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Food hygiene aspects of leptospirosis and the current situation in Ireland

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1 Introduction

Leptospirosis is a zoonotic disease caused by any of the pathogenic serovars of the genus *Leptospira*. These are small, motile bacteria with the possibility to invade though small abrasions to intact skin, with prolonged survival possible in the environment under certain favourable conditions (Lunn, 2015).

The disease has a worldwide distribution, having been found on all continents except Antarctica. Evidence for carriage has been found in nearly all mammalian species examined (Adler and de la Peña Moctezuma, 2010). The epidemiology of the disease is complex with the importance of certain leptospiral strains and host species varying by geographic location, with a more complex picture being found in tropical and subtropical regions (Terpstra, 2003).

The aim of this study is to give an overview of the present knowledge of the disease in general, its impact on both animal and human health, its diagnosis in food producing animals and especially to examine the ways that the disease can be transmitted to humans during the animal production process with a focus on the particular situation in Ireland. From this perspective, leptospirosis has primary importance in cattle, small ruminants and pigs. Although not generally classified as a food-borne disease (Toldra, 2010), leptospirosis has many potential ways to infect humans during animal production from the farm to the abattoir.

Disease in humans was traditionally seen as an occupational disease of farmers and farm workers, veterinarians, livestock producers and abattoir workers (Faine *et al.* 1999) but is now increasingly encountered as recreational disease contracted by those exposed to water contaminated with urine from infected domestic animals or wildlife (Vijayachari *et al.*, 2008). Clinical effects may range from inapparent infection to multiple organ failure and death (Lunn, 2015). The severity of disease will vary by host species and infecting serovar but there are many common aspects across the species.

In Ireland, economic losses in the livestock industry are dominated by chronic *Leptospira* Hardjo infection in breeding cattle herds. Losses due to *Leptospira* Bratislava infection in breeding pig herds are also thought to be significant (Williams, 2015b).

2 Aetiology

Leptospira belong the family *Leptospiraceae* in the order *Spirochaetales*, along with two other families of veterinary significance - *Spirochaetaceae* and *Brachyspiraceae*. All of these are spiral or helical bacteria sharing several important morphological and functional features. The genus *Leptospira* within the *Leptospiraceae* family, as well as the *Borrelia*, *Brachyspira* and *Treponema* genera contain pathogens of importance to both human and animal medicine. Each family also has some non-pathogenic genera (Quinn *et al.*, 2012).

All pathogenic spirochaetes are difficult to culture, requiring specialised culture media with some requiring liquid media. Organisms in the group are classified according to genetic relatedness. Serological methods are used for both epidemiology and clinical diagnosis (Quinn *et al.*, 2012).

2.1 *Leptospira*

Leptospires are motile helical bacteria with a size of 0.1×6 to 12 μm. They have characteristic hook shaped ends, which gave rise to the species name of *Leptospira interrogans* (Maxie *et al*., 2007). Genetic material is held within two circular chromosomes. They are aerobic, fastidious, slow growing and move with a characteristic corkscrew-like motility (Lunn, 2015). Leptospires can become greatly elongated if subjected to adverse nutritional conditions and coccoid forms of about 1.5 to 2 μm may emerge in conditions of high salt concentration or aging cultures (Ellis, 2012).

Leptospires are cultured in liquid media at 30°C. They are classified as Gram-negative owing to their cytochemical make-up, however they are not visualised well with conventional bacteriological dyes. Visualisation is usually achieved with the aid of a dark-field microscope. Immunological staining and silver impregnation techniques are used to show leptospires in histological sections. Molecular methods are also frequently used for diagnosis (Quinn *et al.*, 2012).

Leptospires can survive in ponds, rivers, surface waters, moist soil and mud when environmental temperatures are moderate. They may produce systemic infections in a wide range of animal species. Pathogenic leptospires can persist in the renal tubules or in the genital tract of carrier animals and are shed in the urine. Although indirect transmission can occur if

environmental conditions are favourable, transmission occurs most effectively by way of direct contact (Quinn *et al.*, 2012). Under ideal conditions, for example in water-logged soil or stagnant water, leptospires may survive for weeks or months. Under adverse conditions, survival is a matter of minutes (Maxie *et al*., 2007).

The taxonomic classification of *Leptospira* has been modified in recent years owing to advances in genomic analysis. Traditionally, *Leptospira* were divided into two groups; the pathogenic *Leptospira* were all classified as members of *Leptospira interrogans* and the saprophytic *Leptospira* were classified as members of *Leptospira biflexa*. Within each of these species, many leptospiral serovars were recognized based on their surface antigens, with more than 250 pathogenic serovars identified throughout the world. The serovars are often grouped into antigenically related serogroups. With the increased use of genomic information for the classification of bacteria, the genus Leptospira was reorganized into 21 recognized genomospecies of leptospires, including both pathogenic, intermediate, and non-pathogenic organisms. Pathogenic leptospires are now identified in 9 species of *Leptospira*, with 6 species being regarded as intermediate in pathogenicity, and 6 being non-pathogenic. Some of the common leptospiral pathogens of domestic animals now have different species names. For example, *Leptospira interrogans* serovar Grippotyphosa is now *Leptospira kirschneri* serovar Grippotyphosa. The two types of serovar Hardjo have been formally split into two species: serovar Hardjo type hardjo-bovis (found in the USA and much of the world) is now *Leptospira borgpetersenii* serovar Hardjo and the less common serovar Hardjo type hardjo-prajitno found primarily in the UK is now *Leptospira interrogans* serovar Hardjo (Lunn, 2015).

Serological classification remains clinically important because particular serovars are associated with specific host animals and cross immunity between serovars is minimal, therefore identification and understanding of the infecting serovar is essential for understanding and controlling leptospiral infections (Quinn *et al.*, 2012).

3 Epidemiology

Leptospires have a worldwide distribution, however many of the pathogenic serovars have a limited geographic spread. Generally, serovars have a relationship with particular host species - the so called maintenance host(s). A list of leptospiral serovars and their common maintenance hosts is given in Table 1. In these animals, susceptibility to infection is high but disease is usually mild or subclinical and is commonly followed by a long period of renal excretion of leptospires in the urine. Acute disease is usually absent and economic losses in food producing animals are generally due to chronic reproductive disease (Ellis, 1994). These animals are the most important source of environmental contamination and direct transmission of that serovar to other animals. Transmission of leptospires within such maintenance host species generally results in an endemic nature of transmission within that species (Radostits *et al.*, 2006).

Table 1. Common Maintenance Hosts of the Pathogenic Leptospires Associated with Disease in Domestic Animals in the USA and Canada (Lunn., 2015)

Leptospiral serovar	Maintenance hosts

If leptospires infect an animal other than the maintenance host of that serovar (an incidental host), these animals will typically have a relatively low susceptibility to infection but if infected may exhibit severe disease. They are, however, inefficient transmitters of leptospirosis to other animals (Quinn *et al.*, 2012). Disease in these incidental host species, if present, will tend to be of an acute nature and transmission within the species will be of a sporadic, occasionally epidemic nature. There is usually a marked antibody response to infection in incidental hosts and this leads to the generally good efficacy of vaccination in these animals (Radostits *et al.*, 2006).

Previous nomenclature classified leptospiral serovars as either host-adapted or non-host-adapted with these terms being equivalent to the maintenance and incidental host terminology generally now used (Radostits *et al.*, 2006).

The true incidence and prevalence of leptospiral disease is undetermined for most countries and regions. Serological surveys are frequently undertaken but these may be flawed for many reasons including the choosing of antigens of serovars not circulating in that country, because seropositivity does not necessarily indicate the significance of disease, because sampling is often undertaken due to convenience instead of as part of a carefully designed study, and because antibody titres in the Microscopic Agglutination Test deemed significant (usually >100 or greater) may underestimate true seroprevalence for some host adapted strains (Maxie *et al.*, 2007).

Diagnosis of leptospirosis in maintenance hosts is made more difficult due to the fact that they generally have a fairly low antibody response with few organisms present in the affected tissues. This may be the case for example in serovar Bratislava infection in pigs or in serovar Hardjo infection in cattle (Lunn, 2015). The low antibody response seen in maintenance hosts also leads to the low efficacy of vaccination in the prevention of infection (Radostits *et al.*, 2006).

Incidental hosts typically exhibit a marked antibody response to infection and large numbers of organisms are present in tissues, for example in the case of *Leptospira* Icterohaemorrhagiae infection in cattle or pigs. The classification into maintenance and incidental hosts however should not be viewed too strictly. For example, in the case of swine or cattle infected with serovar Pomona, they behave as a host intermediate between the two forms, with the organism persisting in the kidneys but the host also showing a marked antibody response to infection (Lunn, 2015).

According to genomic studies, environmental survival of pathogenic leptospires is variable with some serovars in the *Leptospira borgpetersenii* species unable to survive in the environment whereas serovars in the *Leptospira interrogans* species may exhibit prolonged survival in a suitable environment such as surface water (Xue *et al.*, 2009).

4 Pathogenesis

Leptospira generally only cause significant disease where particular serovars infect incidental host species. Exceptions are found when leptospires infect immature animals of maintenance host species and especially in the special case of infecting foetuses (Quinn *et al*., 2012). Chronically infected animals may show no signs other than slow weight gain (Jensen *et al.*, 2004).

Leptospires invade though moist, softened skin or through mucous membranes. The corkscrew-like motility of *Leptospira* may aid their invasion through intact skin, a feature thought to be unique to the *Leptospira* genus, although successful invasion probably requires small abrasions to the surface layers of the skin (Zhang *et al.*, 2012). Prolonged immersion in water may make the skin more easily penetrated by leptospires even in the absence of skin abrasions (Faine *et al.*, 1999).

After a variable incubation period of between four and twenty days, leptospires enter the blood and replicate in many tissues including the liver, kidneys, lungs, genital tract and central nervous system for between seven and ten days (Lunn, 2015). After a period lasting around ten days, antibodies will appear in the blood and leptospires will disappear from the circulation. The organisms may be able to evade the immune response and persist in certain tissues of the animal for extended periods of time with the most important site of persistency being the renal tubules. Additional sites of persistency include the uterus, the eye and the meninges of the brain and spinal cord (Quinn *et al.*, 2012).

The natural reservoir of leptospires is in the proximal convoluted tubules of the kidneys in addition to the genital tract in some serovars infecting their maintenance hosts (especially *L* Hardjo in cattle and *L* Bratislava in pigs) where there may be persistence in the oviducts, uterus and vagina in females or the epididymis, prostate gland and seminal vesicle in males (Maxie *et al.,* 2007).

