREATMENT, PREVENTION, AND CONTROL

Treatment of *M. bovis*-associated otitis is usually coupled with that of treatment of respiratory disease. Commonly used antibiotics include enrofloxacin, ampicillin, penicillin, spectinomycin, trimethoprim/sulfamethoxazole,

and rifampin (Francoz et al., 2004). Ceftiofur and oxytetracycline have also been indicated as antibiotic therapies (Walz et al., 1997).

Preventative strategies should mirror those of respiratory disease prevention. There seems to be a seasonal trend in cases of otitis with more cases occurring in late winter and spring (Lamm et al., 2004). Similar trends have been made in incidence of *M. bovis*-associated mastitis (Jasper et al., 1979). With correlations made between mastitis in cows and otitis in calves, reduction and/or elimination of *M. bovis*-associated mastitis may be necessary.

CHAPTER 2

DETERMINING SEROLOGICAL AND COLOSTRAL RESPONSE IN LATE GESTATION HOLSTEIN COWS VACCINATED WITH A *MYCOPLASMA BOVIS* BACTERIN

Introduction

Mycoplasma bovis, a bovine bacterial pathogen, has been implicated as a necessary and/or essential cause of contagious mastitis, respiratory disease, arthritis, otitis media and interna, and a host of other disease processes within cattle populations. First identified as a contagious bovine mastitis pathogen in 1961 (Hale et al., 1962), much effort has been made towards establishing proper diagnostic, treatment, prevention, and control measures.

Mycoplasma bovis associated diseases are costly to both beef and dairy production systems within North America as well as worldwide. Cattle producers incur costs to treat as well as replace those infected animals culled from the production facility. Dairy farmers may incur additional costs as decreased premium or perhaps penalty in bulk tank sales. Like other *Mycoplasma* spp., *M. bovis* lacks a typical bacterial cell wall (Boone et al., 2001). Traditional bacteriocidal antibiotics, such as ceftiofur and procaine penicillin G which inhibit bacterial cell wall synthesis, have no efficacy. Therefore, when faced with treating a *M. bovis*-associated respiratory disease in their cattle, producers may perceive that a treatment failure has occurred.

Vaccinating dams prior to calving against respiratory pathogens such as infectious bovine rhinotracheitis (IBRV), parainfluenza 3 virus (PI3V), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), Mannhaemia hemolyica, and Pasteurella multocida, has resulted in increases in specific immunoglobulin G (IgG) levels against these pathogens. This IgG is then passively transferred to calves via colostrum.

The purpose of this controlled trial is to determine if vaccinating cows during late gestation against *Mycoplasma bovis* will result in adequate levels of *M. bovis*-specific IgG_1 in these cows' serum, colostrum, and milk.

Materials and Methods

Experimental animals and design

A total of 77 Holstein cows with confirmed expected parturition dates were used. The study population originated from and was housed at the University of Missouri-Columbia Foremost Dairy. Cows had been dried off from their previous lactation 0-6 days prior to enrollment. Upon initial enrollment, a coin flip was used to allocate the initial cow into its respective study group with binary allocation into the two study groups following. Cows assigned to the vaccinate group (VAC) were given 2ml PulmoGuard™ MpB bacterin (Boehringer Ingelhiem Vetmedica, Inc., 2621 North Belt Highway, St. Joseph, MO 64506) subcutaneously in the right neck area. Simultaneously, a 10ml blood sample was collected aseptically via the coccygeal vein. Cows assigned to the control

group (CTRL) received no vaccination. A blood sample was collected from these cows in the same fashion. Two weeks later, a booster vaccination of the same product was given to the VAC group and a second blood sample was taken in the same fashion. A blood sample was taken from the CTRL group members at the same time and in a same fashion. Cows were monitored for impending parturition by the farm management. At time of calving, a third blood sample was taken. All blood samples were centrifuged with the serum sample being decanted into a 5ml serum collection tube. Cow number, sample number, and collection date only identified samples. Samples were frozen at -10°C until serological analysis was performed. At calving, a 10ml composite sample of colostrum was taken from each cow in an aseptic fashion and collected into a 10ml Falcon tube. Colostrum samples were frozen at -10°C until serological analysis was performed. A composite milk sample was collected in a similar way at 7-13 DIM.

Serological, colostrum, and milk analysis

Analysis of serum, colostrum, and milk samples were performed by American Animal Health Laboratory in Grand Prairie, Texas. Serum samples were analyzed for *Mycoplasma bovis*-specific IgG_1 concentration. Colostrum and milk samples were analyzed for *Mycoplasma bovis*-specific IgG_1 and IgA concentrations. The technical staff was blinded to which study group each sample belonged.

ELISA

An ELISA was developed and used for analysis of submitted samples. *Mycoplasma bovis* coated plates prepared by AAH were used. Plates were blocked with gelatin. Bovine serum samples were diluted 1:100 with PBS/Tween 20. One hundred µl of sample was used. Serum collected from *Mycoplasma bovis* infected cattle at two times serial dilution starting at 1:100 were used as positive controls. FBS at a 1:100 dilution was used as a negative control. Samples were incubated at 37°C for 1-2 hours. Samples were then washed three times with dH_20 or PBS/Tween 20. One hundred µl of anti-bovine IgG_1 for serum, milk, and colostrum analysis or 100 µl of anti-bovine IgA for milk and colostrum analysis were added with HRPO conjugate (Bethyl Lab). Samples were again incubated for 1-2 hours at 37°C. Samples were washed 6 times with dH_20 or PBS/Tween 20. One hundred µl of TMB substrate (Sureblue, KPL) was added and left to develop for 10 minutes. One hundred 0.5 M H_2SO_4 was added to stop the reaction. Optical density was read with a microplate reader with duel wavelength of 450 and 630 nm. To minimize ELISA plate-to-plate variability, *S/P* ratios were calculated for each sample by using the following formula:

S/P ratio = [(OD_{450} sample – OD_{450} negative control) ÷ (OD_{450} positive control – OD_{450} negative control)]

Statistical analysis

Anti-*Mycoplasma bovis* immunoglobulin G_1 antibody responses are indicated as mean *S/P* ratios ± standard error of the mean (S.E.M.). Variance

ratio tests were performed for each comparison between groups. Statistically significant differences in variance were adjusted using Welch's approximation. Mean serum, colostrum, and milk IgG_1 antibody response were compared between the two groups by Student's *t*-test. Mean serum IgG_1 antibody response within groups were compared using paired *t*-tests (Zar, 1999).

Linear regression models that predicted maternal anti-*Mycoplasma bovis* IgG_1 *S/P* ratios at parturition as a function of maternal serum anti-*Mycoplasma bovis* IgG_1 *S/P* ratios 39 days prior to parturition were constructed. Linear regression models that predicted colostral anti-*Mycoplasma bovis* IgG_1 *S/P* ratios as a function of maternal serum anti-Mycoplasma bovis IgG_1 *S/P* ratios 39 days prior to parturition and at actual date of parturition were also constructed (Kutner et al., 2004). Variables with associated P values ≤ 0.05 were included. The regression coefficient, *P* value, and correlation coefficient were reported for each significant regression model.

Responses were indicated as significant if $P < 0.05$. All statistical analyses were performed by using specialized software (Intercooled Stata for Windows, version 9.0; Stata Corporation, College Station, Texas, USA).

