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Pasteurella multocida and its role in porcine pneumonia

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Abstract

Pasteurella multocida has been recognized as a contributor to debilitating and fatal porcine pneumonia for at least 120 years and there continues to be sustained, unabated high prevalence of the organism in cases submitted for diagnostic work up. Understanding of its role in disease has been limited, in part because of difficulty in reproducing the disease experimentally with capsular type A strains of *P. multocida*, the predominant type associated with porcine pneumonia. This limitation has stymied the development of improved methods for disease control. In this review, the reports of efforts to reproduce the disease are compared. Reports have indicated induction of pneumonia in combined infections with agents such as hog cholera virus, pseudorabies virus and *Mycoplasma hyopneumoniae*. Pneumonia has been induced with intratracheal or endobronchial inoculation of anesthetized swine using capsular type A strains. Substantial recent progress in understanding the putative virulence attributes and molecular genetics of *P. multocida* will likely lead to better understanding of the host–parasite and parasite–parasite interactions in porcine pneumonia associated with this organism. In particular, it seems important to consider the role of biofilm formation in the pathogenesis of this disease. Ultimately, this understanding should provide a foundation for better methods for induction of the experimental disease, development of improved diagnostics, development of better therapeutic/prophylactic pharmaceutical approaches and development of immunoprophylactic products.

Keywords: *Pasteurella multocida*, pneumonia, porcine and swine

Introduction

Pneumonia was apparently recognized as an important disease in swine in antiquity by Aristotle (circa 343 BC) who described three diseases of swine; one disease chiefly involved the jaws and windpipe but extended to and strained the lungs, followed by death. Virgil (circa 29 BC) described a disease in swine characterized by a wracking cough, short quick breathing and suffocation. Beginning about 125 years ago, the work of Bollinger, Hueppe, Kitt and Nocard incriminated what later became known as *Pasteurella multocida* as an important factor in swine pneumonia (cited in Friedberger and Frohner's *Veterinary Pathology* translated by Hayes, 1908). Schutz (1886) described cases of pneumonia in pigs and incriminated an organism identical to Loeffler's bipolar bacteria. Hayes (1908) also indicated that Bollinger and Kitt had independently reproduced this disease experimentally in swine.

The original reports by Bollinger indicate that the disease outbreaks were very serious. Indeed, Hueppe (1886) coined the term 'septicaemia haemorrhagica' for a constellation of similar diseases occurring in horses, cattle, pigs, rabbits and fowl. *Bacillus suiseptica* (probably *P. multocida*) was incriminated in the swine disease. There was undoubtedly difficulty in discerning that cases of septicaemia haemorrhagica under investigation were distinct from what was at the time known as 'schweinepest' or classical swine fever (hog cholera), a disease subsequently shown to be caused by a virus (de Schweinitz and Dorset, 1903; Hutyra, 1907). Thus,

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the limited experimental work with the incriminated *B. suiseptica* could also have involved the hog cholera virus and/or other agents now known to be involved in swine pneumonia. Hutyra (1907) and Glasser (1910) characterized a catarrhal bronchopneumonia in pigs that they distinguished from hog cholera. It was clear that while *B. suiseptica* was often involved, it was difficult to be certain that the organism was the sole cause of the disease. Glasser (1910) attributed the disease to *B. suiseptica* but also recognized that predisposing factors were involved in this disease. Based upon the description of lesions observed it is likely that the diseases Hutyra and Glasser were working with could also have involved agents such as *Mycoplasma hyopneumoniae*, unknown at that time. The lack of certainty about the exact nature or the type of *B. suiseptica* involved, the potential likelihood of there being other agents involved, and the small number of experimental animals utilized make it impossible to ascertain the significance of the reported experimental reproduction of swine pasteurellosis in early reports.

Sustained prevalence of *P. multocida* in swine lungs

With the development of understanding of the viral natures of hog cholera (de Schweinitz and Dorset, 1903) and swine influenza (Shope, 1931), the focus on pasteurellosis or hemorrhagic septicemia in swine was diminished during the first half of the 20th century. Nevertheless, certain studies indicate that chronic catarrhal pneumonia (Hutyra, 1907; Glasser, 1910) associated with *B. suiseptica* infection continued as a major factor in swine disease during that time; Spray (1922) isolated *B. suisepticus* from 54% of pneumonic porcine lungs of slaughter swine in the US and Magnusson (1931) reported 'a prevalence among other virulent pasteurella' in 42% of pneumonic porcine lungs in cases from Sweden from 1915 to 1929. Schofield and Ingle (1942) reported that acute lobar pneumonia associated with *Pasteurella suiseptica* infection was one of the commonest diseases among swine brought to the Ontario Veterinary College. They also described involvement of this organism in a chronic form that followed the acute attack and less frequently, septicemic and enteric forms. The report of a separate, widespread chronic pneumonia of swine which was initially called Virus Pneumonia of Pigs (VPP) (Betts, 1952) and subsequently shown to be caused by a mycoplasma named *M. hyopneumoniae* (Goodwin *et al.*, 1965; Mare and Switzer, 1965) coincided with and was followed by more comprehensive investigations of the microbial flora of pneumonic lungs in swine, including bacteria, mycoplasmas and viruses. Scott (1938) reported isolation of *P. multocida* from 'all cases of stockyard influenza, from a case of influenza occurring in garbage fed pigs and an outbreak of influenza associated with hog cholera' and Betts (1953) described *P. multocida* infection as a common secondary infection in VPP. Ryu (1954), L'Ecuyer *et al.* (1961), Gois *et al.* (1975) and Morrison *et al.* (1985) are examples of reports that document the continuation of *P. multocida* as a dominant bacterial isolate from pneumonia in swine.

Roberts *et al.* (1962) assessed the histopathologic character of 86 lungs examined by L'Ecuyer *et al.* (1961) with naturally occurring pneumonia; they found a more marked neutrophilic reaction throughout the lung with septal cell proliferation when *P. multocida* was isolated. Similar histopathologic assessments of swine pneumonia were reported from Taiwan by Liu *et al.* (1972). Straw *et al.* (1996) summarized microbiological findings from 21 studies of porcine pneumonia published from 1922 to 1990. They found that *P. multocida* was the most frequent microbial agent isolated. The relative risk ratio for this organism was second only to that for *A. pleuropneumoniae* but *A. pleuropneumoniae* was isolated from less than 2% of pneumonic lungs in the 21 reports, whereas *P. multocida* was isolated from 31%. Halbur *et al.* (2006), in an analysis of agents isolated from cases of porcine respiratory disease complex (PRDC) submitted to the Iowa State University Veterinary Diagnostic Laboratory over the 12 year period from 1994 to 2005, found that detection of *P. multocida* in porcine pneumonia roughly doubled in frequency. Numbers of cases of *A. pleuropneumoniae* pneumonia declined by about 50% over the same time period. Most striking, the numbers of cases with involvement of porcine reproductive and respiratory syndrome virus (PRRSv), swine influenza virus and porcine circovirus type 2 increased 3-, 4- and 20-fold, respectively. Clearly, viral respiratory infections in swine have assumed a much greater importance over the past 1–2 decades. These infections probably predispose to much greater involvement of bacteria such as *P. multocida*.

