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Review

A Review of *Listeria monocytogenes* and Listeriosis

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SUMMARY

Following the initial isolation and description in 1926 *Listeria monocytogenes* has been shown to be of world-wide prevalence and is associated with serious disease in a wide variety of animals, including man. Our knowledge of this bacterial pathogen and the various forms of listeriosis that it causes has until recently been extremely limited, but recent advances in taxonomy, isolation methods, bacterial typing, molecular biology and cell biology have extended our knowledge. It is an exquisitely adaptable environmental bacterium capable of existing both as an animal pathogen and plant saprophyte with a powerful array of regulated virulence factors. Most cases of listeriosis arise from the ingestion of contaminated food and in the UK the disease is particularly common in ruminants fed on silage. Although a number of forms of listeriosis are easily recognized, such as encephalitis, abortion and septicaemia, the epidemiological aspects and pathogenesis of infection in ruminants remain poorly understood. The invasion of peripheral nerve cells and rapid entry into the brain is postulated as a unique characteristic of its virulence, but relevant and practical disease models are still required to investigate this phenomenon. This review offers an up to date introduction to the organism with a description of virulence determinants, typing systems and a detailed account of listeriosis in animals. Experimental and field papers are reviewed and further sections deal with the diagnosis, treatment and control of listeriosis in animals. A final part gives an overview of listeriosis in man.

KEYWORDS: Listeriosis; epidemiology; pathogenesis; control; diagnosis.

INTRODUCTION

Listeric infections, caused by micro-organisms of the genus *Listeria*, occur worldwide and in a variety of animals including man. Listeriosis was first recognized as a disease of animals and although the link between silage feeding and infection in farm animals has been known for decades it was the recognition in the 1980s of listeriosis as a food-borne human disease that prompted intense research activity. This review is intended to provide critical details of *Listeria monocytogenes* and of listeric infections which occur in animals. Whilst by no means comprehensive it is intended that sufficient detail will be provided such that the reader has ready access to information contained in significant reviews and original work. It is our desire to provide an up to date and accurate understanding of the organism, the infections it causes and to indicate those areas where further research is necessary.

THE GENUS *LISTERIA*

Taxonomy

Originally *L. monocytogenes* was the only species within the genus but *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri* and *L. seeligeri* are now all separately recognized (Seeliger & Jones, 1986; McLauchlin, 1987). On the basis of phenotypic and genomic studies it is controversial whether *L. murrayi* and *L. grayi* should be included in the genus (Stuart & Welshimer, 1974; Wilkinson & Jones, 1977; Rocourt *et al.*, 1987a), although

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recent evidence (Rocourt *et al.*, 1992) has indicated that they should be considered a single species and referred to as *L. grayi*. The single isolate of *L. denitrificans* has now been ascribed to the genus *Jonesia* (Rocourt *et al.*, 1987b). Clinical infections are caused primarily by *L. monocytogenes* although *L. ivanovii* is also pathogenic, being particularly associated with abortion in ruminants. The remaining species are regarded as non-pathogenic.

Characterization

L. monocytogenes was described originally by Murray *et al.* (1926) and Pirie (1927). It is a small, Gram-positive rod, 1–2 μm in length and 0.5 μm wide, with bacteria often lying parallel to each other in palisades, and thread-like forms present in some cultures. Growth occurs between 3 and 45°C but the optimum temperature range is 30–37°C. The organism multiplies readily in aerobic or microaerophilic conditions at pH values as high as 9.6. Growth is absent or scant in complete anaerobic conditions and multiplication is inhibited by pH values lower than 5.6. The bacterial colonies are small, smooth, slightly flattened and milky white by reflected light (Gray & Killinger, 1966). When illuminated by obliquely transmitted light the colonies exhibit a characteristic blue/green colour (Henry's lamp technique).

Organisms grown at 37°C show little or no motility (Gray & Killinger, 1966; Seeliger & Hohne, 1979; Seeliger & Jones, 1986) but a characteristic tumbling motility can be demonstrated by incubation of cultures at room temperature with motile organisms commonly possessing many peritrichious flagellae (Peel *et al.*, 1988). Although relatively inactive biochemically, *Listeria* species produce catalase, are positive in the Voges–Proskauer reaction and hydrolyse aesculin. They are indole- and oxidase-negative, do not hydrolyse urea or reduce nitrates and there is no liquefaction of gelatin. The different *Listeria* species can be distinguished on the basis of a few simple biochemical tests (Rocourt *et al.*, 1983) and recently a rapid ten test strip identification system has been developed (Bille *et al.*, 1992). Included in the routine identification the presence and appearance of haemolysis is important for species differentiation with only *L. monocytogenes*, *L. ivanovii* and *L. seeligeri* being haemolytic (Table I). The haemolysis shown by *L. monocytogenes* can be enhanced by culture with *Staphylococcus aureus* (Brzin & Seeliger, 1975), the so-called CAMP phenomenon, whilst *Rhodococcus equi* markedly enhances the double zone of haemolysis typically shown by *L. ivanovii* (Rocourt *et al.*, 1983). Although some texts report no synergistic CAMP effect *R. equi* weakly enhances the haemolysis shown by *L. monocytogenes* strains (McKellar, 1994).

Typing methods

Agglutination and absorption experiments with hyperimmune serum formed the basis of early serological studies (Seastone, 1935; Webb & Barber, 1937; Julianelle, 1941). Paterson (1939, 1940a) demonstrated somatic 'O' and flagellar 'H' antigens and described four serological types. This basic scheme was extended to include more antigenic variants (Seeliger, 1961; Donker-Voet, 1966; Seeliger & Hohne, 1979) and the currently accepted Seeliger/Donker-Voet scheme, pre-

Table I
Identification of *Listeria* spp.
(according to Rocourt *et al.*, 1983 and Bille *et al.*, 1992)

	DIM[1] *test*	*Haemolysis of horse blood*	*CAMP test*		*Production of acid from:*		
			Staphyloccocus aureus	Rhodococcus equi	*L-rhamnose*	*D-xylose*	*Alpha-methyl D-mannoside*
L. monocytogenes	–[2]	+	+	(+)[3]	+	–	+
L. ivanovii	+	+++	–	+	–	+	–
L. innocua	+	–	–	–	v	–	+
L. welshimeri	+	–	–	–	v	+	+
L. seeligeri	+	v	–	–	–	+	v

+, Positive; –, negative; v, variable reaction; +++, strong positive.
[1]DIM, Differentiation of *innocua* and *monocytogenes*.
[2]All *L. monocytogenes* isolates are DIM negative but occasional isolates of the other species may be negative.
[3]Enhanced haemolysis may be apparent (McKellar, 1994).

sented in Table II, includes 16 serovars of the genus. A major disadvantage of serotyping is that it does not correlate with the species distinctions and a number of serovars are common to different species. Serotyping is also of limited value in epidemiological investigations as only three serovars (1/2a, 1/2b and 4b) are usually involved. The importance of *L. monocytogenes* as a food-borne pathogen has prompted the development of reliable and highly discriminating typing methods for epidemiological purposes and the investigation of alternative typing methods.