Results

Serological results

The two groups consisted of 40 control cows (CTRL) and 38 vaccinated cows (VAC). Mean *S/P* ratio values between the CTRL and VAC groups were similar upon enrollment of the study and concurrent primary vaccination (0.2813

± 0.012 and 0.2514 ± 0.021 respectively, $P = 0.2254$). Mean *S/P* ratio values between the CTRL and VAC groups were statistically different at the time of second vaccination three weeks following enrollment (0.3228 ± 0.019 and 0.5535 ± 0.031 respectively, $P < 0.0001$). Mean *S/P* ratio values between the CTRL and VAC groups were statistically different at the time of freshening as well (0.2321 ± 0.023 and 0.3090 ± 0.030 respectively, $P = 0.047$). The mean dry period ± standard deviation for the cows in the CTRL and VAC groups were 53.51± 20 and 55.00 ± 20.54 days respectively, $P = 0.75$ (Figure 1).

When comparing changes in mean *S/P* ratio values over time, there was significant difference in mean *S/P* ratio values in both the CTRL and VAC groups between 39 and 60 days prior to expected parturition and between parturition and 39 days prior to expected parturition (Table 1). There is also a statistical difference between 60 days prior to expected parturition date and actual date of parturition for both CTRL and VAC groups ($P = 0.04$ and $P = 0.0257$, respectively).

Colostrum results

Colostrum samples from 28 CTRL cows and 26 VAC cows were available for analysis. Mean colostrum IgG_1 *S/P* ratio values were lower in the CTRL cow study group (Figure 2). Mean *S/P* ratio values between the CTRL and VAC groups were significantly different between the two study groups (0.3546 ± 0.050 and 0.5661 ± 0.087 respectively, $P = 0.0106$).

Milk results

Milk samples from 32 CTRL cows and 27 VAC cows were available for analysis. Mean milk IgG_1 *S/P* ratio values were lower in the CTRL cow study group (Figure 2). However, mean S/P ratio values between the CTRL and VAC groups were not significantly different between the two study groups (0.5585 ± 0.047 and 0.6511 ± 0.079 respectively, $P = 0.3215$). A significant difference in mean S/P ratio was found between colostrum and milk of cows in the CTRL group ($P = 0.0129$) but not in cows in the VAC group ($P = 0.4365$).

Regression modeling

Predicting maternal anti-*Mycoplasma bovis* IgG_1 *S/P* ratios at parturition as a function of maternal serum anti-*Mycoplasma bovis* IgG_1 *S/P* ratios 39 days prior to parturition yielded a significant linear model (Figure 3), as did predicting colostral anti-*Mycoplasma bovis* IgG_1 *S/P* ratios as a function of maternal serum anti-*Mycoplasma bovis* IgG_1 *S/P* ratios 39 days prior to expected parturition (Figure 4). Predicting colostral anti-*Mycoplasma bovis* IgG_1 *S/P* ratios as a function of maternal serum anti-*Mycoplasma bovis* IgG_1 *S/P* ratios at actual date of parturition yielded no significant linear did not yield a significant linear model. Y-intercepts, regression coefficients, correlation coefficients, and *P*-values are reported in Table 2.

Discussion

Based on herd records, cases of *Mycoplasma* spp. mastitis have not been recorded and to date random bulk tank cultures for *Mycoplasma* spp. have been negative. To the author's knowledge, cows enrolled in this controlled trial had never received a prior *Mycoplasma bovis* bacterin. As expected, all cows entering this trial had statistically similar anti-*Mycoplasma bovis* S/P ratios. At the time of booster vaccination, there was a substantial larger mean serum S/P ratio within the vaccinate group versus the control group. This trend continued though parturition. There is a noticeable decline in mean serum S/P ratios at time of calving. If this parameter is used to try to gauge the overall immune status of the periparturient dam, we find that these findings are consistant with other studies (Ishikawa, 1987, Kashiwazaki et al., 1985, Kehrli et al., 1989). These alterations of immune status in the dairy cow begin approximately three weeks prior to calving and continue until three weeks after calving (Mallard et al., 1998). Serum IgG_1 concentrations tend to decrease at time of parturition (Detilleux et al., 1995, Kehrli et al., 1989). While our study only evaluates levels of mean anti-*Mycoplasma bovis* IgG_1, there is a difference between mean IgG_1 concentrations at calving versus enrollment sixty days prior to freshening within both groups, with vaccinated cows having higher responses at calving.

Predicting the level of anti-*M. bovis* IgG_1 in the serum of cows at freshening can be made by knowing serum levels approximately five to six previously. In this positive correlation, we can say that higher serum levels at the previous measurement leads to higher serum levels at freshening. The same

correlation can not be made between serum levels at freshening and colostrum levels.

Although the scope of this paper is not determine the effective level of immunity against *Mycoplasma bovis* needed to ward off associated disease, we feel that our findings are of importance. Vaccinated cows tend to have higher serum anti-*Mycoplasma bovis* IgG_1 at calving than their non-vaccinated herdmates. This trend follows into the lactation phase as well.

Colostrogenesis begins about 15 days prior to parturition. This event is marked by active and selective transport of immunoglobulin, generally of the IgG_1 subclass, into the mammary gland (Concha, 1986, Watson, 1980). Colostral quality and subsequent calf health can be influenced by vaccination status of the dam, exposure to infectious diseases, production of protective antibodies, and antibody transport into the mammary gland (Mallard et al., 1998). In our study, vaccinated cows produced colostrum higher in anti-*Mycoplasma bovis* IgG_1 than their non-vaccinated herdmates. Again, the scope of this paper is not to determine the effective level of colostrum needed to protect calves against *Mycoplasma bovis*.

Analysis of milk, however, yielded no statistical differences between the two study groups. With the purpose of vaccinating cows in late gestation for the sole benefit of promoting immunity in neonatal calf stock, milk anti-*Mycoplasma bovis* IgG_1 are of little benefit. Calf feeding practices within modern dairy production systems usually involve feeding a balanced milk replacer rather than dump milk from lactating cows. However, the relevant increases in milk anti-

Mycoplasma bovis IgG_1 versus colostrum anti-*Mycoplasma bovis* IgG_1 are unusual. Higher IgG_1 concentrations are expected in colostrum rather than milk. Further investigation into the colostrum and milk ELISA used in this study is needed to further validate their practical usage.

CHAPTER 3

PASSIVE TRANSFER OF *MYCOPLASMA BOVIS*-SPECIFIC ANTIBODIES TO CALVES BORN TO DAMS VACCINATED WITH A *MYCOPLASMA BOVIS* BACTERIN

Introduction

Calves have low serum immunoglobulin concentrations at birth (Weaver et al., 2000). Immunoglobulin requirements for the calf are met through ingestion of colostrum, a process termed passive transfer of maternal immunity. The extent of this passively derived immunity is reliant on the amount of antibodies ingested and absorbed (Fulton et al., 2004). Colostrogenesis begins about 15 days prior to parturition. This event is marked by active and selective transport of immunoglobulin, generally of the IgG_1 subclass, into the mammary gland (Concha, 1986, Watson, 1980). Colostral quality and subsequent calf health can be influenced by vaccination status of the dam, exposure to infectious diseases, production of protective antibodies, and antibody transport into the mammary gland (Mallard et al., 1998).

