Review

Evolving views on bovine respiratory disease: An appraisal of selected key pathogens – Part 1

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ABSTRACT

Bovine respiratory disease (BRD) is one of the most commonly diagnosed causes of morbidity and mortality in cattle and interactions of factors associated with the animal, the pathogen and the environment are central to its pathogenesis. Emerging knowledge of a role for pathogens traditionally assumed to be minor players in the pathogenesis of BRD reflects an increasingly complex situation that will necessitate regular reappraisal of BRD pathogenesis and control. This review appraises the role of selected key pathogens implicated in BRD pathogenesis to assess how our understanding of their role has evolved in recent years.

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Introduction

Bovine respiratory disease (BRD) is one of the most commonly diagnosed causes of morbidity and mortality in cattle, both within large feedlots and traditional, smaller, pasture-based husbandry systems (Edwards, 2010; Murray et al., 2016a). The pathogenesis of BRD is often driven by complex interactions of factors associated with the animal, the pathogen and the environment which creates significant challenges in its control (Edwards, 2010).

The traditional model of primary viral infection followed by secondary bacterial opportunism has increasingly been challenged as being overly simplistic in failing to acknowledge the primary role of some pathogens that were previously considered of minor importance. The use of approaches in pathogen identification such as metagenomics has revealed the presence of some of these pathogens in bovine lungs which were rarely, or not previously, detected (Ng et al., 2015). Emerging knowledge of a potential role for these 'minor' players (e.g. bovine coronavirus, bovine rhinitis A virus) in the pathogenesis of BRD coupled with the polymicrobial nature of many cases, and the detection of many recognised BRD pathogens in the nasopharynx or lungs of healthy cattle (Caswell, 2014), complicates BRD diagnosis and blurs the precise role played by specific pathogens in eliciting disease (Virtala et al., 1996; Murray et al., 2016a). Determining the likely significance of detection of these

pathogens in diseased bovine lungs is important in interpreting pathologic analyses, and in initiating control measures, for the veterinary practitioner and veterinary pathologist alike.

This review appraises the role of key pathogens implicated in BRD pathogenesis. These pathogens have been selected on the basis of a traditional assumption of their lesser importance in BRD pathogenesis; an assumption which has been called into question by recent evidence.

Histophilus somni

Histophilus somni (formerly *Haemophilus somnus*) is a non-encapsulated gram negative bacterium associated with a number of clinical syndromes, including respiratory disease, in cattle maintained in feedlot (Gagea et al., 2006) and traditional, smaller, pasture-based husbandry systems (Murray et al., 2016a). *H. somni* pneumonia is not distinguishable clinically from other causes of BRD (Apley, 2006). Gross post-mortem findings in pneumonic animals include fibrinosuppurative bronchopneumonia often in conjunction with severe diffuse fibrinous pleuritis, although pleuritis can also occur as a solitary lesion (Saunders et al., 1980; Andrews et al., 1985). Histologically, purulent bronchiolitis, vasculitis, fibrin thrombi and haemorrhage are typically recorded in acute cases (Andrews et al., 1985).

The prevalence of *H. somni* detection post-mortem in pneumonic bovine lungs can vary considerably, with up to 40% recorded in some years (Welsh et al., 2004; Murray et al., 2016a). *H. somni* strains differ in their ability to induce pneumonia in calves (Groom

et al., 1988), may be found commensally in the genital (Kwiecien and Little, 1992) and respiratory tract, and can persist in the lung for long periods in the absence of clinical disease (Gogolewski et al., 1989). Although a nasal prevalence of 42% has been recorded in beef cattle prior to export (Moore et al., 2015), once established it appears that H. somni survives more readily in the bronchoalveolar area than on the nasal mucosa (Gogolewski et al., 1989). Following introduction into appropriate sites under favourable conditions, individual strains can change from being a commensal to either opportunistic pathogens or primary pathogens (Sandal and Inzana, 2009). In respiratory disease cases, H. somni can be found alone or in concert with other respiratory pathogens (Corbeil, 2007; Murray et al., 2016a). Synergism between H. somni and bovine respiratory syncytial virus (BRSV; Gershwin et al., 2005) has previously been reported. Interestingly, the order of infection appears to be critical with prior H. somni infection up-regulating the antiviral response of respiratory epithelial cells. This effect was mediated by lipooligosaccharide in a dose-dependent manner. This surprising result has raised the hope that nasal inoculation of at-risk cattle with isolates of H. somni from other subclinical carriers may, in the future, play a role in decreasing susceptibility to respiratory disease in a similar manner to that of gut probiotics such as Lactobacillus acidophilus (Lin et al., 2016).

While a role for H. somni in BRD has been long recognised (Gogolewski et al., 1987), emerging awareness of the virulence factors which aid its pathogenesis has highlighted the potential importance of that role. H. somni can adhere to endothelial cells causing the activation of platelets which promotes thrombus formation while lipooligosaccharide production can induce apoptosis of endothelial cells (Sylte et al., 2001; Kuckleburg et al., 2008) and help the pathogen to evade host defences through phase variation (Inzana et al., 1992). Immunoglobulin binding proteins (Ibps), which are secreted from the surface of the pathogen, bind the Fc region of IgG2b (Bastida-Corcuera et al., 1999) and may also facilitate bacterial dissemination from the lungs across the alveolar barrier; Agnes et al. (2013) proposed a mechanism for dissemination which was mediated by retraction of alveolar type 2 cells, in response to Ibp A, and degradation of the basement membrane.

Biofilm formation by H. somni has also been recognised in vitro in both commensal and pathogenic strains. Bacterial biofilms are highly structured and organised aggregates of bacteria connected by an extracellular matrix which enable bacteria to colonise and persist in sites, enhancing their resistance to antibiotics and host defence mechanisms. As there are substantial differences in the amount and architecture of biofilm formed by commensal and pathogenic strains it has been suggested that the differences in biofilm structure may correlate with pathogenicity (Sandal et al., 2007). Polymicrobial biofilms of commensal H. somni and Pasteurella multocida have been described (Elswaifi et al., 2012). Transferring-binding proteins, which allow H. somni acquire iron from host components (Ekins et al., 2004), histamine release, which causes vasoconstriction and increased permeability (Ruby et al., 2002), and the resistance to killing by phagocytes (Siddaramppa and Inzana, 2004), also contribute to H. somni survival. This latter attribute, together with its ability to destroy macrophages within hours in vivo (Gogolewski et al., 1987), has led to the suggestion by Corbeil (2007) that H. somni behaves more like an extracellular parasite than a facultative intracellular parasite which multiplies over time inside macrophages. Animal defence against H. somni appears to rely on antibody responses, particularly IgG$_2$ (Gogolewski et al., 1989; Corbeil et al., 1997), however, IgE responses are also sometimes induced and were suggested by Gershwin et al. (2005) to have a role in the pathogenesis of the more severe BRD in some animals. In studies using Western blotting, Corbeil et al. (2006) demonstrated that the predominant H. somni antigen recognised by IgE was major outer membrane protein (MOMP), a virulence factor of H. somni, the precise

functional role of which is still uncertain, but a role similar to bacterial porins has been proposed (Ueno et al., 2014).