Phage-typing is reproducible and discriminatory (Audurier *et al.*, 1984; McLauchlin *et al.*, 1986), but is limited in its application by the low percentage of typable strains present in serogroup 1/2 (around 50%) and by the lack of phages for some of the rarer serovars. The overall percent typability of *L. monocytogenes* strains has ranged from 52–78% in various studies, but a new set of phages and a modified procedure have been described enabling the typing of most serogroup 1/2 strains and the majority of isolates of serovar 4b (Loessner, 1991; Estela & Sofos, 1993).

Multilocus enzyme electrophoresis (MEE) analysis, a form of iso-enzyme typing, allows the definition of electrophoretic types (ETs) and a comparison of the genetic relationships among the ETs. For *L. monocytogenes*, two primary divisions have been delineated, with serovars 1/2b, 3b and 4b falling into one division and serovars 1/2a, 1/2c and 3a falling into the other (Piffaretti *et al.*, 1989; Bibb *et al.*, 1989; Bibb *et al.*, 1990; Norrung & Skovgaard, 1993). The technique appears to be useful in either confirming or eliminating a common source as the cause of an outbreak of food-borne listeriosis, although the results must be interpreted cautiously if an isolate pair (e.g. patient–food) is of a commonly occurring ET, in contrast to a situation in which a less common ET is involved and a causal link or association can be made with more confidence (Bibb *et al.*, 1990).

Direct analyses of the genome have resulted in the application of several different techniques to the typing problem. Restriction enzyme analysis (REA) has been used to characterize strains of *L. monocytogenes* recovered from outbreaks of listeriosis (Nocera *et al.*, 1990; Wesley *et al.*, 1990; Baloga & Harlander, 1991), and has shown that isolates from major human epidemics each exhibit a unique restriction enzyme pattern (Wesley & Ashton, 1991). However, recording and comparison of the complex, multiple band patterns generated can be difficult. Pulsed-field gel electrophoresis produces fewer and larger fragments of DNA which are more easily identified and although the technique requires specialized equipment it has proved to be an extremely powerful discriminatory technique (Carriere *et al.*, 1991; Brosch *et al.*, 1991; Howard *et al.*, 1992; Brosch *et al.*, 1994).

Table II
Serovars of the genus *Listeria*, (according to Seeliger & Jones, 1986)

Designation		*O antigens*	*H antigens*
Paterson	*Seeliger–Donker-Voet*		
1	1/2a	I II (III)[a]	A B
	1/2b	I II (III)	A B C
2	1/2c	I II (III)	B D
3	3a	II (III) IV	A B
	3b	II (III) IV (XII XIII)	A B C
	3c	II (III) IV (XII XIII)	B D
4	4a	(III) (V) VII IX	A B C
	4ab	(III) V VI VII IX X	A B C
	4b	(III) V VI	A B C
	4c	(III) V VII	A B C
	4d	(III) (V) VI VIII	A B C
	4e	(III) V VI (VIII IX)	A B C
	5	(III) (V) VI (VIII) X	A B C
	7	(III) XII XIII	A B C
	6a(4f)	(III) V (VI VII) (IX) XV	A B C
	6b(4g)	(III) (V VI VII) IX X XI	A B C

()[a], Not always present.

Random amplification of polymorphic DNA is rapid (Mazurier & Wernars, 1992) and recently has shown considerable promise in distinguishing *Listeria* species (Black *et al.*, 1995). Ribotyping has failed to discriminate particularly among *L. monocytogenes* serovar 4b isolates (Saunders *et al.*, 1989; Wesley *et al.*, 1990; Baloga & Harlander, 1991; Nocera *et al.*, 1993) which appear to be of a close genetic relationship in comparison to serovar 1/2 strains (Nocera *et al.*, 1993; Norrung & Gerner-Smidt, 1993). Plasmid typing is not likely to be of value as most clinical strains of *L. monocytogenes* do not appear to carry plasmids and monocine typing has been of little value. Typing based upon biochemical differences, although useful for species identification, cannot be used to discriminate amongst strains (Seeliger, 1961) and although pyrolysis mass spectrometry (Freeman *et al.*, 1991; Low *et al.*, 1992) is rapid and potentially capable of resolving strain differences the technique is limited and has received little attention.

VIRULENCE DETERMINANTS IN *L. MONOCYTOGENES*

The nature, structure and biological functions of many of the virulence determinants for *L. monocytogenes* have recently been clarified (Portnoy *et al.*, 1992). The organism is capable of multiplication within cells of the monocyte–macrophage series (Mackaness, 1962; Armstrong & Sword, 1964, 1966) and is capable of entering the cell, escaping from the phagosome, multiplying within the cytoplasm and spreading between cells. Crucial to the virulence of the organism is its ability to escape intracellular killing within macrophages by lysis of the phagosomal membrane and escape into the cytoplasm, which is mediated by its secretion of a haemolysin, listeriolysin O (LLO). The importance of LLO as a virulence determinant has been clearly established in the elegant work of Gaillard *et al.* (1986); Geoffroy *et al.* (1987); Kathariou *et al.* (1987).

Several other virulence determinants have been identified which are involved or associated with bacterial survival. The majority of the genes involved are arranged around the haemolysin gene forming a virulence cluster which is also present in other haemolytic *Listeria* species (Gouin *et al.*, 1994). The *prfA* gene positively regulates expression of LLO in pathogenic strains and encodes a protein of 27 kDa molecular mass which has no significant homology to other bacterial regulatory proteins (Leimeister-Wachter *et al.*, 1990). Further studies have shown the *prfA* gene to regulate its own synthesis and to be a pleotrophic regulator of virulence determinants involved in invasion, intracellular bacterial motility and cell-to-cell spread (Mengaud *et al.*, 1991a; Chakraborty *et al.*, 1992). The phosphatidylinositol-specific phospholipase C (PI-PLC) gene encodes a protein of approximately 36 kDa molecular mass (Camilli *et al.*, 1991; Leimeister-Wachter *et al.*, 1991; Mengaud *et al.*, 1991b) which may act in concert with LLO for lysis of the phagosomal membrane (Camilli *et al.*, 1991). However, mutations within the gene exert a polar effect upon a regulatory gene (*prfA*) and it is therefore difficult to assess the true role of PI-PLC in virulence (Camilli *et al.*, 1991; Leimeister-Wachter *et al.*, 1991; Mengaud *et al.*, 1991b).