The clinical signs of acute leptospirosis will appear during the period of leptospiraemia. These signs will vary by infecting serovar and affected species. As the organisms are cleared from the blood and most of the tissues, the clinical signs of acute leptospirosis will begin to resolve, although damaged organs may take some time to return to their normal function. In some cases, severely damaged organs may not recover, leading to chronic disease or death. This is especially

true of the kidneys. Although similar up to this point, the pathogenesis of maintenance and incidental hosts will then diverge. In incidental hosts, the leptospires will only remain in the kidney tubules and thus be shed in the urine for a short period of time – a period of between a few days and several weeks. The situation in maintenance hosts is quite different in that leptospires may persist in the renal tubules, the genital tract and occasionally in the eye in spite of the presence of antibodies in the blood. In this case, leptospires can be shed in both the urine and the genital secretions of these persistently infected animals for a period ranging from months to years. It is these animals who serve as reservoirs of infection and have primary importance in the transmission of infection both to other maintenance hosts and to incidental hosts, thus initiating cases of clinical disease (Lunn, 2015). In some cases, for example as has been demonstrated for *Leptospira* Copenhageni infection in rats, infection may initially cause little or no damage to the renal tubules in spite of renal excretion of leptospires from day nine post infection (although it can cause an interstitial nephritis from one month post infection). In these rats, renal excretion generally persisted for the life of the rat (Nally *et al.*, 2008).

In susceptible animals, leptospires can damage endothelial cells and the membranes of red blood cells in addition to causing hepatocellular injury leading to the main clinical signs of acute leptospirosis – those being haemolytic anaemia, haemoglobinuria, haemorrhages and icterus. Pathogenic leptospires also contain haemolysins which may be partly responsible for these lesions (Quinn *et al.*, 2012). Haemolysis is thought to initially be caused by these haemolysins with later haemolysis caused by antibodies reacting with leptospiral products coating red blood cells. In acute disease, capillary injury is caused by inflammatory cytokine release (Maxie *et al*., 2007).

Leptospires reach the kidney from the blood and migrate randomly with a brief period of persistence in the interstitial spaces. They will enter the tubules at all levels of the nephron however, after the antibody response of the host they will localise in the proximal convoluted tubules from where they will multiply. The physiological changes to the glomerular filtrate that happen lower in the nephron and in the urine damage the leptospires (Maxie *et al*., 2007).

The most important factor in the epidemiology of leptospirosis is the ability of leptospires to persist in the renal tubules in spite of a specific immune response by the host. The reasons for this persistence are not completely clear. Proposed mechanisms include the downregulation of antigenic protein expression on the surface of leptospires or differential protein expression in the course of chronic disease. Virulence factors have not been fully characterised but it seems

that leptospires do not produce specific exotoxins. Being a Gram-negative bacterium, the cell wall of *Leptospira* species contains endotoxin, however the lipopolysaccharide of leptospires seems to induce a much lower endotoxic effect than that of most other Gram-negative bacteria (Quinn *et al.*, 2012).

Leptospira are the leading cause of recurrent uveitis in horses, especially in the case of *L* Pomona infection. This condition is not common in ruminants or pigs but is a possible, if rare, consequence of leptospiral infection in these species (Maxie *et al*., 2007).

5 Disease in cattle

Leptospirosis is a common disease in both dairy and beef herds with economic importance due to reproductive losses and abortion, infertility and reduction in milk yield in addition to its zoonotic importance (Cockcroft, 2015). Infection of cattle usually arises as a result of contact with infected urine or the products of abortion with infection most commonly occurring in the spring and summer months while cattle are at pasture. Although possible, venereal transmission of *L* Hardjo is not thought to adversely affect reproduction as the organisms are killed by the uterine defences during oestrus (Williams, 2015a). Abortion is often the only clinical sign observed in a herd, except in lactating cattle where signs of agalactia, mastitis, fever, haemolytic anaemia, haemoglobinuria and icterus may be seen (AFBI/DAFM, 2013). Leptospirosis is noted as a leading cause of milk drop syndrome in dairy herds (Pearson *et al.*, 1980).

Cattle can be infected with *Leptospira* Pomona or *Leptospira* Icterohaemorrhagiae leading to a severe, perhaps fatal septicaemia with associated pyrexia, icterus and haemoglobinuria however the most important serovar in the UK and Ireland is serovar Hardjo (Scott *et al.* 2011).

Costs due to infertility, abortions and drop of milk yield have been calculated at between £68 - £106 per cow in an affected herd or in terms of cost per litre of milk, this works out at a loss of 0.91-1.41 pence per litre (Owen, 2003).

5.1 Risk factors

Known risk factors for cattle herds contracting the disease include the buying in of cattle, the use of natural service as opposed to artificial insemination, having cattle grazing alongside sheep, and access to watercourses (Owen, 2003). An increasing herd size is also an additional risk factor (Williams and Winden, 2014). Access to cattle by feral animals or wildlife can also potentially transmit the disease (Ward *et al.*, 2006) although in the UK, *Leptospira* Hardjo is not believed to be shed by vermin or wildlife however sheep can carry and excrete *L* Hardjo therefore mixed grazing with sheep is a risk factor for contracting this serovar (Williams, 2015a).

Ryan *et al.* investigated the herd level risk factors for *Leptospira* Hardjo infection in Irish suckler herds (Ryan *et al.*, 2012a) in addition the describing the seroprevalence as discussed

below. As in the seroprevalence study, to be eligible for the analysis herds had to be unvaccinated and contain ≥8 breeding animals of beef breeds which were more than 12 months of age. The results of the seroprevalence study were used in conjunction with results obtained from a questionnaire targeted at farm demographic and management factors. Of 320 questionnaires sent out 157 were returned completed or partially completed, a response rate of 49 per cent. All of the herds which were vaccinating (n=21) against leptospirosis responded to the questionnaire suggesting that the previous presence of the disease in their herds contributed to the decision of these farmers to respond. The 21 vaccinating herds were excluded from the study and 7 more were excluded due to having ≤8 breeding animals. One was excluded due to insufficient data leaving 128 herds in the final risk factor dataset. The prevalence distribution of the 128 risk factor herds was found to be representative of the overall herd prevalence of the 288 herds involved in the seroprevalence study. The risk factors chosen to be included in the questionnaire were mainly based on those sent to dairy herds in previous studies. The aim was to have a majority of questions as unambiguous as possible so that they were answered in a "Yes" or "No" fashion. The 163 herds that did not respond to the questionnaire were contacted by telephone to establish their vaccination status. The vaccinating herds showed many differences with the non-vaccinating herds included in the risk analysis. 52 per cent of these vaccinating herds had a history of leptospirosis, as opposed to only 3 per cent in the unvaccinating herds and they had a much higher incidence of abortions, stillbirths, weak calves and apparent infertility. Vaccinating herds also had a much higher mean breeding herd size and 57 percent of these herds were operating an open herd policy and were buying in animals. They were also more likely to have a part of their grazing land flooded each year. The key result of this study was the clear association between breeding herd size and herd leptospirosis status when using a multivariate model. Following a univariate statistical analysis, 5 variables showed a statistically significant association with herd leptospirosis status ($P<0.05$). Those were region, breeding herd size, the use of a stock bull, grazed acreage and percentage of wet land grazed. Although there was a high regional variation in herd leptospirosis, the authors of this study believed the association, particularly in the South East of Ireland, was due to the larger size of suckler herds in that region. They also believed that the major risk factor in Irish suckler herds was the presence of a number of carrier animals in a herd which would correlate well with the significance attributed to breeding herd size in their study. The lack of association of true animal seroprevalence with age or sex is in contrast to findings of other studies, mostly of dairy herds, in which age and sex were important risk factors (Alonso-Andicoberry, 2001; Ellis, 1994). In the previous studies, heifers were generally immunologically naïve to *L* Hardjo on entering the

milking herd. The authors believed that the lack of these associations was due to heifer and bull suckler calves being reared alongside carrier cows and therefore being exposed to *Leptospira* Hardjo from a young age.

Other studies have also shown a statistically significant association between breeding herd size and seroprevalence for *Leptospira* Hardjo infection in cattle (Lilenbaum and Santos, 1996; Ellis, 1994).

In other parts of the world many other risk factors that are not present in Ireland or the UK seem to play a role in the transmission of bovine leptospirosis. A Brazilian study found that the main risk factor leading to *L* Hardjo seropositivity was co-grazing with other species, mostly pigs. In a study in the United States, higher mean annual temperatures and longer breeding seasons were associated with greater seropositivity to *Leptospira borgpetersenii* serovar Hardjo (Wikse, 2007).

5.2 Clinical signs

The most common clinical signs of leptospirosis in cattle in the UK and Ireland are caused by *Leptospira* Hardjo. Cattle are maintenance hosts of this serovar and leptospires can persist in the genital tract of both infected cows and bulls. The most common clinical sign attributable to *L* Hardjo infection is sporadically occurring abortion when naïve pregnant cows are infected for the first time (AFBI/DAFM, 2011). Serovars Grippotyphosa, Bratislava, Icterohaemorrhagiae, and Canicola can cause occasional incidental disease (Divers, 2015a).

In non-pregnant and non-lactating cattle, leptospirosis is often of a subclinical nature with severe disease possible in young animals infected by incidental serovars. Chronic disease is usually manifested by reproduction losses including abortion and still birth. Decreased fertility involving prolonged calving intervals and increased services per conception is associated with persistent colonisation of the uterus and the oviducts by *L* Hardjo (Divers, 2015a).

Acute disease is generally caused by incidental serovars, especially with *L* Pomona infection and less commonly with *L* Icterohaemorrhagiae. Clinical signs occurring during the period of leptospiraemia may include pyrexia, haemolytic anaemia, haemoglobinuria, icterus and pulmonary congestion. Meningitis may occur occasionally (Divers, 2015a).

The most severe form of the disease in cattle is the infection of calves by incidental serovars, especially *L* Pomona. Haemorrhages into the renal tubules may lead to haematuria and blood clots forming in the urinary tract. Haemoglobinuria may be the first sign noticed and can last for two or three days or may be more transient. In fatal cases the urine will have a port-wine colour (Maxie, 2007). Infection of lactating cows by incidental serovars may result in almost complete agalactia with only small amounts of blood-tinged milk being produced (Williams, 2015a).

In lactating cows infected by *L* Hardjo, a kind of "milk drop syndrome" may occur but it less severe than that caused by incidental serovars and may occur as an isolated clinical sign without any other evidence of disease (Divers, 2015a). The drop in milk yield is sudden and occurs from two to seven days after infection in susceptible cows. The udder will become soft and flabby (so called "flabby bag") with colostrum-like secretions and blood-tinged milk in all quarters (Williams, 2015a).

Chronic disease in pregnant cows can result in infection of the foetus and abortion or stillbirth. Live calves may be born weak and/or prematurely although sometimes they may be born healthy. Abortion or stillbirth may be the only clinical sign noted but a period of disease may have passed unnoticed up to 6 weeks earlier in the case of *L* Pomona infection or 12 weeks earlier in the case of *L* Hardjo. *L* Hardjo caused abortion is generally sporadic and occurs in mid to late gestation whereas in the case of incidental host abortions, they may be late term and in groups or as part of an abortion storm (Divers, 2015a). Retention of foetal membranes can also occur (AFBI/DAFM, 2011). The foetus is frequently decomposed, indicating death some time before the abortion event (Maxie *et al.*, 2007).