O'Toole and Sondgeroth (2016) outlined three approaches to H. somni control in cattle: mass medication with antimicrobials, vaccination for H. somni or vaccination for other agents of the BRD complex that predispose to BRD. Mass medication with antimicrobials is discussed in detail in Part 2 of this review (Murray et al., 2016b). Relevant to mass medication is the challenge potentially posed by an apparent trend of decreasing susceptibility of H. somni and other BRD pathogens to most of the antimicrobials used for the treatment and control of BRD (DeDonder and Apley, 2015). Antimicrobial resistance in BRD pathogens, including H. somni, mediated by plasmids and other integrative and conjugative elements (ICE) was evaluated by Klima et al. (2014) in a study of isolates from US feedlots. Four of 10 H. somni lung isolates harboured multiresistant genes. They demonstrated the presence of ICE in H. somni and the capacity for transfer of ICE from H. somni to P. multocida. Although plasmids have not been frequently isolated from H. somni, 15% of H. somni isolates from pneumonic lungs in one study in Denmark contained plasmids (Fussing and Wegener, 1993). In contrast, no plasmids were observed in any of 606 nasal isolates of H. somni from randomly selected healthy animals in a feedlot study by D'Amours et al. (2011). This led them to suggest that strains having plasmids may have virulence genes although this has not yet been proven. Portis et al. (2012) recorded a decrease in the proportion of susceptible H. somni isolates, over a 10-year period from 2000 to 2009, which was most notable with enrofloxacin, florfenicol and tetracycline. Their report, which examined isolates from both sick and deceased animals, suggested that H. somni resistance was relatively low. This apparent divergence from the findings of Klima et al. (2014) may be, in part, due to selection bias towards resistant pathogens in studies based on samples from BRD fatalities.

Although vaccination against H. somni is widely practised in the U.S. the efficacy of H. somni vaccination is uncertain or unproven (Larson and Step, 2012; O'Toole and Sondgeroth, 2016). Most H. somni vaccines use killed cells or specific outer membrane proteins to induce immunity. Research to identify new, and effective, vaccine epitopes continues with some encouraging results. Geertsema et al. (2011) reported significantly reduced gross and microscopic lung lesion scores in calves inoculated twice with the Ibp A direct repeat (DR) 2 subunit following experimental inoculation with pathogenic H. somni strain 2336. This subunit also induced lower IgE antigen-specific responses than Ibp A3 and Ibp A5, which were also assessed. The findings of Lo et al. (2012) also suggested that Ibp A DR2 would be a useful addition to H. somni vaccines.

An additional challenge in controlling H. somni-induced BRD is the identification of clinically infected animals; however, recent developments in this area are particularly noteworthy. Exopolysaccharide (EPS) is a major component of the H. somni biofilm matrix and is most abundantly produced when a biofilm is formed but is also produced under growth-restricting stress conditions which are likely to occur during the disease process or during colonisation of mucosal epithelia (Sandal et al., 2011; Pan et al., 2014). As EPS antigen is expressed predominantly during active disease, Pan et al. (2014) have recently used purified EPS in a direct ELISA to differentiate animals (both naturally and experimentally infected) with H. somni disease from healthy commensally infected animals; sensitivity of 90.5% and specificity of 92.5% were reported 3 weeks post infection. Lo et al. (2012) have also recently reported on IbpA subunit antigens which could potentially be deployed in a similar manner.

In conclusion, H. somni is now recognised as a significant BRD pathogen of relatively high prevalence which poses many challenges to the implementation of effective BRD control measures. Recent research addressing the diagnostic challenges in the

live animal is likely to be of benefit in future BRD control, however, further research addressing the efficacy of vaccination is required.

Pasteurella multocida

P. multocida is a gram-negative coccobacillus, classified into five capsular serogroups (A, B, D, E, F; Carter, 1955, 1961) and 16 sero-types (Heddleston et al., 1972). It has been isolated from many different species, is potentially zoonotic (Miyoshi et al., 2012) and has been implicated in a number of different diseases, including BRD. *P. multocida* isolates from serogroup A may exist as either commensals or pathogens in the bovine respiratory tract and A: 3 is most commonly isolated from respiratory disease with a smaller isolation rate for D: 3 (Harper et al., 2006; Sellyei et al., 2015). Gross post-mortem findings in animals with *P. multocida* pneumonia are typically described as acute bronchopneumonia characterised by well-demarcated deep red consolidation with an anteroventral distribution. Abscessation can occur which is potentially responsible for poor antimicrobial efficacy and may act as a source for recrudescence of infection. Histological lesions in acute cases typically include the infiltration of neutrophils into the alveolar and bronchiolar air spaces and extensive yet scattered areas of alveolar oedema. Fibrin may be abundant in the alveoli with occasional multinucleate giant cells also visible in the alveolar space (Dagleish et al., 2010). Although considered highly infectious, *P. multocida* is not considered to be highly contagious (Taylor et al., 2010).

Reported respiratory prevalence of *P. multocida* in various published studies varies considerably due to differences in the disease status, age and enterprise type of the study animals, the sample type harvested, and the testing method employed. Autio et al. (2007), using bacteriological culture of tracheobronchial lavage samples of dairy calves aged less than 3 months, isolated *P. multocida* from approximately 42% of diseased calves and 26% of healthy calves; Hotchkiss et al. (2010) recorded 17% prevalence in nasal swabs of healthy beef calves aged less than 10 weeks using polymerase chain reaction (PCR), while Murray et al. (2016a) in a post-mortem survey of weanlings, both dairy and beef, between 6 and 12 months of age identified *P. multocida* in 37.9% of lungs with lesions and 14.3% of those without, using a combination of bacteriological culture and PCR. Welsh et al. (2004) reported an apparent increasing prevalence of *P. multocida* in a survey of lungs examined post-mortem over 8 years although the findings of McClary et al. (2011) did not support this.

Co-infections of *P. multocida* with other respiratory pathogens in diseased animals are commonly recorded (Autio et al., 2007; Murray et al., 2016a). Synergism with *Mycoplasma bovis* has been proposed (Virtala et al., 1996) and frequent detection of *P. multocida* with *Mycoplasma*-like organisms or bovine parainfluenza virus-3 has been reported (Hotchkiss et al., 2010; Murray et al., 2016a).

There has been some debate regarding the ability of *P. multocida* to assume a primary role in BRD pathogenesis. The plausibility of such a role is supported by the discovery of several virulence factors which help it to evade host defences including outer membrane proteins (e.g. OmpA) and type IV fimbriae which may be responsible for adherence to host cells (Glorioso et al., 1982; Dabo et al., 2003), lipopolysaccharide (LPS) which plays a role in the disease process by interacting with innate host immune defences through toll-like receptors (Harper et al., 2011), and a capsule which plays a role in resisting phagocytosis by host cells and complement-mediated lysis (Harmon et al., 1991). Biofilm can be formed by *P. multocida*, and polymicrobial biofilms with *H. somni* are also reported (Elswaifi et al., 2012). *P. multocida*, in common with other gram-negative bacteria, possesses outer membrane vesicles containing virulence factors such as toxins, enzymes (including β-lactamases) and adhesions which can be released into the surrounding medium by commensal and pathogenic strains (Sellyei et al., 2009; Amano et al., 2010). Among other roles, these outer membrane vesicles are considered to play a part in biofilm formation (Schooling and Beveridge, 2006).

Although various studies have recorded generally mild clinical disease following experimental intra-tracheal inoculation of calves with *P. multocida* (Gourlay et al., 1989; Dagleish et al., 2010), such studies, which circumvent upper respiratory tract defences while challenging animals with a pathogen load which may exceed what is likely in the natural infection, do not provide sufficient evidence of a primary role for *P. multocida* in BRD. Recently, Taylor et al. (2015) recorded a decreased risk of treatment for BRD, yet also modestly decreased performance, in calves from which *P. multocida* was detected in the upper respiratory tract. They suggested that these conflicting results might be due to the reliance of *P. multocida* on the presence of other agents or risk factors in BRD pathogenesis. Autio et al. (2007) also found that an association between the isolation of *P. multocida* from tracheobronchial lavage samples from dairy calves in BRD outbreaks in Finland and clinical respiratory disease was not significant when other pathogens were absent. In contrast Nikunen et al. (2007) identified an association between the presence of *P. multocida* in tracheobronchial lavage fluids and both clinical signs of BRD and raised acute phase proteins, in natural outbreaks among dairy breed calves in dairy herds and fattening units in Finland. They concluded that the association indicated a strong pathogenic role for *P. multocida* in BRD when other recognised pathogens were absent. Such an association does not prove causation and although a significant role for *P. multocida* in BRD appears undeniable, conclusive evidence of a primary role for *P. multocida* is still awaited.

