Actinobacillus Species and their Role in Animal Disease

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SUMMARY

Actinobacillus species are Gram-negative bacteria responsible for several quite distinct disease conditions of animals. The natural habitat of the organisms is primarily the upper respiratory tract and oral cavity. A. lignieresii is the cause of actinomycosis (wooden tongue) in cattle: a sporadic, insidiously-developing granulomatous infection. In sharp contrast is A. pleuropneumoniae which is responsible for a rapidly spreading often fatal pneumonia, common among intensively reared pigs. Detailed investigation of this organism has provided a much clearer picture of the bacterial factors involved in causing disease. A. equuli similarly causes a potent septicaemia in the neonatal foal; growing apparently unrestricted once infection occurs. Other members of the genus induce characteristic pathogenesis in their preferred host, with one, A. actinomycetemcomitans, being a cause of human periodontal disease. This article reviews recent understanding of the taxonomy and bacteriology of the organisms, and the aetiology, pathogenicity, diagnosis and control of animal disease caused by Actinobacillus species. © 2000 Harcourt Publishers Ltd

KEYWORDS: Actinobacillus, animal disease, pleuropneumoniae, lignieresii, equuli.

INTRODUCTION

Actinobacillus is a genus of Gram-negative bacteria which has remained relatively obscure until recently. In part, this must be due to there having been no recognized medical pathogen in the group to provoke research interest. The type species, A. lignieresii, has been recognized for many years as the cause of actinobacillosis in cattle and sheep. Latterly, the group has been growing with the recognition of A. actinomycetemcomitans as a significant cause of periodontal disease and the transfer from Haemophilus of A. pleuropneumoniae, an economically significant cause of respiratory disease in the pig. Through the intense research interest focused on these two species, a much improved understanding of the bacteria has been acquired.

In this article, we will review the history and bacteriology of the genus and examine the current understanding of the pathogenicity characteristics of each species as veterinary pathogens, their pathogenesis and the immune responses they invoke.

HISTORY AND DEVELOPMENT OF ACTINOBACILLUS

The first accounts of the genus Actinobacillus were made by Lignières and Spitz (1902), after studying multiple subcutaneous abscesses in the head and neck of cattle in Argentina. These chronic lesions were similar to those of the well known actinomycosis, and both infections yielded small granules in the pus. However, in the majority of cases the infectious agent was distinct from Actinomyces bovis, and the lesions yielded a small Gram-negative rod which they named Actinobacillus. A. actinomycetemcomitans was first described in lesions of actinomycosis...
cosis in man where it was considered as a secondary pathogen (Klinger, 1912). Only quite recently has its role in periodontal disease become fully appreciated.

In 1918, an organism resembling Actinobacillus was reported from pneumonia in calves (Smith, 1918). This was first termed Bacillus actinoides and then Actinobacillus actinoides. The species is not recognised today and is almost certainly what we now term Haemophilus suis.

It was not until 1960 that Baynes and Simmons (1960) reported another organism which could be included in the genus. This was Actinobacillus suis isolated from epididymitis in three rams in Australia. Although it has been proposed as a cause of infertility in rams (Heath et al., 1991), it has not become a properly recognised member of the genus. A. suis was described from pigs in 1962 (VanDorssen & Jaartsveld, 1962), as was A. capsulatus from joint infections in rabbits (Arseculeratne, 1962). A. salpingitidis was reported as a cause of salpingitis and peritonitis in chickens (Bisgaard, 1975), however DNA hybridization studies now suggest that this organism is not closely related to the other members of the genus and will probably be transferred from the actinobacilli in time. There are six members of the genus which today are recognized as significant causes of disease in animals: A. lignieresii, A. suis, A. equuli, A. seminis, A. pleuropneumoniae and A. capsulatus. Others: A. rossii, A. muris, A. hominis and A. ureae are minor species of little veterinary impact. A. actinomyctecomitis is only considered as a significant pathogen of humans.

**BACTERIOLOGY**

The collective characteristics of the group Actinobacillus are that they are pleomorphic, non-motile rods which are able to grow on MacConkey agar, produce β-galactosidase and ferment carbohydrates without production of gas. Wild strains often have a characteristic sticky or waxy texture to the small, grey colonies. When cultured in liquid medium, the growth can be so cohesive as to have the consistency of processed cheese. This can make them difficult to handle in the laboratory, but colonies of a soft form, which are more easily manipulated, are usually segregated spontaneously. These do not appear to be altered in pathogenic ability. The molecular basis for this transition has never been reported, but comparison of isogenic soft and waxy forms suggests that it may be related to a subtle change in the Lipid A – core region of the lipopolyaccharide. Isolates of Actinobacillus show a variable reaction in catalase and oxidase tests. They do not convert tryptophan to indole but they do reduce nitrate-to-nitrite. Actinobacillus strains do not survive well in the laboratory and are usually non-viable 7–10 days from first culture, even after storage at 4°C, although some strains will persist for considerably longer.

**ACTINOBACILLUS LIGNIERESII**

Although this is the type species of the genus, A. lignieresii produces a very different pattern of infection from other actinobacilli. It appears to be a commensal of the oral cavity and pharynx of ruminants, particularly cattle and sheep. It has also been isolated from the rumen (Phillips, 1961). It causes disease usually after direct inoculation into the submucosal tissue during abrasion by rough feed or sharp object; the infection is therefore sporadic. However, one report challenging this assumption is an account of infection of several animals with A. lignieresii by a veterinary surgeon (deKruif et al., 1992). Granulomatous lesions followed caesarian sections in a number of cattle. In some cases the infection became disseminated to the viscera.