Co-infections with *P. multocida* and various bacteria have been documented repeatedly in studies of naturally occurring cases of porcine pneumonia. In addition to *P. multocida* and *A. pleuropneumoniae*, bacteria commonly isolated include *Hemophilus parasuis*, *Salmonella choleraesuis*, *Streptococcus suis*, *Actinobacillus suis*, *Bordetella bronchiseptica*, and *Mycoplasma hyorhinis* (Ryu, 1954; L'Ecuyer *et al.*, 1961; Gois *et al.*, 1975; Morrison *et al.*, 1985; Straw *et al.*, 1996). Comprehensive evidence of a specific pulmonary interaction between *P. multocida* and most of these bacterial species has not been reported. The interaction of toxigenic *P. multocida* with *B. bronchiseptica* in atrophic rhinitis is well documented (Chanter *et al.*, 1989). Dugal *et al.* (1992) demonstrated that preinfection of porcine tracheal rings with *B. bronchiseptica* enhanced adherence to the tracheal epithelium by both toxigenic and non-toxigenic capsular types A and B *P. multocida*.

The continuing documentation of the importance of *P. multocida* in pneumonic pasteurellosis (Pijoan in *Diseases of Swine*, 2006, and previous editions) indicates

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that the organism is very common in the final stage of enzootic pneumonia and what is known today as PRDC. It is remarkable that *P. multocida*, one of the first agents specifically identified in pneumonic swine lung, continued throughout the past over 100 years as a major agent in porcine pneumonia, often mixed with viral agents, and continues today without any evidence that it has been diminished.

Classification, serotyping and strain diversity of *P. multocida*

Over the course of many years, different systems for classification of the organism have been reported. With the work of Carter (1955) and Heddleston *et al.* (1972), a prevailing logic for classification evolved. Carter utilized serological typing on the basis of capsular antigens and recognized four different serotypes, A–D; one additional type, E, was added. Heddleston *et al.* utilized somatic cell antigens that placed the organism in 16 different serotypes. These two systems have been applied routinely by many workers over the past 50 years. Additional work to define the taxonomy of this group was provided in the excellent paper by Mutters *et al.* (1985). In that work, three subspecies were defined within *P. multocida* (*P. multocida* subsp. *multocida*, *P. multocida* subsp. *septica* and *P. multocida* subsp. *gallicida*). One of the more comprehensive applications of this 1985 system of identification was the work by Blackall *et al.* (1997) who characterized 150 isolates of *P. multocida* from Australian pigs. They recognized seven different biochemical biovars among these isolates; 91% of the isolates belonged to subsp. *multocida* and 9% to subsp. *gallicida*.

Strains of *P. multocida* isolated from porcine pneumonia have generally been Carter capsular type A (Pijoan *et al.*, 1983, 1984; Choi *et al.*, 2001; Davies *et al.*, 2003; Jordan *et al.*, 2006; Pijoan, 2006). However, given the explosiveness and severity of outbreaks reported from Europe during the late 19th and early 20th centuries, it is conceivable that at least some of the cases of swine pneumonia/septicemia observed that were reported and reproduced experimentally could have been caused by what is known today as *P. multocida* type B (Hudson, 1959). There is no way to know the capsular serotypes of strains isolated during early years. According to Hueppe (1886) and Hutyra (1907), this disease occurred also in deer, cattle and buffalo (presumably *Bubalis bubalis*) in Europe. Further, Hutyra speculated on the substantial variation in virulence of strains involved in acute and chronic cases of swine pneumonia. More recently, strains of serotype B have occasionally been implicated as a cause of a pneumonic and septicemic disease in swine in India (Murty and Kaushik, 1965; Verma, 1988), Sri Lanka (Gamage *et al.*, 1995) and Vietnam (Townsend *et al.*, 1998). Recently, other serotypes have also been reported to cause more severe pneumonia along with septicemia. A serotype D strain of *P. multocida* was implicated in an outbreak of septicemia with multifocal thrombosis and necrosis in the liver, lung and brain in Australia by Mackie *et al.* (1992). *P. multocida* subspecies *gallicida* (capsular serotype A) was isolated from the lungs of pigs from an extensive outbreak of acute hemorrhagic septicemia in Australia (Cameron *et al.*, 1996).

Toxigenic serotype D strains of *P. multocida* are a major cause of turbinate atrophy in swine (Chanter *et al.*, 1989; de Jong, 2006). In a survey of 113 pneumonic porcine lungs conducted in Minnesota, Pijoan *et al.* (1984) showed that 87.5 and 12.5% of *P. multocida* isolated from pneumonia in pigs were respectively serotype A and serotype D; of these, 80 and 18.2%, respectively of serotypes D and A were toxigenic. Choi *et al.* (2001), in a survey of 230 isolates from porcine pneumonia in Korea found that 87% were capsular type A, 4% were capsular type D and 9% were not typable. The *tox*A gene was present in 13% of the isolates. Further characterization of *P. multocida* isolated at Iowa State University has indicated a shift in types of isolates from pneumonic porcine lungs; 'less than 2% of isolates were serotype D in 1996; in 2003, over 33% of isolates were serotype D' (Jordan *et al.*, 2006).

Several more sophisticated molecular approaches have been utilized to characterize porcine isolates of *P. multocida* in recent years. Molecular approaches have included ribotyping (Zhao *et al.*, 1992; Bowles *et al.*, 2000), plasmid and restriction endonuclease patterns (Zhao *et al.*, 1992; Rubies *et al.*, 2002), capsular PCR typing (Davies *et al.*, 2003, 2004), SDS Page of outer membrane proteins (Davies *et al.*, 2003, 2004), single enzyme amplified fragment length polymorphism (SE-AFLP; Moreno *et al.*, 2003), and multilocus sequence analysis (Davies *et al.*, 2004). These approaches have been utilized to characterize *P. multocida* isolates classified according to more traditional means (e.g. Carter–Heddleston methods) to study diversity or lack of diversity among isolates in a given area, and to attempt to provide a foundation for molecular epidemiologic studies of the organism (Rubies *et al.*, 1996; Blackall *et al.*, 2000). Djordjevic *et al.* (1998), using restriction endonuclease analysis, found that among Australian strains of *P. multocida*, toxigenic isolates from cases of porcine infectious atrophic rhinitis were very similar or clonal, whereas those from pneumonia were very heterogeneous. In a study of *P. multocida* isolated from swine in Brazil, Paixao *et al.* (2004), using SE-AFLP and restriction fragment length polymorphism, also found that type A strains were quite heterogeneous. Fussing *et al.* (1999) utilized ribotyping to document that a single type of toxigenic strains of *P. multocida* was involved in an outbreak of atrophic rhinitis involving several herds in Denmark. It would seem that there is good potential to utilize these methods as well to identify isolates that have unique pathogenic characteristics, or to help identify the molecular basis of pathogenicity, particularly the

