BOVINE LEPTOSPIROSIS: METHODS AIDING TREATMENT AND DIAGNOSIS

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Adam Martin discusses the various types of leptospirosis, and argues that more research is needed to achieve an effective national control strategy.

LEPTOSPIROSIS is an economically important and zoonotic disease, which is caused by spiral-shaped bacteria of the genus *Leptospira*.

In cattle, leptospirosis has been associated with reproductive losses (infertility, abortions and stillbirths – Figure 1), as well as non-reproductive losses (milk drop syndrome, nephritis and septicaemia). Leptospirosis is believed to be the most widespread zoonotic disease in the world, and considerable effort and money has been spent in an attempt to reduce the number of occupationally acquired infections worldwide.

Historically, pathogenic leptospires were named *Leptospira interrogans*, while the saprophytic strains isolated from the environment were classified as *L biflexa*. Individual serotypes were then determined according to the bacteria's reaction to various laboratory tests. The phenotypic classification system has now been replaced by a genotypic one, and at least 16 different genomospecies of *Leptospira* exist.

Both pathogenic and nonpathogenic serovars can be found in the same species. Many of the different strains of *Leptospira* species have evolved and are adapted to individual maintenance host species (Table 1). The patterns and consequences of infection vary depending on whether the serovar has infected a maintenance host or an accidental host.
Hosts

Maintenance hosts are readily susceptible to infection, which is often endemic in the host species. Transmission between maintenance hosts occurs readily, and any disease caused by infection is generally chronic – losses are often insidious in nature. Maintenance hosts are persistently infected frequently, with the pathogen often colonising the urino-genital tract.

Generally, immune responses to the infection (be it naturally occurring, or as the result of vaccination) are mild. In contrast, accidental hosts are relatively insusceptible to infection by a particular serovar.

However, when infection does occur, the resulting disease is often acute and severe. It elicits a considerable immune response from the infected animal. Infection is generally shortlived, and onward transmission of the infection to other individuals occurs only sporadically.

Types

In UK cattle practice, the most significant Leptospira serotypes are L borgpetersenii serovar hardjo and L interrogans serovars hardjo-prajitno, icterohaemorrhagiae and canicola. Of these, the two most important serovars are L borgpetersenii serovar hardjo and L interrogans serovars hardjo-prajitno, which are now referred to as L hardjo-bovis and L hardjo-prajitno, respectively, or L hardjo collectively.

Cattle are regarded as the maintenance hosts for both serovars of L hardjo. However, the type of disease seen with each serovar is generally distinct. L hardjo-prajitno is a highly virulent serovar that colonises reproductive tracts in cattle. In comparison, L hardjo-bovis colonises the urinary tract of cattle and is typically of lower virulence. The two serovars are indistinguishable from each other serologically.

Interestingly, L hardjo-bovis has a worldwide distribution, while L hardjo-prajitno has primarily been isolated from the UK.

In 1999, a study of bulk milk samples reported that 75 per cent of herds in the UK had antibodies to Leptospira species. However, there are no current, reliable studies on the prevalence of the different serovars in the UK cattle population. This makes an evidence-based assessment of the disease situation in the UK impossible, as it is not known whether the highly virulent (reproductive) or lowly virulent (urinary) form of the disease predominates. Establishing the prevalence of the differing serovars is important for making an accurate risk assessment of the probability of zoonotic disease transmission from cattle. Humans are more susceptible to L hardjo-bovis infection than L hardjo-prajitno.

Infection most commonly results after exposure to urine containing leptospires. However,
contaminated milk, foetal membranes and aborted foetuses also represent potential sources of infection. Vertical and venereal transmission of the disease can also occur. Leptospires have the ability to cross intact mucous membranes. They then cause a bacteraemia, during which acute signs of infection may be seen. The immune response to the *Leptospira* species is predominantly humoral in nature, and the development of a significant circulating antibody response takes approximately 10 days.

During the bacteraemia, leptospires localise in the renal tubules and reproductive tract. Leptospires can be found in the milk and urine of the infected animal after approximately two to three weeks.

Infection often persists for a considerable period in individual animals. Persistent infection of animals is common. The reported persistence of leptospires in the mammary gland is three months. However, excretion has been reported to occur in urine for more than 500 days in individual animals, although the mean time is just more than seven months. Excretion can be intermittent, which makes testing for infected animals difficult. Urinary excretion of *L. hardjo-bovis* (urinary form) is of considerably higher levels than *L. hardjo-prajitno* (reproductive form) excretion. *L. hardjo* has been detected in utero for up to five months following infection. After calving, the vaginal discharges of infected animals have been shown to contain leptospires for up to eight days postpartum.

The existence of intermittently excreting, clinically normal and persistently infected animals poses considerable problems for disease control. It is believed that leptospirosis is introduced to herds via the purchase of persistently infected animals.

Definitive leptospirosis diagnosis involves the bacteriological culture of infected urine or tissues. However, this approach is problematic, as the bacteria are only shed via being excreted intermittently in urine. Furthermore, the culture of leptospires is difficult and time consuming. Serological testing of an individual animal prior to its introduction into the herd is of limited diagnostic value, and in fact may often mislead. The standard serological test for leptospirosis is the microscopic agglutination test (MAT), which primarily detects IgM antibodies. These are produced in the initial response to infection and tend to decrease rapidly. Consequently, if an animal was infected several months before testing, it could produce a negative test result, despite it excreting bacteria in its urine.

An enzyme-linked immunsorbant assay (ELISA) technique is available for leptospirosis. This test detects the presence of IgG antibodies, which are detectable three to four weeks after infection and remain high for a considerable period of time. Interpretation of serological results is further complicated by crossreactivity between antibodies, widespread vaccination and a lack of consensus on what titres are diagnostic of active infection.