Strains of A. lignieresii are reported to vary in their ability to cause disease, both in natural and experimental infections. Cattle, particularly young animals, are very susceptible following experimental subcutaneous inoculation, and lesions (small abscesses) begin to develop after a few days. Lesions begin with an initial leukocytosis followed by formation of a granulomatous reaction with epithelioid cells and some giant cells in the centre. At the centre of the lesion, purulent foci develop which are bounded by concentric layers of connective tissue which become a thick fibrous wall surrounding the lesion. Where microcolonies of the bacteria develop in the centre of the structure, they are surrounded by the characteristic club-shaped clusters of organisms (Fig. 1). These are characteristically seen when the small cheese-like granules in the pus from actinobacillosis lesions are squashed to yield the distinctive club-shaped clusters.

Progress of the granulomatous lesions is slow and chronic. The lesions, which contain odourless pus, are located in the subcutaneous soft tissues of the head and neck, particularly the parotid region between the jaws, in the gums and cheeks and sometimes the tongue (wooden tongue in cattle). Infection can disseminate to the lymphatics and may sometimes spread to deep organs such as the
lungs. It has been suggested that lesions of the rumen and reticulum may be more common than is generally appreciated because such disease is infrequently diagnosed. Lesions distant from the head and neck may be confused with neoplasms (Rebhun et al., 1988). Antibodies to A. lignieresii are common in cattle serum, and levels increase in diseased animals. However, agglutinating antibody is not protective or associated with recovery. The granulomatous type of lesions indicates that a cell-mediated immune response is also evoked.

**ACTINOBACILLUS PLEUROPNEUMONIAE**

Previously known as *Haemophilus parainfluenzae* (Pattison et al., 1957), *Haemophilus parahaemolyticus* and then *Haemophilus pleuropneumoniae*, this organism was first identified as the cause of lung disease by Pattison et al., (1957) and Matthews and Pattison (1961). Following an outbreak of severe respiratory disease in pigs in Argentina, Shope made a comprehensive description of the infection and organism (Shope, 1964; Shope et al., 1964). The two papers describing the causal agent and the pathogenesis of the disease were very influential, but shed little light on the pathogenicity characteristics of the organism. Its original inclusion with the haemophili was due to its V-factor dependence. It will not usually grow on Blood agar alone and requires exogenous NAD or culture on heated blood agar. Alternatively, culture in the presence of a ‘nurse’ streak of a staphylococcus can be used, and this has the advantage of demonstrating the co-haemolysin activity if the staphylococcus is a β-toxin producer. A second biotype of *A. pleuropneumoniae* has been recognized (Pohl et al., 1983). These are NAD-independent for growth, are subdivided into two serotypes and have a minor role in pig respiratory disease (Fodor et al., 1989).

**Taxonomy of *A. pleuropneumoniae***

Examination of the taxonomic relationship of a large number of *Haemophilus* strains concluded that *H. parahaemolyticus* isolates from pigs formed a distinct group from those of the same name found in humans (Kilian, 1976a). A new species, *H. pleuropneumoniae* (the name originally used by Shope) was proposed as the agent of porcine pleuropneumonia (Kilian et al., 1978). Since the English isolate was lost, the isolate of Shope (strain 4074) became the type strain of the new species. In 1983, based on a variety of characteristics the organism was transferred from *Haemophilus* to the genus *Actinobacillus* (Pohl et al., 1983).

**Serotyping**

During the 1970s, an antigenic typing scheme was developed by Nicolet in Switzerland, Gunnarsson in Sweden and Nielsen in Denmark. This scheme was used to conduct original work on natural cross-protection and vaccination against the disease, and has led to the recognition of 13 distinct serotypes: 1–12 with 5a and 5b (Nielsen, 1986a, b). Certain serotypes are now recognized to be present in particular parts of the world with serotypes 1 and 5 particularly dominant in North America and serotypes 2, 3 and 9 in Western Europe.

Serotyping of strains was originally conducted using tube agglutination (Gunnarsson et al., 1977). Immunoprecipitation tests were not specific enough due to cross-reactivity of non-serotype
specific antigens (Gunnarsson et al., 1978), but using phenol-water extraction of the bacteria a more specific antigen preparation was obtained (Gunnarsson, 1979). The type-specific antigen was presumed to be capsular polysaccharide and this has now become accepted. More recently, improved serotyping was reported using co-agglutination (Mittal et al., 1983). This group was able to show the presence of antigen in lung lesions, providing a rapid diagnostic method for pleuropneumonia in the field. It was also recognized that among strains of serotype 1 there exists two types: those carrying a serotype 1 antigen which is heat-labile after boiling or autoclaving, and those with a heat-stable serotype 1 antigen (Mittal et al., 1983). An extensive study of serotyping methods has been conducted and reviewed by Mittal et al. (1992).

Serodiagnosis

Many herds of pigs are chronically infected with A. pleuropneumoniae but show no clinical signs and have a low level of pulmonary lesions. A successful serological test would be a most valuable tool in assisting diagnosis in the live animal: in screening animals before their movement to herds which are free of the disease and in implementation of eradication policies. Serodiagnosis cannot be relied upon to recognize all individual animals which are exposed to the organism or carrying it in the tonsil, and a representative proportion of the animals in a herd must be sampled.

Several methods for serodiagnosis of infection in herds and carrier animals have been attempted. The complement fixation (CF) test has become the standard test in the field (Gunnarsson, 1979; Jones, 1984) and is still used in serodiagnosis. It has a relatively low sensitivity and its reliability has been questioned.