Within their environment, neonatal calves are exposed to numerous disease pathogens. Passively acquired immunity may provide protection against disease caused by bovine herpesvirus-1 (Mechor et al., 1987), bovine viral diarrhea virus (Bolin and Ridpath, 1995, Cortese et al., 1998, Howard et al., 1989), and bovine respiratory syncytial virus (Belknap et al., 1991). Passively acquired immunity may decrease the severity of disease caused by bovine rotavirus (Parreno et al., 2004). However, researchers have debated whether

the presence of maternally-derived immunity competes with development of the calf's endogenous antibodies to exposed antibodies. Early work indicated that this blockage did indeed exist (Husband and Lascelles, 1975). However, other studies have suggested exposure to immunogens by calves induces immunologic memory that is not susceptible to maternal antibody regulation (Brar et al., 1978, Menanteau-Horta et al., 1985).

Neonatal calf exposure to *Mycoplasma bovis* varies across the world. Approximately 20% of calves become seropositive to *Mycoplasma bovis* within the first two weeks of life in some endemic areas (Van Donkersgoed et al., 1993). The primary disease processes associated with *Mycoplasma bovis* in young calfstock include otitis media/interna and respiratory disease processes such as enzootic pneumonia.

Enzootic pneumonia (EP), a disease occurring in dairy calves one to five months of age (Curtis et al., 1988) can infect 80-90% of calves within a population and may yield mortality rates of up to 5% (Bryson, 1985, Kiorpes et al., 1988). Occurrence and severity of EP depends on a series of complex interactions between several different infectious pathogens, environmental factors, and the calf's immunological status (Bryson, 1985, Curtis et al., 1988, Kiorpes et al., 1988, Waltner-Toews et al., 1986).

The purpose of this prospective cohort study is to determine the serological response in calves born to dams vaccinated with a *Mycoplasma bovis* bacterin during late gestation.

<u>Materials and Methods</u>

Experimental animals and design

A total of 30 Holstein heifer calves were enrolled. In this cohort study, exposure was determined by *Mycoplasma bovis* vaccination status of its dam [vaccinate or control] with calves being either exposed [born to a *M. bovis* vaccinated dam] or non-exposed [born to a non-vaccinated dam]. A total of 12 heifers (CTRL) born to non-vaccinated dams and 18 heifers (VAC) born to vaccinated dams were enrolled. Heifer calves within the VAC group were born to dams that were vaccinated with 2ml PulmoGuard™ MpB bacterin (Boehringer Ingelhiem Vetmedica, Inc., 2621 North Belt Highway, St. Joseph, MO 64506) at 60 and 39 days prior to date of expected parturition. Serum samples from vaccinated and non-vaccinated dams were collected at 60 and 39 days prior to expected date of parturition. A third serum sample and a composite colostrum sample were collected from each dam at time of parturition. Enrollment of heifer calves into this cohort study occurred at birth. A 10ml blood sample was collected aseptically via the jugular vein at birth from all calves [0 hours]. Heifer calves were offered up to 3L colostrum via bottle within 15 minutes of birth. Heifer calves were then offered up to 3L colostrum via bottle at 12 hours of age. A second blood sample was then taken in a similar fashion from all calves at 48 hours of age. Calves were then moved to individual hutches. A third blood sample was collected from all calves at 30 days of age. All blood samples were centrifuged with the serum sample being decanted into a 5ml serum collection

tube. Calf number, sample number, and collection date only identified samples. Samples were frozen at -10°C until serological analysis was performed.

Serological analysis

Analysis of serum samples were performed by American Animal Health Laboratory in Grand Prairie, Texas. Serum samples were analyzed for *Mycoplasma bovis*-specific IgG_1 concentration.

ELISA

An ELISA was developed and used for analysis of submitted samples. *Mycoplasma bovis* coated plates prepared by AAH were used. Plates were blocked with gelatin. Bovine serum samples were diluted 1:100 with PBS/Tween 20. One hundred µl of sample was used. Serum collected from *Mycoplasma bovis* infected cattle at two times serial dilutions starting at 1:100 were used as positive controls. FBS at a 1:100 dilution was used as a negative control. Samples were incubated at 37°C for 1-2 hours. Samples were then washed three times with dH_20 or PBS/Tween 20. One hundred µl of anti-bovine IgG_1 for serum analysis was added with HRPO conjugate (Bethyl Lab). Samples were again incubated for 1-2 hours at 37°C. Samples were washed 6 times with dH_20 or PBS/Tween 20. One hundred µl of TMB substrate (Sureblue, KPL) was added and left to develop for 10 minutes. One hundred 0.5 M H_2SO_4 was added to stop the reaction. Optical density was read with a microplate reader with duel

wavelength of 450 and 630 nm. To minimize ELISA plate-to-plate variability, *S/P* ratios were calculated for each sample by using the following formula:

S/P ratio = [(OD_{450} sample – OD_{450} negative control) ÷ (OD_{450} positive control – OD_{450} negative control)]

Total immunoglobin G analysis

Serum IgG concentration was determined via radial immunodiffusion (Holloway et al., 2002). Assay plates used to measure serum IgG concentration were prepared by dissolving 1% agarose (Sigma Chemical Co., St. Louis, MO) in sodium barbital buffer (Sigma Chemical Co., St. Louis, MO) containing 0.1% sodium azide (Sigma Chemical Co., St. Louis, MO). Rabbit anti-bovine IgG (Organon Technika Corp., West Chester, PA) (1%) was added to the agarose solution; this solution was transferred to 10-cm Petri dishes (11ml/dish). After the agarose solidified, 3-mm diameter wells were cut in the agar. Serum samples were diluted 1:100 with sodium barbital buffer; 5 µL aliquots of diluted serum samples were added to wells in the assay plates. Plates were incubated at 23°C for 72 hours. The diameter of each observed zone of precipitation was measured. Serum IgG concentrations of study samples were determined with a standard curve (r^2 = 0.99) generated by assay of serial dilutions of a commercially available standard solution of bovine IgG (Sigma Chemical Co., St. Louis, MO).

Calf passive transfer status was assessed on the basis of serum IgG concentration at 48 hours of age. Failure of passive transfer was defined as

serum IgG concentration < 1,000 mg/dL. Calves with serum IgG concentration of ≥ 1,000 mg/dL were considered to have had adequate passive transfer.

Statistical analysis

Anti-*Mycoplasma bovis* immunoglobulin G_1 antibody responses are indicated as mean *S/P* ratios ± standard error of the mean (S.E.M.). Variance ratio tests were performed for each comparison between groups. Statistically significant differences in variance were adjusted using Welch's approximation. Mean anti-*Mycoplasma bovis* immunoglobulin G_1 antibody responses were compared among groups by Student's *t*-test and within a groups using paired *t*-tests. Mean total calf serum IgG concentrations, parity, calf weight, and total volume of colostrum ingested were compared among groups by Student's *t*-test (Zar, 1999).

Linear regression models that predicted calf serum anti-*Mycoplasma bovis* IgG_1 *S/P* ratios at 48 hours and 30 days of age as a function of total calf serum IgG concentrations at 48 hours and 30 days of age, parity of dam, calf weight, and total volume of colostrum fed. Inclusion of predictor variables into the two regression models (48 hours and 30 days of age) was based on backward stepwise modeling. Linear regression models that predicted calf serum anti-*Mycoplasma bovis* IgG_1 *S/P* ratios at 48 hours and 30 days of age as a function of maternal serum anti-*Mycoplasma bovis* IgG_1 *S/P* ratios 39 days prior to parturition and at actual date of parturition and colostral anti-*Mycoplasma bovis*

IgG_1 *S/P* ratios were also constructed (Kutner et al., 2004). Variables with associated P values ≤ 0.05 were included. The regression coefficient, *P* value, and correlation coefficient were reported for each significant regression model.