6 Disease in sheep

In comparison to cattle and swine, small ruminants seem to be relatively resistant to infection by pathogenic *Leptospira* with only a few serovars appearing to cause disease. Seroprevalence is fairly low. The main importance of the disease in sheep is to act as maintenance hosts for *L* Hardjo thereby transmitting the disease to cattle in shared husbandry situations. Occasional outbreaks of incidental host disease may be seen resulting in haematuria, haemoglobinuria, icterus and perhaps death in lambs as well as occasional abortions in pregnant females (Divers, 2015a).

Although considered less clinically important in sheep compared with cattle, infection by *L* Hardjo can result in infertility, abortion, and the birth of weak or non-viable lambs. Abortion is typically seen late in gestation. Agalactia may be seen in recently lambed ewes (Maxie *et al.*, 2007).

Acute disease in lambs may occasionally be seen and is similar to that described above in calves (Maxie *et al.*, 2007).

7 Disease in pigs

Leptospirosis is an important cause of reproductive losses in pigs and is present worldwide with disease most visible in the intensive pig industries of the developed world. Although endemic infection may only produce subclinical disease, new infection of a naïve breeding herd may produce significant losses by way of abortion, stillbirth and the birth of live piglets with reduced viability in addition to reduced fertility in breeding animals. Persistence of leptospires may occur in both the kidney tubules as well as the genital tract and they may be excreted in both the urine and in genital secretions. Direct or indirect contact with carrier animals facilitates transmission of the disease with the most important factor in the transmission of the disease in most situations being shedding from carrier pigs (Ellis, 2012).

Only a small proportion of infected pigs will exhibit clinical illness, and this will usually pass as an unrecognised episode of transient fever, anorexia and depression (Maxie *et al*., 2007).

Costs due to leptospiral infections in pig herds may vary widely. In one calculation of an outbreak in a 300 sow herd lasting for four months and causing a 7 per cent reduction in the farrowing rate in addition to deceased livebirths and increased neonatal mortality, the cost was put at £14 000 (Williams, 2015b).

Several species and serovars of *Leptospira* can infect swine including *L interrogans* serovars Pomona, Icterohaemorrhagiae, Canicola, Hardjo and Bratislava, *L borgpetersenii* serovars Sejroe and Tarassovi and *L kirschneri* serovar Grippotyphosa. Swine are maintenance hosts of serovars Pomona and Bratislava and incidental hosts of the others (Divers, 2015b).

In principle, swine may be infected by any of the pathogenic serovars making the epidemiology very complex, however in practice only a few serovars are of real importance in any one region. *L* Pomona and the closely related *L* Kennewicki are the most commonly isolated serovars worldwide (Ellis, 2012). A recent review in Germany found that the most common serovars found on serological testing of pigs during the past 20 years have been *L* Bratislava (41.8 percent), *L* Pomona (16.3 per cent) and *L* Tarassovi (2.9 per cent) (Strutzberg-Minder and Kreienbrock, 2011).

7.1 Leptospira Pomona

Infection of pigs by *Leptospira* Pomona does not fit into an exact maintenance host - incidental host scheme as this serovar is of intermediate pathogenicity in pigs.

Acute clinical signs may be seen in young pigs and pregnant sows may abort, often in groups. These are signs suggestive of an incidental host nature. However, pigs infected by serovar Pomona can also remain infected and shed serovar Pomona for up to a few months and in this case can lead to high rates of pig-to-pig transmission in confined husbandry arrangements (Divers, 2015b). As *L* Pomona may be carried by animals other than pigs, for example skunks or opossums, contact with these animals may transmit infection to pigs. The move to indoor housing arrangements makes this mode of transmission less important. Indirect contact is also an important way of transmission of *L* Pomona by way of contact with infected effluent, water or soil (Ellis, 2012).

If a naïve herd is infected by *L* Pomona, initially all ages of pigs may show clinical signs of disease. After the disease has become established, an endemic cycle of transmission will become established with piglets having protection from maternally derived antibodies in their mother's colostrum (Bolt and Marshall, 1995). Once the endemic cycle has become established, clinical disease is usually only found in those gilts raised in isolation since weaning or bought in from an uninfected herd (Ellis, 2012).

In a study of four non-vaccinating herds of grower pigs in New Zealand, three of which were known to be endemically infected, evidence of infection with *Leptospira* became evident by 12 weeks of age with the intensity of excretion in the urine greatest in the first three to four weeks of infection (Bolt and Marshall, 1995). This study looked at factors affecting the cultural and serological prevalence of leptospirosis in the piglets and found that the most important factors were standard of hygiene and the antibody titre in the dam, with higher dam titres affording better protection for the piglets for a longer period of time. Mixing of infected and susceptible grower pigs encouraged disease transmission resulting in epidemic outbreaks in individual pens.

7.2 *Leptospira* Bratislava

In *Leptospira* Bratislava infection, pigs will only rarely show signs of acute disease but disease will instead be manifested by reproductive failure and infertility, with sporadic abortions being the most common clinical sign. Venereal transmission may occur in serovar Bratislava infection (Divers, 2015b).

The roles of *L* Bratislava and *L* Muenchen are relatively poorly understood due to the difficulties in isolating these serovars (Maxie *et al.*, 2007).

Within the *L* Bratislava serovar, different strains are contained, some that are more adapted to pigs and others that are only found in wild animals. Furthermore, within these pig isolates, some are more associated with disease than others (Ellis *et al.*, 1991). These different strains can have differing serological profiles depending on the husbandry conditions. In sows kept under indoor conditions and excluded from contact with wildlife, many sows may have low positive titres but a few will have titres of 1:100 or greater in the Microscopic Agglutination Test. These sows will probably have been infected at coitus. In sows kept under outdoor conditions however, more than 50 per cent of sows may have MAT titres of greater than 1:100 with infection probably as a result of contact with infected rodent urine. Urinary excretion of *L* Bratislava is relatively low in comparison to *L* Pomona and transmission inside the fattening house is thought to be relatively poor (Ellis, 2012). The upper genital tract of both sows and boars have also been identified as important sites of carriage of *L* Bratislava (Bolin and Cassells, 1992).

L Bratislava infection in breeding pigs can cause increased returns to service both at regular three week intervals and at abnormal times. It can also be seen as a mucopurulent discharge occurring two to three days before return to oestrus and abortions, especially in late gestation. Additional clinical signs include an increase in the number of weak piglets born along with stillbirths and mummification. Where cases do occur, abortions may be limited to gilts suggesting that in endemically infected herds, sows may achieve a certain degree of immunity (Williams, 2015b).

7.3 Other serovars

More classical incidental host type disease can occur in the case of infection by serovars Grippotyphosa, Icterohaemorrhagiae, and Canicola with acute clinical signs involving pyrexia, haemolytic anaemia, haemoglobinuria and icterus, although this is rare (Divers, 2015b).

Both *L* Icterohaemorrhagiae and *L* Copenhageni are maintained by the brown rat (*Rattus norvegicus*) and infection may be transmitted to pigs via environmental contamination by infected rat urine. These serovars may cause sporadic disease in young pigs (Williams, 2015b) but transmission between pigs is not thought to be significant in the epidemiology of the disease. There has only been limited isolation of these serovars in developed countries with widespread seroprevalence attributable to vaccination, although high titres have been found in Brazil which may well relate to clinical disease (Ellis, 2012).

Leptospira Canicola is known to be maintained by dogs and perhaps also by wild animals (Paz-Soldán *et al.,* 1991). Long periods of urinary excretion and the ability of this serovar to survive in urine for up to six days makes pip-to-pig transmission quite possible (Ellis, 2012).

Leptospira Grippotyphosa is known to be maintained by wildlife species including raccoons, skunks and voles (Lunn, 2015). Widespread but low seroprevalence has been reported in central and eastern Europe and in the United States (Ellis, 2012).

Leptospira Hardjo may infect pigs and cause disease but this seems limited in importance to situations of shared keeping arrangements with cattle. Transmission within swine populations seems to be limited (Ellis, 2012).

8 Human disease

Leptospirosis in humans is caused by one of several pathogenic serovars of *Leptospira*. They induce biphasic symptoms with both phases including acute febrile episodes. The second phase may include hepatic, renal and meningeal disease (Bush and Perez, 2014). It is a widespread and occasionally fatal zoonosis and is endemic in many tropical countries with widespread outbreaks often occurring after heavy rainfall (Haake and Levett, 2015).

Infection in humans is generally acquired by direct contact with urine or tissues from infected animals or by indirect means by way of contact with contaminated soil or water. Although many animals can potentially transmit the disease to humans, the brown rat (*Rattus norvegicus*) is the most important source of infection. People living in urban slums in the developing world with inadequate housing and sanitation are at the greatest risk of disease by way of exposure to rat urine (Haake and Levett, 2015). As humans are generally considered as incidental hosts of leptospirosis, transmission within the human population is not considered important in the epidemiology of the disease (Lunn, 2015). In certain ecosystems however, there is evidence of humans acting as maintenance hosts of both pathogenic and intermediate leptospiral serovars with persistent renal colonisation and shedding in people without either clinical signs or serological evidence of infection. This seems to happen in hyperendemic regions of high disease transmission as described in the Peruvian Amazon (Ganoza *et al.*, 2010).

Outbreaks of leptospirosis often follow exposure to contaminated flood water. The usual way of entry is through exposed mucous membranes (conjunctival, nasal or oral) or abraded skin. Leptospirosis in humans can be considered either as an occupational disease of farmers, slaughterhouse workers, pet traders, veterinarians, rodent catchers and sewer workers (Hartskeerl at al., 2009) or a recreational disease of those engaging in activities exposing them to contaminated waters. Other likely sources of infection may include infected dogs and rats (Bush and Perez, 2014).

8.1 Occurrence and risk factors

Leptospirosis has a very wide geographical distribution with disease occurring in tropical, subtropical and temperate regions. Reported incidences are from 0.1-1 per 100 000 population per year in temperate zones, >10 cases per 100 000 population in humid topical or subtropical zones and >100 cases per 100 000 population in outbreak situations (Terpstra, 2003).

Leptospirosis is probably the most widespread and prevalent zoonotic disease in the world. Climate change is likely to favour an increase in its global incidence (Hartskeerl *et al.*, 2011).

Incidence of the disease in developed countries has decreased substantially in recent years with most cases now attributed to recreational exposure although the incidence appears to be increasing in the developing world (Vijayachari *et al.*, 2008). Epidemic outbreaks in recent years include Nicaragua in 2007, Sri Lanka in 2008, and the Philippines in 2009 with each outbreak affecting thousands of people and causing hundreds of deaths (Hartskeerl *et al.*, 2011).