In an effort to improve serological diagnosis, ELISA methods have been developed using EDTA-extract and various components of the bacteria (outer membrane, lipopolysaccharide and capsular polysaccharide) purified by gel filtration (Nicolet et al., 1981). Since infection may be with highly pathogenic or less pathogenic serotypes, it is valuable to determine which serotype is involved in the disease (Nicolet et al., 1981; Bossé et al., 1990; Nielsen et al., 1991; Gottschalk et al., 1997). Cross-reactivity between antigens (including LPS and certain envelope proteins) which are common to strains of different serotypes means that positive serological results can occur with more than one serotype antigen. Since they carry almost identical O antigen, infection with strains belonging to serotypes 1, 9 or 11 will generate antibody which is cross-reactive in assays using serotype 1 antigen (Gottschalk et al., 1994; Rodríguez-Barbosa et al., 1996). Similarly, there is cross-reactivity between LPS of serotypes 4 and 7, and serotypes 3, 6 and 8 (Nakai et al., 1992; Rodríguez-Barbosa et al., 1995). To take account of this, a more comprehensive serotyping scheme for A. pleuropneumoniae, which includes designation of both the K (capsular) and O-side chain lipopolysaccharide, has been proposed (Beynon et al., 1992).

Some investigators consider the best antigen for serodiagnostic purposes is highly purified capsular polysaccharide from which LPS and membrane proteins have been removed (Fenwick et al., 1996). However, long-chain LPS may also be suitable for serodiagnosis in ELISA (Gottschalk et al., 1994, 1997). Unfortunately, the preparation and standardization of such antigens is a difficult procedure and may vary between labs.

As an alternative to surface antigens, assays based on the detection of serum antibody to the Apx toxins have been attempted. In view of the prevalence of antibody which is cross-reactive with the Apx toxins in pigs, perhaps arising from infection with Actinobacillus suis (secreting ApxI var. suis and ApxII var. suis) or haemolytic Escherichia coli (producing HlyA), serological tests based on this have not been successful (Devenish et al., 1990c). In addition, LPS appears to bind strongly to the Apx toxins, causing cross-reactivity with that antigen unless recombinant Apx toxin is used (A.N. Rycroft, unpublished data). Detection of neutralizing antibody may prove a better alternative, but such assays are not easy to perform in a routine setting (particularly ApxI and ApxIII), and the sensitivity of the test may be inadequate.

Detection of the organism

Subclinical infection with A. pleuropneumoniae may also be determined with cultural or non-cultural methods to detect the bacteria in the tonsil. Selective media have been designed (Jacobsen & Nielsen, 1995) which improve the isolation of A. pleuropneumoniae from sites with a mixed flora. In addition, PCR-based methods have been developed in Denmark which are claimed to have a high level of sensitivity and specificity (Gram et al., 1996; Gram & Ahrens, 1998). Also, an immunomagnetic separation method was considered to be 1000-fold more sensitive than culture and highly effective in detection of A. pleuropneumoniae from tonsils (Gagne et al., 1998).
Pathogenicity

Outbreaks of disease caused by Actinobacillus pleuropneumoniae are usually associated with intensive production of pigs. In conditions of high stock density, rapid growth, poor ventilation etc., the disease can spread rapidly among animals with no immunity (Nicolet, 1993). Many animals can die in such an outbreak, while others will recover only partially, having residual lesions in the lung. Scarring can predispose to future infections with other bacteria, and pleural adhesions may inhibit normal respiratory function and cause poor growth. As herd immunity among survivors increases, the acute disease becomes less common, but animals continue to harbour the agent. Colostral antibody derived from infection in the sow is transferred to her piglets (Nielsen, 1975). It was also reported that protection lasts for no longer than 3 weeks, although antibody may persist for several weeks, during which time piglets are exposed to the organism and active immunity develops at this time. Therefore, further outbreaks of infection in a herd tend to occur in groups of animals where insufficient immunity is present (Nielsen & Mandrup, 1977).

The acute disease was systematically studied by Liggett et al. (1987) and by Bertram (1988, 1990): it is a necrotizing, fibrohaemorrhagic pneumonia with pleurisy. There is severe congestion in the lung and haemorrhage and exudation of serosanguinous fluid into the pulmonary parenchyma (Fig. 2). Sites of bacterial growth are rapidly infiltrated with neutrophils which then rapidly degenerate. Septicaemia is a rare, usually terminal complication of the infection. For many years, the primary mediator of damage was considered to be endotoxin (Sebunya & Saunders, 1983). While a role for endotoxin has not been excluded, and indeed the endotoxin of A. pleuropneumoniae is considered to be unusually puissant, intensive research has revealed a primary role for the protein Apx toxins in the invasive ability (Dom et al., 1992; Udeze & Kadis, 1992a; Jansen et al., 1995) and lesion production (Tascón et al., 1994; Kamp et al., 1997) by this organism.