Movement within the host cell cytoplasm follows encapsulation of the organism by actin filaments which propel the bacterium into neighbouring cells (Tilney & Portnoy, 1989; Mounier *et al.*, 1990). A gene, termed *actA*, included in the virulence cluster encodes a surface protein with a calculated molecular mass of 67 kDa (Kocks *et al.*, 1992) and although the protein's function is obscure it is clearly involved in actin assembly since strains having mutations within the *actA* gene do not polymerize actin and have markedly reduced virulence (Kocks *et al.*, 1992; Domann *et al.*, 1992). The operon which incorporates the *actA* gene also includes two other virulence-associated genes one of which encodes a 29 kDa molecular mass, zinc-dependent phospholipase possessing lecithinase activity (Geoffroy *et al.*, 1991). This is apparently involved in lysis of the double cell membrane which forms a barrier to cell-to-cell spread (Vazquez-Boland *et al.*, 1992a). Immunoblotting of *L. monocytogenes* culture supernatant fluids has identified, in addition to the lecithinase, an antigenically related protein which is now recognized as the enzyme precursor. The first gene of the same operon, encodes a metalloprotease (Mengaud *et al.*, 1991c), involved in maturation of the precursor to the mature 29 kDa protein (Raveneau *et al.*, 1992).

Though these mechanisms of intracellular multiplication have been clarified the means for bacterial adherence to cells are unknown. Entry of *L. monocytogenes* to both phagocytic and epithelial cells has been demonstrated *in vitro* and is possibly associated with production of a 80 kDa molecular

mass protein 'internalin' (Gaillard *et al.*, 1991). Sequencing of the internalin gene predicts a protein with a cytoplasmic membrane anchor and a structure reminiscent of *Streptococcus pyogenes* M protein. The gene is part of a gene family regulated by *prfA* and under nonstringent conditions a gene probe has detected several related genes in *L. monocytogenes*, *L. ivanovii* and *L. innocua*. A protein, termed P60, may also have a role in invasion (Kuhn & Goebel, 1989) as rough forms of *L. monocytogenes* lacking P60 are unable to enter cells. However, the protein is unrelated to internalin (Gaillard *et al.*, 1991) and there are contradictory reports of its requirement for cell invasion (Kathariou *et al.*, 1990).

Superoxide dismutase and catalase activities do not correlate with virulence (Welch, 1987; Leblond-Francillard *et al.*, 1989) but a monocytosis-producing agent (MPA) has been extracted from *L. monocytogenes* (Stanley, 1949; Galsworthy, 1987) and the failure of similar extracts from *L. innocua* to stimulate a monocytosis suggests MPA may have a role in virulence. Although the crude cell walls of *L. monocytogenes* have many biological effects (Mara *et al.*, 1974; Petit & Unanue, 1974; Rodriguez *et al.*, 1974; Cohen *et al.*, 1975; Campbell *et al.*, 1976; Baker & Campbell, 1978; Saiki *et al.*, 1982; Hether & Jackson, 1983; Paquet *et al.*, 1986), the experimental studies upon these extracted cell wall products are unrepresentative of the natural disease and the role of their varied, and at times conflicting, immunological effects remains unclear.

LISTERIOSIS IN ANIMALS

The bacterium now known as *L. monocytogenes* was first isolated from an epidemic disease of rabbits and guinea-pigs in a laboratory animal breeding unit (Murray *et al.*, 1926), and causes infectious disease in both animals and man (Murray, 1955; Seeliger, 1961; Gray & Killinger, 1966; Radostits *et al.*, 1994). Infection is truly widespread having been recorded in more than 40 species of wild and domesticated animals and in countries in over six continents (Seeliger, 1961). Listeriosis is of major veterinary importance in cattle, sheep and goats but in the United Kingdom it is most common in sheep (Anon., 1992). A number of conditions are associated with infection with encephalitis and uterine infections being most frequently identified (Wilesmith & Gitter, 1986). However, it is the exception for different listeric conditions to occur in the same flock or herd (Ladds *et al.*, 1974; Jubb & Huxtable, 1992) and Wilesmith and Gitter (1986) recorded overlap of the different clinical manifestations in only seven of 75 affected flocks.

Encephalitis

The first description of listeric encephalitis was as a widespread neurological disease of sheep in New Zealand known locally as 'circling disease' (Gill, 1931). *L. monocytogenes* was isolated from the lesions (Gill, 1933) and listeric encephalitis of sheep, cattle and goats, almost invariably associated with *L. monocytogenes* infection, has since been described throughout the world (Jungherr, 1937; Graham, 1939; Biester & Schwarte, 1939; Pillai, 1962; Gitter *et al.*, 1965; Vandegraff *et al.*, 1981; Istvan, 1982). The clinical signs of infection are a consequence of the lesions in the brain stem (Rebhun & deLahunta, 1982) and although individual cases vary, common symptoms include dullness, turning or twisting of the head to one side and walking in circles. Unilateral facial nerve paralysis causes drooping of the eyelid and ear, and the animal drools saliva because of partial pharyngeal paralysis. In sheep and goats recumbency and death occur within 2 or 3 days but in cattle the duration of illness is often longer. Depending on the stage of infection the rectal temperature may be normal or elevated. Incubation periods vary but evidence from experimental studies and field cases suggest that they are in the order of 2 to 6 weeks.

Gross pathological lesions of the brain are rarely observed but there are patho- gnomonic histological lesions which are predominantly unilateral and consistently most severe in the medulla oblongata and pons. Lesions are less frequent in the cerebellum, cervical spinal cord and diencephalon, and when present in these sites are of lesser severity. The characteristic lesion is a focus of inflammatory cells with adjacent perivascular cuffs consisting predominantly of lymphocytes with histiocytes, plasma cells and occasional neutrophils. In severe cases lesions may coalesce to affect large areas of brain tissue. Meningitis is often present, developing secondary to the parenchymal lesions, but the ependyma and choroid plexuses are rarely affected (Cordy & Osebold, 1959; Ladds *et al.*, 1974; Charlton & Garcia, 1977; Jubb & Huxtable, 1992). Rare cases of limb paralysis as a result of listeric myelitis have been

described in sheep (Gates *et al.*, 1967; Seaman *et al.*, 1990), and there exists a single description of a mild encephalopathy associated with *L. innocua* infection (Walker *et al.*, 1994). In encephalitis cases *L. monocytogenes* is almost invariably isolated only from the brain (Urbaneck, 1962a; Charlton & Garcia, 1977).