8.1.1 Abattoir workers

Abattoir workers have long been known to be at significantly increased risk of contracting leptospirosis due to their frequent contact with potentially infected urine from livestock. All plants take steps to minimise worker exposure. Where inverted dressing procedures are used in particular, for example in some deer abattoirs, increased attention needs to be given to worker safety. Some plants bag and secure the pizzle with a rubber ring, as is standard practice for the bung, to reduce worker exposure from hinds releasing urine (Jensen *et al.*, 2004).

A recent study examined the seroprevalence and risk factors for contracting Leptospira in New Zealand abattoir workers (Dreyfus *et al*., 2014). Leptospirosis is a widespread disease of livestock in New Zealand with 60 per cent of deer herds, 92 per cent of beef cattle herds and 91 per cent of sheep flocks showing seropositivity (Dreyfus *et al*., 2011). The sera of 567 abattoir workers were tested by the Microscopic Agglutination Test for antibodies to Leptospira interrogans serovar Pomona and Leptospira borgpetersenii serovar Hardjobovis, the two most common serovars present in New Zealand. Previous studies in New Zealand had shown both farmers and meat plant workers to be at higher risk of contracting leptospirosis (Thornley *et al*., 2002) and that 63 per cent of farmed deer (Ayanegui-Alcerreca *et al*., 2010) and 5.7 per cent of lambs (Dorjee *et al*., 2008) sampled in abattoirs showed seropositivity to either or both of serovars Hardjobovis and Pomona. It was estimated, based on serology and culture methods, that each abattoir worker was exposed to 5-9 deer or 5-26 sheep carcasses actively shedding leptospires per day (Dorjee *et al*., 2011). A species specific multivariable analysis was used to determine associations between seroprevalence and risk factors. Overall, 11 per cent of the abattoir workers had antibodies to one or both of the serovars tested. Workers from four sheep abattoirs were tested with an average seroprevalence of 10-31 per cent, from two deer abattoirs with a seroprevalence of 17-19 per cent and two beef abattoirs with a seroprevalence of 5 per

cent. In the sheep and deer abattoirs, work position was found to be a strong risk factor, with the highest risk found to be stunning and hide removal, followed by the removal of the bladder and kidneys. The wearing of personal protective equipment seemed to afford no protection against infection. Home slaughtering, farming or hunting were not significant risk factors for seropositivity.

8.2 Clinical signs

Disease in humans is characteristically biphasic with an incubation period ranging between 2 to 20 days (usually between 7 to 13 days). The leptospiraemic phase starts abruptly with clinical signs including headache, severe myalgia, chills, fever, cough, pharyngitis, chest pain and occasionally haemoptysis. There are usually suffusions in the conjunctiva starting from the third to fourth day. Splenomegaly and hepatomegaly appear uncommonly. The leptospiraemic phase lasts between 4 to 9 days with recurring chills and fevers that often spike above 39° C. The fever will then abate (Bush and Perez, 2014).

The immune phase of the disease begins between the sixth and seventh day following the appearance of clinical signs and corresponds to the appearance of antibodies in the blood. Fever will then return along with the above mentioned clinical signs. Meningitis may also develop. Infrequently occurring clinical signs include iridocyclitis, optic neuritis and peripheral neuropathy. Leptospirosis may result in abortion if acquired during pregnancy, even in the convalescent period (Bush and Perez, 2014). Development to a more severe form of the disease depends on the epidemiological conditions, host susceptibility, and the virulence of the pathogen (Haake and Levett, 2015).

8.3 Weil's disease

Weil’s disease is the name given to the severe form of leptospirosis in humans that presents with icterus, normally together with azotaemia, in addition to anaemia, diminished consciousness and persistent fever. The onset is similar to that seen in the less severe forms of the disease. Haemorrhagic conditions relating to capillary damage then develop including epistaxis, petechiae, purpura and ecchymosis and can lead to haemorrhages in the subarachnoid space, the adrenal glands and the gastrointestinal tract. Thrombocytopenia may develop. There may be signs relating to hepatocellular injury and renal dysfunction from the third to the sixth

day. Renal disease may induce proteinuria, pyuria, haematuria and azotaemia. Lasting hepatocellular damage is minimal and complete healing is usually achieved (Bush and Perez, 2014). Elevated bilirubin levels may be observed in patients with acute disease both due to hepatocellular injury and disruption of intercellular junctions between neighbouring hepatocytes which can result in leakage of bile out or the bile canaliculi. Patients with severe forms of the disease experience a cytokine storm characterised by high levels of IL-6, TNF-α and IL-10 (Haake and Levett, 2015).

In patients that do not develop the icteric form of the disease mortality is nil. In Weil's disease the mortality is between 5 and 10 per cent (Bush and Perez, 2014). Mortality increases with age, particularly in patients older than 60. High levels of leptospiraemia are associated with poorer clinical outcomes. This is probably related to poor recognition of leptospiral lipopolysaccharide by human TLR-4 (Haake and Levett, 2015).

Diagnosis in humans is attained by blood culture and serology. In suspected cases, both acute and convalescent serum samples taken three to four weeks apart should be tested for the presence of antibodies. Disease in humans should be differentiated from viral meningoencephalitis, hantavirus caused haemolytic fever with renal syndrome, other spirochaetal infections, influenza virus, and hepatitis (Bush and Perez, 2014) in addition to dengue fever in susceptible populations (Haake and Levett, 2015). The characteristic biphasic fever may aid in the differentiation of leptospirosis from these other conditions. A neutrophil count of above 70 per cent helps to differentiate leptospirosis from diseases caused by viruses. If a patient has a history of possible exposure, leptospirosis should be considered in any patient with a fever of unknown origin (Bush and Perez, 2014).

Confirmation of leptospirosis in humans requires isolation from fluid or tissue samples, a fourfold increase or greater in in the agglutinating titre in the Microscopic Agglutination Test or an antibody titre of 1:800 or greater in patients with appropriate clinical signs (Bush and Perez, 2014).

8.4 Severe pulmonary haemorrhage syndrome

Severe pulmonary haemorrhagic syndrome is an extreme form of leptospirosis in humans with a case fatality rate of greater than 50 per cent resulting from widespread alveolar haemorrhage (Haake and Levett, 2015). SPHS first emerged in China and South Korea but now has a

worldwide occurrence. In some parts of the world it has replaced Weil's disease as the leading cause of death among human leptospirosis cases (Gouveie *et al.*, 2008).

Onset of disease is sudden and associated with a rapidly rising fever of up to 40.5 ºC, headache, myalgia and an initially dry cough which becomes streaked with blood after two to three days. Fine crepitations initially at the bases and then more extensively can be noted on auscultation of the lung fields in addition to tachycardia and tachypnoea. Massive haemoptysis can lead to death by asphyxiation (Vijayachari *et al.*, 2008).

9 Diagnosis

Diagnosis of leptospirosis in incidental hosts may be aided by the clinical signs of acute disease together with a history of possible exposure to contaminated urine. Diagnosis can be more difficult in maintenance hosts and may require screening tests (Quinn *et al.*, 2012).

Diagnostic tests can be performed both to detect the organism in tissue or body fluids and to detect the antibody response of the animal. It is generally recommended to include both serological testing and a method to detect the agent for a good diagnosis (Lunn, 2015).

As leptospiral organisms will die rapidly in tissues or body fluids unless kept at 4°C, samples are recommended to be submitted to laboratories in leptospiral transport medium (Maxie *et al*., 2007). Liquid culture medium or 1% bovine serum albumin solution containing 5-fluorouracil at 100– 200 µg/ml should be used as transport medium for the submission of samples (Ellis, 2014).

Organisms can be detected in fresh urine using dark field microscopy but this is a relatively insensitive method and is rarely used in practice. Tissue samples including kidney and liver samples can be used to demonstrate leptospires in tissues using either the Fluorescent Antibody Test or by silver impregnation (Quinn *et al.*, 2012).

Isolation techniques may be performed on blood during the early leptospiraemic phase or from urine from about two weeks post infection. Techniques involve the use of either a liquid culture medium at 30°C or by way of animal inoculation. Serovars vary in their speed of growth with *Leptospira* Hardjo, a slow growing serovar, taking approximately six weeks to grow in liquid media. The fastidious nature of leptospires require special culture media containing both 1 per cent bovine serum albumin and long chain fatty acids. The importance of the albumin is in adsorbing the long chain fatty acids releasing them slowly over an extended period of time, as they would be toxic to the leptospires at the given concentration. Tween 80 and EMJH culture media are commonly used (Quinn *et al.*, 2012). Culture will rarely be positive after the initiation of antibiotic therapy. Because of the need for specialised culture medium and the fastidious and slow growing nature of leptospires, samples are rarely cultured and culture is of little use in clinical cases (Lunn, 2015).

Isolates can be identified with the aid of serotyping and molecular methods. Many different techniques have been tried for molecular identification of leptospires. The current gold standard

test uses genomic macrorestriction with rare cutting endonucleases followed by pulsed field gel electrophoresis. (Cerqueira and Picardeau, 2009). There is generally a good agreement between pulsed gel field electrophoresis results and serotyping, with occasional discrepancies (Quinn *et al.*, 2012).

For the demonstration of leptospires in tissues, typically liver or kidney samples, the Fluorescent Antibody Technique is most commonly used, in addition to silver impregnation (Quinn *et al.*, 2012).

patterns of cross reacting antibodies vary between host species. Paradoxical reactions in the MAT with a marked response to a leptospiral serovar different to the infecting serovar may occur early in the course of an acute infection in incidental hosts. For the various reasons mentioned, the MAT cannot reliably identify the infecting serovar as this may not be the serovar to which the animal develops the highest titre. The MAT retains significant utility however in establishing numerical titres that allow comparisons to be made between acutely infected and convalescent animals (Lunn, 2015).

The widespread vaccination of animals can complicate the serological diagnosis of leptospirosis. Vaccinated animals will generally show low agglutinating antibody titres of between 1:100 to 1:400 which will persist for between 1 to 4 months after vaccination. In some cases, however a high titre may be provoked which can persist for 6 months or longer (Lunn, 2015).

When accompanied with consistent clinical signs, especially in incidental hosts, titres above 1:400 or a fourfold rise in paired serum samples are considered diagnostically significant (Quinn *et al.*, 2012). The lack of consensus regarding a diagnostic titre for leptospirosis is due to the fact that a low titre in serological tests does not necessarily exclude leptospirosis, especially in maintenance host infections, and because titres can often be low in the early stages of acute disease. A fourfold rise in antibody titre in paired serum samples taken seven to ten days apart is often seen in cases of acute leptospirosis. Caution is warranted when diagnosis is to be based on a single serum sample. With compatible clinical signs and vaccination greater than three months previously, a titre of between 1:800 and 1:1600 or greater is good evidence for infection. Acute and convalescent samples should be taken where possible. Titres usually persist for a few months after infection and will decline gradually over time (Lunn, 2015).