Responses were indicated as significant if *P* < 0.05. All statistical analyses were performed by using specialized software (Intercooled Stata for Windows, version 9.0; Stata Corporation, College Station, Texas, USA).

Results

The two groups consisted of 12 control heifer calves (CTRL) and 18 heifer calves (VAC). Of the 30 heifer calves in this study, all had total serum IgG concentration ≥ 1,000 mg/dL. However, there were no statistical differences (Table 3) between mean total serum IgG concentration (mg/dL) between the CTRL and VAC groups at 48 hours of age (1985.719 ± 155.942 and 1749.034 ± 211.962 respectively, *P* = 0.3697), parity of calf's dam (3.111 ± 0.512 and 3.125 ± 0.398 respectively, P = 0.9835), calf weight (kg) at birth (44.1 ± 2.424 and 43.15 ± 2.112 respectively, P = 0.7744), and total volume of colostrum (L) fed to the heifer calves was statistically similar between the CTRL and VAC groups (5.668 ± 0.165 and 4.979 ± 0.361 respectively, *P* = 0.09).

Serological results

Mean *S/P* ratio values between the CTRL and VAC groups were similar upon enrollment of the study (0.0018 ± 0.001 and 0.0589 ± 0.040 respectively, *P*

= 0.1711). Mean *S/P* ratio values between the CTRL and VAC groups were not statistically different at 48 hours of age (0.5217 ± 0.035 and 0.5441 ± 0.051 respectively, P = 0.7864). Mean *S/P* ratio values between the CTRL and VAC groups were not statistically different at 30 days of age as well (0.3975 ± 0.057 and 0.4165 ± 0.050 respectively, P = 0.8062, Figure 5).

When comparing changes in mean *S/P* ratio values over time, there was significant difference in mean *S/P* ratio values in both the CTRL and VAC groups between the 0 and 48 hour samples and between the 48 hour and 30 day samples (Table 4).

Regression modeling

Backward stepwise regression analysis that predicted calf serum anti-M. bovis *S/P* ratios as a function of calf total serum IgG concentrations (mg/dL), parity of calf's dam, calf weight (kg) at birth, and total volume of colostrum fed (L) yielded only one predictor (calf weight) of statistical significance at 48 hours of age (Table 5) and no predictors of statistical significance at 30 days of age (Table 6).

Backward stepwise regression analysis that predicted calf anti-*M. bovis* IgG_1 serum *S/P* ratios at 48 hours and 30 days of age as a function of maternal anti-*M. bovis* IgG_1 serum levels thirty-nine days prior to and date of actual parturition and colostral anti-*M. bovis* IgG_1 levels are reported in Tables 7 and 8.

Linear regression models predicting calf anti-*M. bovis* IgG_1 serum *S/P* ratios at 48 hours of age as a function of maternal anti-*M. bovis* IgG_1 serum *S/P* ratios at parturition (Figure 6), predicting calf anti-*M. bovis* IgG_1 serum *S/P* ratios

at 48 hours of age as a function of maternal anti-*M. bovis* IgG_1 serum *S/P* ratios thirty-nine days prior to expected parturition (Figure 7), and predicting calf anti-*M. bovis* IgG_1 serum *S/P* ratios at 30 days of age as a function of maternal anti-*M. bovis* IgG_1 serum *S/P* ratios at parturition (Figure 8) all yielded significant linear relationships.

Discussion

In this cohort study, no statistical difference was found in serum anti-*M. bovis* IgG_1 *S/P* ratios at 48 hours of age (post-colostrum ingestion) between heifer calves born to vaccinated dams versus heifer calves born to non-vaccinated dams. The same can be said for comparisons made at 30 days of age. Although on-farm historical medical records were not included in this study, there have been historical cases of calfhood pneumonia fitting *Mycoplasma bovis*-like clinical signs described elsewhere (Rund et al., 1986, Stipkovits et al., 2000, Waltner-Toews et al., 1986). Management protocols at the university farm where this cohort study was performed indicated that calves that exhibit clinical signs consistent pneumonia are moved from their respective hutches into the maternity barn. This move is made to facilitate closer observation and isolation from neighboring calves in the hutch area. Newborn calves also occupy the same maternity barn until time warrants they be moved into the calf hutch area. Although there is an alley physically separating isolated calves from neonatal calves, pathogen transfer is certainly a point of contention. Therefore, although

properly documented, a reservoir of *Mycoplasma bovis* may be contained within the maternity facility.

As expected, dramatic increases in serum anti-*M. bovis* IgG_1 *S/P* ratios are seen both study groups when comparing pre- and post-colostral ingestion samples. This increase reflects the passive transfer of immunity in both groups of calves via colostral ingestion. Since no calves were classified as having failure of passive transfer based on total IgG serum concentrations taken at 48 hours of age, there is no evidence of confounding bias based on some calves having lower than normal total IgG serum concentrations.

Using regression analysis to predict calf serum anti-*M. bovis* IgG_1 *S/P* ratios at 48 hours of age as a function of maternal serum anti-*M. bovis* IgG_1 *S/P* ratios at parturition yielded a significant linear relationship with a positive correlation. When the same model is used to predict serum anti-M. bovis IgG_1 levels in 30 day old heifer calves, colostrum anti-*M. bovis* IgG_1 and maternal serum anti-*M. bovis* IgG_1 levels at parturition, when combined, yield a significant relationship. The validity of these relationships is central to the concept that neonatal calf health depends on adequate passive transfer of antibodies. In turn, the quality of antibodies found in colostrum depends on the health and the immune status of the dam.

Measures of association and measures of effect were not calculated in this cohort study. The lack of a statistical significant relationship between serum anti-*M. bovis* IgG_1 *S/P* ratios in the two study groups indicates that risk of acquiring effective levels of protection due to cow vaccination is equal between

groups. It is hypothesized that a background level of *M. bovis* exposure is prevalent on the study farm, thus providing a pre-existing level of immunity against *M. bovis* in adult cows. Therefore, vaccination of cows in late gestation probably does not add any benefit in terms of additional *M. bovis*-specific IgG_1 being passively transferred to calves via colostrum. Again, this scenario is specific to the study farm. Vaccination of *M. bovis* naïve cows may yield an added benefit in other dairy production systems with varying lower levels of *M. bovis* prevalence.

APPENDIX

Table 1. Comparing changes in mean serum S/P ratio values over time in CTRL and VAC cow groups

	Group	
Days prior to expected parturition	**CTRL**	**VAC**
60 days and 39 days	$P = 0.0134$	$P < 0.0001$
39 days and 0 days	$P = 0.0002$	$P < 0.0001$
60 days and 0 days	$P = 0.04$	$P = 0.0257$

Table 2. Results of linear regression models predicting maternal anti-*M. bovis* IgG_1 *S/P* ratios at parturition as a function of maternal serum anti-*M. bovis* IgG_1 *S/P* ratios 39 days prior to parturition; predicting colostral anti-*M. bovis* IgG_1 *S/P* ratios as a function of maternal serum anti-*M. bovis* IgG_1 *S/P* ratios 39 days prior to parturition; predicting colostral anti-*M. bovis* IgG_1 *S/P* ratios as a function of maternal anti-*M. bovis* IgG_1 *S/P* ratios at parturition. Y-intercepts, regression coefficients, and correlation coefficients are not reported for non-statistically significant linear models.