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Table 1. Attempts to induce pneumonia in swine with *Pasteurella multocida* capsular Type A

Author, year	Capsular serotype	Route of inoculation	Inoculum	Amount or cell numbers	Co-infecting agent or condition[1]	Result with pure *P. multocida*	Result with co-infection or other agent
Scott, 1938	Unknown	Intranasal (IN)	24 h culture	20–25 ml	Swine influenza virus	Negative	Enhanced pulmonary disease
Schofield and Ingle, 1941	Unknown	*Per os*, intratracheal (IT), tampon in nasal passage, sub-cutaneous (SQ), intracardiac (IC), intrapulmonary (IPu); ten experiments	Lung tissue, tampons soaked with lung tissue from fresh cases, filtered lung homogenate, culture of *P. suiseptica*	Not given	Chilling in ice bath in 3/10 experiments with pigs anesthetized; hemolytic *Streptococcus* with *P. suiseptica* in one experiment	2/6 pigs inoculated with homogenate of lungs with purulent pneumonia; 1/8 with congestion when inoculated with culture IT and chilled in ice bath. More pigs with acute congestion of lungs or consolidation when culture given IC or IPu	Two pigs inoculated IC with *P. suiseptica* and *Streptococcus* died showing acute enteritis and congestion of lungs
Luke, 1947	Unknown	IP, SQ, IN	Culture	1 ml	None	Negative	
Switzer, 1956	Unknown	IN	Crude turbinate/ *P. multocida*	1 ml	Pigs hand-reared after removal from sow a few hours after birth	2/3 pigs with pneumonia	NA
	Unknown	IN	Chick embryo amnio-allantoic fluid/*P. multocida*	1 ml	Pigs hand-reared after removal from sow a few hours after birth	3/4 pigs with small areas of pneumonia	NA
	Unknown	IN	Tryptose broth	1 ml	Pigs hand-reared after removal from sow a few hours after birth	Negative	NA
Kojnok *et al.*, 1959	Unknown	IN	Not given	5–20 ml	Pseudorabies virus	Negative	Enhanced pulmonary disease
Pijoan and Ochoa, 1978	Unknown	IT	Ten fresh isolates	3 ml with 5.6×10^9 cfu	Live hog cholera virus vaccine	2/3 pigs with lesions	Enhanced pulmonary disease
Ose *et al.*, 1973	Unknown	IN	Lung homogenate with *P. multocida* and *Corynebacterium pyogenes*	10^6–10^7 cfu *P. multocida* and 10^7–10^8 cfu *C. pyogenes*	Forced exercise	37 of 71 pigs with pneumonia; 19 of the 71 died	NA
Smith *et al.*, 1973a	A	IN	Nutrient broth culture	125×10^6 cfu	Experiments in gnotobiotic piglets; adenovirus, enterovirus	Minimal, except serofibrinous pleuritis	Serofibrinous pleuritis or arthritis in some pigs

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Smith *et al.*, 1973b	A	Nebulized, IN or IT	Nutrient broth culture	24×10^{3} cfu	Experiments in gnotobiotic piglets. *Mycoplasma hyopneumoniae* given in combination with *P. multocida* in some	1 of 3 pigs with consolidation, 6 with no gross lesions in a second experiment; 2 with either pericarditis or arthritis; in a third experiment 11/12 IT inoculated pigs had mild to severe pneumonia	Enhanced pulmonary disease
Raynaud *et al.*, 1977	A	Nebulized and IT	Serum broth	2.3×10^{10} cfu	Pigs specific pathogen free (SPF) *Ascaris suum* and prednisolone	NA	Enhanced pulmonary disease and death
Pijoan *et al.*, 1986; Fuentes and Pijoan, 1987	A and D	IN with perforated cannula	Cultures grown in brain heart infusion 6 h	10^{9} cfu each serotype	Pseudorabies virus, anesthesia	Negative	Enhanced pulmonary disease
Pijoan and Fuentes, 1987	A and D	IN		2 ml 10^{9} cfu; three type A strains	Pseudorabies virus	NA	Variably enhanced pulmonary disease with pleuritis; type D strain induced no disease
Neumann *et al.*, 1987a,b	A	IV	Bouillon	5×10^{10}–8×10^{10}	50 or 100 ppm ammonia and thermomotor stress		Enhanced disease with ammonia exposure
Rafai *et al.*, 1987	A	IN	Broth	5 ml with 2×10^{9} or 6×10^{8}	Two levels of low ambient temperature	6/8 with histologic findings of mild subacute pneumonia	No gross pulmonary changes; no change from results with pure culture
Ciprian *et al.*, 1988	A	Nebulizer with aerosol chamber	Brain heart infusion broth	22 ml per animal group; 30 min of 10^{8} cfu ml^{-1}	*M. hyopneumoniae*	Negative	Enhanced pulmonary disease
Baekbo, 1988	Nontoxigenic A and D; toxigenic A and D	IN with a nebulizer	Not given	2–4 ml with 10^{9}–10^{10} cfu/ml	Pigs SPF *M. hyopneumoniae*	1/8 inoculated with nontoxigenic strain with small lesion	Enhanced pulmonary disease; nontoxigenic and toxigenic strain effects equivocal
Hall *et al.*, 1990	A	IT with endotracheal tube under anesthesia	Brain heart infusion broth	10^{10} cfu in either 2–3 ml per pig or 8 ml/kg	Large volume of saline and anesthesia	2.7% of lung with pneumonia when given in 2–3 ml	14% of lung with pneumonia when given at the rate of 8 ml/kg

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Table 1 (Continued)