A. pleuropneumoniae has always been known to be haemolytic, as visualized by the CAMP effect (Christie et al., 1944). Indeed, this remains one dependable means of recognizing the organism (Kilian, 1976b). In 1980, Soren Rosendal and colleagues in Guelph published work reporting the discovery that lung lesions could be induced in pigs using cell-free culture fluid (Rosendal et al., 1980). They followed this with a landmark paper describing how heat-labile, cell-associated and heat-stable, cell-free extracts of a virulent isolate of H. pleuropneumoniae were toxic to porcine cells, particularly alveolar macrophages (Bendixen et al., 1981). This, together with the rapidly increasing problem of pleuropneumonia in growing pigs, was a potent stimulus for further examination of the causal agent. Investigations of the nature and role of the haemolysin then began. Early suggestions that the haemolysin may be involved in the disease came from Nakai et al. (1983, 1984), who reported a heat-stable haemolytic activity in H. pleuropneumoniae. They expanded upon this to reveal that the haemolytic substance was also cytotoxic for porcine macrophages, and that it was carbohydrate in nature (Kume et al., 1986). Another group reported the haemolysin to be related to the group of toxins typified by Streptolysin S and the haemolysin of Serpula hyodysenteriae (Martin et al., 1985). They found that haemolytic activity was protease-sensitive and heat-labile, but was extremely variable and unreliable, and appeared to depend on the presence of RNA as a carrier molecule. However, only certain batches of RNA were effective and, in hindsight, this may have been due to unrecognized contamination with calcium ions required for haemolytic activity by the Apx toxins. Similarly, Maudsley and Kadis (1986) reported a heat-labile haemolysin detectable in cultures from H. pleuropneumoniae serotype 3. Crucially, they included 10 mM calcium ions in their assay buffer, and the results they obtained were more consistent than those of Martin et al. (1985).

The first report of purification of the haemolysin which is now recognized to be ApxI was made by Frey and Nicolet (1988). It was shown to be a protein of approximately 105 kDa whose activity was highly unstable, and therefore difficult to handle and study. Simultaneously, Rosendal et al. (1988) reported a detailed study of the haemolytic and cytotoxic activity of the organism, and Van Leengoed et al. (1989) studied the cytotoxic action of A. pleuropneumoniae of serotype 9 on porcine alveolar macrophages. They were unable to demonstrate cytotoxic activity in whole-cell extracts, but recovered toxic activity in cell-free supernatant. Notably, Rosendal et al. showed a neutrophil toxicity that was not always present in strains which were haemolytic. Devenish and Rosendal (1989) then confirmed the size of the haemolysin by demonstrating in situ haemolytic activity on electrophoretically separated material.
immobilized on nitrocellulose. Other work showed the haemolysin production was dependent on free calcium ions in the environment of the bacteria, and that these acted on gene expression at the transcriptional level (Frey and Nicolet, 1988). At this time, there was every reason to suppose that the haemolytic and cytotoxic activities were facets of the same molecule. However, Frey and Nicolet (1988) showed that polyclonal antibody raised to haemolysin of serotype 1 did not inactivate the haemolysin of serotype 2. Furthermore, its production was not improved by calcium ions, but the activity of the haemolysin did require calcium indicating, at least, that there were two distinct haemolysins.

A major advance in understanding the haemolytic and cytotoxic activities came from work by Kamp and colleagues at CVI in Holland. Using antibody raised to supernatants from cultures of different serotypes of *A. pleuropneumoniae*, they were able to demonstrate that some of the haemolysin and cytotoxin activities were serologically distinct (Kamp and Van Leengoed, 1989). This also implied that there was more than one haemolytic/cytotoxic activity in *A. pleuropneumoniae* and that different serotypes produced different substances. This was unexpected in view of the fact that all serotypes of *A. pleuropneumoniae* are known to cause disease with identical pathogenesis.

In 1990 came the first reports of a distinct cytotoxic protein of 120 kDa (Kamp et al., 1990; Rycroft and Cullen, 1990a). Using mutants of a serotype 2 strain, distinct haemolysin and cytotoxin molecules were recognised and these were identified as 109 and 120 kDa, respectively, and the non-haemolytic protein was named pleurotoxin (Rycroft et al., 1991a). This was supported by the demonstration of three distinct haemolytic and cytotoxic proteins by Western blotting using monoclonal antibodies (Kamp et al., 1991) and the recognition of the sec-

**Fig. 2.** Lung pathology in acute porcine pleuropneumonia due to *Actinobacillus pleuropneumoniae*. (a) normal lung tissue with open structure and patent bronchiole; (b) in experimental pleuropneumonia due to *A. pleuropneumoniae* serotype 2 the parenchymal tissue is consolidated with infiltrating macrophages and neutrophils and the bronchiole is obstructed with inflammatory cells (H & E ×75); (c) at higher power the inflammatory cells filling the airway can be seen as neutrophils; (d) a bronchiole filled primarily by fibrin deposition (H & E, ×300). (Reduced to 40% for reproduction.)
ond, rather weaker, haemolysin (ApxII) in serotype 1 strains (Frey et al., 1992).

Attempts to identify the genetic elements encoding haemolysin or cytotoxin did not begin well with the accidental cloning of a gene, later termed hlyX, which is in fact a global regulator element analogous to FNR which activates expression of a latent haemolytic activity in E. coli. (Lian et al., 1989; MacInnes et al., 1990). Similarly, a report describing the cloning of the CAMP cohaemolysin gene (cfp) has now been recognized as incorrect, and is probably the same sequence as hlyX (Frey et al., 1989). Isolation of the haemolysin genes came shortly afterwards (Frey et al., 1990). The work of many laboratories towards the understanding of these toxins was then brought successfully together.

To rationalize the disparate nomenclature which had arisen through different laboratories' use of different names and gene designations, it was proposed to redesignate the haemolysin and cytotoxin molecules of A. pleuropneumoniae ApxI, ApxII and ApxIII (Frey et al., 1993). The work of many laboratories towards the understanding of these toxins was then brought successfully together.

