Leptospirosis Diagnosis - Part 1: The Individual Animal

Of the 6 endemic serovars in NZ*, only Pomona, Hardjo, Ballum and Copenhageni are associated with disease in cattle, sheep and deer.

- **Pomona** causes abortion and milk drop in cows, and outbreaks of haemoglobinuria and death in young calves\(^1\) and lambs\(^4\).
- **Copenhageni** and **Ballum** causes acute nephritis and death in calves\(^5\).
- **Hardjo** while a common cause of abortion, milk drop, still or weak births, and recently, infertility and early embryonic death globally\(^6\)\(^-\)\(^7\) has not been shown to be a pathogen in sheep or cattle in NZ. In deer it is associated with reduced reproduction and weight gain in deer\(^8\).

Part 1 of this Hardfacts discusses individual animal diagnosis due to these serovars. Part 2 discusses herd level diagnosis.

A diagnosis of Leptospirosis depends on good clinical and vaccination history and the thoughtful use of diagnostic tests. The ease of diagnosis often depends on the serovar involved.

All current diagnostic tests have their strengths and weaknesses (See Table 1) and generally no single test can lead to a definitive serovar specific diagnosis. To maximize the likelihood of reaching a diagnosis contact the laboratory prior to submission to ensure suitable samples are appropriately collected.

### Haemoglobinuria, Nephritis or death in young animals due to Pomona or Copenhageni.

History, presentation, gross post-mortem and histological findings are often strongly suggestive of Leptospirosis. The history usually includes a lack of vaccination, recent introduction of stock and exposure to surface water. Post-mortem may reveal jaundice, dark urine (NB can confirm as haemoglobinuria by dipstick), swollen dark kidneys with diffuse haemorrhagic and pale areas. The liver is often swollen and yellowish.

High antibody titers (≥ 1:1600) in serum or fluids can be considered confirmatory. Lower titres are suggestive; PCR of urine, kidney or pleural fluid can be used for confirmation. Note asymptomatic infection with either Pomona or Copenhageni is reported\(^9\). Antibody determination of dams, recent arrivals and other in-contact animals may be used to shed some light on the source of the infection.

Note if MAT titres are determined early in the serological response, cross reaction between serovars is common, and the highest titer may not be the infecting serovar. It is best to repeat testing in samples taken 2-3 weeks later and sample in contact animals to confirm the serovar involved.

*See Leptospirosis in NZ Dairy Herds Technical Bulletin for details.

### Table 1: Summary of leptospiral diagnostic methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Samples</th>
<th>Material detected</th>
<th>ID serovar</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark-field microscopic</td>
<td>Blood and urine</td>
<td>Live leptospires</td>
<td>no</td>
<td>Rapid, but low sensitivity, and requires a high degree of skill.</td>
</tr>
<tr>
<td>Culture</td>
<td>Blood, urine, csf, kidney or other tissues</td>
<td>Live leptospires</td>
<td>yes</td>
<td>Slow (weeks to months).</td>
</tr>
<tr>
<td>Polymerase chain reaction (PCR)</td>
<td>Urine, tissues e.g. kidney placenta, foetal fluids</td>
<td>Leptospiral DNA</td>
<td>no</td>
<td>Very specific and sensitive. False-negatives due to inhibitors in tissues preventing DNA amplification. False-positives as exquisitely sensitive to contamination with leptospiral DNA.</td>
</tr>
<tr>
<td>Microscopic agglutination test (MAT)</td>
<td>Serum, fluid from tissues or body cavities</td>
<td>Agglutinated antibodies</td>
<td>yes</td>
<td>Highly specific. In acute cases animals may die before they seroconvert.</td>
</tr>
<tr>
<td>Histology +/- Silver staining</td>
<td>Any body organ e.g. kidney, placenta.</td>
<td>Intact leptospires</td>
<td>no</td>
<td>Non-specific and silver staining will identify intact organisms only. Poor sensitivity.</td>
</tr>
</tbody>
</table>

\(^{1}\)–\(^{7}\) See Leptospirosis in NZ Dairy Herds Technical Bulletin for details.
Stillbirth or Abortion

Pomona and Hardjo (although not confirmed in NZ) can cause abortion at any stage of pregnancy, however usually they induce abortion in the last ½ of gestation. Aborting dams are often asymptomatic, however fever, anorexia, agalactia, malaise, dark urine, jaundice and anaemia are reported\(^\text{10}\). The interval from infection to abortion varies with serovar, with Pomona induced abortion occurring within 1-2 weeks; whereas Hardjo infected animals may abort several months after infection. MAT titres are very variable. A Pomona titre ≥1:1600 or a Hardjo titre ≥1:200, along with typical signs and history are considered strongly suggestive; however, Hardjo titres are often lower or negative (i.e. <1:50) as infection can occur months before abortion. A NZ researcher states the arbitrary cutoff of 1:50 used by labs is too high for Hardjo and suggested a dilution of 1:25 should be used\(^\text{11}\). Paired sera are also often not diagnostic as the serological peak has occurred before the animal aborts. Furthermore, as many animals in the same mob may have similar titres without evidence of abortion and titres due to natural infection can last for up to two years, confirmation of diagnosis should be made by PCR or culture of urine, or if available renal tissue collected at slaughter. Ideally, urine should be collected after the injection of furosemide, as the increased glomerular filtration flushes more leptospires from the kidneys and the dilute urine enhances their survival\(^\text{12}\). In interpreting a positive urine sample, note that some cattle have been shown to excrete Hardjo for 542 days and it has been speculated for life\(^\text{13}\).

Postmortem examination of foetuses usually reveals only non-specific autolytic findings, although subcutaneous jaundice, foci of renal tubular necrosis and vascular lesions in many organs are observed occasionally\(^\text{14}\). Placenta, if available, is histologically non-diagnostic, however some researchers have found the placenta the most likely organ to be MAT or PCR positive\(^\text{13}\). Identification of leptospires by silver staining in aborted tissues is diagnostic, but not serovar specific. Pooled foetal fluid from the pericardial, thoracic and abdominal cavities, or pooled foetal kidney, adrenal gland, lung and placenta are useful tissues to examine for leptosporal antigen by culture or PCR. For Hardjo, a prominent researcher states for MAT on foetal fluids dilutions should be started a 1:10, in contrast to the usual starting dilution of 1:50\(^\text{12}\).

Reproductive failure and other production related diseases associated with Hardjo

As reproductive or production loss associated with Hardjo generally manifests subclinically, and in most unvaccinated herds the within herd seroprevalence is high, it is difficult to diagnose Leptospirosis as the cause. Conformation of endemic Hardjo within a herd is the first step (See Part 2). Secondly, showing evidence of seroconversion during mating or lactation is useful. Lastly, response to Leptavoid\textsuperscript{®} vaccination has been used in NZ deer\(^\text{8}\) and lactating cattle in the UK\(^\text{15}\) as a means of gauging the impact of infection.

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References