Serological diagnosis is more difficult in maintenance hosts due to the relative lack or delay of antibody response to infection. This is of special importance in cattle infected with *Leptospira* Hardjo where prolonged urinary excretion of leptospires may occur in the absence of a significant serological titre. In other cases of maintenance host infection, by the time clinical signs are apparent titres may be low or falling (Quinn *et al.*, 2012).

Several ELISA tests have been developed in some countries based on the predominant serovars circulating in those countries (Quinn *et al.*, 2012).

9.1 Diagnosis in cattle

9.2 Diagnosis in pigs

Diagnosis may need to performed for many reasons apart from following an occurrence of clinical disease such as; assessment of the herd status for the purposes of control or eradications programs, epidemiological studies or the assessment of the infection status of an individual animal for the purposes of trade. As signs of acute disease often pass undetected, diagnosis typically relies on laboratory methods (Ellis, 2012).

When used as a herd test, at least 10 per cent or 10 animals (whichever is greater) should be chosen for serology by the Microscopic Agglutination Test. Serology is very useful to diagnose acute infection in an individual animal, with rising antibody titres in paired acute and convalescent serum samples being diagnostic (Ellis, 2012).

Caution should be applied when interpreting serological tests on a herd level. In the case of reproductive problems arising in a herd, serology is frequently performed which will often find antibody titres as high as 1:200 in some animals but these results may well not be of significance. Low titres may be present in normal herds and cross reactions are common with the many serovars present in and around pig farms from infected rodents, badgers, foxes hedgehogs, etc. (Williams, 2015b). A retrospective diagnosis of leptospiral abortion may be made when the majority of affected animals have antibody titres of 1:1000 or greater (Ellis, 2012).

Demonstration of leptospires in abortion products or in the genital tract at postmortem by way of the Fluorescent Antibody Test provides strong evidence for a diagnosis (Williams, 2015b). The demonstration of antibodies in foetal serum is diagnostic of leptospiral abortion but immunofluorescence is the method of choice for diagnosing leptospirosis in swine foetuses (Ellis, 2012).

Isolation of leptospires is difficult and not commonly attempted. If leptospires are isolated from the internal organs or body fluids of animals showing signs of disease, this provides evidence of acute infection. In the absence of signs of generalised disease, isolation from the genital tract of either males or females or in the urine gives evidence of chronic infection. Renal excretion may be intermittent and the failure to demonstrate leptospires in the urine does not rule out the carrier state (Ellis, 2012).

Leptospirosis in pigs is thought to be clinically over-diagnosed, at least in the UK, with many resources wasted on antibiotic therapy without an adequate diagnosis (Williams, 2015b).

10 Treatment and Control

The principles of treatment and control are applicable across the species. Biosecurity and vaccination based on serovars prevalent in that region are the most important preventative control measures although vaccines have variable efficacy in incidental hosts. Appropriate antibiotics may be used both to treat the disease and to end the carrier state.

10.1 Cattle

With the large increase in risk associated with herds buying in cattle, a closed herd management strategy is suggested if possible (Williams and Winden, 2014). Although biosecurity measures can help to reduce the risk of exposure to infection, it would be very difficult to completely eradicate bovine leptospirosis in the UK because of the high percentage of herds infected. In practical terms, vaccination will often be the best control option (Owen, 2003).

As a part of herd screening programs, a bulk tank ELISA test can be used for surveillance in a naïve herd. Pooled samples from first lactation heifers may also be used (Williams, 2015a).

Steptomycin is added to bull semen collected at Artificial Insemination centres as a precautionary method to control the disease (Williams, 2015a).

10.2 Pigs

In cases of confirmed disease, whole herd antibiotic treatment is generally appropriate. This can take the form of either single or double streptomycin treatment of the whole herd, injection of females at service with streptomycin or potentiated sulphonamides, in-feed medication using tetracyclines or by regular treatment of boars with streptomycin, for example every six weeks (Williams, 2015b). Streptomycin is the most useful drug for both control and treatment but its veterinary use is no longer permitted in some countries (Ellis, 2012).

There is conflicting information on whether streptomycin therapy alone can eliminate renal carriage but oxytetracycline or erythromycin therapies have been shown to be effective, at least in the case of *L* Pomona (Ellis, 2012).

Bacterins are commonly used in breeding operations to reduce the prevalence of abortions but these only afford serovar specific protection and will not eliminate the infections in animals who already carry the disease (Divers, 2015b). Although vaccination against *L* Pomona is widely practised in countries like Australia and New Zealand, no vaccine to control *L* Bratislava is currently available in the UK (Williams, 2015b). Swine leptospirosis vaccines are relatively poor compared with those used to control *L* Hardjo in cattle with none approaching year-long protection. Although vaccination may markedly reduce the prevalence of disease in a herd, it cannot be relied upon alone to completely eliminate infection or renal excretion (Ellis, 2012).

As other animal species may serve as reservoirs of infection for pigs, limiting contact with these animals may be of benefit. Of particular concern in the UK and Ireland are rats and hedgehogs.

Limiting hedgehog contact in outdoor pigs is probably not feasible. Due to the possible sexual transmission of *Leptospira* Bratislava, the choice of artificial insemination over natural mating may help to reduce the prevalence of the disease (Williams, 2015b).

11 Pathological findings and meat hygiene

Although suspected as possibly being a food-borne pathogen, leptospirosis is not currently classified as such due to a lack of reports and a lack of isolation methods and research, although this may change with improved technologies and surveillance. These features are shared with many Gram-negative bacteria such as *Citrobacter, Edwardsiella, Enterobacter, Klebsiella, Hafnia, Kluyvera, Proteus, Providencia, Morganella, Serratia, Vibrios and Pseudomnas* and the Gram-positives *Corynebacterium, Streptococcus* and some species of *Bacillus* and *Clostridium* and miscellaneous organisms including *Brucella, Mycobacterium, Coxiella burnetti, Erysipelothrix rhusiopathiae* and *Francisella tularensis* (Toldra, 2010).

Leptospires may be found in the milk in acute cases, however they do not survive for long periods of time (Herenda et al., 1994). Pasteurisation effectively destroys all leptospiral organisms excreted in the milk (Williams, 2015a).

11.1 Antemortem findings

Acute and subacute forms of the disease may be marked by a transient fever, anorexia, agalactia in lactating cows and mastitis. Milk produced may be yellow, clotted and frequently blood-tinged. In animals suffering from severe disease, icterus may be present together with anaemia and there may be a pneumonia. Abortion may occur along with retained foetal membranes. Young calves severely affected by the disease may show yellowish discolouration of the mucous membranes and reddish-brown urine (Herenda *et al.,* 1994).

Chronic disease may be subclinical or only have mild clinical signs, although abortion may be seen. In the case of meningitis, affected animals may demonstrate ataxia, ptyalism and muscle stiffness (Herenda *et al.,* 1994).

11.2 Postmortem findings

The leading postmortem findings are anaemia and icterus (Herenda *et al.,* 1994). It is a haemolytic icterus brought about by red blood cell destruction (Collins and Huey, 2015). Other lesions include haemorrhages in the subserosa and submucosa and ulceration. Haemorrhages in the mucosa of the abomasum may also be seen (Herenda *et al.,* 1994). In rare cases there may

be pulmonary oedema or emphysema. A focal, non-purulent, interstitial nephritis may be observed in pigs and septicaemic signs may also be seen (Herenda *et al.,* 1994). The spleen may be blackish-red and soft.

Interstitial nephritis may be caused by all serovars and may be especially severe in pigs. Indeed, the majority of cases of interstitial nephritis observed at slaughter in pigs are thought to be leptospiral in origin. Localisation of leptospires after the period of leptospiraemia is associated with either a focal or diffuse interstitial nephritis and with an acute, transient degeneration of the tubules. Renal failure as is often noted in leptospirosis in dogs is not a significant feature in food producing animals (Maxie *et al.*, 2007).

11.2.1 Cattle

The postmortem picture of acute leptospirosis in cattle is dominated by severe anaemia together with mild icterus. There may or may not be haemorrhage. The lungs are typically pale, oedematous and expanded with dilated septa filled with bile stained fluid. The liver is enlarged, friable, anaemic and stained with bile. It may contain haemorrhages and small necrotic zones around the central veins which are not necessarily visible on gross pathology. Haemoglobinuria is not always present. The kidneys will be swollen and initially dark but later, pigmented foci will be restricted to small groups and may give the appearance of small haemorrhages. So called "white-spotted kidney" may be the result of congenital infection by *L* Hardjo (Maxie *et al.*, 2007).

Histopathology in bovine leptospirosis shows neither prominent nor specific changes. Oedema may be seen in the lungs and severe anoxic changes in the liver brought about by anaemia may manifest as periacinar zonal necrosis. In cases of protracted haemolytic disease, the bile canaliculi may be distended with bile (Maxie *et al.*, 2007).

In cases of acute disease, appropriate stains, for example silver impregnation, may demonstrate leptospires in both the liver and kidney. In the kidney they will frequently appear as clusters in the tubular lumen (Maxie *et al.*, 2007).

During the recovery phase or in the course of subclinical disease, the leptospires organise as microcolonies in the kidneys and will occur as intratubular clumps and will only rarely be seen in the interstitium. The renal localisation will be associated with a widespread focal interstitial

nephritis. The non-suppurative inflammation will be mostly confined to the renal cortex and will be mostly comprised of lymphocytes and plasma cells. The inflammatory reaction will subside very slowly over time and focal lesions will scarify (Maxie *et al.*, 2007).

11.2.2 Pigs

In the case of abortions caused by *L* Pomona as well as incidental serovars, the litter will normally be aborted one to three weeks before term will some foetuses stillborn and others dying shortly after delivery. Leptospires may be recoverable from the fresher foetuses. Straw covered effusions may be found principally in the pleural cavity. Both the liver and spleen will be enlarged and areas of tan-coloured focal necrosis 2-5mm in diameter may be seen, especially near the margins. Haemorrhages may be seen in the pleura, epicardium, renal cortex and elsewhere (Maxie *et al*., 2007).

As in cattle, histopathological changes are inconstant with the most consistent changes seen in those piglets dying at or soon after birth. Acute hepatitis may be seen with neutrophils and lymphocytes infiltrating the portal areas. These areas may be surrounded by foci of coagulative necrosis. Inflammatory cells may infiltrate beneath the epicardium and endocardium. Many small foci of interstitial nephritis may be seen in the kidney in addition to large areas of circumscribed infiltration of mononuclear cells in the peripelvic parenchyma, which may also involve the papilla (Maxie *et al*., 2007).

Infections of pigs by *L* Bratislava or *L* Muenchen tend to show similar but more subtle pathological changes compared to those seen in *L* Pomona infection (Maxie *et al*., 2007).

11.3 Judgement

Animals showing signs of leptospirosis must not be slaughtered for human consumption. Any diagnosed form of the disease, whether acute or chronic, generalised or localised, is to be deemed unfit for human consumption.