Author, year	Capsular serotype	Route of inoculation	Inoculum	Amount or cellnumbers	Co-infecting agent or condition[1]	Result with pure *P. multocida*	Result with co-infection or other agent
Müller and Heilmann, 1990	A	Intrabronchial	Apparently a 24 h broth culture (see Berndt *et al.*, 2002)	$\sim 10^{11}$ cfu	Pigs SPF anesthesia	88% of 43 pigs with low grade pneumonia	NA
Müller *et al.*, 1993	A	Intrabronchial	Apparently a 24 h broth culture (see Berndt *et al.*, 2002)	8.8×10^{10} cfu	Anesthesia	Prior exposure to aerosol resulted in reduced pneumonia following challenge with *P. multocida*	NA
Amass *et al.*, 1994	A	IT	Not given	5 ml of 3.1×10^{8} cfu	*M. hyopneumoniae*	Minimal coughing only	Enhanced pulmonary disease
Berndt and Müller, 1995	A	IT	Bovine blood agar culture	4.8×10^{9} cfu	Pigs SPF	Documented changes in immune cell surface antigens	NA
Carvalho *et al.*, 1997	A (two strains)	IN	Blood agar culture	10^{9} cfu	PRRSv Pseudorabies virus	NA	Enhanced disease with pseudorabies virus but not with PRRSv
Marquette *et al.*, 1999	Not given	Intrabronchial intubation	Brain heart infusion broth	10^{6}–10^{7} cfu in 50 ml saline	Large volume of saline and anesthesia	Limited foci of pneumonia in 3/7 pigs	NA
Andreasen *et al.*, 2000	A (toxigenic)	Nebulizer with aerosol chamber	Blood agar	$\sim 10^{9}$ cfu 12 ml over 20 min	50 and 100 ppm ammonia		No enhancement of pulmonary disease
Brockmeier *et al.*, 2001	A	IN	Cultures grown on dextrose starch agar and suspended in tryptose broth	$2 \times 10^{8.4}$ cfu	PRRSv	Negative	No enhancement of pulmonary disease
	A	IN	Same as above	$2 \times 10^{8.8}$ cfu	*B. bronchiseptica* and PRRSv	Negative	Enhancement of colonization by *P. multocida* and enhanced pulmonary disease
Berndt *et al.*, 2002	A	Intrabronchial	Broth (Hofer *et al.*, 1982. *Arch Exp. Vet. Med.* **36**: 565–575)	5 ml of 4.8×10^{9} cfu	Anesthesia	Changes in spatial distribution of cytokine mRNA-expressing cells in lung tissue documented during this model disease	NA

Müller *et al.*, 2003	A	Intrabronchial	Broth	4×10^{10} cfu	Anesthesia	Further documented cytokine levels in model disease	NA
Ono *et al.*, 2003	A	IN or IT; exact method for IT not given	Cultures grown on dextrose starch agar and suspended in Mueller–Hinton broth	Groups with 10^6–10^9 cfu	CDCD pigs and SPF pigs	3/29 IT inoculated pigs with gross lesions; no IN inoculated pigs with lesions	NA
Halloy *et al.*, 2005a	A	IT through skin mid trachea	Tryptose broth with 10% horse serum	5 ml of $>2\times10^9$ cfu	Endotoxin and anesthesia	Size of lesions in control and *P. multocida* only pigs very similar	Enhanced pulmonary disease with increasing dose of endotoxin
Halloy *et al.*, 2005b	A	IT through skin mid trachea	Tryptose broth with 10% horse serum	5 ml of $>2\times10^9$ cfu	Fumonison and anesthesia	Size of lesions in control and *P. multocida* only pigs very similar	Enhanced pulmonary disease

[1]Unless specified, pigs were of conventional origin or assumed to be of conventional origin.
NA=not applicable, CDCD=Caesarian-derived, colostrum-deprived.

host–parasite and parasite–parasite interactions that lead to disease development with *P. multocida*.

Induction of experimental pneumonia with *P. multocida*

Thus, in spite of all of the work that has been done with failed bacterins and dubious field trials with antibiotics commonly used to treat porcine pneumonia that *in vitro* assays suggest should be efficacious against *P. multocida*, the organism continues to be associated with huge economic losses. Development of understanding of the disease and, most particularly, development of efficacious vaccines or other prophylactic or therapeutic approaches have been thwarted because of limited progress in development of methods for induction of experimental disease with capsular type A strains of the organism, the predominant isolate in porcine pneumonia in many swine raising areas. As indicated, capsular type B strains clearly cause a primary pneumonia as part of the hemorrhagic septicemia in southeastern Asia. Rhoades *et al*. (1967) and Heddleston *et al*. (1967) and Bentley and Farrington (1980) have documented that a primary pneumonia can be induced in swine using type B strains isolated from bison (*Bison bison*) in North America.

Scott (1938), Schofield and Ingle (1942), Luke (1947), Kojnok *et al*. (1959), Pijoan *et al*. (1986), Fuentes and Pijoan (1987), Ciprian *et al*. (1988) and Baekbo (1988), have indicated that inoculation of *P. multocida* alone will not induce pneumonia or only infrequently results in lesions of pneumonia in swine. In a series of experiments on atrophic rhinitis, Switzer (1956) reported that crude turbinate suspension from which only *P. multocida* was isolated and the same strain grown in embryonated hen's eggs induced pneumonia (Table 1). Details regarding many of these experiments are summarized in Table 1. Smith *et al*. (1973a) reported that intranasal (nebulized) inoculation of *P. multocida* type A in gnotobiotic piglets resulted in only 'doubtful inflammatory lesions of the lungs' in three of four pigs; the fourth pig had a lethal fibrinous pneumonia and septicemia. Smith *et al*. (1973b) found that repeated intranasal inoculation of the same strain, again in gnotobiotic piglets, resulted in slightly more disease in some piglets. In unpublished work, we (Ross, Hoffman and Thacker, unpublished, 2004) failed to induce significant lesions of pneumonia with *P. multocida* capsular type A given intratracheally according to the procedure of Bentley and Farrington (1980). Cultures were grown on dextrose starch agar, a medium known to enhance production of hyaluronic acid capsule of this organism, or encased in agarose beads or pigs were pretreated intranasally with 1% acetic acid.

While toxigenic strains of *P. multocida*, particularly serotype D, have been well accepted as a major

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contributor to porcine infectious atrophic rhinitis, efforts to document that the toxin plays any role in lower tract disease have failed (Pijoan and Fuentes, 1987; Baekbo, 1988). In a study of non-toxigenic type A and type D strains isolated from pneumonia in Australia using restriction endonuclease profiles, Djordjevic *et al*. (1998) concluded that *P. multocida* strains with the *tox*A gene either 'do not have a competitive survival advantage in the lower respiratory tract or that toxin production does not play a role' in pneumonia, or both.

Inoculation of *P. multocida* in combination with other known porcine respiratory pathogens has clearly resulted in enhanced lesion development. As summarized in Table 1, the organism has clearly been shown to enhance severity of pneumonia when in combination with swine influenza virus (Scott, 1938), hog cholera virus vaccine (Pijoan and Ochoa, 1978), and pseudorabies virus (Kojnok *et al*., 1959; Pijoan *et al*., 1986; Fuentes and Pijoan, 1987; Pijoan and Fuentes, 1987; Carvalho *et al*., 1997). Pijoan and Fuentes (1987) isolated capsular type A *P. multocida* from field cases of severe pleuritis and lung abscesses. They were able to reproduce pleuritis and abscessation with sequential infection using pseudorabies virus followed by the *P. multocida* isolated from field cases. Using experimental exposure, Carvalho *et al*. (1997) were unable to show any clear evidence of interaction between PRRSv and *P. multocida*. Brockmeier *et al*. (2001) also found that prior infection with PRRSv did not predispose pigs to infection with *P. multocida*. Prior infection of pigs with both *B. bronchiseptica* and PRRSv enhanced colonization of the lungs and resulted in pneumonia when a nontoxigenic serotype A *P. multocida* was utilized (Brockmeier *et al*., 2001). The same authors found that prior infection with *B. bronchiseptica* alone did not enhance lower tract colonization or result in pneumonia when pigs were subsequently inoculated with the *P. multocida*. Interaction of *B. bronchiseptica* and *P. multocida* (toxigenic, serotype D) in colonization and induction of turbinate atrophy in swine is well documented, where damage caused by the *B. bronchiseptica* cytotoxin enhances colonization with toxigenic *P. multocida* (Chanter *et al*., 1989; de Jong, 2006).