Comparison	**Y-intercept (anti-*M. bovis* IgG_1 *S/P* ratio)**	**Regression coefficient (anti-*M. bovis* IgG_1 *S/P* ratio)**	r^2	***P* value**
Maternal serum 39 days prior to and at actual parturition	0.0735	0.4597	0.273	< 0.0001
Maternal serum 39 days prior to expected parturition and colostrum	0.0946	0.8690	0.131	< 0.01
Maternal serum at actual parturition and colostrum				0.401

Table 3. Comparison of total serum IgG concentration at 48 hours of age, parity of calf's dam, calf birth weight, and total volume of colostrum fed to heifer calves between two study groups, control (CTRL) and vaccinated (VAC). Heifer calves within the VAC group were born to dams vaccinated with commercially available Mycoplasma bovis bacterin at sixty and thirty-nine days prior to expected date of parturition. Heifer calves within the CTRL group were born to dams not vaccinated.

Parameter	**Mean ± S.E.M. (CTRL group)**	**Mean ± S.E.M. (VAC group)**	***P* value**
Total serum IgG concentration (mg/dL) at 48 hours of age	1985.719 ± 155.942	1746.034 ± 211.962	0.3697
Parity of dam	3.111 ± 0.512	3.125 ± 0.398	0.9835
Calf weight at birth (kg)	44.1 ± 2.424	43.15 ± 2.112	0.7744
Total volume colostrum fed to calf (L)	5.668 ± 0.165	4.979 ± 0.361	0.0914

Table 4. Comparing changes in mean serum anti-*M. bovis S/P* ratio values over time in CTRL and VAC heifer calf groups.

	Group	
Age of heifer calf	**CTRL**	**VAC**
0 hours and 48 hours	$P = 0.0001$	$P < 0.0001$
48 hours and 30 days	$P = 0.0002$	$P < 0.0001$

Table 5. Results of backward stepwise regression analysis of models that predict mean calf serum S/P ratio at 48 hours of age as a function of serum IgG concentration, parity, total volume colostrum fed, and calf weight. Variables with associated P values ≤ 0.05 were included. Y-intercepts, regression coefficients, and correlation coefficients are not reported for non-statistically significant linear models.

Parameter	Y-intercept	Regression coefficient	r^2	P value
Serum IgG concentration (mg/dL)				0.974
Parity				0.4944
Total volume colostrum (L)				0.1034
Calf weight (kg)	-0.3282	0.0189	0.4769	0.004

Table 6. Results of backward stepwise regression analysis of models that predict mean calf serum IgG_1 *S/P* ratio at 30 days of age as a function of total serum IgG concentration (mg/dL), total volume colostrum fed (L), calf weight at birth (kg), and parity of calf's dam. Variables with associated *P* values ≤ 0.05 were included. Y-intercepts, regression coefficients, and correlation coefficients are not reported for non-statistically significant linear models.

Parameter	***P*-value**
Serum IgG concentration (mg/dL)	0.979
Total volume colostrum (L)	0.8254
Calf weight (kg)	0.5170
Parity	0.0825

Table 7. Results of backward stepwise regression analysis of models that predict mean calf serum IgG_1 *S/P* ratio at 48 hours of age as a function of maternal serum anti-*M. bovis* IgG_1 at thirty-nine days prior to expected parturition, maternal serum anti-*M. bovis* IgG_1 at parturition, and colostral anti-*M. bovis* IgG_1 (*S/P* ratios). Variables with associated *P* values ≤ 0.05 were included. Y-intercepts, regression coefficients, and correlation coefficients are not reported for non-statistically significant linear models.

Parameter	Y-intercept	Regression coefficient	r^2	*P* value
Materal serum anti-*M. bovis* IgG_1 (*S/P* ratio) thirty-nine prior to expected parturition				0.4686
Colostral anti-*M. bovis* IgG_1 (*S/P* ratio)				0.5837
Materal serum anti-*M. bovis* IgG_1 (*S/P* ratio) at parturition	0.3442	0.6451	0.4720	< 0.001

Table 8. Results of backward stepwise regression analysis of models that predict mean calf serum IgG_1 *S/P* ratio at 30 days of age as a function of maternal serum anti-*M. bovis* IgG_1 at thirty-nine days prior to expected parturition, maternal serum anti-*M. bovis* IgG_1 at parturition, and colostral anti-*M. bovis* IgG_1 (*S/P* ratios). Variables with associated *P* values ≤ 0.05 were included. Y-intercepts, regression coefficients, and correlation coefficients are not reported for non-statistically significant linear models. Overall correlation coefficient for regression model is 0.4192.

Parameter	**Y-intercept**	**Regression coefficient**	***P* value**
Materal serum anti-*M. bovis* IgG_1 (*S/P* ratio) thirty-nine prior to expected parturition			0.7732
Colostral anti-*M. bovis* IgG_1 (*S/P* ratio)		-0.1825	0.034
Materal serum anti-*M. bovis* IgG_1 (*S/P* ratio) at parturition		0.6051	0.002
Model	0.3264		0.003

Figure 1. Mean serum S/P ratios between CTRL (n = 40) and VAC (n = 38) groups at 60 and 39 days prior to expected parturition date. Zero days represents actual date of parturition. Significance designations: **a** = $p < 0.05$ compared to CTRL group; **b** = $p < 0.05$ compared to prior measurement for CTRL group; **c** = $p < 0.05$ compared to prior measurement for VAC group; **d**= $p < 0.05$ compared to -60 days prior to expected parturition for CTRL group; **e** = $p < 0.05$ compared to -60 days prior to expected parturition for VAC group.

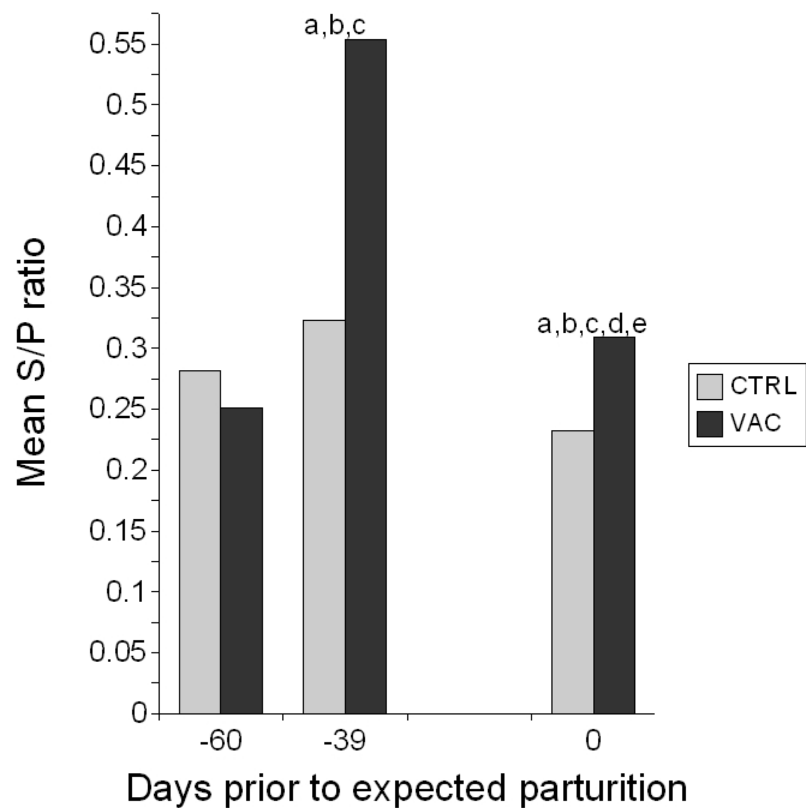


Figure 2. Mean colostrum S/P ratios between CTRL (n = 28) and VAC (n = 26) and mean milk S/P ratios between CTRL (n = 32) and VAC (n = 27). Significance designations: **a** = $p < 0.05$ compared to CTRL group; **b** = $p < 0.05$ compared to prior measurement for CTRL group.