11.4 Differential diagnosis

Both acute and subacute forms should be differentiated from babesiosis, anaplasmosis, rape and kale poisoning, bacillary haemoglobinuria, post parturient haemoglobinuria and acute haemolytic anaemia in calves. Blood-tinged milk is a characteristic clinical sign and may help in the differential diagnosis of other infectious agents (Herenda et al., 1994).

Acute intravascular haemolysis in young calves and lambs should be distinguished from *Clostridium perfringens* type A infection, bacillary haemoglobinuria and chronic copper poisoning (Maxie *et al.*, 2007).

12 Occurrence in Ireland

Leptospirosis is an important and common disease of food producing animals in Ireland. The most important affected species from an economic perspective is cattle. Human disease is known to be high among farmers and outbreaks have been recorded in those engaging in water sports.

The highly complex epidemiology seen in warmer regions of the world (Terpstra, 2003) is not as marked in Ireland however wildlife, including rats, badgers and hedgehogs play an important role in maintaining the disease.

12.1 Cattle

A 2004 study in the Republic of Ireland examined bulk milk tank samples from 347 dairy herds through seven milk recording organisations for the presence of antibodies to *Leptospira interrogans* serovar Hardjo using an ELISA test (Leonard *et al.*, 2004). These herds had not vaccinated against leptospirosis within the previous five years. As the majority of herds were spring calving, samples were collected during the summer months to try to get as representative a sample as possible of all the lactating cows in the herd. Two hundred and seventy-three (79 per cent) of these herds had a positive ELISA sample. Both the probability of a herd being seropositive and the antibody level of the bulk milk tank sample were affected by the province ($P<0.05$ and $P<0.01$, respectively) and the herd size category ($P<0.05$ and $P<0.01$, respectively). Herds in the north and east (Ulster and Leinster) were more likely to have a positive titre (78/88 herds) or a high titre. In addition, large herds of greater than sixty cows were more likely to have a positive titre (55/61 herds) or a high titre. This was the first study in the Republic of Ireland to investigate the true seroprevalence of leptospirosis in the national dairy herd. Previous studies had been carried out by questionnaire surveys without confirmatory laboratory testing or in samples selected from herds with a history of abortions.

A more recent study in the Republic of Ireland examined the seroprevalence of leptospirosis in Irish sucker herds, which had previously not been examined. It described the seroprevalence both at herd level and at animal level (Ryan *et al.* 2012b). This study clearly showed the endemic nature of leptospirosis in Irish cattle and highlighted important differences relating to region and breeding herd size. The study was performed in conjunction with a study to estimate

the prevalence of paratuberculosis in Ireland. For the purposes of the study, the 26 counties of the Irish Republic were divided into 6 regions from which a representative sample of herds were selected to be sampled. Each region contained roughly 200,000 suckler cows and shared broadly similar husbandry practices and farmland type. Using previous UK and Irish studies estimating a seroprevalence of around 70 percent, it was calculated that 320 herds would need to be sampled to estimate the prevalence of leptospirosis to within 5 percentage points. The number of herds sampled in each region was proportionate to the percentage of the national herd contained in that region. Herds registered as a part of the National Brucellosis Eradication Scheme served as a base for the sampling where at least one calf had been registered into the herd on the Cattle Movement Monitoring System in the year 2003. One thousand herds of mixed suckler and dairy type were randomly selected from an eligible total of 96,173 herds. A herd was considered eligible for sampling if it was not vaccinating against leptospirosis and if it contained ≥ 8 animals of beef breeds greater than 12 months of age. The individual animal eligibility were unvaccinated females and bulls of beef breeds greater than 12 months of age. In total, 288 herds were eligible for inclusion in the seroprevalence dataset, 21 herds had been excluded due to vaccination and 11 herds had less than nine breeding animals. Serological testing was performed using a commercially available monoclonal antibody capture ELISA with an assumed sensitivity and specificity of 100 per cent and 86.67 per cent respectively which detected an antibody response to a lipopolysaccharide outer envelope epitope common to both *Leptospira borgpetersenii* serovar Hardjo and *Leptospira interrogans* serovar Hardjo. This test compared unfavourably to the Microscopic Agglutination Test, the "gold standard" as recognised by the OIE, although reinterpretation of the results using more recent data from the manufacturer yielded improvements in specificity. A probabilistic approach was used to classify herds as either "free from infection" or "infected" using the epidemiological software tool *FreeCalc2.0* based on the serological results obtained, the likely minimum herd prevalence assuming infection, and the limitations of the diagnostic test. Of particular concern in this study was the relatively low specificity of the serological test leading to many false positive results. Using this classification, 237 herds were classified as being infected equating to a herd level prevalence of 82.29 per cent (89.9 per cent according to the new manufacturer data). The South West and South East regions had the highest herd level prevalence with the regional effects being largely mirrored by herd size. Using the epidemiological software tool *TruePrev* and accounting for the sensitivity and specificity of the test as well as the number of animals tested, the true animal level prevalence on a national level was calculated at 41.75 per cent, although with the new data from the manufacturer this rose to 46 per cent. There was a statistically

significant regional trend in the animal level prevalence, being highest in South East Leinster and South West Munster and lowest in West Connaught and North Leinster/South Ulster ($P<0.001$). The regions with the highest herd level prevalence also had the largest breeding herd size. When categorised into quartiles, there was a statistically significant influence of breeding herd size on individual animal prevalence with true animal level seroprevalence increasing with increasing breeding herd size. There were statistically significant differences ($P<0.05$) in breed seroprevalence between Aberdeen Angus and Belgian Blue, between Aberdeen Angus and Charolais and between Aberdeen Angus and Limousin with Belgian Blue having the highest seroprevalence followed by Limousin and Charolais and with Aberdeen Angus having the lowest seroprevalence. These findings with respect to breed had not been found in previous studies. There was no statistically significant difference in true animal seroprevalence according to age or sex.

The herd level prevalence of leptospirosis appears to be higher in the Republic of Ireland than in many other countries. It is estimated at around 11 per cent in Spanish beef herds (Alonso-Andicoberry *et al.*, 2001). 42 per cent of suckler herds in the United States had evidence of infection with *Leptospira borgpetersenii* serovar Hardjo (Wikse *et al.*, 2007). Disease caused by *Leptospira* Hardjo is endemic in the UK with serological surveys suggesting that more than 75 per cent of UK cattle have been exposed (Owen, 2003). A market report in 2008 indicated that 35 per cent of UK suckler herds had confirmed or suspected leptospirosis. Figures from 1648 herds tested in 2011 found that 43 per cent of herds had been exposed to leptospirosis, however in these surveys the distinction between seroconversion and clinical disease resulting in production losses was not stated clearly. A recent survey in the UK examined bulk milk tank samples from 1088 dairy herds for antibodies to *Leptospira* Hardjo in addition to testing for BVDV and BHV-1 and used data from questionnaires collected under the DairyCheck scheme (MSD Animal Health) to gather information on farm demographic and management practices. The herd level prevalence of L Hardjo infection was found to be 71.9 per cent, roughly consistent with the results of similar recent surveys (Williams and Winden, 2014).

At 42 per cent, animal level prevalence in the Irish study (Ryan *et al.*, 2012b) was also much higher than those results obtained in England, where animal level seroprevalence figures of 24.2 per cent (Pritchard *et al.*, 1989) and 18 per cent (Pritchard *et al.*, 1987) have been published.

In aborted foetuses submitted to veterinary laboratories in the Republic of Ireland in 2013, 7.4 per cent tested positive for *L* Hardjo. The corresponding figure for Northern Ireland during this period was 5.3 per cent (AFBI/DAFM, 2013). Figures for the three years up to 2013 are listed in Table 2. Diagnosis is based on antibody titre reaching a defined threshold or positivity on the Fluorescent Antibody Test.

Table 2. Percentage positivity for Leptospira Hardjo *(numbers positive) in bovine foetuses tested by DAFM (Republic of Ireland) and AFBI (Northern Ireland) in the years 2011-2013 (from AFBI/DAFM, 2011;AFBI/DAFM, 2012;AFBI/DAFM, 2013)*

Year	DAFM	AFBI
2011	1.6% (21)	4.5% (24)
2012	1.9%	7.2%
2013	7.4% (122)	5.3% (28)

The significant differences in positivity found between Northern Ireland and Republic of Ireland laboratories are not thought to accurately represent real differences in the prevalence of leptospirosis but are instead the result of the poor sensitivity of the diagnostic tests and disagreement as to their interpretation (AFBI/DAFM, 2012). The figures shown in Table 2 do not allow for an exact comparison across the two jurisdictions of Ireland during these years as the two authorities disagreed as to the appropriate antibody titre indicative of infection. In 2011, for example, AFBI would make a positive diagnosis of leptospirosis if an antibody titre of greater than 1:30 was detected in foetal fluids whereas the threshold used by DAFM was 1:100 (AFBI/DAFM, 2011).

12.2 Sheep

Endemic infection of sheep by *L* Hardjo is present in both the United Kingdom and Ireland (AFBI/DAFM, 2013).

Of aborted ovine foetuses and stillbirth cases submitted to veterinary laboratories in Northern Ireland in the year 2013, 7.9 per cent tested positive for *Leptospira* species. No diagnoses of leptospirosis were made in aborted ovine foetuses in the Republic of Ireland during this period. The diagnoses in Northern Ireland were made using the Fluorescent Antibody Test for antigen detection in the aborted foetuses. This test was not routinely employed in cases of ovine abortion in Republic of Ireland laboratories during that time (AFBI/DAFM, 2013).

In 2012, twenty-three positive diagnoses for leptospirosis were made in ovine abortion cases submitted to AFBI equating to 6.0 per cent of cases investigated. These results were based on

serological testing of the dam using the Microscopic Agglutination Test. No positive MAT results were recorded in the Republic of Ireland for ovine abortion cases during this period (AFBI/DAFM, 2012).

12.3 Pigs

Although the most common serotype found in most of the major pig producing regions outside of Western Europe, *L* Pomona infection of pig herds has not been recorded in the United Kingdom or Ireland (Williams, 2015b).

In Ireland, *Leptospira* Bratislava seroprevalence is thought to be high in outdoor pigs but there is little direct evidence of reproductive losses attributable to this serovar (AFBI/DAFM, 2011).

Of 12 cases of porcine abortion or stillbirth submitted to veterinary laboratories in Northern Ireland in the year 2013, one was found to have leptospires in foetal tissue (AFBI/DAFM, 2013). Of thirteen abortion cases submitted in 2011, two were found to be caused by leptospirosis (AFBI/DAFM, 2011).

A 1986 survey of swine abortions and stillbirths in Northern Ireland isolated leptospires from 55 of 78 litters examined. Of these, 91 per cent were members of the Australis serogroup including *L* Bratislava and *L* Muenchen with the other belonging to *L* Icterohaemorrhagiae, *L* Hebdomadis and *L* Autumnalis (Ellis *et al.,* 1986).