Control of *P. multocida* in Europe is complicated by the absence of a licensed vaccine for cattle. In the US, where vaccination against *P. multocida* has been performed, reports of proven field efficacy are weak or inconsistent (Perino and Hunsaker, 1997; Larson and Step, 2012). The role of vaccination as a control measure for BRD is discussed further in Part 2 of this review (Murray et al., 2016b). Mass medication of animals with antimicrobials (i.e. metaphylaxis or prophylaxis) is also employed in the control of BRD associated with *P. multocida*. Relevant to this are reports of the prevalence of resistant isolates which also differ between North America and Europe. Although the occurrence of resistant isolates of bacterial pathogens associated with BRD appears to be steadily increasing (DeDonder and Apley, 2015) antimicrobial susceptibility of *P. multocida* for almost all licensed antibiotics for BRD in Europe is very high (de Jong et al., 2014). In the US, a survey of isolates from necropsied animals over an 8 year period from 1994 to 2002 reported a decline in *P. multocida* susceptibility to florfenicol from 100% to 86% in the first 6 years after it came into use (Welsh et al., 2004). Portis et al. (2012) reported a decrease in the numbers of *P. multocida* isolates (from clinically ill and necropsied animals) in US and Canadian diagnostic laboratories which were susceptible to tilmicosin between 2000 and 2009 and a three-fold increase in the MIC_{90} for tulathromycin over a 6 year period from 2004 to 2009. Resistance of *P. multocida* to the aminoglycosides and tetracyclines has been regularly reported (de Jong et al., 2014; Jamali et al., 2014). It is interesting to note that ceftiofur, a third generation cephalosporin that is resistant to the β-lactamases produced by *P. multocida*, has maintained its effectiveness since its introduction in 1988 (Watts and Sweeney, 2010). Kadlec et al. (2011), in publishing the first report on the genetics of macrolide, triamilide and lincosamide resistance in *P. multocida*, observed that the three genes implicated were also present in other bacteria which supported the assumption that *P. multocida* is able to acquire plasmid-borne resistance genes from other gram-negative bacteria.

In conclusion, *P. multocida* is an important pathogen in BRD but its primary role remains unproven. Nevertheless, while assuming

an opportunistic role for this pathogen but also recognising its many virulence factors and prevalence in BRD co-infections, the identification of suitable antigen candidates for effective vaccines is still of significant importance if control is to be achieved.

Bovine coronavirus

Bovine coronavirus (BCoV) is sometimes shed by healthy cattle (Crouch et al., 1985) but is also associated with three recognised clinical syndromes: calf enteritis, adult winter dysentery and BRD. While studies have identified antigenic and genetic differences between bovine enteric coronaviruses and bovine respiratory coronaviruses (Hasoksuz et al., 1999a), all strains belong to a single serotype (Hasoksuz et al., 1999b) and there is cross protection between strains (Cho et al., 2001). BCoV strains appear to be diverging over time from an enteric tropism to a dual enteric and respiratory tropism (Kanno et al., 2007; Park et al., 2007; Fulton et al., 2013).

Seasonal variation in the incidence of respiratory BCoV infections has been reported with peak incidence recorded between November and May in Ireland (O'Neill et al., 2014). Stress plays a significant role in the dissemination of infection, with stressors such as the comingling or transport of cattle (Fulton et al., 2011) identified as triggers for viral replication and shedding. Shedding of BCoV can be prolonged, particularly from the faeces, but shedding does not necessarily indicate transmission potential (Oma et al., 2016). Mortality associated with BRD outbreaks in which BCoV has been identified can be high (Storz et al., 2000; Decaro et al., 2008).

The precise role of BCoV in BRD pathogenesis remains the subject of much debate. Storz et al. (2000), in a study of 26 fatalities (25 of which shed nasal BCoV) and 18 clinically normal controls, which were BCoV isolation-negative, claimed that Evan's criteria (Evans, 1976) for causation were satisfied. Published studies since then have supported some of the criteria on which this claim is based – the virus has been identified at high rates from respiratory secretions and lung samples during the pathogenesis of BRD (Hick et al., 2012; O'Neill et al., 2014); animals with high serological titres to BCoV tend to shed BCoV at a lower rate (Thomas et al., 2006) and the primary virus specific immune response to respiratory BCoV is IgM with higher IgG2 responses after the first week of infection while in fatal cases only IgM responses were detected (Lin et al., 2000). However, their claims that BCoV was not isolated from clinically normal cattle have been contradicted by the findings of Hasoksuz et al. (1999a) and Fulton et al. (2011). Furthermore, demonstrating that the elimination of the virus factor prevents or decreases the severity of disease has also proved elusive and still awaits the development of an effective vaccine. O'Connor et al. (2001) in a study of 852 animals on arrival at three feedlots found that higher BCoV arrival titres but not titre changes after arrival reduced BRD risk. They suggested that arrival titres to BRD pathogens could be interpreted as evidence of healthy animals capable of mounting an effective immune response rather than evidence of BCoV-specific protection per se. Furthermore, they argued that if increased weight, in terms of causal inference, was given to exposures that occurred concurrent to, rather than prior to, disease occurrence, the findings of Storz et al. (2000) could be explained by the confounding effect of other pathogens, rather than evidence of causality.

Since the report by Storz et al. (2000), other studies have contributed evidence of a role for BCoV in BRD although the centrality of that role remains somewhat unclear. In a study of 837 calves from four feedlots, Lathrop et al. (2000) reported that animals that shed BCoV were at increased odds of pulmonary lesions (OR 2.2, 95% CI 1.12–4.32) and that seroconversion to BCoV during the first 28 days was associated with reduced odds (0.59; 0.42–0.84) of treatment

for BRD but only in those animals that did not shed BCoV. Plummer et al. (2004), in a randomised trial with 414 heifers, examined the effect of intranasal modified live BCoV vaccination, nasal BCoV shedding and BCoV serological titres on entry to the feedlot on the risk of treatment for BRD. Intranasal vaccination was found to be protective ($P = 0.008$) and nasal shedding of BCoV increased BRD treatment risk ($P = 0.009$).

Results from natural outbreaks have also supported a role for BCoV in BRD pathogenesis. Hick et al. (2012) detected BCoV by reverse transcriptase quantitative PCR (RT-qPCR) in 9-month-old weanlings presenting with BRD in a paddock-based feedlot system; BCoV was identified in nasal samples from 10/30 acutely affected animals and in the lungs of 2/15 with BRD lesions at slaughter. Other viruses were not detected in these animals and bronchointerstitial pneumonia was diagnosed on histopathology. Decaro et al. (2008) reported on four outbreaks involving young calves, both dairy and beef breeds, aged 2–3 months in Italy. BCoV was detected by RT-PCR on a high proportion of nasal swabs from all four outbreaks; no other BRD virus or *Mycoplasma* spp. was identified in three of the four outbreaks. In two of these outbreaks concurrent respiratory and enteric signs were recorded with nasal and faecal detection of BCoV.