Structure and distribution of Apx toxin genes
The operon structure of the apx genes, like those of other RTX genes is CABD (Jansen et al., 1994). The C gene encodes an activation enzyme thought to acylate the protoxin, encoded by the A gene (Issartel et al., 1991). The functions of the B and D genes is to export the active toxin molecule across the inner and outer membranes (Welch & Pellet, 1988) using the C-terminal target sequence of the toxin molecule for recognition (Stanley et al., 1991).

The distribution of the Apx toxins among the serotypes is consistent among the large number of field isolates tested. Despite the fact that the functional activity of the Apx toxins are different, contagious pleuropneumonia caused by different serotypes is clinically and pathologically indistinguishable. Not all strains carry entire operons for each toxin they secrete, and the distribution of the different apx genes, and the Apx toxins themselves, is shown in Fig. 3.

Other factors are thought to contribute to disease. The polysaccharide capsule, while apparently different on each of the 12 serotypes, is considered as a virulence determinant (Fig. 4). Bacterial capsules usually reduce the extent to which phagocytic activity is effective in the absence of specific antibody. Phagocytic action was considered a difficult parameter to measure in the presence of potent Apx cytotoxins, but phagocytic killing of intact encapsulated A. pleuropneumoniae was demonstrated using an ApxI-/ApxIII-double-negative mutant which did not kill the phagocytic cells before their function could be examined (Cullen & Rycroft, 1994). Capsular material from a serotype 5 strain was found to be non-toxic in pig lung (Fenwick et al., 1986).

Resistance of A. pleuropneumoniae to killing by serum complement was first described by Inzana et al. (1988). A. pleuropneumoniae was found to be resistant even when homologous convalescent serum was used. Analysis of the resistance, using sublethal polymyxin B to permeate the outer membrane and sensitize the bacteria, showed that A. pleuropneumoniae was serum-resistant through a quite different mechanism from that seen in strains of E. coli (Rycroft & Cullen, 1990b). Further investigations showed that the mechanism of resistance involved interference by blocking antibody to lipopolysaccharide and other antigens (Udeze & Kadis, 1992b; Ward & Inzana, 1994).

A role for lipopolysaccharide endotoxin was implied by the results of the experiments conducted by Bendixen et al. (1981). Work by Fenwick et al. (1986) supported this. They found the rough LPS (lacking O-antigen side chains) to be more toxic than smooth LPS, and they demonstrated that LPS prepared by phenol-water extraction induced lesions in pigs lung similar to those dying from acute pleuropneumonia. Furthermore, vaccination with a strain of Escherichia coli was shown to have a protective effect against lethal H. pleuropneumoniae challenge, perhaps through antibody to Lipid A (Udeze et al., 1987). Maudsley et al. (1986) found the LPS of a serotype 2 strain to be smooth and similar in potency to that derived from H. influenzae or E. coli, and Lallier et al. (1987) reported a factor which induced dermal oedema in
**Fig. 3.** Diagrammatic presentation to show the presence of genes from the apx operons and the expression of the Apx toxins in the 12 serotypes of *A. pleuropneumoniae*. [Complied using data from Frey et al. (1993). Dark boxes indicate the presence of genes from the apx operon which lead to expression and secretion of the corresponding active Apx toxin. Light boxes show the presence of genes from a specific apx operon which do not result in the expression of that toxin.]

**Fig. 4.** (a) Transmission electron micrograph of *A. pleuropneumoniae* type strain 4074, immunostabilized with homologous antiserum to show the capsular polysaccharide (×100,000); (b) *A. pleuropneumoniae* cells visualized in pulmonary tissue from a pig infected with *A. pleuropneumoniae* showing capsule production in vivo (×75,000). From: M. Jacques, Foiry, B. Higgins R. Mittal, K.R. Journal of Bacteriology 170, 3314–8; reproduced with permission. (Reduced to 44% for reproduction.)
rabbits which was unchanged in material from a haemolysin-and cytotoxin-negative mutant (AN Rycroft, unpublished observations). This may have been endotoxin and we have no reason to suppose that LPS endotoxin of A. pleuropneumoniae cannot contribute to production of lesions. However, recent demonstration of the ability of isolated, recombinant, Apx toxins to induce typical pulmonary lesions (Kamp et al., 1997) supports the contention that LPS is not essential to lesion production. The immunomodulating activity now associated with bacterial endotoxin (induction of tumour necrosis factor, Interleukin-1 etc.) may explain the similarity of the lesions induced by different bacterial products.

The mechanism of adhesion of A. pleuropneumoniae to mucosal surfaces has been an area of controversy. Utlera and Pijoan (1991) reported fimbriae from cultures of A. pleuropneumoniae taken from pig respiratory tract without serial passage on artificial media. However, these results have not been confirmed in other laboratories, and the ‘hairlike projections’ seen by Inzana et al. (1988) are consistent with dehydrated capsular material rather than true surface appendages. A stronger candidate for the major adhesive component is the LPS which effects adhesion to porcine tracheal cells and respiratory tract mucous (Bélanger et al., 1990; Paradis et al., 1994). The role of adhesion in colonization, invasion and long-term persistence of the organism remains to be investigated.

Another factor which enables A. pleuropneumoniae to invade is its ability to acquire iron from the host environment. Under conditions where iron is scarce, the organism has been shown to produce new outer membrane proteins which specifically bind transferrin and allow the bacteria to utilize the iron for growth (Gonzalez et al., 1990; Gerlach et al., 1992).