12.4 Humans

An epidemiological review in 2000 surveyed the clinical and serological incidence of leptospirosis in the Republic of Ireland (Pate *et al.*, 2000). Diagnosed cases according to Hospital In-Patient Enquiry (HIPE) data are shown in Table 3.

Table 3. Confirmed leptospirosis cases in the Republic of Ireland at the time of discharge from hospital in the years 1990-1996 (from Pate et al., 2000)

Health Board	Population (1991)	Cases (1990-1996) millions	Mean annual incidence per million
Eastern	1 245 225	18	2.1
Midland	202 984	5	3.5
Mid-Western	310 728	16	7.4
North-Eastern	300 183	9	4.3
North-Western	208 174	5	3.4
South-Eastern	383 188	33	12.3
Southern	532 263	20	5.4
Western	342 974	15	6.2
IRELAND	**3 525 719**	**121**	**4.9**

Between 1990 and 1996, the serological incidence of leptospirosis in the South-Eastern Health Board area was 10.4 cases per million per year. This was statistically significantly higher than the national serological incidence of 6.0 per million. The absolute incidence of *Leptospira* Hardjo disease in the South-Eastern area was almost 3.0 per million which was double the national average and seven times the incidence in Great Britain. The leptospirosis incidence due to undetermined serovars was also elevated in the South-Eastern area.

The sex distribution in this survey was 95 per cent male, an even higher figure than the 93 per cent figure for England & Wales and 86 per cent for Scotland.

With information from the Department of Agriculture, the incidence of human cases of leptospirosis in the Health Board areas was compared to the number of cattle per head of population and the number of cattle per square kilometre. The South East region was found to have the highest density of cattle per square kilometre (3.3 versus a national average of 1.96). The figures for England & Wales and Scotland were 0.14 and 0.04 respectively. It was found that the mean annual incidence according to the hospital discharge data was strongly correlated with the head of cattle in each health board region. There was no association between discharge diagnosis and the numbers of sheep, pigs, horses, goats, deer or donkeys. There was an association with the total number of livestock (cattle, sheep and pig) but this trend was not as strong as for cattle alone. With the positive relationship between both hospital discharge diagnosis and serological incidence of leptospirosis with the number of cattle in each health board region, this at least suggests an association between human and cattle leptospirosis cases in Ireland.

At the time of this study the only information available relating to bovine leptospirosis in the Republic of Ireland was a national questionnaire survey of Irish dairy farmers which found

confirmed or suspected cases of leptospirosis in 40 per cent of herds. More detailed information, particularly as it related to geographical location, was not available. More detailed and unbiased studies of dairy and suckler herds to establish the true seroprevalence of leptospirosis in the national cattle herd were not available until later (Leonard *et al.*, 2004; Ryan *et al.*, 2012b). As will be discussed below, a 2012 study of suckler herds in particular showed the concentration of bovine leptospirosis in the South East of the country, although it should be noted that this area is not identical to the health board area at that time, in addition to the significant time gap between the studies.

The reported incidence of human leptospirosis in the Republic of Ireland increased threefold between the years 1995-1999 and 2004-2009. Although there may have been a true increase in the incidence of clinical disease in humans, most of this rise seems to be attributable to improved reporting. Occupational exposure though exposure to livestock and recreational exposure, in particular kayaking, are the most important risk factors (Garvey *et al.* 2014).

A cluster of human leptospirosis cases caused by *L* Icterohaemorrhagiae occurred following a white-water rafting competition that took place on the River Liffey in November 2001 (Boland *et al.* 2004). After a possible case was notified to the Department of Health, an outbreak control team conducted an epidemiological investigation in which they found that 6 of the 65 participants who participated in the event had contracted the disease – an attack rate of 9.2 per cent. Participants who reported swallowing more than one mouthful of water had a higher risk of developing disease. Possible contributory factors including the increased rainfall prior to the event and the release of water from a hydroelectric dam upstream.

A 1990 survey of farmers in Northern Ireland examined 382 farmers for antibody to *Leptospira interrogans* by the Microscopic Agglutination Test finding an overall seropositivity of 8.1 per cent (Stanford *et al.*, 1990). The results by serovar are given in Table 4.

Table 4. Seropositivity to Leptospira in Northern Irish farmers (from Stanford et al., 1990)

Serovar	Number (%) seropositive
Leptospira interrogans	**31 (8.1)**
Hardjo	15 (3.9)
Icterohaemorrhagiae	10 (2.6)
Canicola	0 (0)
Pomona	0 (0)
Ballum	3 (0.7)
Autumnalis	0 (0)
Bratislava	6 (1.5)

Thirty-one farmers (8.1 per cent) had antibody to *Leptospira interrogans*, the most common being to serovar Hardjo. Antibody to this serovar was present in 6.4 percent of dairy farmers, 2.9 per cent of beef farmers and 1.9 per cent of mixed or arable farmers. None of the six farmers with Bratislava antibody were keeping pigs at the time of the study.

13 Conclusion

Leptospirosis has an importance across the world and across a huge range of animal species in addition to humans. The epidemiology of the disease is complex and there are many possible transmission pathways between animals and humans.

The general principles of the epidemiology, pathogenesis, clinical signs, diagnosis, treatment and control are largely applicable across the various affected species. The severity of the disease can vary greatly due to the infecting serovar, affected species, and individual animal or human factors.

Some important parts of the pathogenesis remain to be uncovered, in particular in relation to the ability of the organisms to evade the immune response and persist in certain parts of the body for long periods of time.

Correct diagnosis, especially in the case of chronic infections, can be difficult to attain with under-diagnosis thought to be common, especially in cattle herds chronically infected by *Leptospira* Hardjo. Over-diagnosis may also be a problem, particularly in pig herds with unexplained reproductive problems. Clinicians must be aware in particular of the vagaries of interpreting the results of serological tests in chronic cases and the need to include multiple diagnostic tests in addition to a good clinical examination.

Economic importance in the livestock industry is almost exclusively due to chronic disease impinging on reproduction performance with acute illness being an important cause of mortality in susceptible humans.

Infection of the human population in Ireland is common, with disease found to be high among certain risk groups such as farmers and those engaging in outdoor activities. The prevalence of the disease in humans and the known potential for severe illness and indeed death highlights the need for increased knowledge of leptospirosis among high risk groups with appropriate preventative steps being taken where feasible.

14 Summary

In this thesis, I discussed the importance of leptospirosis in food producing animals and in humans with particular focus on the situation in Ireland. The aetiology and epidemiology of this disease are complex and sometimes confusing and many animal species, both wild and domestic, may be involved in transmission cycles, even in temperate zones like Ireland. The classification into incidental and maintenance host infections should not be considered as absolute but remains a useful framework for discussing the pathogenesis, clinical signs and epidemiology of the disease. Diagnosis of acute disease is generally straightforward but in chronic cases, representing the bulk of the economic cost, is more difficult to achieve. Serology retains prime importance for diagnosis but requires a judicious approach to its interpretation with care taken to avoid over diagnosis in the face of positive results. Treatment is often rewarding but efforts should be made to identify the source of infection and to prevent or eliminate the carrier state in chronically infected animals. Leptospirosis remains a common disease of food producing animals in Ireland and is likely to remain so for the foreseeable future as eradication programs are not considered feasible. Human disease follows direct or indirect contact with urine from infected animals and in Ireland is found sporadically in people who work alongside livestock and as occasional epidemics in those engaging in water sports. Control is difficult, although preventative measures and education targeted at high risk groups may reduce its prevalence and aid in its early diagnosis and treatment. Reducing infection in livestock will both improve animal health and lessen the opportunities for transmission to humans.

15 References

AFBI/DAFM Veterinary Laboratories (2011): All-island Animal Disease Surveillance Report 2011. p. 31-32; 47; 55

AFBI/DAFM Veterinary Laboratories (2012): All-island Animal Disease Surveillance Report 2012. p. 19; 44

AFBI/DAFM Veterinary Laboratories (2013): All-island Animal Disease Surveillance Report 2013. p. 22-23; 46; 75-76

Adler B.; de la Peña Moctezuma A. (2010): Leptospira and leptospirosis. Vet Microbiol. 140 (3-4) p. 287-296

Alonso-Andicoberry C.; Garcia-Pena F. J.; Pereira-Bueno J.; Costas E.; Ortega-Mora L. M. (2001): Herd level risk factors associated with Leptospira spp. Seroprevalence in dairy and beef cattle in Spain. Prev Vet Med 52 p. 109-117

Ayanegui-Alcerreca M.; Wilson P. R.; Mackintosh C. G.; Collins-Emerson J. M.; Heuer C.; Midwinter A. C.; Castillo-Alcala F. (2010): Regional seroprevalence of leptospirosis on deer farms in New Zealand. New Zealand Veterinary Journal 58 p.184–189

Boland M.; Sayers G.; Coleman T.; Bergin C.; Sheehan N.; Creamer E.; O'Connell M.; Jones L.; Zochowski W. (2004): A cluster of leptospirosis cases in canoeists following a competition on the River Liffey. Epidemiol Infect. 132(2) p. 195–200

Bolin C. A.; Cassells J. A. (1992): Isolation of Leptospira interrogans serovars bratislava and hardjo from swine at slaughter. J Vet Diagn Invest 4 p.87-89

Bolt I.; Marshall R. B. (1995): The epidemiology of Leptospira interrogans serovar pomona in grower pig herds. New Zealand Veterinary Journal 43 (1) p. 10-15

Bush L. M.; Perez M. T. (2014): Leptospirosis. MSD Manual Professional Edition. http://www.msdmanuals.com/en-gb/professional/infectious-diseases/spirochetes/leptospirosis, visited 15/10/15

Cerqueira G. M.; Picardeau M. (2009): A century of Leptospira strain typing. Infect Genet. Evol. 9 (5) p. 760-768

Cockcroft P.D. (2015): Bovine Medicine, Third Edition. Oxford, UK. John Wiley & Sons, Ltd. p. 491

Collins D. S.; Huey R.J. (2015): Gracey's Meat Hygiene, Eleventh Edition. Chichester, West Sussex, United Kingdom. John Wiley & Sons, Ltd p. 209

Dhaliwal G. S.; Murray R. D.: Downham D. Y.:Dobson H (1994): The effect of vaccination against Leptospira interrogans serovar hardjo infection at the time of service on pregnancy rates in dairy cows. Veterinary Research, BioMed Central 25 (2-3) p.271-274

Divers T. J. (2015): Leptospirosis in Ruminants. The Merck Veterinary Manual. http://www.merckvetmanual.com/mvm/generalized_conditions/leptospirosis/leptospirosis_in_ruminants.html, visited on 05/11/15

Divers T. J. (2015): Leptospirosis in Swine. The Merck Veterinary Manual. http://www.merckvetmanual.com/mvm/generalized_conditions/leptospirosis/leptospirosis_in_swine.html, visited on 12/11/15