Presence of both *P. multocida* and *M. hyopneumoniae* in lesions of pneumonia has been a common finding by many investigators (Morrison *et al*., 1985; Straw *et al*., 1996). Smith *et al*. (1973b) demonstrated that simultaneous intranasal or intratracheal inoculation of gnotobiotic piglets with mixtures of *P. multocida* type A and *M. hyopneumoniae* resulted in relatively extensive pulmonary lesions in 4 out of 6 pigs inoculated (Table 1). Ciprian *et al*. (1988), Baekbo (1988) and Amass *et al*. (1994) reported similar findings with sequential experimental *M. hyopneumoniae*–*P. multocida* infections. We were unable to demonstrate that *P. multocida* (capsular type A) grown in chicken embryo allantoic sacs was able to enhance pneumonia induced by *M. hyopneumoniae* (Thacker *et al*., unpublished, 2002).

Both swine lung worms and ascaris larvae are known to complicate respiratory diseases caused by other agents in swine. Ose *et al*. (1973) induced pneumonia in pigs with *Ascaris suum* ova followed 5–10 days later by intranasal inoculation of lung homogenate from field cases of swine pneumonia containing *P. multocida* and *Corynebacterium pyogenes*. Raynaud *et al*. (1977) reported successful development of a model for induction of a combined infection using nebulized *A. suum* ova and prednisolone, followed 8 days later with nebulized cultures of type A *P. multocida*. The ensuing disease was that of severe respiratory symptoms, hyperthermia, loss of weight and an acute bronchopneumonia. The acute bronchopneumonia frequently resulted in death 4–8 days after exposure to the *Pasteurella*.

Experimental pneumonia has been induced using 10^{10} *P. multocida* type A in a large bolus of saline (Hall *et al*., 1990 – Table 1). Marquette *et al*. (1999) used pigs to develop an animal model for ventilator-acquired pneumonia. Pigs in this study were put under general anesthesia and intubated with endotracheal low-pressure cuff tubes, and mechanically ventilated with a volume controlled respirator. Seventeen of 18 pigs developed gross lesions of pneumonia that, in the majority of cases, exceeded 30% of the lungs. The pneumonia was polymicrobic, involving organisms assumed to be from the oronasal flora. *P. multocida* was a frequent isolate from the lesions that developed. Other pigs were given pure cultures of *P. multocida*, with very limited success (Table 1). The authors concluded that prolonged mechanical ventilation was essential to development of the pneumonia in this model, the microbial agents being mainly opportunists and not capable of inducing a primary pneumonia.

Recently, Ono *et al*. (2003) inoculated 8–14-week-old caesarean-derived, colostrum-deprived (CDCD) or specific pathogen free pigs 8–14 weeks of age intranasally or intratracheally with a strain of *P. multocida* capsular type A. Cultures were grown on dextrose starch agar. Gross lesions of pneumonia were observed in 3/29 intratracheally inoculated pigs, whereas most pigs had histologic lesions of exudative bronchpneumonia. Intranasally inoculated pigs had only histologic lesions; 8/29 had exudative bronchopneumonia. The exact means of intratracheal inoculation was not given. Pleural adhesions and arthritis were observed in some of the intratracheally inoculated pigs. The potential synergism of *E. coli* endotoxin and *P. multocida* type A for induction of pneumonia was evaluated by Halloy *et al*. (2005a). Using a range of doses of endotoxin, they found that the highest dose used, $400\ \mu g\ kg^{-1}$, given intratracheally with a bronchoscope followed one day later with *P. multocida* intratracheally (mid trachea, through the skin), both under anesthesia, resulted in progressively more severe cough, hyperthermia, inflammatory cell influx into the airways and increased expiratory breathing pattern measured by the Penh index. Lung lesions reported were

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exudative subacute alveolar and insterstitial pneumonia with pleurisy and pericarditis. Surprisingly, *P. multocida* was not recovered from the lung lesions. Necropsies were done 13 days after inoculation with *P. multocida*. In followup work, Halloy *et al.* (2005b) reported that administration of fumonison intratracheally for 6 days, followed by intratracheal inoculation of *P. multocida* type A leads to delayed growth, increased coughing and increased size of macroscopic lesions in the lungs. Again, *P. multocida* was not reisolated from the lungs of the pigs.

Elevated environmental ammonia levels are often detected in swine rearing facilities and are implicated as predisposing factors in respiratory disease. Neumann *et al.* (1987a,b) demonstrated reduced systemic and local (pulmonary) resistance to infection when pigs were exposed continuously to 50 and 100 ppm ammonia, followed by intravenous challenge with *P. multocida* type A and thermomotor stress. However, Andreasen *et al.* (2000) found that aerosol exposure to 50 and 100 ppm ammonia had no influence on extent of pneumonia or turbinate atrophy when pigs were exposed by aerosol to *M. hyopneumoniae* followed by aerosol exposure to toxigenic *P. multocida* type A. Done *et al.* (2005) exposed 960 weaned pigs from a commercial herd to various concentrations of ammonia and dust. Assessments of pigs were done by necropsy, or slaughter inspection, after 5 weeks exposure, after 7 weeks exposure and at time of slaughter; only minimal gross pathology was detected in both treated and control pigs. *P. multocida* and other common respiratory pathogens were isolated from the upper respiratory tracts from similar numbers of pigs in both treated and control pigs. The organism was isolated only rarely from the tracheas or lungs. Seemingly, the pigs were unaffected by the pollutants. Rafai *et al.* (1987) found that cold stress had no effect on susceptibility of piglets to experimental *P. multocida* infection (Table 1).

Perhaps the most sustained use of an experimental model has been carried out by Müller and Heilmann (1990), Müller *et al.* (1993), Berndt and Müller (1995), Berndt *et al.* (2002) and Müller *et al.* (2003). They reported use of an intrabronchial inoculation procedure to induce pneumonia in anesthetized 8-week-old swine (Table 1). The procedure entails preparation of a *P. multocida* capsular type A strain that is washed and resuspended in a phosphate buffer, then a standardized dosage is administered through an endobronchial tube. In their study, the experimental disease was characterized by 'a predominant exudative and an additional proliferative interstitial component as well as histologically detectable abscess formation in the lung'. The work carried out by this group may provide a basis for a study utilizing molecular approaches to further study the interaction between *P. multocida*, other co-infections and porcine respiratory tract cells.