**Immune response**

The nature of protective immunity to contagious pleuropneumonia is not clear. Nielsen (1979, 1984) reported cross-protection between serotypes after natural infection, although this has been challenged by more recent experiments, which found some cross-protection but not universal cross-protection (Cruijisen et al., 1995a; Hæsebrouck et al., 1996). The humoral immune response is thought to be a key part of the host’s protection against A. pleuropneumoniae, with IgG playing a major role. Passive transfer of immune swine serum produces protection against A. pleuropneumoniae (Inzana et al., 1988; Bossé et al., 1992). Levels of IgA also increase after infection with A. pleuropneumoniae (Bossé et al., 1992; Hensel et al., 1995). An increase in secretory IgA (sIgA) levels following oral administration of an antigen is a well characterized event. Produced in the respiratory mucosa, sIgA is thought to have a protective role, possibly preventing colonization of the mucosal surface by A. pleuropneumoniae.

The contribution of a cell-mediated immune response (CMI) is not so well defined, but a recent study reported that high delayed type hypersensitivity responses (as a measure of CMI) and antibody responses were associated with protection (Furesz et al., 1997).

It is our experience, and that of others, that neutralizing antibody to the Apx toxins is present in convalescent serum and is associated with protection against pleuropneumonia (Devenish et al., 1990b; Cruijisen et al., 1995b).

**Control by vaccination**

Pigs surviving a natural infection with A. pleuropneumoniae develop immunity to the organism and are protected against further infections of homologous and heterologous serotypes (Nielsen, 1979, 1984; Inzana, 1991). However, the antigens which produce this solid protective immunity have not been clearly identified and commercially available vaccines based on whole killed bacteria, which include capsular polysaccharide, lipopolysaccharide and outer membrane proteins, do not produce complete protection. These vaccines reduce mortality but they do not prevent disease or the development of chronic lesions (Higgins et al., 1985; Fenwick & Osburn, 1986). As the commercially available vaccines do not prevent the economic losses associated with the disease, work continues to elucidate the mechanism for complete protection.

The ability of several of the known virulence factors in A. pleuropneumoniae to induce an immune response has been investigated. Pigs and mice vaccinated with only capsular polysaccharides from A. pleuropneumoniae have been shown to be partially protected against challenge with a homologous serotype (Nielsen, 1984; Rosendal et al., 1986; Bhatia et al., 1991). In addition, vaccines containing outer membrane proteins have been shown to provide some degree of cross-immunity (Rapp & Ross, 1986, 1988; Deneer & Potter, 1989; Chiang et al., 1991). Neutralizing antibodies to the 104 kDa
haemolysin are found in pigs convalescent from pleuropneumonia (Rosendal et al., 1988), and Devenish et al. (1990c) reported complete protection from homologous challenge in pigs immunized with pure haemolysin, when high neutralizing antibody titres were achieved.

The use of recombinant protein vaccines could result in an improvement in the protection provided by all of these elements. Indeed, Rosic-Campos et al. (1992) showed that vaccination of pigs with a recombinant cytolysin protein provided partial protection, but that the level of protection was increased by the combination of the recombinant cytolysin with transferrin binding-protein. An alternative vaccination strategy is the use of viable or inactivated A. pleuropneumoniae for oral immunization of pigs, particularly by aerosol administration. Partial protection has been reported, but environmental considerations oblige limitations on field usage (MacInnes & Rosendal, 1988; Delventhal et al., 1992; Hensel et al., 1995). Attempts to commercialize defined acapsular mutants (Ward & Inzana, 1996) and other defined mutants (Hodgson et al., 1996) as live attenuated vaccines, which secrete active or genetically detoxified Apx toxins, are also underway.

Despite the extensive work on the protection afforded by the known antigens in A. pleuropneumoniae, it remains true that natural infection with A. pleuropneumoniae results in complete protection but killed bacterin vaccines produce only partial protection against infection. By comparing the antibody- and cell-mediated immune responses produced with low-dose aerosol infection and a commercial bacterin, Furesz et al. (1997) proposed that the immune response produced from a natural infection is different from that produced by current bacterins. Significant increases in serum antibody responses were recorded in the low-dose challenge group, most notably to Hlyl. Hence, it may be beneficial to modify the current bacterins to elevate the serum antibody response produced.

**ACTINOBACILLUS EQUULI**

A. equuli is the cause of the rare, but frequently fatal, sleepy foal disease. This is a septicaemia of neonatal foals seen throughout the world. Early estimates suggested that as many as 29% of all fatalities among newborn foals were due to this disease (Miller, 1950; Platt, 1973). The occurrence is sporadic, but the infection can affect a number of neonatal foals delivered on the same premises over a period of time. Infection of the foal is thought to be via the mouth, respiratory tract or umbilicus during or immediately following birth. The source of infection is the mare, where the organism may be carried as a commensal in the mouth, upper respiratory tract and alimentary tract. It is rarely found in the genital tract. Another proposal that the organism may infect the foal in utero, perhaps by transplacental invasion by the larvae of Strongylus vulgaris, has not yet been substantiated. The organism invades many organs of the body but purulent nephritis is the most commonly seen lesion (Fig. 5). The organism is also a cause of joint-ill and other infections of the foal, and occasionally causes disease (septicaemia, peritonitis and abortion) in mature animals. A recent survey of lower airway inflammation in the adult horse has recognized the involvement of A. equuli-like organisms in lower respiratory tract infection (Wood et al., 1993).

Disease in the newborn foal is strongly associated with colostrum deprivation, and therefore components of colostrum from the mare (probably immunoglobulins) are thought to be passively protective to the foal. Supporting evidence is limited, but in a recent case of fatal sleepy foal disease, the serum antibody from the mare and the foal was compared by immunoblotting against the infecting organisms (Rycroft et al., 1998). This showed high levels of antibody specific to surface bacterial antigens in the mare, while it was virtually undetectable in the foal. However, the antigen specificity of the immunoglobulins which are required for protection is not known.

