New Zealand is free from the major causes of abortion in sheep, such as *Chlamydia abortus* and *Salmonella abortus ovis* that cause considerable losses overseas (West 2002). In New Zealand the agents most commonly diagnosed in sheep abortion are campylobacters, toxoplasmas and *Salmonella* Brandenburg. West (2002) reported that these three agents have accounted for approximately 80% of sheep abortions where a causal agent is identified. However the agents causing abortion are often not determined.

Several, often large, abortion outbreaks with similar liver lesions, and with no evidence for the involvement of common agents, have been seen in sheep flocks in Canterbury, Otago and Southland since the early 1990s. Investigations into the potential aetiological agent were largely unrewarding (Hutton 1992, Smart and Gill 1999). In 2009, a Southland veterinarian informed MPI via the 0800 exotic disease and pest hotline of an abortion storm affecting a large commercial flock of approximately 4,000 ewes. Aborted lambs had enlarged livers, some with pale foci resembling those seen in Campylobacter abortions however, no Campylobacter were isolated. Histopathology and serology excluded toxoplasma infection. As part on an MPI-led abortion outbreak investigation, ELISA testing of acute and convalescent sera excluded exotic agents Q-fever and *Chlamydia abortus*. Bacterial culture and molecular testing performed on fresh abortion submissions and ewe vaginal swabs found no evidence of Q-fever, *Chlamydia abortus*, *Salmonella* or Brucella species.

An aetiological agent was pursued. Histopathology identified large areas of coagulative necrosis. Silver staining showed up variable numbers of curved rods along the sinusoidal margins of intact hepatocytes. Electron microscopy was undertaken and within the biliary canaliculi, moderate numbers of a slightly curved rod with a spiral periplasmic membrane were visible (Gill 2010, Figure 1). A breakthrough came with the link that this ultrastructure was consistent with that of the bacterium *Flexispira rappini* (subsequently Helicobacter spp. with flexispira morphology). This organism had been reported as a cause of sporadic ovine abortions in the US and UK (Kirkbride *et al*. 1985, Kirkbride 1993, Crawshaw and Fuller 1994). A provisional diagnosis of abortion associated with Helicobacter spp. was made (Bingham 2010).

In an attempt to definitely identify and clarify the causative agent, communication took place with Massachusetts Institute of Technology (MIT, USA) a laboratory with extensive experience in the culture and characterisation of Helicobacter species. Follow up liver samples from ten aborted foetuses (from six Southland farms) were collected in Spring 2011. Samples were from farms on which the known common causes of abortion (toxoplasma, campylobacter, salmonella) had been excluded. These were collected into brain-heart infusion (BHI) broth with 20% glycerol to ensure travel did not compromise laboratory work in the USA. Of the liver samples sent to the collaborating laboratory for culture and molecular testing, Helicobacter spp. bacteria were detected using molecular techniques in two samples. Helicobacter spp. could however only be cultured from a single sample.

This assessment confirmed the presence of Helicobacter spp. with flexispira morphology in aborted lambs from New Zealand sheep flocks. The finding of Helicobacter bacteria in flocks experiencing...
abortion, and in which common endemic agents of abortion had been excluded, was an important milestone. Further research was required and in partnership with Gribbles Veterinary Pathology, MIT and The Forsythe Institute (TFI, USA) a survey was carried out to assess the association of Helicobacter with abortion outbreaks. The study also sought to determine the Helicobacter strain types present, and attempted to assess the importance of Helicobacter spp. as a potential cause of sheep abortion in New Zealand.

The collaboration has solved some of the mystery around unexplained sheep abortion in New Zealand – findings which may be applicable to other countries. Development of a diagnostic assay is underway to be implemented at Gribbles Veterinary Pathology, Invermay, meaning soon an assay will be available at a regional veterinary laboratory for workup of routine abortion outbreaks. Further abortion investigation work and surveys across different sheep rearing regions of New Zealand are required to help us understand the epidemiology and true impact of this organism, whether it presents an emerging condition, and whether research into prophylaxis or treatment is warranted.

Figure 1. Bacteria consistent with Helicobacter spp. identified in biliary caniculi of the liver of an aborted lamb (2009), Bar=500nm
<table>
<thead>
<tr>
<th>Year</th>
<th>Study/Project</th>
<th>Region</th>
<th>Outcome</th>
<th>Collaborators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring 2009</td>
<td>Abortion investigation on single farm</td>
<td>Southland</td>
<td>Investigation made a provisional diagnosis of Helicobacter associated abortion</td>
<td>Gribbles and MPI</td>
</tr>
<tr>
<td>Spring 2011</td>
<td>Small number of samples from farms experiencing abortion and in which common endemic agents had been excluded</td>
<td>Southland</td>
<td>Study confirmed presence of Helicobacter species with flexispira morphology on multiple farms experiencing abortions in southern NZ</td>
<td>Gribbles and MPI and MIT</td>
</tr>
<tr>
<td>Spring 2012</td>
<td>Large number of samples from farms experiencing abortion – Two groups: a selection where endemic agents had been excluded, the other where an endemic diagnosis had been reached</td>
<td>Southland, Otago</td>
<td>Study added to the 2011 findings and suggest Helicobacter species with flexispira morphology as a likely causative agent of abortion outbreaks in southern NZ</td>
<td>Gribbles and MPI and MIT and TFI</td>
</tr>
<tr>
<td>Spring 2013</td>
<td>As for 2012</td>
<td>Southland, Otago</td>
<td>Study added further confidence to findings of 2012 study</td>
<td>Gribbles and MPI</td>
</tr>
<tr>
<td>Spring 2014</td>
<td>Various samples (heart blood, stomach content, liver) from farms experiencing abortion – all where endemic agents had been excluded</td>
<td>Southland, Otago</td>
<td>Stomach content appears to be a more sensitive sample type for detection of Helicobacter spp. using PCR</td>
<td>Gribbles and MPI</td>
</tr>
<tr>
<td>2015</td>
<td>Regional veterinary laboratory setup and validation of molecular assay for Helicobacter spp.</td>
<td>Otago</td>
<td>Diagnostic assay available at the regional veterinary laboratory for routine abortion outbreak investigations</td>
<td>Gribbles and MPI</td>
</tr>
</tbody>
</table>

Gribbles Veterinary Pathology, Invermay, Dunedin, New Zealand; Ministry for Primary Industries, PO Box 40742, Wallaceville, Upper Hutt 5018, New Zealand; Massachusetts Institute of Technology, Cambridge, MA, USA; The Forsyth Institute, Cambridge, MA, USA

Table 1. Studies carried out to assess the role of Helicobacter as a potential agent of sheep abortion in New Zealand

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Proceedings of the Society of Sheep and Beef Veterinarians of the NZVA and Cervetec
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