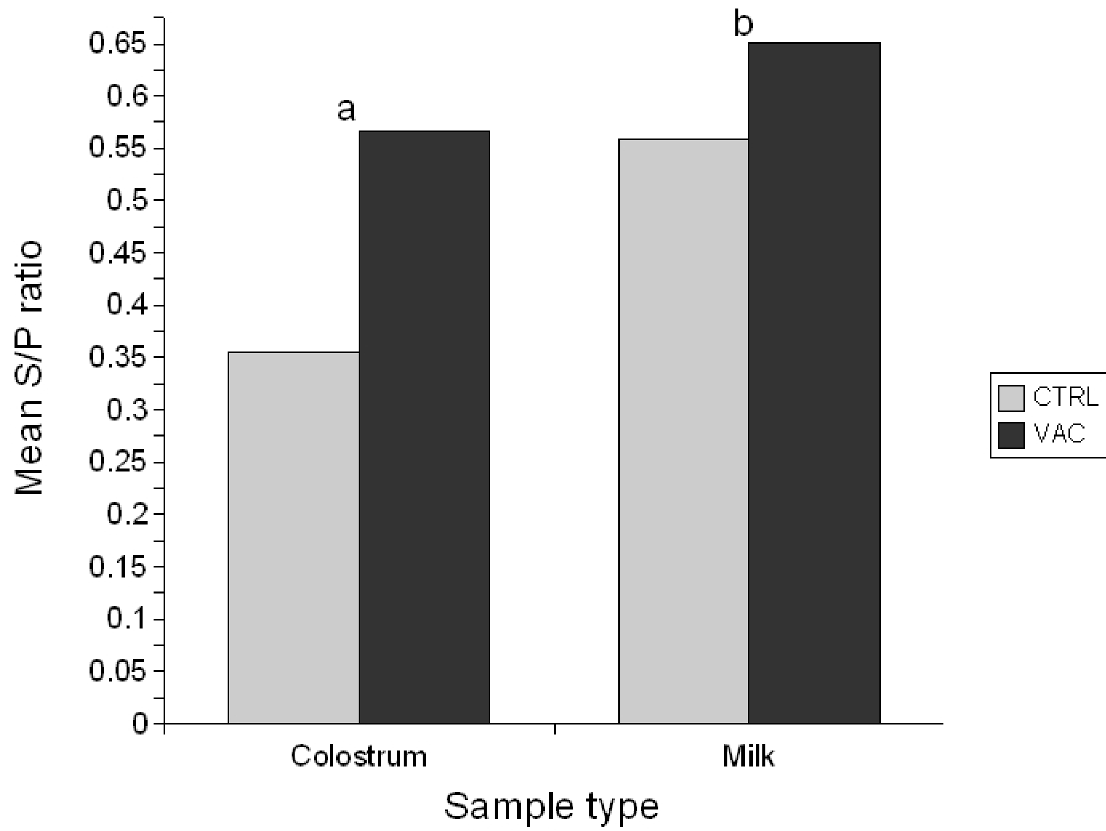


Figure 3. Maternal serum anti-*M. bovis* IgG_1 *S/P* ratios thirty-nine days prior to expected parturition and actual date of parturition (individual cow data points [■]) in 73 Holstein cows. The straight solid line represents the derived linear regression equation: Maternal serum anti-*M. bovis* IgG_1 *S/P* ratios at actual date of parturition = 0.0735 + (0.4597 X maternal serum anti-*M. bovis* IgG_1 *S/P* ratio thirty-nine days prior to expected parturition); $P < 0.001$; $r^2 = 0.273$. The curved solid lines represent the 95% confidence interval of the derived linear regression line-of-best fit.

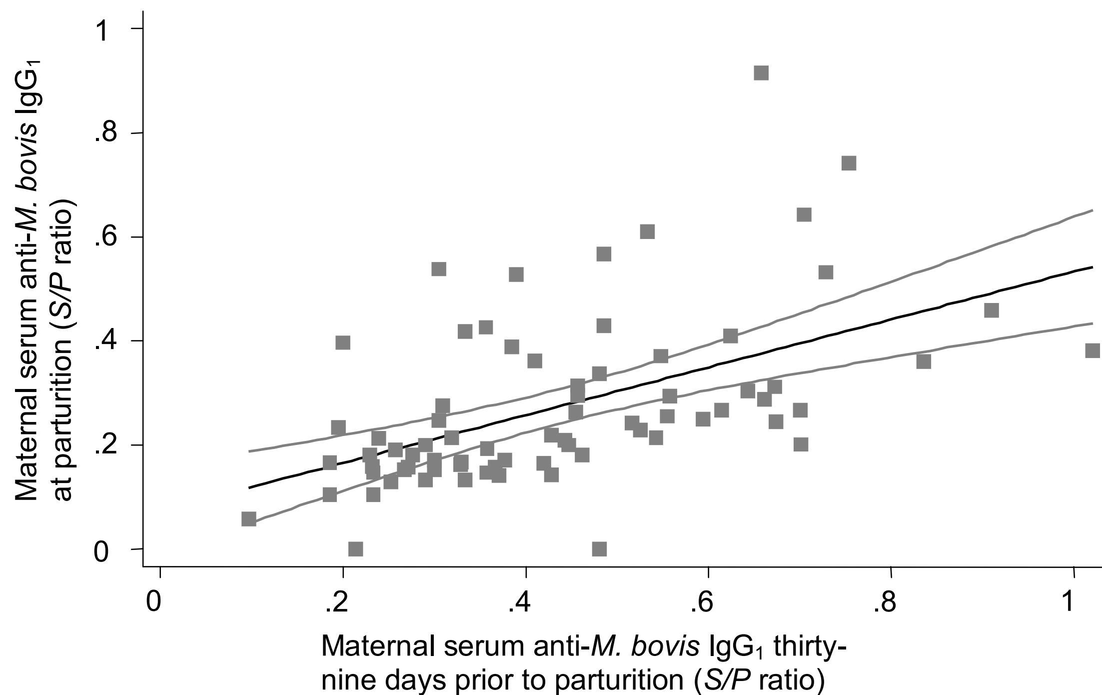


Figure 4. Maternal serum anti-*M. bovis* IgG_1 *S/P* ratios thirty-nine days prior to expected parturition and colostral anti-*M. bovis* IgG_1 *S/P* ratios (individual cow data points [■]) in 51 Holstein cows. The straight solid line represents the derived linear regression equation: Colostral anti-*M. bovis* IgG_1 *S/P* ratio= 0.0946 + (0.869 X maternal serum anti-*M. bovis* IgG_1 *S/P* ratio thirty-nine days prior to expected parturition); $P < 0.01$; $r^2 = 0.131$. The curved solid lines represent the 95% confidence interval of the derived linear regression line-of-best fit.