**Fig. 5.** Microscopic appearance of a lesion in the kidney from a fatal case of A. equuli septicaemia (H&E; Bar = 25 μm).
In a serological study, it was found that *A. equuli* is antigenically heterogeneous: there being at least 28 different antigenic groups based on the immunodominant heatstable surface (lipopolysaccharide) antigen (Kim, 1976). In view of this, vaccination has, to date, been considered to be impractical. Similarly, attempts to use mare’s serum, or an antisem to *A. equuli*, to treat the disease have not been effective (Kim, 1976). This may have been because the reagent was administered too late in the disease or because the protective components were missing from the serum.

No particular sero-groups are associated with disease in foals, and all are thought to be equally capable of producing disease. This is supported by the recent finding of two distinct strains of *A. equuli* in a case of septic foal disease (Rycroft et al., 1998). This suggests *A. equuli* is an opportunistic pathogen of relatively low pathogenicity, which will invade and kill foals when circumstances allow.

No toxins have been reported with *A. equuli*, however RTX-like sequences have been independently detected using DNA hybridization techniques by Burrows and Lo (1992) and Macdonald and Rycroft (1992). Whether this is expressed by the organism as a toxic activity remains to be discovered. It is known that some strains of *A. equuli* are haemolytic (Carter et al., 1971; Hughes & Murphy, 1972). These must therefore produce a haemolytic component from the bacterial cell. Furthermore, anti-haemolysin antibody is present in equine serum, indicating natural exposure to this or a closely related bacterial product. The fact that many strains of *A. equuli* do not elaborate such a haemolysin does not demonstrate that they are not producing a toxin: other members of the Actinobacillus group produce related protein exo-toxins, of which some are clearly haemolytic (ApxI) while others are not (ApxIII). Identification of the bacterial factors responsible for invasive disease in the foal and the antibody specificity of colostrum to prevent this are areas for future investigation.

**ACTINOBACILLUS SUIS**

*A. suis* is a β-haemolytic organism associated with respiratory mucosa of pigs and, to a lesser extent, with horses. Original isolates were from septicaemia and lesions in various organs in young pigs. Later, it was found to cause disease in adult pigs. There has been some debate as to whether the organism is present in healthy pigs, but it is now clear that it may colonize the tonsil and upper respiratory tract of apparently healthy animals without causing disease. However, if introduced to pigs which are naive to the organism, or if immunity levels have declined in the sow, it is likely to initiate outbreaks of sudden death in a few piglets from the same litter. Disease is therefore seen mostly in pigs of high health status (Sanford et al., 1990).

Infection is probably by aerosol into the upper respiratory tract, and invasion is probably through the upper respiratory tract mucosa. Infected emboli spread haematogenously throughout the body adhering to the endothelium of blood vessels or becoming trapped in smaller vessels (Sanford, 1992). *A. suis* infection is seen as petichial haemorrhages in the lungs, kidney and other organs. There is often a serous or fibrinous exudate which may be present in the thorax and pericardium at postmortem. In older animals, *A. suis* septicaemia may produce irregular erythematous lesions in the skin which may be confused with the lesions of erysipelas.

The pathogenicity factors of *A. suis* are not known except, by inference, the 104 kDa protein haemolysin. This is an RTX group cytolsin genetically related to ApxII of *A. pleuropneumoniae* (Burrows & Low, 1992; Kamp et al., 1994) which may perform a similar defensin or aggression role in pathogenesis.

Immunological evidence, however, suggests that *A. suis*-infected pigs develop neutralizing antibody to ApxI. Strains may therefore carry toxins similar to both ApxI and ApxII or the one toxin may have features of these. In support of this, Van Ostaaijen et al. (1997) demonstrated genes homologous with the apxICA and apxIICA genes of *A. pleuropneumoniae* in *A. suis* strains. Thus, the *A. suis* forms of the ApxI and ApxII haemolysins are probably responsible for generating antibodies commonly detected in healthy pigs. This cross-reactive antibody causes interference with serodiagnostic tests for *A. pleuropneumoniae* based on antibody to the Apx toxins (Devenish et al., 1990c). In addition, the antibody may provide protection in normal pigs against *A. suis* disease but may also provide a degree of protection against contagious pleuropneumonia (Fenwick et al., 1996).

Evidence suggesting that porcine isolates of *A. suis*, at least in southwestern Ontario, are a very limited clonal group was reported by Van Ostaaijen et al. (1997). A large number of isolates from diseased and healthy animals reacted with the same
rabbit antiserum to one isolate, suggesting that they represent one serotype. They also showed remarkably little variation in analysis by restriction endonuclease fingerprinting. However, this analysis should be extended to strains from beyond the local sampling area of Canada in order to establish the wider picture of clonality in A. suis.

Elsewhere, there has been confusion over the identity of porcine and equine isolates of A. suis. Haemolytic strains of Actinobacillus from equine disease have been considered to be A. suis, haemolytic A. equuli or haemolytic A. lignieresii (Carter et al. 1971; Hughes & Murphy, 1972; Bisgaard et al., 1984; Nelson et al., 1996). Kim et al. (1976) investigated the serological and biochemical characters of a large group of equine isolates, and concluded that the haemolytic strains formed a group separate from A. equuli which they referred to as A. suis.