For many years the pathogenesis of listeric encephalitis has been the subject of research and speculation. Gill (1931) was of the opinion that *L. monocytogenes* invaded the brain *via* the nasal passages and lymphatics but he provided no explanation for the absence of olfactory lobe lesions. Experimentally, listeric encephalitis could not be reproduced by intracarotid, intracerebral, intravenous, subcutaneous, intramuscular, intradermal injections, conjunctival instillation, or intraruminal inoculation (Gill, 1933; Jungherr, 1937; Biester & Schwarte, 1939; Olafson, 1940; Graham *et al.*, 1940; Olson *et al.*, 1953; Osebold & Inouye, 1954a; Asahi *et al.*, 1957). Osebold and Sawyer (1956) claimed intracarotid injection produced encephalitis typical of natural cases, but the organism was present in the visceral organs and the lesions described included a marked choroiditis. Cordy and Osebold (1959) were of the opinion that encephalitis resulted from haematogenous spread, and that there was a vascular basis to the topographic distribution of lesions in the brain.

Asahi *et al.* (1957) stated that the unilateral nature of the pathological lesions, their topographic distribution and the absence of the organism in tissues other than the brain could not be explained by infection arising from any of the described routes. These workers produced listeric encephalitis in mice by applying *L. monocytogenes* to minute puncture wounds on the lips and oral mucosae. Sequential studies revealed inflammation at the root of the trigeminal nerves within 24 h and in the medulla oblongata by day four. Four of 11 goats exposed in this way developed neurological disease 17 to 28 days after challenge and histological lesions resembled those of the natural disease. The authors suggested that the pathogenic basis of listeric encephalitis was for the organism to reach the brain *via* the trigeminal nerves following invasion of the oral or nasal mucosae.

Barlow and McGorum (1985) inoculated *L. monocytogenes* into the pulp cavities of premolar or incisor teeth of sheep and subsequently, between days 20 and 41, six animals developed neurological signs typical of listeric encephalitis. The observations and the presence and distribution of lesions in the cranial nerves of spontaneous cases of encephalitis (Urbaneck, 1962a; Groch, 1976; Charlton & Garcia, 1977) lend support to the hypothesis of Asahi *et al.* (1957) but, in a recent study, Peters and Hewicker-Trautwein (1994) found no evidence for *L. monocyto- genes* possessing a tropism for neuronal cells and it remains unclear whether the organism is able to travel to the brain *via* centripetal passage through the cranial nerves. No experimental study has conclusively defined the pathogenesis of listeric encephalitis and it must be appreciated that the experimental direct injection of *L. monocytogenes* into the tooth pulp, the facial or trigeminal nerves of sheep (Borman *et al.*, 1960; Urbaneck, 1962b; Schleicher *et al.*, 1968; Barlow & McGorum, 1985) does not exclude the possibility of infection arising *via* haematogenous spread.

Abortion

Listeric abortion caused by *L. monocytogenes* occurs in ruminants and many other species of domesticated animal (Paterson, 1940b; Stockton *et al.*, 1954; Seeliger, 1961; Gray & Killinger, 1966; Njoku & Dennis, 1973; Mason *et al.*, 1980; Kennedy & Miller, 1992; Watson & Evans, 1985; Sturgess, 1989; McLaughlin *et al.*, 1993). *L. ivanovii* is also recorded as a cause of abortion in sheep and cattle but occurs less frequently than *L. monocytogenes* (Ivanov, 1962; MacLeod *et al.*, 1974; Sergeant *et al.*, 1991; Alexander *et al.*, 1992) and is extremely rare as a cause of other conditions. Listeric abortion in the United Kingdom is most common in sheep (Anon., 1992) and although cases are usually sporadic (Kennedy & Miller, 1992) in exceptional circumstances approximately 50% of the flock have been affected (Low & Renton, 1985). Placental lesions are pin-point, yellowish, necrotic foci involving the tips of the cotyledonary villi with a focal to diffuse intercotyledonary placentitis covered in a red/brown exudate. The foetus is usually autolytic and with miliary necrotic foci occasionally visible throughout the liver and spleen. Histologically, these foci show coagulative necrosis and infiltration to variable degrees by macrophages and neutrophils (MacDonald, 1967; MacLeod *et al.*, 1974; Kennedy & Miller, 1992). The causal agent can be readily isolated from foetal and placental lesions by routine bacteriological culture.

Oral infection does not consistently produce abortion (Paterson, 1940b; Gray *et al.*, 1956; Gitter

et al., 1986) but the gravid uterus is highly susceptible to infection and abortion is readily produced by the intravenous injection of pregnant ewes with *L. monocytogenes* or *L. ivanovii* (Paterson, 1940b; Molello & Jensen, 1964; Smith *et al.*, 1968; Njoku *et al.*, 1972). The pathogenesis of foetal infection is considered to be haematogenous spread from the placenta (Molello & Jensen, 1964; Smith *et al.*, 1970; Njoku & Dennis, 1973) and the incubation period is 5 to 12 days (Molello & Jensen, 1964; Smith *et al.*, 1970; Njoku & Dennis, 1973; Ladds *et al.*, 1974; Low & Renton, 1985).

Septicaemia

Septicaemia is relatively uncommon and generally, although not invariable, occurs in the neonate as an extension of intrauterine infection. The most consistent lesion is focal hepatic necrosis with pin-point greyish, white nodules throughout the liver. Lesions are present also in the spleen but rarely in other tissues. The histological appearance is of multiple, focal areas of necrosis with invasion by polymorphs and mononuclear cells. The organism can be readily demonstrated in the lesions and is easily isolated on direct culture (Seeliger, 1961; Gray & Killinger, 1966). Given a sufficiently large inoculum, septicaemia is readily produced by the intravenous injection of *L. monocytogenes* (Murray *et al.*, 1926; Gill, 1937; Jungherr, 1937; Osebold & Inouye, 1954b; Gray & Killinger, 1966) and the incubation period is a matter of 2 to 3 days (Low & Renton, 1985). Occasional massive outbreaks of septicaemia involving pregnant ewes have been described (Low & Renton, 1985), with clinically affected animals being pyrexic and showing profuse diarrhoea. Diagnosis of listeric septicaemia is dependent upon isolation of the organism from the tissues of affected animals. In adult sheep, a marked enteritis with extensive haemorrhage affecting the abomasal folds, ulceration of the abomasal and intestinal mucosae and abscessation of Peyer's patches as a result of infection by *L. monocytogenes* has been recognized (unpublished observations). After diagnosis, the response of clinically affected animals to antibiotic therapy is good although in pregnant animals cases of abortion may follow clinical recovery (Low & Renton, 1985).