Capsular type B strains of *P. multocida*, isolated from bison in the US have been shown to induce pneumonia in swine (Heddleston *et al.*, 1967; Bentley and Farrington, 1980; Ross *et al.*, 1982). Heddleston *et al.* (1967) utilized *P. multocida* strain M-1404 in a series of studies in cattle, sheep and swine. Pigs were exposed by aerosol, instillation of a swab dipped in culture and by intranasal instillation of culture; all developed acute dyspnea, inappetence, depression and elevated temperatures by day 1 after exposure (Heddleston *et al.*, 1967). Four of five inoculated pigs died. At necropsy, pigs had fibrinous pleuritis, extensive pneumonia, and edema. Bentley and Farrington (1980) standardized a protocol for induction of pneumonia in swine with *P. multocida* capsular type B strain MSU7. The culture was grown 4.5 h in tryptose phosphate broth with 5% horse serum. The culture was washed, resuspended in the same broth and adjusted to a standard spectrophotometric standard. The inoculum was given using a rubber veterinary catheter inserted into the trachea, while the pig was restrained head up and with the mouth held open with a speculum. The procedure enabled very quick administration and no anesthesia was used. Mortalities often ensued and, at necropsy, lesions were a fibrinous, exudative bronchopneumonia. We found the procedure useful for evaluating efficacy of liquamycin (Ross *et al.*, 1982) and other antibiotics for therapy of this induced bacterial swine pneumonia (Ross *et al.*, unpublished clinical trials, 1982–1984). Strains of capsular type B *P. multocida* utilized in these studies were derived from bison in Yellowstone National Park. Serotyping has recently distinguished these serotype B strains as B: 3,4 (Rimler and Wilson, 1994), whereas most strains isolated in Asia belong to B:2 or 5.

Diagnosis

Pijoan (2006) has provided a good review of the current status of diagnostic tests for pneumonic *P. multocida* infections in swine. As he stated, 'lesions of *P. multocida* infection are not pathognomonic'. He stated further 'history of the outbreak, histopathology and isolation of the organism should be used to confirm the original presumptive diagnosis'. Pijoan goes on to provide a good description of reliable methods for isolation of *P. multocida*. Recently, methods for PCR for diagnosis of *P. multocida* infections have been well documented (Christensen *et al.*, 2003). Townsend *et al.* (2000) utilized biotyping and REP-PCR to investigate an outbreak of acute swine pasteurellosis in Australia. PCR methods using species-specific primers, capsule type or serogroup-specific primers and multiplex PCR, and for hemorrhagic septicemia and *toxA* genes were applied to characterize *P. multocida* isolated from slaughter pigs in New Zealand (Jamaludin *et al.*, 2005). Register and Dejong (2006) developed a multiplex PCR for genes common to all *P. multocida* and one for toxigenic *P. multocida*. Therefore, it seems that application of recently developed molecularly based methods, particularly PCR, will be useful for study of natural outbreaks as well as studies

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of various recently developed models for induction of pneumonic pasteurellosis caused by *P. multocida* capsular type A. Various molecular approaches to understanding the organism described in the section 'Current approaches to pathogenesis' may help to identify improved targets for PCR or other technologies used in diagnosis of *P. multocida* disease. However, identification of a highly useful specific diagnostic PCR target associated with pneumo-pathogenicity may be difficult. Djordjevic *et al.* (1998), in a sophisticated study of field isolates, demonstrated considerable diversity among *P. multocida* capsular type A strains suggesting that many different types have the potential to play important roles in pathogenesis of naturally occurring porcine pneumonia.

Antimicrobial resistance

Development of antimicrobial resistance is undoubtedly a factor in the continued high prevalence of *P. multocida* in porcine pneumonias. The organism has been repeatedly shown to be susceptible to a variety of antibiotics; however, as will be reviewed in this section, there is evidence of increasing antibiotic resistance of the organism, including to multiple antibiotics. In outbreaks of porcine pneumonia, the clinician must quickly choose an antibiotic therapy. Pijoan (2006) indicated that 'treatment of *P. multocida* field infections is usually difficult or unsuccessful'. As documented in other sections of this review, the prevalence of the organism in porcine pneumonias continues unabated. Thus, in spite of all the work to establish antimicrobial therapies for porcine pneumonias in which *P. multocida* is perceived to be a major factor, antibiotic therapy has frequently been quite inadequate. Pijoan (2006) continues by stating that there is widespread antibiotic resistance in *P. multocida* isolated from swine in the United States. Worldwide, this circumstance has led to extensive effort by microbiologists and diagnosticians to study the antimicrobial resistance profiles of *P. multocida* isolated from porcine pneumonia. Development of multiple drug resistance is putatively one outcome of the long term, extensive usage of antibiotics and a complicating factor in efforts to find solutions to control *P. multocida* infections.

Evidence of multiple drug resistance of *P. multocida* isolated from both cattle and swine was reported by Chang and Carter (1976). They found that 63 of 75 porcine isolates were resistant to at least one of the antibiotics tested. Further, they found that combinations of streptomycin and penicillin and streptomycin and tetracycline accounted for approximately 10% of resistance patterns in *P. multocida*. Yamamoto *et al.* (1990) studied 163 strains of porcine *P. multocida* and reported six resistance patterns among these strains. Their conclusion was that the results indicated that the number of multiple drug-resistant strains of *P. multocida* had increased in swine in Japan. Two strains studied were shown to have R plasmids. Coté *et al.* (1991) also characterized the resistance patterns of porcine *P. multocida*. They found resistance to sulfonamides and streptomycin in 7 of 29 strains studied; these isolates contained R plasmids conferring resistance to streptomycin and sulfonamides. Gutierrez Martin and Rodriguez Ferri (1993) reported on minimum inhibitory concentrations (MICs) determined for 42 antimicrobial agents evaluated against 59 strains of *P. multocida*. Cephalosporins and quinolones were the most effective; however most strains were also susceptible to penicillins, aminoglycosides, tetracyclines and erythromycin. The strains were quite resistant to tylosin, vancomycin, metronidazole, dapsone and tiamulin.

The report of Raemdonck *et al.* (1994) involved a worldwide study to determine MICs of danofloxacin and eight other commonly used antimicrobial drugs used against 969 isolates of *P. multocida* collected from 14 countries. This report, while documenting the high susceptibility levels to danofloxacin, also provided evidence of resistance to lincomycin, oxytetracycline and spectinomycin. Salmon *et al.* (1995) evaluated susceptibility of 515 porcine isolates of various bacterial pathogens from the United States, Canada and Denmark to a variety of antibiotics; overall, ceftiofur and enrofloxacin were the most active. Like other authors, these investigators found evidence of resistance to erythromycin, lincomycin, spectinomycin and tetracycline with some variability between countries. Bousquet *et al.* (1997) reported that strains of *P. multocida* isolated from nasal swabs or pneumonic lungs of diseased swine showed a bimodal resistance to oxytetracycline, 'indicating the emergence of resistant strains in France'. Reporting in 2001 from Japan, Yoshimura *et al.* compared antimicrobial resistance profiles of *P. multocida* isolated mainly from pneumonia in cattle and swine; they found 25% of bovine isolates and 32% of porcine isolates were resistant to one or more major antimicrobial agents in use in that country. The higher percentage resistance in porcine isolates was thought to be related to higher usage or route of administration in swine. The data suggest that multiple drug resistance occurred. Often, it is difficult to ascertain the extent of multiple drug resistance or resistance combinations unless authors specifically analyze their findings for such.