On balance, epidemiological evidence of BCoV induced BRD is persuasive, however, studies demonstrating BCoV antigen in pneumonic lesions are lacking and would provide more conclusive evidence. Park et al. (2007) demonstrated BCoV antigen in the cytoplasm of degenerated and necrotic epithelial cells of the nasal turbinates, trachea and lungs with concurrent interstitial pneumonia using immunohistochemistry (IHC) in calves experimentally infected orally with a winter dysentery strain of BCoV; respiratory clinical signs were not recorded however. Histological changes including multinucleate syncytial epithelial cells identified in the bronchiolar lumina and bronchus associated lymphoid tissue (BALT) hyperplasia have been ascribed to BCoV in a natural outbreak by Hick et al. (2012) based on PCR rather than IHC detection.

Control of the disease is challenging as respiratory BCoV vaccines have not been developed and while Plummer et al. (2004) reported that intranasal use of modified live BCoV vaccine reduced the risk of treatment for BRD in calves, differences between the strain used in the enteric BCoV vaccine and those strains isolated from BRD cases suggest that the vaccine may not be fully protective against the BCoV isolates in circulation (Fulton et al., 2013). Plummer et al. (2004) assumed that the effect of vaccination was mediated by IgA but further research is required to confirm the factors which confer mucosal protection. Considering the apparent changing tropism of BCoV strains (Kanno et al., 2007) coupled with BCoV transmission by either the nasal or faecal-oral route and considering that experimental oral infection can lead to viral infection of respiratory epithelial cells (Park et al., 2007) it is likely that any effective vaccine will need to confer concurrent enteric and nasal mucosal immunity. Control is further complicated by the potential for re-infection of the same animal and persistence of infection in both the upper respiratory tract (Heckert et al., 1991) and in intestinal tissues and lymph nodes (Oma et al., 2016) of infected animals possibly for weeks after infection.

Bovine adenovirus 3

Bovine adenoviruses (BAdV) were first identified by Klein et al. (1959) in the faeces of a healthy cow. Since then, 10 serotypes have been identified in cattle which are distributed between two subgroups – the *Mastadenoviruses* and the *Atadenoviruses* (Benko et al., 2000). Although BAdV-10 is unlike other BAdV species, it displays certain characteristics of *Mastadenoviruses* (Matiz et al., 1998). BAdVs appear to have both respiratory and enteric tropisms (Mattson et al.,

1977; Reed et al., 1978) but many serotypes are of uncertain significance as pathogens (Sibley et al., 2011). Shedding of multiple BAdV genotypes by cattle is prevalent such that, considering the environmental stability of the AdV virion, BAdVs have been suggested as conservative indicators of the presence of viral or faecal contamination in aqueous environments (Sibley et al., 2011).

Transmission of BAdV-3 is facilitated by enhanced survival in aerosols in conditions of low temperatures and high relative humidity (Elazhary and Derbyshire, 1979) and within infected herds seropositivity to BAdV-3 is often high (Mattson et al., 1988; Sibley et al., 2011). Indeed, the apparent low incidence of clinical disease, coupled with the ability of BAdV-3 to infect both dividing and non-dividing cells and to induce both humoral and cell mediated immunity, has led to a focus on the potential use of BAdV-3, with genetic modification of capsid proteins, as a vaccine delivery vehicle in cattle and other animals (Ayalew et al., 2015).

The pathogenic significance of BAdV-3 remains uncertain. In a study of feedlot calves during the first 56 days on feed, Mattson et al. (1988) reported an association between BAdV-3 natural infection and seroconversion with pyrexia, but not with weight gain. Ng et al. (2015), using viral metagenomics on nasopharyngeal swabs taken from 100 dairy calves aged between 4 and 8 weeks in a BRD case–control study, showed that the detection of BAdV-3 was significantly ($P < 0.0001$) associated with BRD. In Turkey, Ceribasi et al. (2014), using direct fluorescent antibody technique and immunoperoxidase staining of pneumonic lungs from 247 cattle identified with lung lesions at slaughter, recorded BAdV3 antigen specific staining in infiltrating peribronchiolar mononuclear cells and in bronchiolar and alveolar epithelial cells generally in the pneumonic areas of the lung. No immunopositive or fluorescence staining was detected in the negative controls. Degeneration of bronchial and bronchiolar epithelium and mononuclear cell infiltration have been recorded as consistent histological features in these diseased lungs.

The apparent subclinical infection of some animals coupled with the sometimes severe pathological changes observed in others has led to uncertainty regarding the co-factors responsible for BAdV-associated BRD in cattle. Immunological or genetic factors may be implicated in the progression of disease. Based on experimental inoculation, differences in immune system development (i.e. development of cell-mediated immunity) between neonatal and older calves appear to strongly influence immunopathological reactions to BAV-3 (Narita et al., 2002; Yamada et al., 2003).

In summary, recent studies have added weight to claims of a role for BAdV-3 in some BRD outbreaks but further investigation of the potential immunological or genetic factors that influence the development or severity of disease is warranted.

Influenza D virus

Hause et al. (2013) described an influenza virus isolated from swine in 2011 with moderate homology to Influenza C virus which was provisionally designated Influenza D virus (IDV; Hause et al., 2014). IDV is a single strand, negative sense RNA virus belonging to the family *Orthomyxoviridae*. Since 2011, IDV has been identified in many countries (Hause et al., 2013; Jiang et al., 2014; Chiapponi et al., 2016) with a homology between strains which suggests that IDV has a global distribution (Collin et al., 2015). Cattle have been proposed as the natural host of the virus (Ferguson et al., 2016) while evidence of infection has been detected in other species, including humans (Hause et al., 2013; Quast et al., 2015; White et al., 2016). Studies of transmission dynamics of IDV have shown that IDV can be transmitted efficiently between cattle by direct contact (Ferguson et al., 2016). IDV appears to be present in cattle populations for some time and the seroprevalence of IDV in cattle is relatively high with 15.9% prevalence reported by Ferguson et al. (2015) in a retrospective survey (2004–2006) of 6–8 month old calves

and cows in the US. They also showed that antigenically distinct clusters of IDV may be detected co-circulating in cattle populations at the same time.

Collin et al. (2015) proposed a primary role for IDV in BRD pathogenesis based on five of 10 IDV quantitative real-time PCR (qRT-PCR) positive clinical BRD samples (swabs or lung tissue) having no other BRD virus detected; however, bacteriology was not reported in this study nor were control animals used for comparison. Ferguson et al. (2015) recorded nasal or nasopharyngeal swab prevalence by qRT-PCR of 29.1% among sick and 2.4% among healthy 6–9 month old beef calves but acknowledged that the contribution of IDV to the BRD recorded was unknown. Ferguson et al. (2016) recorded only mild disease associated with experimental infection of 4 month old male dairy calves. They suggested that their findings, together with those of Ng et al. (2015), which identified IDV at a high prevalence (14%) using viral metagenomics in 4–8 week old dairy calves with BRD but not in 50 healthy controls, supported a facilitator role rather than a primary role for IDV in BRD. In the lungs, they detected IDV nucleic acid by qRT-PCR but not IDV antigen using IHC which was suggestive of IDV causing an upper respiratory tract infection.

In conclusion, research into IDV pathogenesis and its role in BRD continues. Early indications suggest a relatively prevalent virus in cattle populations which is unlikely to play a primary role in BRD outbreaks but which may facilitate disease caused by other BRD pathogens.

Bovine rhinitis A virus

Bovine rhinitis A virus (BRAV), a single-stranded positive-sense RNA virus, is a species of the genus Aphthovirus in the family *Picornaviridae*. Two serotypes have been identified – BRAV 1 and BRAV 2.