Other listeric infection

Iritis and keratoconjunctivitis caused by *L. monocytogenes* have been recorded occasionally in both cattle and sheep (Kummeneje & Mikkelsen, 1975; Morgan, 1977; Watson, 1989; Blowey & Weaver, 1991; Walker & Morgan, 1993). The condition is often unilateral and usually occurs during winter in silage-fed animals. The response to antibiotic therapy is poor although combined corticosteroid and antibiotics early in the course of the condition is an effective treatment. Bovine mastitis has been reported but with few documented cases (Gitter *et al.*, 1980; Sharp, 1989; Farber *et al.*, 1990; Fedio *et al.*, 1990; Vishinsky *et al.*, 1993). The condition may present as subclinical or clinical mastitis, and in all the cases reviewed the response to antibiotic treatment was poor and the organism was excreted for prolonged periods. Culling of such infected animals is therefore to be recommended.

In monogastric animals, listeriosis is uncommon although septicaemia and meningoencephalitis have been reported (Gray & Killinger, 1966; Radostits *et al.*, 1994). Infection in birds causes a septicaemia and myocardial necrosis (Seastone, 1935). Infection of rabbits with sublethal doses characteristically induces a marked monocytosis (Murray *et al.*, 1926; Osebold & Inouye, 1954b; Gray & Killinger, 1966) and in laboratory animals and rodents a septicaemia as originally described by Murray *et al.* (1926). At post mortem examination, lesions are similar to those seen in ruminants and isolation of the organism is relatively easy.

IMMUNITY TO *LISTERIA*

The classical work of Mackaness has established the importance of the monocyte–macrophage and thymus-derived (T-cell) lymphocyte in the protective immune response to this facultative intracellular parasite (Mackaness, 1962; 1964; Lane & Unanue, 1972; Mitsuyama *et al.*, 1978) and it has been clearly established that intracellular growth is a prerequisite for the development of this cell-mediated immunity (Kaufmann, 1984a; Berche *et al.*, 1987). The ability of different T-cell subsets to provide adoptive protection in mice has been extensively studied (Hahn & Kaufmann, 1981; Kaufmann & Hahn, 1982; Bortolussi *et al.*, 1984; Kaufmann, 1984b), but the role and relevance of T-cell subsets in listeric infections of ruminants is currently unknown. In mice, cytotoxic T-cells which recognize antigen associated with MHC class I molecules are of paramount importance and when stimulated by exogenous interleukin 2

cause target cell cytolysis (Kaufmann *et al.*, 1986). The release of listeria by the cytolysis of infected cells may not be detrimental to the host as cytolytic T-cells will be active only in infective foci where activated macrophages take up and destroy the released organisms.

Genetically determined differences in innate resistance to listeric infections exist among various inbred strains of mice (Cheers & McKenzie, 1978; Kongshavn *et al.*, 1980: Kongshavn, 1985) and although there exists the possibility of genetic variation in the innate resistance of ruminants its importance to infection is currently unrecognized. In humans, age and pregnancy-related defects in cell-mediated immunity have been described (Bortolussi *et al.*, 1984) which may explain the prevalence of listeric infections in neonates and during pregnancy. Similar mechanisms may be involved in listeric infections of ruminants.

EPIDEMIOLOGY

In sheep, which are the most commonly affected species in United Kingdom, the pattern of disease changed in the 1980s from sporadic cases to flock outbreaks (Anon., 1983; Wilesmith & Gitter, 1986) and the incidence recorded by Veterinary Investigation Centres increased considerably (Anon., 1992). In a survey of 60 flocks Wilesmith and Gitter (1986) reported a variable number of encephalitis cases with a mean attack rate of 2.5% in adults and a range of 0.1–13.3% (Graham, 1939; Olafson, 1940; Vandegraff *et al.*, 1981). Other than being extremely rare under 6 weeks of age, there is no exclusive age incidence (Olafson, 1940; Vandegraff *et al.*, 1981; Wardrope & MacLeod, 1983; Wilesmith & Gitter, 1986). Barlow and McGorum (1985) noted most cases in lambs up to 4 months old or in adults of 2 years or more. Scott (1993) recorded a preponderance of cases in 2-year-old ewes and Green and Morgan (1994) identified the condition frequently in lambs from 6 to 12 weeks of age. The condition in the United Kingdom, has a seasonality, and although abortion is obviously associatied with pregnancy, the majority of encephalitis cases occur in February and March (Anon., 1983; Anon., 1992). The reason for this seasonal distribution is obscure but the peak of infection is coincidental with late pregnancy when there are fundamental changes in the animals' immune status.

Rocourt and Seeliger (1985) state that the natural habitat for all *Listeria* species, including *L. monocytogenes*, is the environment and that the organism is widespread, having been isolated from surface soils (Welshimer & Donker-Voet, 1971; Weis, 1975), decaying vegetation and pasture herbage (Welshimer, 1968; Welshimer & Donker-Voet, 1971; Weis, 1975), silage (Gray, 1960), sewage sludge (Watkins & Sleath, 1981; Fenlon, 1985), factory effluents and river waters (Watkins & Sleath, 1981). Although the organism is ubiquitous listeric infections have been particularly associated with silage feeding for many years (Olafson, 1940; Gray, 1960), and Wilesmith and Gitter (1986) found that the incidence of listeriosis increased in flocks when silage feeding was introduced. Much of this association is attributable to the high bacterial numbers present in silage, which can contain more than 10^7 *L. monocytogenes* c.f.u. kg^{-1} (Gray, 1960; Low & Renton, 1985; Fenlon, 1986). The seasonal occurrence of listeriosis together with the increase in both severity and numbers of outbreaks may be explained by the increasing popularity and extensive use of silage as an animal feed. Since the organism is widespread in the environment it is likely that silage will be commonly contaminated with *L. monocytogenes*. Within a few inches of the surface of a silage bale or clamp the aerobic environment provides suitable conditions for growth, whereas deeper within the silage anaerobic conditions and fermentation of the natural sugars leads to acidification and an inhibitory environment (Fenlon, 1985). Multiplication of the organism from the presumably low levels initially present is dependent upon the quality of fermentation. Welshimer (1960) found that at 6°C the peak of logarithmic growth occurs in 10 to 11 days and the elapse of several months between silage making and its use allows ample time for considerable multiplication which is usually close to the surface. Multiplication after opening of the bale or clamp may also occur. Although attempts to model the deterioration of silage and to predict the growth of *Listeria* spp. have been made (Ruxton & Gibson, 1993; Kelly *et al.*, 1995) these studies have indicated a need for further field and experimental research to develop a better understanding of the growth of the organism within a complex matrix such as a silage bale or clamp.