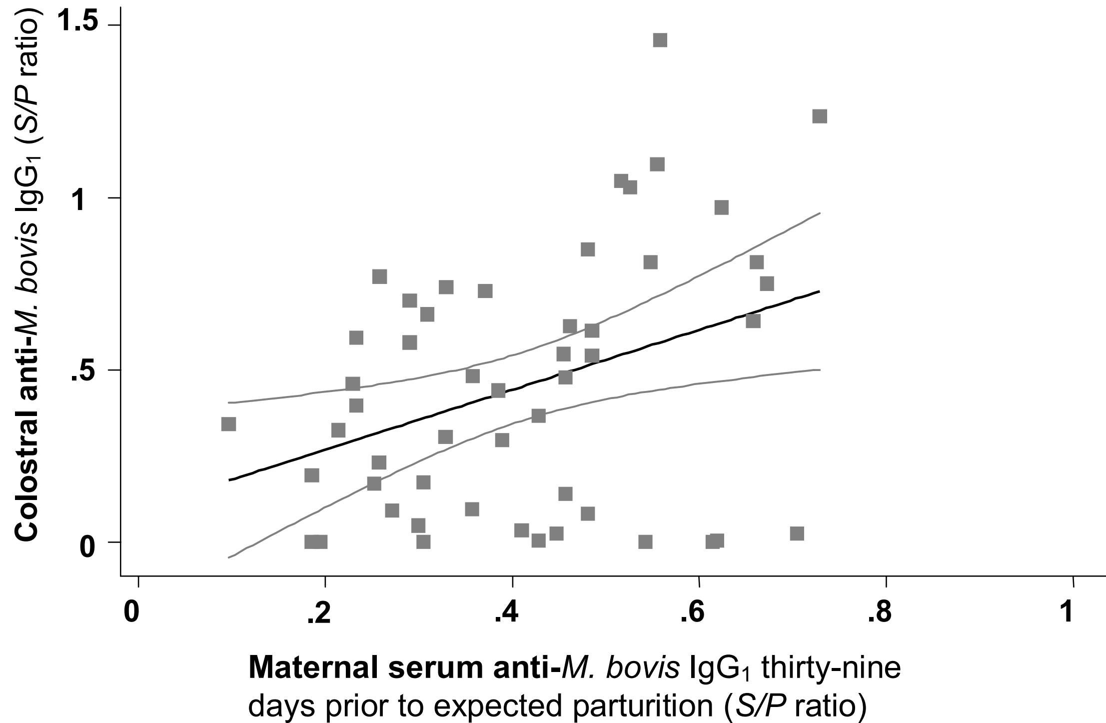


Figure 5. Mean serum S/P ratios between heifer calves in CTRL (n = 12) and VAC (n = 18) groups at 0 hours, 48 hours (2 days), and 30 days of age. Zero days represents mean serum S/P ratio of pre-colostrum sample and 2 days represents mean serum S/P ratio of post-colostrum sample. Significance designations: **a** = $p < 0.05$ compared to prior measurement for CTRL group; **b** = $p < 0.05$ compared to prior measurement for VAC group.

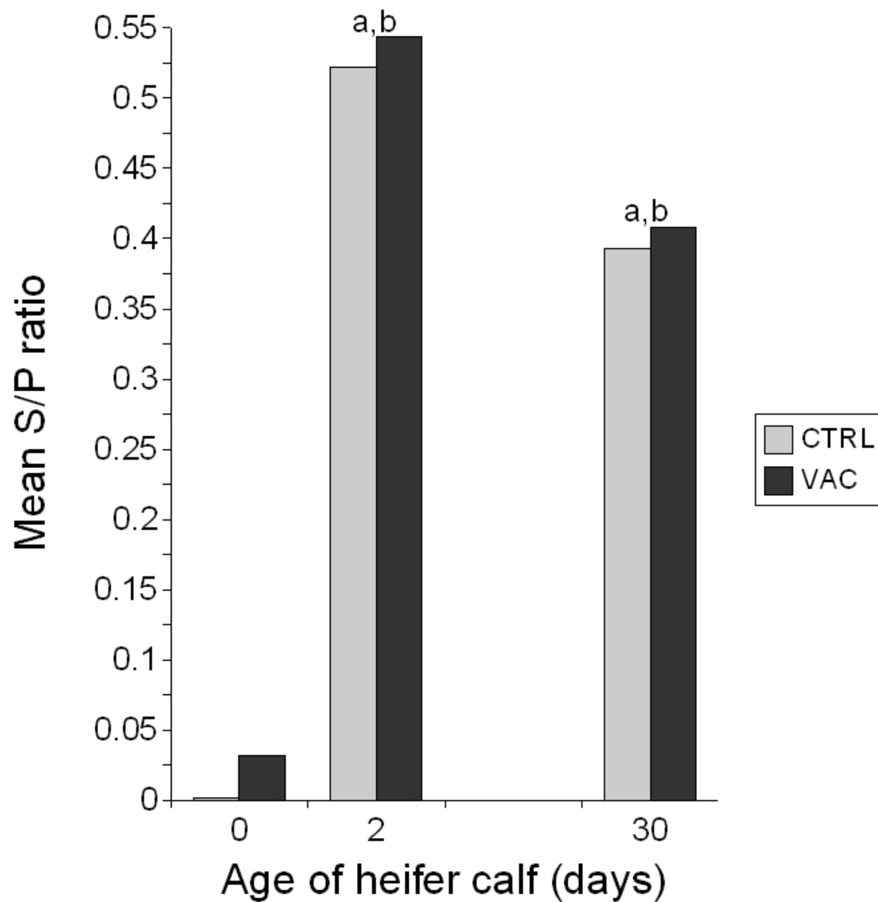


Figure 6. Maternal serum anti-*M. bovis* IgG_1 *S/P* ratios at parturition and calf serum anti-*M. bovis* IgG_1 *S/P* ratios at 48 hours of age (individual calf data points [■]) in 25 heifer calves. The straight solid line represents the derived linear regression equation: Calf serum anti-*M. bovis* IgG_1 *S/P* ratios at 48 hours of age = 0.3667 + (0.5401 X maternal serum anti-*M. bovis* IgG_1 *S/P* ratio at parturition); $P < 0.002$; $r^2 = 0.361$. The curved solid lines represent the 95% confidence interval of the derived linear regression line-of-best fit.