Although BRAV was first identified in 1962, relatively little is known about its role in BRD pathogenesis (Hause et al., 2015). Hussain and Mohanty (1979) recorded mild respiratory disease following the experimental inoculation of 6–8 week old dairy bull calves by multiple routes with the virus. Isolation of the virus from healthy animals has also been recorded (Mohanty and Lillie, 1968), however, Ng et al. (2015) reported a significant association between the detection of BRAV in nasal secretions of dairy calves and BRD; a nasal prevalence of 30% was recorded while recognised BRD viruses were not detected. Hause et al. (2015) recorded bovine rhinitis virus [either BRAV or bovine rhinitis B virus (BRBV)] in 6.4% of nasopharyngeal or lung samples from BRD cases and through metagenomic sequencing of a subset of these showed that co-infections with multiple serotypes of bovine rhinitis are common in cattle. Seroprevalence studies, though few, have recorded high prevalence of antibodies to BRAV among cattle (Mohanty and Lillie, 1968).

Much work remains in determining the significance of these findings. Hause et al. (2015) readily acknowledged that further research is needed to determine if bovine rhinitis viruses are pathogenic or commensal viruses.

Conclusions

In light of evolving knowledge regarding the role of specific pathogens in BRD pathogenesis we need to regularly reappraise our understanding of BRD pathogenesis and control. Emerging knowledge of pathogens traditionally assumed to play minor roles in BRD shows us that the 'playing field' for BRD is more crowded than previously thought and that the potential role of these pathogens, either alone or in concert with other BRD pathogen infections, should not be discounted.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Agnes, J.T., Zekarias, B., Shao, M., Anderson, M.L., Gershwin, L.J., Corbeil, L.B., 2013. Bovine respiratory syncytial virus and *Histophilus somni* interaction at the alveolar barrier. Infection and Immunity 81, 2592–2597.

Amano, A., Takeuchi, H., Furuta, N., 2010. Outer membrane vesicles function as offensive weapons in host-parasite interactions. Microbes and Infection 12, 791–798.

Andrews, J.J., Anderson, T.D., Slife, L.N., Stevenson, G.W., 1985. Microscopic lesions associated with the isolation of *Haemophilus somnus* from pneumonic bovine lungs. Veterinary Pathology 22, 131–136.

Apley, M., 2006. Bovine respiratory disease pathogenesis, clinical signs, and treatment in lightweight calves. Veterinary Clinics of North America Food Animal Practice 22, 399–411.

Autio, T., Pohjanvirta, T., Holopainen, R., Rikula, U., Pentikainen, J., Huovilainen, A., Rusanen, H., Soveri, T., Sihvonen, L., Pelkonen, S., 2007. Etiology of respiratory disease in nonvaccinated, non-medicated calves in rearing herds. Veterinary Microbiology 119, 256–265.

Ayalew, L.E., Kumar, P., Gaba, A., Makadiya, N., Tikoo, S.K., 2015. Bovine adenovirus-3 as a vaccine delivery vehicle. Vaccine 33, 493–499.

Bastida-Corcuera, F.D., Bastida-Corcuera, F.D., Nielsen, K.H., Corbeil, L.B., 1999. Binding of bovine Ig2a and Ig2b allotypes to Protein A, Protein G and *Haemophilus somnus* IgBPs. Veterinary Immunology and Immunopathology 71, 143–149.

Benko, M., Harrach, B., Russell, W.C., 2000. Family Adenoviridae. In: Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R., et al. (Eds.), Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, New York, pp. 227–238.

Carter, G.R., 1955. Studies on *Pasteurella multocida*. I. A hemagglutination test for the identification of serological types. American Journal of Veterinary Research 16, 481–484.

Carter, G.R., 1961. A new serological type of *Pasteurella multocida* from Central Africa. Veterinary Record 73, 1052.

Caswell, J.L., 2014. Failure of respiratory defenses in the pathogenesis of bacterial pneumonia of cattle. Veterinary Pathology 51, 393–409.

Ceribasi, A.O., Ozkaraca, M., Ceribasi, S., Ozer, H., 2014. Histopathologic, immunoperoxidase and immunofluorescent examinations on natural cattle pneumonia originated from Parainfluenza type 3, Respiratory Syncytial virus, Adenovirus type 3 and Herpesvirus type 1. Revue de Médecine Véterinaire 165, 201–212.

Chiapponi, C., Faccini, S., De Mattia, A., Baioni, L., Barbieri, I., Rosignoli, C., Nigrelli, A., Foni, E., 2016. Detection of influenza d virus among swine and cattle, Italy. Emerging Infectious Diseases 22, 352–354.

Cho, K.O., Hasoksuz, M., Nielsen, P.R., Chang, K.O., Lathrop, S., Saif, L.J., 2001. Cross-protection studies between respiratory and calf diarrhea and winter dysentery coronavirus strains in calves and RTPCR and nested PCR for their detection. Archives of Virology 146, 2401–2419.

Collin, E.A., Sheng, Z., Lang, Y., Ma, W., Hause, B.M., Li, F., 2015. Cocirculation of two distinct genetic and antigenic lineages of proposed influenza D virus in cattle. Journal of Virology 89, 1036–1042.

Corbeil, L.B., 2007. *Histophilus somni* host-parasite relationships. Animal Health Research Reviews 8, 151–160.

Corbeil, L.B., Bastida-Corcuera, F.D., Beveridge, T.J., 1997. *Haemophilus somnus* immunoglobulin binding proteins and surface fibrils. Infection and Immunity 65, 4250–4257.

Corbeil, L.B., Arnold, K.F., Kimball, R., Berghaus, L., Gershwin, L.J., 2006. Specificity of IgG and IgE antibody responses to Haemophilus somnus infection of calves. Veterinary Immunology and Immunopathology 15, 191–199.

Crouch, C.F., Bielefeldt Ohmann, H., Watts, T.C., Babiuk, L.A., 1985. Chronic shedding of bovine enteric coronavirus antigen-antibody complexes by clinically normal cows. Journal of General Virology 66, 1489–1500.

de Jong, A., Thomas, V., Simjee, S., Moyaert, H., El Garch, F., Maher, K., Morrissey, I., Butty, P., Klein, U., Marion, H., et al., 2014. Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased cattle and pigs across Europe: The VetPath study. Veterinary Microbiology 172, 202–215.

Dabo, S.M., Confer, A.W., Quijano-Blas, R.A., 2003. Molecular and immunological characterization of *Pasteurella multocida* serotype A:3 OmpA: Evidence of its role in *P. multocida* interaction with extracellular matrix molecules. Microbial Pathogenesis 35, 147–157.

Dagleish, M.P., Finlayson, J., Bayne, C., MacDonald, S., Sales, J., Hodgson, J.C., 2010. Characterization and time course of pulmonary lesions in calves after intratracheal infection with *Pasteurella multocida* A:3. Journal of Comparative Pathology 142, 157–169.

D'Amours, G.H., Ward, T.I., Mulvey, M.R., Read, R.R., Morck, D.W., 2011. Genetic diversity and tetracycline resistance genes of *Histophilus somni*. Veterinary Microbiology 150, 362–372.

Decaro, N., Campolo, M., Desario, C., Cirone, F., D'Abramo, M., Lorusso, E., Greco, G., Mari, V., Colaianni, M.L., Elia, G., et al., 2008. Respiratory disease associated with bovine coronavirus infection in cattle herds in Southern Italy. Journal of Veterinary Diagnostic Investigation 20, 28–32.

DeDonder, K.D., Apley, M.D., 2015. A literature review of antimicrobial resistance in pathogens associated with bovine respiratory disease. Animal Health Research Reviews 16, 125–134.

Edwards, T.A., 2010. Control methods for bovine respiratory disease for feedlot cattle. Veterinary Clinics of North America Food Animal Practice 26, 273–284.