The faeces of healthy animals often contain *L. monocytogenes* (Gray & Killinger, 1966; Kampelmacher & van Noorle Jansen, 1969;

Gronstol, 1979a; Rocourt & Seeliger, 1985; Skovgaard & Morgen, 1988; Husu, 1990) and the faecal carriage rate of *Listeria* species has been examined on a number of occasions (Gronstol, 1979a, 1979b, 1980a, 1980b; Loken *et al.*, 1982; Skovgaard & Morgan, 1988; Husu, 1990). It is uncertain whether the excretion rate is associated with the prevalence of the organism in feedstuffs or if the organism is maintained for long periods in the bowel. However, the tendency is for excretion rates to be lower in grazing animals (Husu, 1990).

Clinical responses after the subcutaneous injection of sheep or goats with *L. monocytogenes* (Gill, 1933; Olafson, 1940; Olson *et al.*, 1950; Osebold & Inouye, 1954a; Osebold & Sawyer, 1956) or after oral challenge with high doses of virulent organisms (Gill, 1933; Osebold & Inouye, 1954a; Gray & Killinger, 1966; Low & Donachie, 1991) are minimal. Since oral dosing of mice results in the development of immunity after invasion and replication in the spleen and liver (Audurier *et al.*, 1980, 1981; Macdonald & Carter, 1980) and similar immune responses develop in sheep (Low & Donachie, 1991; Miettinen & Husu, 1991; Lhopital *et al.*, 1993) it is likely that during silage feeding subclinical infections commonly occur and that animals become protected against the septicaemic form of listeriosis but these responses are not wholly effective in protecting against encephalitis (Low & Donachie, 1991). Although the pathogenesis and epidemiology of listeric encephalitis remain unclear, an explanation for the rarity with which encephalitis and abortion are recorded in the same flock, and why encephalitis occurs more frequently than abortion or septicaemia, is that many silage fed animals acquire protection against the latter forms of listeriosis through ingestion of the organism.

The role of intercurrent disease and immunosuppression in the development of listeric disease is unclear. Vandegraff *et al.* (1981) describe a number of conditions associated with listeriosis such as hypocupraemia, anaemia and intestinal parasitism but Wilesmith and Gitter (1986) found no evidence to support any interaction within intercurrent disease. In a series of experiments marked for their lack of success in producing clinical illness, no evidence was produced that immunosuppressive drugs (Gronstol, 1980c) infection by *Eperythrozoon ovis* (Gronstol & Overas, 1980a) or tick-borne fever (Gronstol & Overas, 1980b) could predispose to listeric infections. Although inclement winter weather may precipitate listeriosis (Gitter *et al.*, 1965; Vandegraff *et al.*, 1981; Loken & Gronstol, 1982; Wardrope & MacLeod, 1983), this may be an indirect effect as animals are forced to eat silage contaminated by *L. monocytogenes* (Loken & Gronstol, 1982; Wardrope & MacLeod, 1983) or are exposed to heavy environmental contamination by the organism (Vandegraff *et al.*, 1981). Low and Renton (1985) described a severe listeriosis outbreak in ewes protected from the weather by housing.

The use of molecular methods of bacterial typing has enabled the discrimination of individual strains of *L. monocytogenes* but there is little detail regarding the distribution and temporal occurrence of these strains particularly in the veterinary field. It is also unclear whether there is an epidemiological connection between faecal carriage causing the contamination of silage, feedstuffs or bedding and thus clinical disease (Green & Morgan, 1994; Widemann *et al.*, 1994). Different strains may be epidemiologically linked simply as a result of the diversity of strains and through a lack of understanding of the population dynamics of strains in the environment and foodstuffs. Since the incubation period for the encephalitic form of the disease is long, the temporal link between clinical disease and contaminated silage may also be unclear and difficult to establish. Identical strains have occasionally been isolated from silage and clinical cases in animals (Vasquez-Boland, 1992b; Wiedmann *et al.*, 1994) and the identification of single strains in flock outbreaks may be the consequence of exposure to a single dominant strain. Conversely, simultaneous exposure to multiple strains may result in the isolation of unrelated strains, as described in human infections by Schwartz *et al.* (1989), and from silages and clinical cases in single flocks as reported in veterinary studies (Nicolas *et al.*, 1988; Baxter *et al.*, 1993; Green & Morgan, 1994). Investigation of the population dynamics of *L. monocytogenes* is crucial to the understanding of the epidemiology of infections and outbreaks, and until then it must be assumed that all *L. monocytogenes* strains are capable of causing disease, and that for this environmental organism there has been no selection of a virulent clone.

DIAGNOSIS OF LISTERIC INFECTIONS

Listeriosis can be diagnosed in the laboratory by cultivation of the organism, demonstration of the

infectious agent or its products in tissues or body fluids, detection of a specific immune response or, in the encephalitic form of the disease, only by demonstration of the pathognomonic histological lesions.

Cultivation of the organism from a normally sterile site is usually relatively easy with growth occurring readily on simple laboratory media. However, primary isolation can be difficult when the organism is present in low numbers or if culture is attempted from a heavily contaminated environment (Seeliger, 1961; Gray & Killinger, 1966). Isolation from brains may be difficult (Biester & Schwarte, 1939; Gray *et al.*, 1948) but success rates can be improved either by macerating sections (Gill, 1933) or by using the 'cold enrichment' technique (Gray *et al.*, 1948). Selective media have been described for the isolation of *L. monocytogenes* from contaminated samples such as faeces, water, and human or animal feedstuffs (Kampelmacher & van Noorle Jansen, 1969; Watkins & Sleath, 1981; Fenlon, 1985; Doyle & Schoeni, 1986; Lovett *et al.*, 1987). These methods have been reviewed by Farber & Peterkin (1991) and their use may be adopted for the cultural examination of clinical specimens as demonstrated by Eld *et al.* (1993).

Fluorescent antibody techniques (Eveland, 1963; Biegeleisen, 1964; Watson & Eveland, 1965) and peroxidase anti-peroxidase staining methods exist (Domingo *et al.*, 1986) but results from the use of polyclonal sera must be regarded with caution because of the likely abundance of cross-reactions with other bacteria. Specific monoclonal antibodies may be useful (McLauchlin *et al.*, 1988) but these have not been used in veterinary studies. McLauchlin and Samuel (1989) and Samuel *et al.* (1990) described the use of an ELISA to detect soluble antigen in cerebrospinal fluid (CSF) samples from human cases of listeric meningitis but neither report suggests that these assays are entirely reliable. Oligonucleotide primers based on the *L. monocytogenes* invasion-associated P60 protein (iap) gene (Kuhn & Goebel, 1989; Kohler *et al.*, 1990) and LLO gene (*hylA*) (Wiedmann *et al.*, 1994) with amplification by polymerase chain reaction (PCR) have been used to detect very low numbers of bacteria. However, the results have to be confirmed as these methods have not been extensively used. Examination of the CSF may be indicative of listeric encephalitis (Rebhun & deLahunta, 1982; Scott, 1992; Scott, 1993), with increases in protein concentration >0.4 g l^{-1} and white cell counts above 1.2×10^7 l^{-1}. These changes are not present in all cases of listeric encephalitis and thus there is no accurate ante mortem diagnostic method for confirming listeric encephalitis.