Resistance of *P. multocida* to tetracyclines has been associated with at least three *tet* genes, with *tet*(H) being most common (Hansen *et al.*, 1993, 1996; Kehrenberg *et al.*, 2001a,b). Kehrenberg *et al.* (2003) reported a multiresistance gene cluster on a plasmid from a US turkey isolate of *P. multocida* comprising genes for sulfonamide, streptomycin and tetracycline resistance. They comment that 'when such resistance gene clusters are located on plasmids, they are easily spread among strains, especially, and sometimes even genera'. Kehrenberg *et al.* (2004) found that porcine isolates of *P. multocida* exhibited no change in susceptibility to florfenicol over ten years usage in Germany. However, Kehrenberg and

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Schwarz (2005) reported the first evidence of resistance to florfenicol in a bovine isolate of *P. multocida*. In a more recent report, Vera Lizarazo *et al.* (2006), compared *P. multocida* isolated from porcine pneumonia in Spain in 1987–1988 with those isolated in 2000–2004, and revealed a substantial increase in resistance to sulfachlorpiridazine, sulfadimethoxine, sulfathiazole and trimethoprim-sulfamethoxazole. The authors also reported several multiple drug resistance patterns, most frequently among isolates recovered in the 2003–2004 time frame. Although there were slight increases in resistance to penicillins and tetracyclines, Vera Lizarazo *et al.* (2006) found that a high proportion of isolates collected in Spain were susceptible to both of these classes of antibiotics. Wallmann (2006) reported lower antimicrobial resistance rates for *P. multocida* from swine than reported previously from Germany.

Kehrenberg *et al.* (2001a) presented an excellent review of antimicrobial resistance in *Pasteurella*, including information on the genetic basis of resistance to β-lactam antibiotics, tetracyclines, aminoglycosides, sulfonamides and chloramphenicol. They also elaborated on the roles of plasmids and transposons in the spread of resistance genes among the *Pasteurellaceae*. Kehrenberg *et al.* (2001a) categorize resistance genes found in *Pasteurella* as (a) those widely distributed in *Enterobacteriaceae* and other Gram-negative bacteria, (b) those which link between Gram-negative and Gram-positive bacteria and (c) those that appear to be restricted to the *Pasteurella*, such as *tet*(H). The authors emphasize that occurrence of these resistance genes in a wide range of bacteria 'implies that *Pasteurella* and *Mannheimia* isolates have access to large gene pools within which an interchange of resistance genes takes place'. Further, they make the assumption 'that resistance development in *Pasteurella* and *Mannheimia* isolates is a continuous process in which novel resistance genes may be acquired or developed under the selective pressure imposed by the use of new drugs'. McDermott *et al.* (2002) provide a similar perspective stating that 'the ease and speed with which bacteria can acquire antimicrobial resistance genes is disturbing'. Because of the importance of antimicrobial resistance to both animal and human health, it is widely accepted that continued surveillance of this resistance is necessary.

Resistance of bacteria to antimicrobials used in livestock has become a major issue of concern worldwide. Not only is this resistance a factor in unsatisfactory treatment of disease in swine and other livestock, there is legitimate fear of spread of the resistance to bacteria causing disease in humans. This concern is documented in a report on the food safety aspects of antibiotic resistance by McDermott *et al.* (2002) and is a part of the basis in the United States for an interagency task force plan to combat antimicrobial resistance (Centers for Disease Control, 2005). As described by Kehrenberg *et al.* (2001a), the European Commission has established a multi-nation program for the purpose of monitoring antimicrobial resistance (FAIR-CT97-3654, 1998). Wallmann (2006) reported an ongoing national antimicrobial resistance monitoring program in Germany.

Search for virulence attributes in *P. multocida*

In vitro methods that have been utilized in attempts to gain understanding about possible pathogenetic interactions between *P. multocida* and porcine lower respiratory tract tissues included demonstration of attachment of *P. multocida* to porcine tracheal epithelial cells (Jacques, 1987), porcine tracheal and nasal epithelial cells (Jacques *et al.*, 1988, 1993; Pijoan and Trigo, 1990), lung cells (Vena *et al.*, 1991), porcine respiratory mucus (Letellier *et al.*, 1991; Jacques *et al.*, 1993), and enhancement of adherence to porcine tracheal rings preinfected with *B. bronchiseptica* (Dugal *et al.*, 1992). Trigo and Pijoan (1988) demonstrated pili on strains of *P. multocida*. Isaacson and Trigo (1995) confirmed the finding of pili on strains of *P. multocida* isolated from pigs with atrophic rhinitis, but were unable to demonstrate that these organisms attached to red blood cells or immobilized porcine respiratory tract mucus. Finally, Mogollon *et al.* (1998) were unable to demonstrate that *P. multocida* capsular type A attached to porcine tracheal rings. In unpublished work, we (Ross *et al.*, unpublished, 2003) were also unable to confirm that porcine capsular type A strains from field cases of pneumonia attached to immobilized mucus or to porcine tracheal rings collected from newborn piglets. Dabo *et al.* (2003) utilized a cloned gene for an OmpA protein from *P. multocida* serotype A to demonstrate that this protein may be involved in adherence of the organism to host cells by way of heparin and/or fibronectin bridging. The strain utilized was *P. multocida* serotype A3, a common isolate from fibrinous pneumonia in cattle. Jacques *et al.* (1993) observed that capsule of toxigenic capsular type D strains decreased binding to respiratory tract mucus and adherence of these strains to porcine tracheal rings maintained in culture. They speculated that capsule masked outer membrane components involved in adherence. However, they also found that a capsulated isolate induced more severe turbinate lesions. Suffice to say at this juncture, it appears that more work is needed to clearly understand the role of attachment of *P. multocida* to porcine lower tract cells and its importance in pneumonia. As reviewed by Chanter (1990), the organism produces a neuraminidase and type B strains produce hyaluronidase, which might also be involved as virulence factors in at least some aspects of disease caused by the organism.