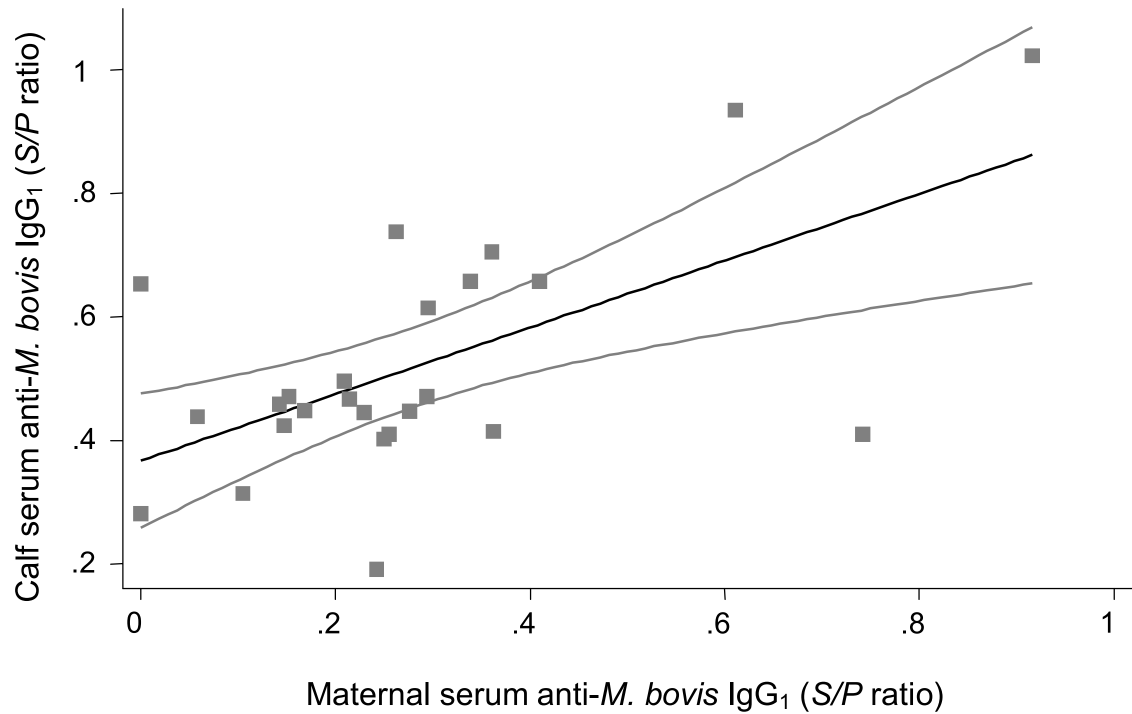


Figure 7. Maternal serum anti-*M. bovis* IgG_1 *S/P* ratios thirty-nine days prior to expected parturition and calf serum anti-*M. bovis* IgG_1 *S/P* ratios at 48 hours of age (individual calf data points [■]) in 26 heifer calves. The straight solid line represents the derived linear regression equation: Calf serum anti-*M. bovis* IgG_1 *S/P* ratios at 48 hours of age = 0.4467 + (0.3137 X maternal serum anti-*M. bovis* IgG_1 *S/P* ratio thirty-nine days prior to expected parturition); $P < 0.04$; $r^2 = 0.165$. The curved solid lines represent the 95% confidence interval of the derived linear regression line-of-best fit.

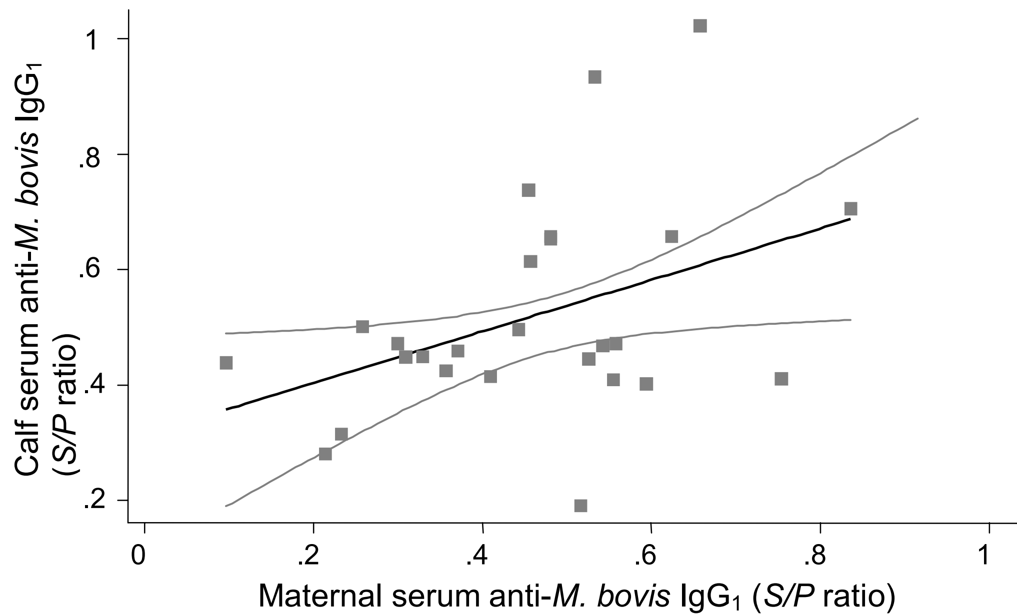
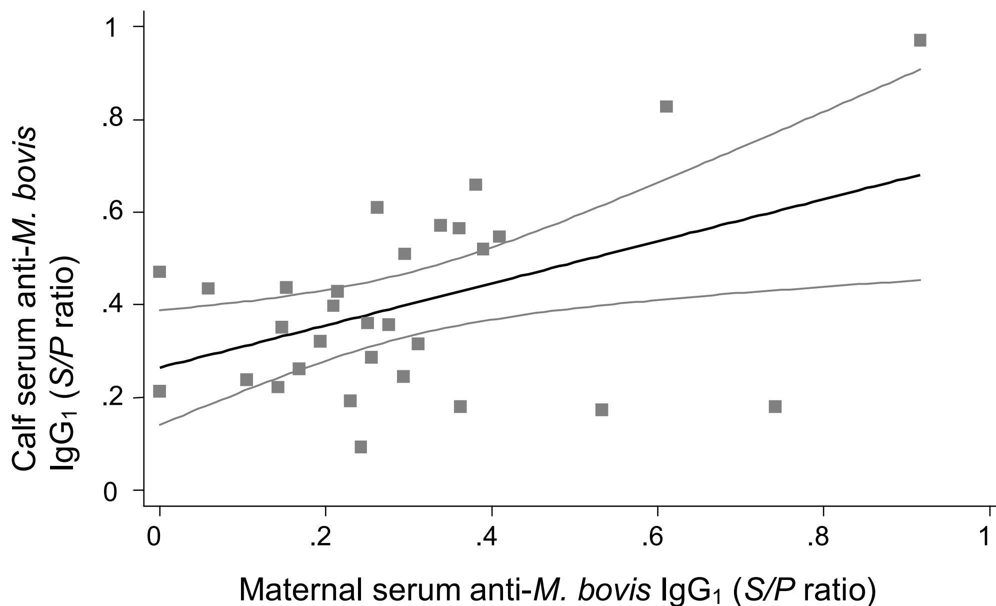


Figure 8. Maternal serum anti-*M. bovis* IgG_1 *S/P* ratios at parturition and calf serum anti-*M. bovis* IgG_1 *S/P* ratios at 30 days of age (individual calf data points [■]) in 30 heifer calves. The straight solid line represents the derived linear regression equation: Calf serum anti-*M. bovis* IgG_1 *S/P* ratios at 30 days of age = 0.4540 + (0.2646 X maternal serum anti-*M. bovis* IgG_1 *S/P* ratio at parturition); $P < 0.02$; $r^2 = 0.203$. The curved solid lines represent the 95% confidence interval of the derived linear regression line-of-best fit.



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