Ekins, A., Bahrami, F., Sijercic, A., Maret, D., Niven, D.F., 2004. *Haemophilus somnus* possesses two systems for acquisition of transferrin-bound iron. Journal of Bacteriology 186, 4407–4411.

Elazhary, M.A.S.Y., Derbyshire, J.B., 1979. Aerosol stability of bovine adenovirus type 3. Canadian Journal of Comparative Medicine 43, 305–312.

Elswaifi, S.F., Scarratt, W.K., Inzana, T.J., 2012. The role of lipooligosaccharide phosphorylcholine in colonization and pathogenesis of *Histophilus somni* in cattle. Veterinary Research 43, 49–62.

Evans, A.S., 1976. Causation and disease: the Henle-Koch postulates revisited 1. Yale Journal of Biology and Medicine 49, 175–195.

Ferguson, L., Eckard, L., Epperson, W.B., Long, L.P., Smith, D., Huston, C., Genova, S., Webby, R., Wan, X.F., 2015. Influenza D virus infection in Mississippi beef cattle. Virology 486, 28–34.

Ferguson, L., Olivier, A.K., Genova, S., Epperson, W.B., Smith, D.R., Schneider, L., Barton, K., McCuan, K., Webby, R.J., Wan, X.-F., 2016. Pathogenesis of Influenza D Virus in Cattle. Journal of Virology 90, 5636–5642.

Fulton, R.W., Step, D.L., Wahrmund, J., Burge, L.J., Payton, M.E., Cook, B.J., Burken, D., Richards, C.J., Confer, A.W., 2011. Bovine coronavirus (BCV) infections in transported commingled beef cattle and sole-source ranch calves. Canadian Journal of Veterinary Research 75, 191–199.

Fulton, R.W., Ridpath, J.F., Burge, L.J., 2013. Bovine coronaviruses from the respiratory tract: Antigenic and genetic diversity. Vaccine 31, 886–892.

Fussing, V., Wegener, H.C., 1993. Characterization of bovine *Haemophilus somnus* by biotyping, plasmid profiling, REA-patterns and ribotyping. Zentralblatt fur Bakteriologie 279, 60–74.

Gagea, M., Bateman, K., van Dreumel, A., McEwen, B.J., Carman, S., Archambault, M., Shanahan, R.A., Caswell, J.L., 2006. Diseases and pathogens associated with mortality in Ontario beef feedlots. Journal of Veterinary Diagnostic Investigation 18, 18–28.

Geertsema, R.S., Zekarias, B., La Franco Scheuch, L., Worby, C., Russo, R., Gershwin, L.J., Herdman, D.S., Lo, K., Corbeil, L.B., 2011. IbpA DR2 subunit immunization protects calves against Histophilus somni pneumonia. Vaccine 29, 4805–4812.

Gershwin, L.J., Berghaus, L.J., Arnold, K., Anderson, M.L., Corbeil, L.B., 2005. Immune mechanisms of pathogenic synergy in concurrent bovine pulmonary infection with *Haemophilus somnus* and bovine respiratory syncytial virus. Veterinary Immunology and Immunopathology 107, 119–130.

Glorioso, J.C., Jones, G.W., Rush, H.G., Pentler, L.J., Darif, C.A., Coward, J.E., 1982. Adhesion of type A *Pasteurella multocida* to rabbit pharyngeal cells and its possible role in rabbit respiratory tract infections. Infection and Immunity 35, 1103–1109.

Gogolewski, R.P., Leathers, C.W., Liggitt, H.D., Corbeil, L.B., 1987. Experimental *Haemophilus somnus* pneumonia in calves and immunoperoxidase localization of bacteria. Veterinary Pathology 24, 250–256.

Gogolewski, R.P., Schaefer, D.C., Wasson, S.K., Corbeil, R.R., Corbeil, L.B., 1989. Pulmonary persistence of *Haemophilus somnus* in presence of specific antibody. Journal of Clinical Microbiology 27, 1767–1774.

Gourlay, R.N., Thomas, L.H., Wyld, S.G., 1989. Experimental *Pasteurella multocida* pneumonia in calves. Research in Veterinary Science 47, 185–189.

Groom, S.C., Little, P.B., Rosendal, S., 1988. Virulence differences among three strains of *Haemophilus somnus* following intratracheal inoculation of calves. Canadian Journal of Veterinary Research 52, 349–354.

Harmon, B.G., Glisson, J.R., Latimer, K.S., Steffens, W.L., Nunnally, J.C., 1991. Resistance of *Pasteurella multocida* A:3,4 to phagocytosis by turkey macrophages and heterophils. American Journal of Veterinary Research 52, 1507–1511.

Harper, M., Boyce, J., Adler, B., 2006. *Pasteurella multocida* pathogenesis: 125 years after Pasteur. FEMS Microbiology Letters 265, 1–10.

Harper, M., Cox, A.D., Adler, B., Boyce, J.D., 2011. *Pasteurella multocida* lipopolysaccharide: The long and the short of it. Veterinary Microbiology 153, 109–115.

Hasoksuz, M., Lathrop, S.L., Gadfield, K.L., Saif, L.J., 1999a. Isolation of bovine respiratory coronaviruses from feedlot cattle and comparison of their biological and antigenic properties with bovine enteric coronaviruses. American Journal of Veterinary Research 60, 1227–1233.

Hasoksuz, M., Lathrop, S., Al-dubaib, M.A., Lewis, P., Saif, L.J., 1999b. Antigenic variation among bovine enteric coronaviruses (BECV) and bovine respiratory coronaviruses (BRCV) detected using monoclonal antibodies. Archives of Virology 144, 2441–2447.

Hause, B.M., Ducatez, M., Collin, E.A., Ran, Z., Liu, R., Sheng, Z., Armien, A., Kaplan, B., Chakravarty, S., Hoppe, A.D., et al., 2013. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. PLoSPathog 9, e1003176.

Hause, B.M., Collin, E.A., Liu, R., Huang, B., Sheng, Z., Lu, W., Wang, D., Nelson, E.A., Li, F., 2014. Characterization of a novel influenza virus in cattle and Swine: Proposal for a new genus in the Orthomyxoviridae family. mBio 5, e00031–14.

Hause, B.M., Collin, E.A., Anderson, J., Hesse, R.A., Anderson, G., 2015. Bovine rhinitis viruses are common in U.S. cattle with bovine respiratory disease. PLoS ONE 10, e0121998.

Heckert, R.A., Saif, L.J., Myers, G.W., Agnes, A.G., 1991. Epidemiologic factors and isotype-specific antibody responses in serum and mucosal secretions of dairy calves with bovine coronavirus respiratory tract and enteric tract infections. American Journal of Veterinary Research 52, 845–851.

Heddleston, K.L., Gallagher, J.E., Rebers, P.A., 1972. Fowl cholera: Gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. Avian Diseases 16, 925–936.

Hick, P., Read, A., Lugton, I., Busfield, F., Dawood, K., Gabor, L., Hornitzky, M., Kirkland, P., 2012. Coronavirus infection in intensively managed cattle with respiratory disease. Australian Veterinary Journal 90, 381–386.

Hotchkiss, E.J., Dagleish, M.P., Willoughby, K., McKendrick, I.J., Finlayson, J., Zadoks, R.N., Newsome, E., Brulisauer, F., Gunn, G.J., Hodgson, J.C., 2010. Prevalence of *Pasteurella multocida* and other respiratory pathogens in the nasal tract of Scottish calves. Veterinary Record 167, 555–560.

Hussain, A., Mohanty, S.B., 1979. Cell mediated immunity to bovine rhinovirus type 1 in calves. Archives of Virology 59, 17–24.

