Bovine paratuberculosis is an enteritis characterized by a chronic and persistent diarrhea, a decrease of the production level (milk, reproduction) and a loss of weight leading to death. These economic and health consequences lead bovine paratuberculosis as a major disease in animal health, thereby justifying the establishment of surveillance and control programs. To do this, different players of veterinary world need several detection tools for:

- Better understanding of the current level of contamination of herds.
- Better control the spread of the disease and secure trades.
- Prioritizing reforms and control introductions.

The diagnosis of paratuberculosis should not be limited to a too late clinical diagnosis of shedders. Whitlock et al. defined classes of animals according to their level of excretion and they highlighted the importance of low and passive shedders in the evolution of the health status of a herd.

**EXISTING PCR PROTOCOLS**

**PCR without concentration**
- 1 to 10g of faeces dilution
- 1/7 in water

**PCR UP with concentration**
- 5µL of DNA
- Grinding and DNA purification
- Centrifiltration of 10ml on Adiafilter
- PCR Amplification on 5µL of DNA

**Level of contamination inside herds according to Whitlock et al.*

- High shedders, Positive +++*, > 8,000 bacteria/gram of faeces
- Intermediate shedders, Positive ++*, 100 to 550 bacteria/gram of faeces
- Low shedders, Positive +*, 5 to 95 bacteria/gram of faeces
- Negative animal, non shedder

**USE OF PCR**

Different tests and different levels of sensitivity are useful to:

- Perform analysis on an isolated animal or at herd level
- Define herd status (infected or not)
- Estimate the prevalence

<table>
<thead>
<tr>
<th>Aim of test</th>
<th>Which test(s)?</th>
</tr>
</thead>
</table>
| 1 Define herd status | - PCR UP on environmental samples (dairy farms)  
- PCR UP on pool of faeces (dairy and meat farms)  
- ELISA |
| 2 To follow a negative or low infected herd managing well paratuberculosis and new cattle introductions | - PCR UP on individual faeces for new animals (trade)  
- PCR UP on bovines <24 months  
- ELISA on bovines >24 months |
| 3 To manage spread of the disease and elimination of shedders.  
- Prioritize reforms | - PCR UP on pool of faeces  
Or PCR on individual faeces  
Or ELISA  
- PCR UP on individual faeces (and young cattle) |
**SPECIFICITY OF PCR**

**Inclusivity**
Inclusivity has been determined against 140 strains of MAP.

<table>
<thead>
<tr>
<th>Number of MAP tested</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>+</td>
</tr>
</tbody>
</table>

All strains of *Mycobacterium avium* subsp. *paratuberculosis* have been detected with ADIAVET™ PARATB REAL TIME.

**Exclusivity**

<table>
<thead>
<tr>
<th>Number of strains tested</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 other micro-organisms</td>
<td>-</td>
</tr>
<tr>
<td>59 mycobacteria + 2 close IS</td>
<td>-</td>
</tr>
</tbody>
</table>

No cross reaction has been observed with 59 other mycobacteria strains different from *Mycobacterium avium* subsp. *paratuberculosis*, 2 other close IS and 58 strains from other micro-organisms.

**SENSITIVITY OF PCR**

**LONGITUDINAL STUDY**

During a longitudinal study, PCR UP allows to check evolution of herds level of infection.

<table>
<thead>
<tr>
<th>Number of cattles</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+4 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>556</td>
<td>399</td>
<td></td>
</tr>
<tr>
<td>1 + 4 months</td>
<td>41</td>
<td>57</td>
<td>525</td>
<td>385</td>
</tr>
</tbody>
</table>

98% of bovines high or intermediate shedders remain high positive after 4 months.

In a contaminated farm, 60% of negative or low shedders bovines remain or become positive.

**PREVALENCE OF POSITIVE PCR RESULTS IN HERDS at different stages of PARATUBERCULOSIS control**

More Paratuberculosis control program moves forward and lower is the prevalence of high and intermediates shedders. A very sensitive diagnostic tool used at the individual animal level makes a lot of sense at the end of a control program to detect low shedders and to check animals before trades and introductions when prevalence is low.