If serology is to be applied to determine if it is likely that an animal is infected with *L. hardjo*, it would be best directed at the herd the animal has been purchased from, although this approach still has
problems. Stock can be purchased from accredited free herds with minimal risk. However, the best avoidance policy is to maintain a strictly closed-herd policy.

Epidemiology

An often-quoted piece of research concerning the epidemiology of leptospirosis is Pritchard et al (1989), which is summarised, slightly inaccurately, to state that the chance of a herd being leptospirosis positive is:

- doubled if the herd is open;
- four times greater if the herd uses natural service (opposed to artificial insemination);
- six times greater if sheep are present on the farm (for all or part of the year); and
- eight times greater if cattle have access to a watercourse.

However, this research should be treated with a degree of caution, because it has never undergone peer review. Furthermore, while the first two management factors mentioned previously (open versus closed herd, natural service versus artificial insemination) did increase the risk of leptospirosis being present in a herd, the finding was not statistically significant (Table 2). Intuitively, it makes good biological sense that these factors are genuine risk factors for higher herd disease prevalence, and they may well be. However, in the study, not enough evidence was found to support the statement that they are risk factors.

The role of sheep in the epidemiology of the disease is interesting. Approximately a quarter of flocks in the UK are thought to be sero-positive for leptospirosis. *L. hardjo-bovis* has been cultured from the urine and kidneys of seropositive sheep at slaughter. Sheep-to-sheep and sheep-to-cattle transmission has been demonstrated. However, the primary cause of reproductive disease in the cattle population is *L. hardjo-prajitno*, so the role of sheep in the transmission of a reproductive disorder is not clear.

Abortion and fertility

Abortion is, perhaps, the sign of disease we most commonly think of with respect to bovine leptospirosis. Abortion, or the birth of weak live calves, typically occurs four to 12 weeks after infection; foetuses in the final four months of gestation appear to be most susceptible. For the reasons mentioned previously, serology of the dam is not useful in confirming a diagnosis of leptospiral abortion. Leptospires or leptospiral DNA can be identified in tissues or certain body fluids using immunofluorescence, PCR assays, culture and histopathology. The collection of appropriate samples is best discussed with a pathologist at the laboratory you will be sending the material to.
The role of leptospirosis in reducing the fertility of a herd has been well documented in the UK. Studies performed in other parts of the world often do not find the effects described here – reduced pregnancy rates (with regular returns to service – Figure 2) and silent heats. However, this is perhaps understandable, as the serovar causing the reproductive form of the disease – \textit{L hardjo-prajitno} – has not been found worldwide. The pathogenic mechanism by which infection reduces fertility has yet to be proven.

**Vaccination programme**

In the UK, control of the reproductive disease is usually achieved by instigating a suitable vaccination programme. However, it has been reported that the primary reason that most farmers opt to vaccinate in the UK is to reduce the risk of zoonotic transmission. Data from DEFRA indicates that between 2003 and 2007 (inclusive), there were 181 reported cases of indigenous leptospirosis in the human population of England and Wales (out of approximately 52 million individuals). Of these, 32 were found in farmers and farm workers (including fish farmers), and only 11 cases were caused by \textit{L hardjo}. It is believed that most cases of leptospirosis are contracted through recreational activities.

The Netherlands has converted from a vaccination-based control policy to a test and cull strategy with great success.

**Looking forwards**

Further research into the genuine prevalence of cattle leptospirosis, and a knowledge of the serovars involved, is necessary to allow for the development of an evidence-based national strategy for disease control. In the longer term, an evidence-based strategy must be better than the application of an annual multi-million pound sticky plaster to prevent a human disease that is rarely seen. Of course, it may be that the zoonosis is rarely seen because of vaccination, but without further research we'll never know.

- References are available on request to the editor.
- Visit [www.vetonline.com](http://www.vetonline.com) to download \textit{VT} articles..
Figure 1. Late-term aborted foetuses can characterise leptospirosis in dairy cattle.
Figure 2. Regular returns to serve are a feature of leptospirosis in the UK.
Table 1. A selection of commonly found *Leptospira* serovars and their maintenance hosts.

<table>
<thead>
<tr>
<th><em>Leptospira</em> serovar</th>
<th>Maintenance host</th>
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<tbody>
<tr>
<td><em>L. hardjo-bovis</em></td>
<td>Cattle</td>
</tr>
<tr>
<td><em>L. hardjo-prajitno</em></td>
<td>Cattle and pig</td>
</tr>
<tr>
<td><em>L. bratislava</em></td>
<td>Horse</td>
</tr>
<tr>
<td><em>L. icterohaemorrhagiae</em></td>
<td>Brown rat</td>
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</tbody>
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Adapted from Radostits et al (2007).

Table 2. Factors influencing chance that herd is infected with leptospirosis

<table>
<thead>
<tr>
<th>Risk factor for leptospirosis infection</th>
<th>Odds ratio</th>
<th>95 per cent confidence interval</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>Purchase of stock</td>
<td>1.97</td>
<td>0.19</td>
</tr>
<tr>
<td>Natural service</td>
<td>3.90</td>
<td>0.75</td>
</tr>
<tr>
<td>Presence of sheep</td>
<td>6.65</td>
<td>1.09</td>
</tr>
<tr>
<td>Presence of water course</td>
<td>7.94</td>
<td>1.28</td>
</tr>
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Factors where a 95 per cent confidence interval excludes the value one are significant. Adapted from Pritchard et al (1989).