Inzana, T.J., Gogolewski, R.P., Corbeil, L.B., 1992. Phenotypic phase variation in *Haemophilus somnus* lipooligosaccharide during bovine pneumonia and after in vitro passage. Infection and Immunity 60, 2943–2951.

Jamali, H., Rezagholipour, M., Fallah, S., Dadrasnia, A., Chelliah, S., Velappan, R.D., Wei, K.S., Ismail, S., 2014. Prevalence, characterization and antibiotic resistance of *Pasteurella multocida* isolated from bovine respiratory infection. Veterinary Journal 202, 381–383.

Jiang, W.M., Wang, S.C., Peng, C., Yu, J.M., Zhuang, Q.Y., Hou, G.Y., Liu, S., Li, J.P., Chen, J.M., 2014. Identification of a potential novel type of influenza virus in Bovine in China. Virus Genes 49, 493–496.

Kadlec, K., Brenner, M.G., Sweeney, M.T., Brzuszkiewicz, E., Liesegang, H., Daniel, R., Watts, J.L., Schwarz, S., 2011. Molecular basis of macrolide, triamilide, and lincosamide resistance in *Pasteurella multocida* from bovine respiratory disease. Antimicrobial Agents and Chemotherapy 55, 2475–2477.

Kanno, T., Hatama, S., Ishihara, R., Uchida, I., 2007. Molecular analysis of the S glycoprotein gene of bovine coronaviruses isolated in Japan from 1999 to 2006. Journal of General Virology 88, 1218–1224.

Klein, M., Earley, E., Zellat, J., 1959. Isolation from cattle of a virus related to human adenovirus. Proceedings of the Society for Experimental Biology and Medicine 102, 1–4.

Klima, C.L., Zaheer, R., Cook, S.R., Booker, C.W., Hendrick, S., Alexander, T.W., McAllister, T.A., 2014. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. Journal of Clinical Microbiology 52, 438–448.

Kuckleburg, C.J., McClenahan, D.J., Czuprynski, C.J., 2008. Platelet activation by *Histophilus somni* and its lipooligosaccharide induces endothelial cell proinflammatory responses and platelet internalization. Shock (Augusta, Ga.) 29, 189–196.

Kwiecien, J.M., Little, P.B., 1992. Isolation of pathogenic strains of *Haemophilus somnus* from the female bovine reproductive tract. Canadian Journal of Veterinary Research 56, 127–134.

Larson, R.L., Step, D.L., 2012. Evidence-based effectiveness of vaccination against *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* in feedlot cattle for mitigating the incidence and effect of bovine respiratory disease complex. Veterinary Clinics of North America Food Animal Practice 28, 97–106.

Lathrop, S.L., Wittum, T.E., Brock, K.V., Loerch, S.C., Perino, L.J., Bingham, H.R., McCollum, F.T., Saif, L.J., 2000. Association between infection of the respiratory tract attributable to bovine coronavirus and health and growth performance of cattle in feedlots. American Journal of Veterinary Research 61, 1062–1066.

Lin, C., Agnes, J.T., Behrens, N., Shao, M., Tagawa, Y., Gershwin, L.J., Corbeil, L.B., 2016. *Histophilus somni* stimulates expression of antiviral proteins and inhibits BRSV replication in bovine respiratory epithelial cells. PLoS ONE 11, e0148551.

Lin, X.Q., O'Reilly, K.L., Storz, J., Purdy, C.W., Loan, R.W., 2000. Antibody responses to respiratory coronavirus infections of cattle during shipping fever pathogenesis. Archives in Virology 145, 2335–2349.

Lo, K.L., Kimball, R.A., Lehmann, J., Gershwin, L.J., Worby, C., Corbeil, L.B., 2012. Antibody responses of calves to *Histophilus somni* recombinant IbpA subunits. Comparative Immunology, Microbiology and Infectious Disease 35, 453–459.

Matiz, K., Ursi, K., Harrach, B., Zadori, Z., Benkö, M., 1998. Sequencing and phylogenetic analysis of the protease gene, and genetic mapping of bovine adenovirus type 10 define its relatedness to other bovine adenoviruses. Virus Research 55, 29–35.

Mattson, D.E., Smith, P.P., Schmitz, J.A., 1977. Isolation of bovine adenovirus type 4 from cattle in Oregon. American Journal of Veterinary Research 38, 2029–2032.

Mattson, D.E., Norman, B.B., Dunbar, J.R., 1988. Bovine adenovirus type-3 infection in feedlot calves. American Journal of Veterinary Research 49, 67–69.

McClary, D.G., Loneragan, G.H., Shryock, T.R., Carter, B.L., Guthrie, C.A., Corbin, M.J., Mechor, G.D., 2011. Relationship of in vitro minimum inhibitory concentrations of tilmicosin against *Mannheimia haemolytica* and *Pasteurella multocida* and in vivo tilmicosin treatment outcome among calves with signs of bovine respiratory disease. Journal of the American Veterinary Medical Association 239, 129–135.

Miyoshi, S., Hamada, H., Miyoshi, A., Ito, R., Hamaguchi, N., Murakami, S., Miyamoto, S., Takeuchi, T., Okura, T., Higaki, J., 2012. *Pasteurella multocida* pneumonia: Zoonotic transmission confirmed by molecular epidemiological analysis. Geriatrics and Gerontology International 12, 159–163.

Mohanty, S.B., Lillie, M.G., 1968. Isolation of a bovine rhinovirus. Proceedings of the Society for Experimental Biology and Medicine 128, 850–852.

Moore, S.J., O'Dea, M.A., Perkins, N., O'Hara, A.J., 2015. Estimation of nasal shedding and seroprevalence of organisms known to be associated with bovine respiratory disease in Australian live export cattle. Journal of Veterinary Diagnostic Investigation 27, 6–17.

Murray, G.M., More, S.J., Sammin, D., Casey, M.J., McElroy, M.C., O Neill, R.G., Byrne, W.J., Earley, B., Clegg, T.A., Ball, H., et al., 2016a. Pathogens, patterns of pneumonia, and epidemiologic risk factors associated with respiratory disease in recently weaned cattle. Journal of Veterinary Diagnostic Investigation In press. doi: 101177/1040638716674757.

Murray, G.M., O Neill, R.G., More, S.J., McElroy, M.C., Earley, B., Cassidy, J.P., 2016b. Evolving views on bovine respiratory disease: An appraisal of selected control measures Part 2. The Veterinary Journal 217, 78–82.

Narita, M., Yamada, M., Tsuboi, T., Kawashima, K., 2002. Immunohistopathology of calf pneumonia induced by endobronchial inoculation with bovine adenovirus 3. Veterinary Pathology 39, 565–571.

Ng, T.F., Kondov, N.O., Deng, X., Van Eenennaam, A., Neibergs, H.L., Delwart, E., 2015. A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. Journal of Virology 89, 5340–5349.

Nikunen, S., Härtel, H., Orro, T., Neuvonen, E., Tanskanen, R., Kivelä, S.L., Sankari, S., Aho, P., Pyörälä, S., Saloniemi, H., et al., 2007. Association of bovine respiratory disease with clinical status and acute phase proteins in calves. Comparative Immunology, Microbiology and Infectious Disease 30, 143–151.

O'Connor, A., Martin, S.W., Nagy, E., Menzies, P., Harland, R., 2001. The relationship between the occurrence of undifferentiated bovine respiratory disease and titer changes to bovine coronavirus and bovine viral diarrhea virus in 3 Ontario feedlots. Canadian Journal of Veterinary Research 65, 137–142.

Oma, V.S., Traven, M., Alenius, S., Myrmel, M., Stokstad, M., 2016. Bovine coronavirus in naturally and experimentally exposed calves; viral shedding and the potential for transmission. Virology Journal 13, 100–111.