A variety of serodiagnostic techniques exists for the detection of an antibody response which is assumed to be a reflection of previous infection by *L. monocyto- genes.* Serum agglutination (Widal test) using heat-killed cell suspensions for the detection of antibodies to somatic (O) antigens and with formalin-treated organisms for flagellar (H) antigens have been described (Seeliger, 1961; Osebold *et al.*, 1965; Larsen *et al.*, 1980). However, early studies were unsatisfactory since the complexity of the antigenic groups was unrecognized and a variety of methods for antigen preparation was used. The major drawback with the serum agglutination test has been its assumed nonspecificity since antibodies to *L. monocytogenes* are frequently found in sera from healthy animals with no histories of listeric infections (Osebold & Sawyer, 1955; Seeliger, 1958; Seeliger, 1961; Osebold, 1965; Aalund *et al.*, 1966; Gray & Killinger, 1966) and serum agglutination tests are considered unsatisfactory for diagnosis and must be used with caution in epidemiological studies. Efforts to improve the specificity of the agglutination test have included the pretreatment of sera with 2-mercaptoethanol (Aalund *et al.*, 1966) but this reduces the sensitivity of the test (Larsen *et al.*, 1974). A complement fixation test has been described (Seeliger, 1961) but it offers no advantage over the serum agglutination test (Potel, 1963). Similarly, haemagglutination and haemagglutination inhibition tests (Rantz *et al.*, 1956; Neter *et al.*, 1960), antibody precipitation (Pease *et al.*, 1972; Jain & Chandiramani, 1978), growth inhibition tests which detect antibodies to somatic antigens and agglutination immobilization assays which detect flagellar antigens (Potel, 1979; Kuhlmann-Berger & Potel, 1985) and ELISA methods (Hudak *et al.*, 1984) have been reported. However, Peel (1987) demonstrated extensive cross-reactions with other Gram-positive organisms and, to date, the use of crude antigens has been unrewarding for serodiagnosis.

Attempts have been made to isolate specific antigens including affinity-purified flagellin of *L. monocytogenes* serovar 4b. However, it is not useful for sero-diagnosis since flagellin is not produced following invasion of the host (Peel, 1987). Delvallez *et al.* (1979) identified a specific surface antigen of *Listeria*, 'antigen 2', but its potential in

serological tests has been described only briefly (Carlier *et al.*, 1980). More recently, the detection of antibodies to purified LLO has been investigated (Berche *et al.*, 1990; Low & Donachie, 1991; Miettinen & Husu, 1991; Low, 1993; Baetz & Wesley, 1995). Measurements of antibody responses to LLO is apparently a reliable indicator of infection and antibody responses arise after oral challenge and systemic infection. In field studies, the detection of antibodies to LLO was useful for diagnosis of both septicaemic and abortion forms of listeriosis but, intriguingly, in cases of listeric encephalitis anti-LLO titres were negative or inconsistent with a recent septicaemic episode (Low, 1993). Loken and Gronstol (1982) and Peel (1987), using crude *Listeria* antigens, also failed to detect any humoral antibody responses in cases of caprine and ovine listeric encephalitis and the accumulated evidence suggests that there is no serological response to listeric antigens in encephalitis cases. The low anti-LLO titres are assumed to be a consequence of the confinement of the organism to the CNS with little antigen presentation to B-cell lymphocytes. Although the finding does not clarify the route of infection the implication is that listeric encephalitis does not develop in those ewes systemically challenged or sensitized by a previous immune response as suggested by Barley (1990).

TREATMENT

Practically all common antibiotics, except cephalosporins, are active against *L. monocytogenes in vitro* (Hof, 1991). However, *in vivo*, a low efficiency may be expected and is probably in part due to the organism's intracellular location. Experimentally, ampicillin and amoxicillin are most active (Hof, 1991), and tetracycline and chloramphenicol are reportedly not the therapeutic agents of choice (Cherubin *et al.*, 1991). Though antimicrobial resistance in clinical isolates is rare (Facinelli *et al.*, 1991) resistance to chloramphenicol, erythromycin, streptomycin and tetracycline has been recorded (Poyart-Salmeron *et al.*, 1990).

In the treatment of human listeriosis, either ampicillin or amoxicillin together with gentamicin is the primary choice of therapy (MacGowan *et al.*, 1990; Hof, 1991). However, the response of ovine encephalitis cases to antibiotic therapy is generally poor (Scott, 1993) and a treatment regime of ampicillin or amoxicillin with an aminoglycoside are recommended with high dosages of antibiotics and prolonged treatment regimes. Currently only those cases which remain ambulatory are likely to recover (Scott, 1992).

Other forms of listeriosis are treated only occasionally as most animals make uneventful recoveries after abortion and individual cases of enteritis or septicaemia are seldom recognized clinically. In flock outbreaks of septicaemia, affected animals respond well to ampicillin or amoxicillin, although encephalitis cases may occur a few weeks later. The clinical response of cases of iritis is markedly improved by the combined use of antibiotics and corticosteroids in subconjunctival injections.

CONTROL

The connection of listeriosis with silage feeding is well established, and it is the responsibility of those feeding silage to sheep to take care in its preparation and use. Contamination of grass with pathogenic species before ensiling is likely to occur as animals and birds can be unaffected carriers of *Listeria*, species and pass the organism in their faeces (Fenlon, 1985). The wide distribution of the organism in nature thus makes it unlikely that silage will be free of *Listeria* and emphasis should be placed upon reducing the likelihood of its multiplication within the clamp or bale. The organism may be present in all silage but is found most frequently in those with pH values greater than 5, particularly where ineffective fermentation has occurred and where there is a concomitant growth of moulds (Fenlon, 1985). The production of good silage with minimal growth of *Listeria* is dependent upon early cutting of grass followed by its effective compaction and sealing in the clamp or bag. A heavy inoculation of organisms from soil or faeces should be avoided and, in exceptional circumstances, it has been recommended that pastures identified for making silage be kept free of grazing animals during the spring.