Biberstein (1990) recognized a group of haemolytic Actinobacillus strains from horses to be A. suis-like, and differentiated them from A. equuli on the basis of biochemical tests (Table I). Samitz and Biberstein (1991) investigated the biochemical and electrophoretic characteristics of 37 strains from horses. A. suis-like organisms and A. suis were similar in many respects and insufficiently different to warrant a separate group. However, they did conclude that aesculin-negative A. suis-like organisms were haemolytic variants of Actinobacillus lignieresii. In order to try to resolve this problem, Bada et al. (1996) studied 50 isolates from pigs and horses. They also found no major biochemical difference among equine and porcine A. suis isolates. Furthermore, tube agglutination tests showed that porcine isolates were antigenically homogeneous, while equine isolates were relatively heterogeneous.

Conversely, strains termed A. equuli and A. lignieresii have been reported from pigs (Windsor, 1973), and both haemolytic and non-haemolytic strains were considered capable of causing disease. Clearly the taxonomic relationship of this group is not well defined and the problem is exacerbated by the unwarranted weight given to haemolysis of these strains in their initial characterization. The group probably represents a range of strains which have diverged over a considerable period of time, with strains able to colonize niches to various extents in both animal species.

**ACTINOBACILLUS SEMINIS**

There is little information about A. seminis. The original isolations made by Baynes and Simmons (1960) were from the semen of rams with epididymitis. Clinically, the disease was reported to

<table>
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<tr>
<th>Table I</th>
<th>Simplified differentiation of selected Actinobacillus species</th>
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<tr>
<td></td>
<td>A. lignieresii</td>
</tr>
<tr>
<td>NAD requirement</td>
<td>-</td>
</tr>
<tr>
<td>Haemolysis (bovine blood)</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>V</td>
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<tr>
<td>Oxidase</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
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<tr>
<td>Acid from:</td>
<td></td>
</tr>
<tr>
<td>Cellobiose</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>V</td>
</tr>
<tr>
<td>Trehalose</td>
<td>-</td>
</tr>
<tr>
<td>Melibiose</td>
<td>-</td>
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<td>Mannitol</td>
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<tr>
<td>Xylose</td>
<td>+</td>
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<tr>
<td>Maltose</td>
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aBiotype 2 is NAD-independent;
bRequires exogenous NAD to grow on blood agar;
V, result variable between isolates.
resemble Brucella ovis disease. Other isolations have been reported (Livingston & Hardy, 1964; Low et al., 1995), and the organism has been suggested as a cause of infertility in rams (Heath et al., 1991). The suggestion was made that isolates of A. seminis were in fact Histophilus ovis (Webb, 1983). H. ovis is recognized as a cause of infertility and was previously isolated from the genital tract of rams (Claxton & Everett, 1966; Low & Graham, 1985). However, Heath et al. (1991) concluded that their A. seminis strains were distinct from H. ovis strains previously isolated in Scotland. This has been supported by recent work using combined PCR-based methodology (Appuhany et al., 1998), and it must be concluded that A. seminis is a close relative of H. ovis which coincidentally occupies a similar niche.

**ACTINOBACILLUS CAPSULATUS**

Actinobacillus capsulatus was described by Arseculeratne in 1962 as a cause of joint disease in laboratory rabbits in Ceylon. The lesion was granulomatous and encapsulated in cellular fibrous tissue. The organism isolated was non-haemolytic and sticky, and sufficiently resembled other members of the Actinobacillus genus to include it with them. Following a detailed examination of the organism, it was considered as a distinct species, but it has not been reported since.

**ACTINOBACILLUS ACTINOMYCETEMCOMITANS**

A. actinomycetemcomitans is not a recognized veterinary pathogen, although it has been isolated from infection in animals such as epididymitis in rams (Bulgin & Anderson, 1983). It is a cause of disease in humans, and was originally described as attendant in actinomycosis infections in humans, from which its name is derived. It was later shown to be a member of the normal human oral flora (Slots et al., 1980), and is now known to play an aetiological role in localized destructive juvenile periodontitis.

At the molecular level, A. actinomycetemcomitans has become the most extensively studied member of this group, and very interesting data on the host-pathogen relationship are accumulating. An RTX leukotoxin (Lkt), related to the \( \alpha \)-haemolysin (Hly) of E. coli, has been recognized for many years (Taichman et al., 1980). This has specificity for human leukocytes and is presumed to play a role in inhibiting neutrophil defence mechanisms at the site of infection. Isolates from healthy individuals do not usually produce the leukotoxin because transcription is suppressed, whereas those from diseased individuals are usually toxic. Highly toxic strains may be associated with increased virulence (Hritz et al., 1996). Furthermore, the organism has immunosuppressive activity by production of a 60 kDa protein which downregulates both T- and B-cell responsiveness (Shencker et al., 1990). It is now known to be capable of invading epithelial cells by receptor-mediated endocytosis (Sreenivasan et al., 1993), following which they escape from the vacuole and spread to neighbouring cells (Meyer et al., 1996).

**MINOR ACTINOBACILLUS SPECIES**

Actinobacillus rossii was described by Sneath and Stevens (1990) from the vagina of post-parturient sows. Actinobacillus urae was previously recognized in the genus Pasteurella and transferred by Mutters et al. (1986). Actinobacillus muris was described from the aerobic pharyngeal flora of healthy white mice (Bisgaard, 1986). Actinobacillus hominis was confirmed as belonging to the Actinobacillus group by Mutters et al. (1984, 1986), and has been implicated as an occasional cause of infections in humans with serious pre-existing pathology. Three new species of Actinobacillus from the pig respiratory tract have been described by Moller et al. (1996). Based on 16S rRNA sequences and other genetic relationships, these V factor-dependent species have been named A. minor, A. porcinus and A. indolicus. They are of little importance in animal health.

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