O'Neill, R., Mooney, J., Connaghan, E., Furphy, C., Graham, D.A., 2014. Patterns of detection of respiratory viruses in nasal swabs from calves in Ireland: A retrospective study. Veterinary Record 175, 351–356.

O'Toole, D., Sondgeroth, K.S., 2016. Histophilosis as a natural disease. Current Topics in Microbiology and Immunology 396, 15–48.

Pan, Y., Fisher, T., Olk, C., Inzana, T.J., 2014. Detection of antibodies to the biofilm exopolysaccharide of Histophilus somni following infection in cattle by enzyme-linked immunosorbent assay. Clinical and Vaccine Immunology 21, 1463–1467.

Park, S.J., Kim, G.Y., Choy, H.E., Hong, Y.J., Saif, L.J., Jeong, J.H., Park, S.I., Kim, H.H., Kim, S.K., Shin, S.S., et al., 2007. Dual enteric and respiratory tropisms of winter dysentery bovine coronavirus in calves. Archives of Virology 152, 1885–1900.

Perino, L.J., Hunsaker, B.D., 1997. A review of bovine respiratory disease vaccine field efficacy. Bovine Practitioner 31, 59–66.

Plummer, P.J., Rohrbach, B.W., Daugherty, R.A., Daugherty, R.A., Thomas, K.V., Wilkes, R.P., Duggan, F.E., Kennedy, M.A., 2004. Effect of intranasal vaccination against bovine enteric coronavirus on the occurrence of respiratory tract disease in a commercial backgrounding feedlot. Journal of the American Veterinary Medicine Association 225, 726–731.

Portis, E., Lindeman, C., Johansen, L., Stoltman, G., 2012. A 10-year (2000–2009) study of antimicrobial susceptibility of bacteria that cause bovine respiratory disease complex–*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*–in the United States and Canada. Journal of Veterinary Diagnostic Investigation 24, 932–944.

Quast, M., Sreenivasan, C., Sexton, G., Nedland, H., Singrey, A., Fawcett, L., Miller, G., Lauer, D., Voss, S., Pollock, S., et al., 2015. Serological evidence for the presence of influenza D virus in small ruminants. Veterinary Microbiology 180, 281–285.

Reed, D.E., Wheeler, J.G., Lupton, H.W., 1978. Isolation of bovine adenovirus type 7 from calves with pneumonia and enteritis. American Journal of Veterinary Research 39, 1968–1971.

Ruby, K.W., Griffith, R.W., Kaeberle, M.L., 2002. Histamine production by *Haemophilus somnus*. Comparative Immunology, Microbiology and Infectious Disease 25, 13–20.

Sandal, I., Inzana, T.J., 2009. A genomic window into the virulence of *Histophilus somni*. Trends in Microbiology 18, 90–99.

Sandal, I., Hong, W., Swords, W.E., Inzana, T.J., 2007. Characterization and comparison of biofilm development by pathogenic and commensal isolates of *Histophilus somni*. Journal of Bacteriology 189, 8179–8185.

Sandal, I., Inzana, T.J., Molinaro, A., De Castro, C., Shao, J.Q., Apicella, M.A., Cox, A.D., St. Michael, F., Berg, G., 2011. Identification, structure, and characterization of an exopolysaccharide produced by *Histophilus somni* during biofilm formation. BMC Microbiology 11, 186–201.

Saunders, J.R., Thiessen, W.A., Janzen, E.D., 1980. *Haemophilus somnus* infections. I. A 10 year (1969–1978) retrospective study of losses in cattle herds in western Canada. Canadian Veterinary Journal 21, 119–123.

Schooling, S.R., Beveridge, T.J., 2006. Membrane vesicles: An overlooked component of the matrices of biofilms. Journal of Bacteriology 188, 5945–5957.

Sellyei, B., Varga, Z., Szentesi-Samu, K., Kaszanyitzky, E., Magyar, T., 2009. Antimicrobial susceptibility of *Pasteurella multocida* isolated from swine and poultry. Acta Veterinaria Hungarica 57, 357–367.

Sellyei, B., Rónai, Z., Jánosi, S., Makrai, L., 2015. Comparative analysis of *Pasteurella multocida* strains isolated from bovine respiratory infections. Acta Microbiologica et Immunologica Hungarica 62, 453–461.

Sibley, S.D., Goldberg, T.L., Pedersen, J.A., 2011. Detection of known and novel adenoviruses in cattle wastes via broad-spectrum primers. Applied Environmental Microbiology 77, 5001–5008.

Siddaramppa, S., Inzana, T.J., 2004. *Haemophilus somnus* virulence factors and resistance to host immunity. Animal Health Research Reviews 5, 79–93.

Storz, J., Lin, X., Purdy, C.W., Chouljenko, V.N., Kousoulas, K.G., Enright, F.M., Gilmore, W.C., Briggs, R.E., Loan, R.W., 2000. Coronavirus and *Pasteurella* infections in bovine shipping fever pneumonia and Evans' criteria for causation. Journal of Clinical Microbiology 38, 3291–3298.

Sylte, M.J., Corbeil, L.B., Inzana, T.J., Czuprynski, C.J., 2001. *Haemophilus somnus* induces apoptosis in bovine endothelial cells in vitro. Infection and Immunity 69, 1650–1660.

Taylor, J.D., Fulton, R.W., Lehenbauer, T.W., Confer, A.W., 2010. Comparison of genotypic and phenotypic characterization methods for *Pasteurella multocida* isolates from fatal cases of bovine respiratory disease. Journal of Veterinary Diagnostic Investigation 22, 366–375.

Taylor, J.D., Holland, B.P., Step, D.L., Payton, M.E., Confer, A.W., 2015. Nasal isolation of *Mannheimia haemolytica* and *Pasteurella multocida* as predictors of respiratory disease in shipped calves. Research in Veterinary Science 99, 41–45.

Thomas, C.J., Hoet, A.R., Sreevatsan, S., Wittum, T.E., Briggs, R.E., Duff, G.C., Saif, L.J., 2006. Transmission of bovine coronavirus and serological responses in feedlot calves under field conditions. American Journal of Veterinary Research 67, 1412–1420.

Ueno, Y., Hoshinoo, K., Tagawa, Y., 2014. Mutations in the major outer membrane protein gene from *Histophilus somni* by an allelic exchange method. Journal of Microbiological Methods 106, 83–92.

Virtala, A.-M.K., Mechor, G.D., Gröhn, Y.T., Erb, H.N., Dubovi, E.J., 1996. Epidemiologic and pathologic characteristics of respiratory tract disease in dairy heifers during the first 3 months of life. Journal of the American Veterinary Medicine Association 208, 2035–2042.

Watts, J.L., Sweeney, M.T., 2010. Antimicrobial resistance in bovine respiratory disease pathogens: Measures, trends, and impact on efficacy. Veterinary Clinics of North America Food Animal Practice 26, 79–88.

Welsh, R.D., Dye, L.B., Payton, M.E., Confer, A.W., 2004. Isolation and antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia: 1994–2002. Journal of Veterinary Diagnostic Investigation 16, 426–431.

White, S.K., Ma, W., McDaniel, C.J., Gray, G.C., Lednicky, J.A., 2016. Serologic evidence of exposure to influenza D virus among persons with occupational contact with cattle. Journal of Clinical Virology 81, 31–33.

Yamada, M., Narita, M., Nakamura, K., Tsuboi, T., Kawashima, K., 2003. Apoptosis in calf pneumonia induced by endobronchial inoculation with bovine adenovirus type 3 (BAV-3). Journal of Comparative Pathology 128, 140–145.