Particularly for sheep, care should be taken in selecting the best silage for feeding, and once the silage clamps or bags are opened any obviously mouldy material should be discarded. The silage a few inches from the front, top and sides of a clamp should not be fed and after feeding any silage remains should be removed. Cattle appear to be less susceptible than sheep to listeriosis and in

many cases silage that has caused disease in sheep has been fed safely to them. However, since listeric infections do occur in cattle there is some risk in adopting this procedure and, in general, the same principles for producing and feeding silage should be adopted as for sheep. Cases of iritis have been particularly common in recent years and many arise when cattle have the opportunity to push their faces into the silage. This may be controlled by altering the silage feeds or by strategic placing of a physical barrier to the cattle.

Fully effective control measures have not been determined and currently no vaccine is available in the United Kingdom. The organism is an intracellular parasite and cell-mediated immunity to *L. monocytogenes* is important in mice, with killed vaccines having been shown to be ineffective (Wirsing von Koenig *et al.*, 1982). Although a live, attenuated vaccine developed in Bulgaria, based upon serovars 1/2a and 4b of *L. monocytogenes* is available in some European countries (Ivanov *et al.*, 1979; Gudding *et al.*, 1985; Gudding *et al.*, 1989; Vagsholm *et al.*, 1991) full details of the composition are unavailable. This vaccine is claimed to be effective in sheep but the results of field trials are equivocal and no experimental model is available to test its efficacy. Listeriosis is a disease of complex aetiology and, despite some reports, further investigations into mechanisms of immunity and their role in the pathogenesis of disease are necessary before effective vaccines can be developed.

LISTERIOSIS IN MAN

L. monocytogenes was first described as a human pathogen in the 1920s. Although relatively rare, human listeriosis is often severe and mortality rates may approach 50%. Infection may present as meningitis or, more rarely, encephalitis. It can also cause a generalized infection or in pregnant women can result in spontaneous abortion, stillbirth or infection of the newborn (Seeliger & Finger, 1983). Minor skin infections, particularly affecting farmers or veterinarians after handling bovine calvings or abortions, are also recognized (McLauchlin & Low, 1994). The infectious dose is unknown but is likely to be high, and host susceptibility is an important factor in infection since most cases of invasive disease involve the immunosuppressed, the elderly or pregnant. Although veterinarians have recognized the association between listeriosis and silage feeding for many years there has been little evidence until recently of the food-borne nature of listeric infections in man.

During the 1980s a number of listeriosis outbreaks were linked with the consumption of contaminated foodstuffs. The first clearly established community outbreak occurred in the Maritime Provinces of Canada in 1981 (Schlech *et al.*, 1983). The outbreak involving seven adults and 34 pregnancies was linked to contaminated coleslaw. In Massachusetts in 1983 an outbreak involved consumption of contaminated pasteurized milk (Fleming *et al.*, 1985) and further outbreaks in California in 1985 (James *et al.*, 1985) and in Switzerland between 1983–7 (Piffaretti *et al.*, 1989) were associated with contaminated soft cheeses. In the United Kingdom a massive increase in the incidence of listeriosis in the period 1987 to 1989 was in part attributed to the consumption of contaminated pâté (McLauchlin *et al.*, 1991; Gilbert *et al.*, 1993) and a similar food product was incriminated in an outbreak which occurred in Western Australia in 1990. In 1992 a French outbreak was traced to the consumption of jellied pork tongues and a more recent outbreak to pork 'rillettes'.

Excluding the major outbreaks, which have separately involved hundreds of individuals, the incidence of listeriosis is difficult to determine. Estimates range from <2 to 12 per million head of population. The Centers for Disease Control estimated 1700 cases per annum in the United States of America with 450 adult and 100 foetal deaths. Although awareness of the condition and improved techniques may have increased diagnosis rates there has been an apparent increase in the incidence of listeriosis in many western populations in the past 20 years. Intriguingly, listeriosis cases in Africa, Asia and South America are rarely reported.

Nosocomial infections and person-to-person spread are recognized but uncommon and there is general agreement that food-borne transmission is the predominant means of infection for sporadic cases which form the majority of listeriosis cases. Clear links with food-borne infection are rare as the incubation period can be many weeks and symptoms of intestinal illness are uncommon. Thus, association of infection with a particular food product is often difficult to prove. Direct contact with animals apparently poses relatively little risk other than to members of susceptible

groups and the World Health Organization reports that animals are not important as direct sources of human infection (Anon., 1988).

Recent studies have confirmed the presence of *L. monocytogenes* in a wide variety of foodstuffs. Of 18 337 food samples examined by the Public Health Laboratory Service in the United Kingdom the organism was found in 1159 overall, and in 42 specimens, numbers exceeded 1000 organisms per gram (McLauchlin & Gilbert, 1990). Milk, soft cheese, ice cream, cook–chill foods, raw meats, ready-to-eat poultry, pâtés, unprocessed vegetables, salads, raw fish, fish products, sandwiches and fried rice were amongst those foodstuffs shown to be contaminated. Pâtés and soft cheese were particularly likely to contain more than 1000 organisms per gram.

Many of the problems of contaminated food products are due to post-processing contamination. The organism has an ability to grow at refrigeration temperatures, is relatively resistant to salt and is capable of long periods of survival. These properties are of particular concern for refrigerated foods which have extended shelf-lives and undoubtedly much of the increase in the incidence of listeriosis is attributable to the popularity of certain convenience food products. As with silage, the organism is capable of multiplying to massive levels within a relatively short period, particularly in foodstuffs with pH values above 5 and soft, surface ripened cheese and pâtés appear to provide conditions which are highly amenable to growth of the organism.

CONCLUSION

There have been major advances in our understanding of the bacterium, its taxonomy and also a molecular definition of its mechanisms of virulence. However, despite these advances there remain major gaps in our understanding of the epidemiology and pathogenesis of listeric infections in farm animals. Improved isolation techniques and methods of bacterial typing have been used by a number of groups but these studies are by no means complete since the diversity and ubiquitous nature of the organism require detailed study to determine the ecological niches in which strains may thrive and mechanisms by which the organism may spread. It is also true that our understanding of the pathogenesis of listeric infections, particularly listeric encephalitis, is dependent upon field observations and limited experimental data. Although the organism has been favoured by immunologists for many years, offering a relatively safe intracellular parasite with which to model mechanisms of cell-mediated immunity, relatively few studies of immune mechanisms have been carried out in ruminants. It is crucial that the skills of veterinarians, microbiologists and immunologists are utilized in an effort to establish the means by which the organism can enter the host and travel to the brain, and the role of the host's humoral and cellular immune responses in the pathogenesis of infection. Only through elucidating these factors involved in pathogenesis and immunity will rational control policies, including effective vaccines, effective treatment regimes and reliable ante mortem diagnostic tests be developed.

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