The answer to questions surrounding mechanisms that *P. multocida* must utilize to establish and play such an important role in porcine pneumonia may derive from understanding gene function in the organism. May *et al.* (2001) reported the complete genomic sequence of *P. multocida*. As noted by these authors, this work

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provides the foundation for research into the mechanisms of pathogenicity of this very important bacterium. Further genomic sequencing utilizing an appropriate strain of porcine origin might also prove useful. Efforts to understand the mechanisms whereby bacterial pathogens initiate and cause disease have focused on functional and topical surface characteristics, changes in composition of membrane proteins during various conditions of growth, particularly infection, mechanisms involved in iron acquisition and changes during various conditions of growth and expression of putative cell injurious substances (e.g., toxins or enzymes such as proteases) (Negrete *et al.*, 1999) and changes during various conditions of growth, including during the process of infection. Factors that have been identified which may contribute to pathogenicity of *P. multocida* include cytoagglutinins, pili, capsule and lipopolysaccharide and the dermonecrotoxin. Choi *et al.* (1989) demonstrated that *P. multocida* strain 1059 that was grown *in vivo* expressed outer membrane proteins that were not expressed during growth *in vitro*. Further work by the same group (Choi-Kim *et al.*, 1991) showed that such proteins were iron-regulated outer membrane proteins (IROMP). Zhao *et al.* (1995) extended the work to show that porcine capsular type A *P. multocida* also expressed IROMPs during iron-restricted conditions. Paustian *et al.* (2001) utilized whole genome microarray analyses for the identification of genes with altered expression profiles when growth conditions were varied. Their work was interpreted to provide a foundation for a more detailed analysis of the molecular mechanisms of iron acquisition and metabolism in *P. multocida*. Prado *et al.* (2005) characterized the heme acquisition system receptor of a bovine (A:3) strain of *P. multocida* and found a surface exposed OMP that is conserved among most *P. multocida* isolates they investigated. Their studies indicated that cattle produced antibodies to IROMPs in the strain investigated, with the 96 kDa HasR protein being an immunodominant IROMP. Further information on the findings of others and the rationale leading up to their study on IROMPs is provided by Prado *et al.* (2005).

Hunt *et al.* (2001) adapted '*in vivo* expression technology' (IVET) to a study of *P. multocida*. They identified numerous genes, expressed during *in vivo* growth in a mouse model. It was speculated that these genes may be useful for study of their products, involvement in pathogenicity and immunity. Fuller *et al.* (2000) utilized signature-tagged mutagenesis in a septicemic mouse model. Their attenuated mutant strains helped them identify potential virulence factors, biosynthetic enzymes, regulatory components and other unknown functions. Ewers *et al.* (2006) evaluated 289 strains of *P. multocida* from various clinically healthy and diseased hosts for capsule biosynthesis genes (*cap*A, B, D, E and F) and 14 virulence-associated genes using PCR and DNA–DNA hybridization. Their culture set tended to have a higher than usual proportion of type D (*cap*D) from porcine pneumonia compared to most publications. They found a combination of genes encoding outer membrane proteins, colonization factors, iron acquisition factors and superoxide dismutases in nearly all isolates. A gene coding for filamentous hemagglutinin, *pfh*A, was strongly associated with the *tox*A gene in swine isolates. Adler *et al.* (1999) charted progress utilizing molecular cloning to identify and characterize outer membrane proteins involved in iron acquisition, pili expressed by the organism, capsule, a hemolytic esterase and other surface proteins.

Current approaches to pathogenesis

Boyce *et al.* (2004) and Boyce and Adler (2006) have provided a series of extremely important studies and have summarized the state of knowledge with respect to pathogenesis of *P. multocida* infections in general. While their particular focus has been on fowl cholera, a septicemic disease, it is likely that many of the principles they have contributed themselves and/or that they have summarized in these reviews have applicability to *P. multocida* infections of the porcine lung. As Boyce and Adler (2006) indicated in their most recent review, some of the most productive recent work has focused on the response of the organism to its host environment transcriptionally with DNA microarrays (Boyce *et al.*, 2002) and protein-expression using proteomics technologies (Boyce *et al.*, 2006). Boyce and Adler (2006) conclude that 'to date, most *P. multocida* genes shown to be upregulated during infection are involved in nutrient acquisition and metabolic processes, indicating that true virulence genes might be constitutively expressed, upregulated only during initial stages of infection or upregulated at levels below current detection levels.' Further use of this approach might shed more information on high probability virulence factors that are important to the pathogenesis of pneumonia in pigs.

Steenbergen *et al.* (2005) have provided evidence that metabolism of sialic acids 'should be included as a potentially essential disease factor in systemic pasteurellosis'. These authors utilized a systemic mouse model of *P. multocida* infection with an attenuated sialate uptake-deficient mutant. They found that this attenuation indicated an important role in the disease process of a sialylation process that 'is dependent on an environmental (host) supply of sialic acid'. Their results were interpreted to indicate that scavenging sialic acid is important in systemic disease. They further speculated that the sialometabolic system might be a factor in the broad host range of *P. multocida*. Finally, as reviewed by Steenbergen *et al.* (2005), sialic acid has recently been shown to be required for normal biofilm formation by *Haemophilus influenzae* (Grenier *et al.*, 2004; Swords

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et al., 2004), a potential key process for bacterial colonization of the mucosa of animals.

Conclusion

The thrust of this review has been focused on pulmonary infections in swine primarily with *P. multocida* type A strains. Clearly, the conditions have not been met for consistent reproduction of the disease without coincident injury to the host tissue that somehow enables the organism to gain a foothold, to colonize and, seemingly, to produce further damage to the porcine lung. Thus, all of the work that has been done to characterize the mechanisms involved in *P. multocida* infections in other species or in the porcine upper respiratory tract with toxigenic strains, may not be entirely applicable to infections with this organism in the porcine lung. It may be important to make comparisons between *P. multocida* infection of the porcine lung and other bacterial infections of the lung such as *Pseudomonas aeruginosa* infection in human cystic fibrosis. It is well established that *P. aeruginosa* infection is a sequel to various types of primary damage to the human lung. As reviewed by Costerton *et al*. (1994) and Hoiby *et al*. (2001), bacteria are now thought to occur in the bodies of animals and humans as floating or planktonic cells or as sessile cells of microcolonies. The microcolonies have a polysaccharide matrix and may be composed of populations of different microbial agents, some dependent upon others. As reviewed by Spoering and Gilmore (2006), multicellular behavior of bacterial populations in these settings includes coordinated control of virulence, luminescence, competence and biofilm formation; all processes that may be regulated by quorum sensing. The thrust of their review was on recent developments showing DNA release and integration into the biofilm, with its linkage to quorum sensing.

Biofilms are characterized by their resistance to innate and immune defense mechanisms, as well as their resistance to antibiotics. It seems probable that *P. multocida* could be growing as a biofilm in the porcine lung. During this process, it is likely that host provided, or signals provided by other agents may be involved. Thus quorum sensing may be involved. Olson *et al*. (2002) have utilized a micro method to demonstrate that *P. multocida* can be grown *in vitro* as a biofilm, thus the capability of the organism to grow in a sessile form in diseased tissue seems likely. It seems important to recognize the likelihood that *P. multocida* utilizes a similar process to establish in the porcine lung, already altered by some prior insult. Hopefully, the use of various molecular methods will lead to a better understanding of the triggers involved in establishing and flourishing of *P. multocida* in the porcine lung. The strain diversity among *P. multocida* involved in serious pulmonary disease (Djordjevic *et al*., 1998) is completely consistent with an opportunist role for *P. multocida* and the establishment of a multicellular, multispecies bacterial biofilm.

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