Dorjee S.; Heuer C.; Jackson R.; West D. M.; Collins-Emerson J. M.; Midwinter A. C.; Ridler A. L. (2011): Assessment of occupational exposure to leptospirosis in a sheep-only abattoir. Epidemiol. Infect. 139 p.797–806

Dorjee S.; Heuer C.; Jackson R.; West D. M.; Collins-Emerson J. M.; Midwinter A. C.; Ridler A. L. (2008): Prevalence of pathogenic leptospira spp. in sheep in a sheep-only abattoir in New Zealand. New Zealand Veterinary Journal 56 p. 164–170

Dreyfus A.; Benschop J.; Collins-Emerson J.; Wilson P.; Baker M. G.; Heuer C. (2014): Sero-Prevalence and Risk Factors for Leptospirosis in Abattoir Workers in New Zealand. Int J Environ Res Public Health 11(2) p. 1756–1775

Dreyfus A.; Verdugo C.; Benschop J.; Collins-Emerson J.; Wilson P.; Heuer C. (2011): Leptospirosis sero-prevalence and associated economic loss in new zealand livestock. In Proceedings of the food safety, animal welfare & biosecurity, epidemiology & animal health management, and industry branches of the NZVA, VetLearn Foundation, Palmerston North, New Zealand: 2011; p. 3.12.11–13.12.10

Ellis W.A. (1994): Leptospirosis as a cause of reproductive failure. Vet Clin North Am Food Anim Pract, 10 p. 463-478

Ellis W. A. (2014): Leptospirosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE. http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.01.09_LEPTO.pdf, visited on 02/11/15

Ellis W.A.: Leptospirosis. In Zimmerman J. J.; Locke A.K.; Ramirez A. Schwartz K. J.; Stevenson G.W. Diseases of Swine, Tenth Edition. Iowa, USA. John Wiley & Sons, Inc. (2012) p. 2818-2849

Ellis W.A.; McParland P. J.; Bryson D.G.; Cassells J. A. (1986): Prevalence of Leptospira infection in aborted pigs in Northern Ireland. Vet Rec. 118(3) p.63-5

Ellis W. A.; Montgomery J. M.; Thiermann A. B. (1991): Restriction endonuclease analysis as a taxonomic tool in the study of pig isolates belonging to the Australis serogroup of Leptospira interrogans. J. Clin. Microbiol. 29 p. 957-961

Faine S.; Adler B.; Bolin C.; Perolat P. (1999): Leptospira and Leptospirosis, Second Edition. Melbourne, Australia. MediSci

Ganoza C. A.; Matthias M. A.; Saito M.; Cespedes M; Gotuzzo E.; Vinetz J. M. (2010): Asymptomatic Renal Colonization of Humans in the Peruvian Amazon by *Leptospira*. PLoS Negl Trop Dis 4(2)

Garvey P.; Connell J.; O'Flanagan D.; McKeown P. (2014): Leptospirosis in Ireland: annual incidence and exposures associated with infection. Epidemiol Infect 142(4) p. 847-55

Gouveie E. L.; Metcalfe J.; de Carvalho A. L. F.; Aires T. S. F.; Villasboas-Bisneto J. C.; Queirroz A.; Santos A. C.; Salgado K.; Reis M. G.; Ko A. I. (2008): Leptospirosis-associated Severe Pulmonary Hemorrhagic Syndrome, Salvador, Brazil. Emerg Infect Dis. 14(3) p. 505–508

Haake D. A.; Levett P. N. (2015): Leptospirosis in Humans. Curr Top Microbiol Immunol. 387 p. 65–97

Hartskeerl R. A.; Collares-Perieira M.; Ellis W. A. (2011): Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clinical Microbiology and Infection 17 (4) p. 494-501

Herenda D. C.; Chambers P. G.; Ettriqui A.; Seneviratna P.; da Silva T. J. P. (1994): Manual on meat inspection for developing countries. Rome, Italy. Food and Agriculture Organization of the United Nations. p. 124-125

Jensen W. K.; Devine C. Dikeman M. (2004): Encyclopedia of Meat Sciences Vol 1-4. Academic Press p. 660; 1263

Leonard, N.; Fee J. F.; Snijders S.; Mackie D. (2004): Prevalence of antibodies to Leptospira interrogans serovar hardjo in bulk tank milk from unvaccinated Irish dairy herds. Irish Veterinary Journal 57 (4) p. 226-231

Lilenbaum W.; Santos M.R.C. (1996): Effect of management systems on the prevalence of bovine leptospirosis. Veterinary Record 138 p. 570-571

Lourdault K.; Aviat F.; Picardeau M (2009): Use of quantitative real-time PCR for studying the dissemination of Leptospira interrogans in the guinea pig infection model of leptospirosis. J Med Microbiol 58 (5) p. 648-655

Lunn K. F. (2015): Overview of Leptospirosis. The Merck Veterinary Manual. http://www.merckvetmanual.com/mvm/generalized_conditions/leptospirosis/overview_of_leptospirosis.html, visited on 03/11/15

Maxie M. G.; Jubb K. V. F.; Kennedy P. C.; Palmer N. (2007): Judd, Kennedy, and Palmer's pathology of domestic animals. Edinburgh, New York. Elsevier Saunders. p. 215;481-489

Nally J. E.; Monahan A. M.; Callanan J. J. (2008): Proteomic analysis of Leptospira interrogans shed in urine of chronically infected hosts. Infect. Immun 76 p. 4952-4958

Owen G. (2003): Bovine leptospirosis. Edinburgh, UK. The Moredun foundation newssheet 3 (20)

Pate G. E.; Hogan M. C.; FitzSimon N.; Mellotte G. J. (2000): A review of the epidemiology of leptospirosis in the Republic of Ireland. Irish Medical Journal 93 (4) p. 117-7

Paz-Soldán S. V.; Dianderas M.T.; Windsor R. S. (1991): Leptospira interrogans serovar canicola: a causal agent of sow abortions in Arequipa, Peru. Trop Anim Health Prod. 23(4) p. 233-40

Pearson J. K. L.; Mackie D. P.; Ellis W. A. (1980): Milk drop syndrome resulting from Leptospira hardjo. Veterinary Record 106 p. 135-136

Pritchard D. G.; Allsup T.N.; Pennycott T.W.; Palmer, N.M.A.; Woolley J.C.; Richards M.S. (1989): Analysis of Risk Factors for Infection of Cattle Herds with Leptospira interrogans serovar Hardjo. Proceedings of the Society for Epidemiology and Preventive Medicine 1989. Exeter University, UK. p. 130–138.

Pritchard D.G.; Allsup N.J.; Penycott T.W.; Stebbings D. (1987): A survey of Leptospiral agglutinating antibody in beef and dairy cattle in Herefordshire, England. Isr J Vet Med 43 p.342–343

Quinn P. J.; Markey B. K.; Leonard F. C.; Hartigan P.; Fanning S. (2012): Veterinary Microbiology and Microbial Disease, Second Edition. Oxford, UK. Wiley-Blackwell. p. 233-235

Radostits O. M.; Gay C. C.; Hinchcliff K. W.; Constable P. D. (2006): Veterinary Medicine, Tenth Edition. Edinburgh, UK. Elsevier Publishing, Ltd.

Rinehart C. L.; Zimmerman A. D.; Buterbaugh R. E.; Jolie R. A.; Chase C. C. (2012): Efficacy of vaccination of cattle with the Leptospira interrogans serovar hardjo type hardjoprajitno component of a pentavalent Leptospira bacterin against experimental challenge with Leptospira borgpetersenii serovar hardjo type hardjo-bovis. Am. J. Vet. Res 73 (5) p. 735-740

Ryan E. G.; Leonard N.; O'Grady L.; Doherty M. L.; More S. J. (2012): Herd-level risk factors associated with Leptospira Hardjo seroprevalence in Beef/Suckler herds in the Republic of Ireland. Irish Veterinary Journal 65 (6)

Ryan E. G.; Leonard N.; O'Grady L.; More S. J.; Doherty M. L. (2012): Seroprevalence of Leptospira Hardjo in the Irish suckler cattle population. Irish Veterinary Journal 65 (8)

Scott P. R.; Penny C. D.; Macrae A. I. (2011): Cattle Medicine. Edinburgh, UK. Manson Publishing Ltd p. 31-32

Stanford C.F.; Connolly J.H.; Ellis W.A.; Smyth E.T.M.; Coyle P.V.; Montgomery W.I.; Simpson D.I.H. (1990): Zoonotic infections in Northern Ireland farmers. Epidemiol Infect 105 p. 566-570

Strutzberg-Minder K.; Kreienbrock L. (2011): [Leptospire infections in pigs: epidemiology, diagnostics and worldwide occurrence]. Berl Munch Tierarztl Wochenschr 124 (9-10) p. 345-59

Terpstra W. J. (2003): Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control. Geneva, Switzerland: WHO

Thornley C. N.; Baker M. G.; Weinstein P.; Maas E. W. (2002): Changing epidemiology of human leptospirosis in New Zealand. Epidemiol. Infect. 128 p. 29–36

Toldra F. (2010): Handbook of Meat Processing. Iowa, USA : Wiley-Blackwell p. 498

Vijayachari P.; Sugunan A. P.; Shriram A. N. (2008): Leptospirosis: an emerging global public health problem. Journal of Biosciences 33 p. 557–569

Ward A. I.; Tolhurst B. A.; Delahay R. J. (2006): Farm husbandry and the risks of disease transmission between wild and domestic mammals: a brief review focusing on bovine tuberculosis in badgers and cattle. Animal Science 82. p.767-773

Wikse S. E.; Rodgers G.M.; Ramachandran S.; Engelken T. J.; Epperson W. B.; Larson R. L.; Mass J.; Richey E; Bolin C. (2007): Herd Prevalence and Risk Factors of Leptospira Infection in Beef Cow/calf Operations in the United States: Leptospira borgpetersenii Serovar Hardjo. Bovine Practitioner 41 p. 15-23.

Williams D.; Winden S. V. (2014): Risk factors associated with high bulk milk antibody levels to common pathogens in UK dairies. Veterinary Record 174 (23) p.23

Williams D. (2015): Leptospirosis in Cattle. National Animal Disease Information Service bulletin. http://www.nadis.org.uk/bulletins/leptospirosis-in-cattle.aspx, visited on 02/11/15

Williams D. (2015): Leptospirosis in Pigs. National Animal Disease Information Service bulletin. http://www.nadis.org.uk/bulletins/leptospirosis-in-pigs.aspx, visited on 02/11/15

Xue F.; Yan J.; Picardeau M. (2009): Evolution and pathogenesis of Leptospira spp.: lessons learned from the genomes. Microbes Infect. 11 (3) p. 328-33

Zhang Y.; Lou X. L.; Yang H. L.; Guo X. K.; Zhang X. Y.; He P.; Jiang X. C. (2012): Establishment of a leptospirosis model in guinea pigs using an epicutaneous inoculation route. BMC Infect. Dis. 12 p. 20

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