THE AIM OF THIS LEISHVET PRESENTATION is to update and discuss the current knowledge on the diagnosis of CanL due to *Leishmania infantum*, focusing on the new challenges raised by the use of vaccines against CanL (Solano-Gallego et al., 2017).

- **WHAT ARE THE VARIOUS PURPOSES FOR WHICH *L. INFANTUM* INFECTION DIAGNOSIS IS PERFORMED?**

The main purpose is to confirm disease in canine patients. However, other purposes are to investigate the presence of infection for screening clinically healthy dogs in endemic areas, including blood donors, breeding dogs, dogs prior to vaccination, dogs heading towards disease progression; or for screening clinically healthy dogs in non-endemic areas (travelling dogs), to avoid importation of infected dogs to non-endemic regions and to monitor response to treatment (Solano-Gallego et al., 2009; Solano-Gallego et al., 2011). Therefore, it is important to distinguish between disease, infection and immune responses induced by vaccination (immunogenicity), and to apply different diagnostic techniques accordingly.

- **HOW CLINICAL CANL IS DIAGNOSED?**

Accurate diagnosis of CanL often requires an integrated approach consisting of a clinicopathological assessment and specific laboratory tests. Pertinent clinical history, a thorough physical examination and several routine diagnostic tests such as CBC, biochemical profile, urinalysis, serum electrophoresis and occasionally other diagnostic techniques like abdominal ultrasound can assist in raising the suspicion index for this disease (Solano-Gallego, 2008).

Numerous diagnostic techniques have been developed to help in the diagnosis of CanL. The detection of *L. infantum* infection in dogs includes parasitological (cytology, histology, immunochemistry and culture of the organism in appropriate medium), molecular (conventional, nested and real-time PCR) and serological methods (qualitative and quantitative antibody tests). In addition, *L. infantum* specific cellular immunity tests have also been developed but they are mainly used only in research settings. Different diagnostic procedures and interpretations of test results might be used accordingly, depending on the purpose of the diagnostic investigation. It is important to understand the basis of each diagnostic test, the limitations and the appropriate clinical interpretation (Solano-Gallego et al., 2009).

Cutaneous lesions, bone marrow, lymph nodes and spleen and less commonly other tissues or body fluids such as joint, cerebrospinal and abdominal fluids are good choice samples to observe *Leishmania* amastigotes in both cytological and histological specimens (Solano-Gallego et al., 2009). Parasites should be suspected in macrophagic, neutrophilic, neutrophilic-macrophagic, or lymphoplasmacytic inflammations in different tissues or in reactive hyperplasia in lymphoid organs on cytological or histological preparations (Solano-Gallego et al., 2009; Solano-Gallego et al., 2011). Definite histopathological identification of parasites within tissue macrophages may be difficult and an immunohistochemical staining method can be employed to detect or verify the presence of *Leishmania* in the tissue (Ferrer et al., 1988). The isolation in culture of parasites from infected tissues is not suitable for rapid diagnosis. Parasite culture is used more often for research purposes (Solano-Gallego et al., 2009).
Detection of parasite-specific serum antibodies should preferably be based on quantitative serological techniques, such as IFAT and ELISA due to the variable diagnostic performance of rapid serological tests (Miro et al., 2008; Solano-Gallego et al., 2009; Solano-Gallego et al., 2011). In non-vaccinated dogs, high antibody levels are usually associated with disease and a high parasite density (Dos-Santos et al., 2008; Reis et al., 2006; Solano-Gallego et al., 2016) and, for this reason, they are conclusive of a diagnosis of clinical leishmaniosis. However, the presence of lower antibody levels is not necessarily indicative of patent disease and needs to be confirmed by other diagnostic method such as PCR, cytology or histology (Solano-Gallego et al., 2009). The challenges of serology include cross-reactivity with other related pathogens and antibodies elicited by vaccination (Moreno et al., 2012; Moreno et al., 2014).

WHAT ABOUT SEROLOGICAL TESTING WHEN LEISHMANIA VACCINES ARE AVAILABLE?

General speaking, the maximum peak of antibodies is found after two weeks of third dose of CaniLeish® vaccine during primary course (Moreno et al., 2012; Moreno et al., 2014). The maximum peak of antibodies is detected after three weeks of first dose of Leishmune®, after three weeks after second dose of Leish-Tec® during primary course (Fernandes et al., 2014) and after two weeks after one dose of LetiFend®(Carcelen et al., 2009). Antibodies reactive with Leishmania antigen have been reported to persist for 4-12 months in dogs vaccinated with CaniLeish® (Moreno et al., 2014; Starita et al., 2016). However, there is a marked decreased of antibody levels during time (Fernandes et al., 2014; Moreno et al., 2014; Starita et al., 2016). Interestingly, antibodies elicited by LetiFend® vaccine does not appear to interfere with qualitative or quantitative serological tests (Carcelen et al., 2009; Iniesta et al., 2016). Very limited information is available regarding antibodies elicited after annual booster of CanL vaccines (Starita et al., 2016).

Considerations regarding antibodies elicited by vaccines include:

- Variable immune response to vaccine by dogs might exist (Starita et al., 2016):
  - Different antigen recognition
  - Differences of duration of antibody response and peak of antibody levels
  - High, low or non-antibody responders

- Diagnostic performance will be different depending on serological test employed and time after vaccination:
  - Serological assays based on recombinant proteins are usually less sensitive than the ones based on whole parasite antigen for the recognition of antibodies elicited by vaccination (Marcondes et al., 2013; Moreno et al., 2014; Starita et al., 2016).
  - Quantitative serological techniques are commonly capable of detecting antibodies elicited by vaccination while rapid serological test are less sensitive (Marcondes et al., 2013).

It is important to develop new serological techniques that will discriminate between naturally-occurring antibodies or antibodies elicited by vaccination. For instance, prototype flow cytometry serology test seems to provide a good diagnostic performance with an absence of false-positive results in vaccinated dogs and minor cross-reactivity against other canine pathogens (Ker et al., 2013).

HOW TO MAKE A DIAGNOSIS OF SICK DOGS WITH CLINICAL LEISHMANIOSIS IF PREVIOUSLY VACCINATED?

Serology will be less useful if there is a recent history of vaccination
  - Diagnostic techniques based on observation of lesion (cytology/histology) and detection of parasite or parasite DNA (PCR) will be employed as first instance.

Serology will be more useful if vaccination has been performed a long time ago
However, diagnostic techniques based on observation of lesion (cytology/histology) and detection of parasite or parasite DNA (PCR) will be employed as first instance due to the fact that some dogs might have persistent antibodies induced by vaccination.

It is essential to know details on vaccination of evaluated dogs in order to make a correct interpretation of the serological test employed. All evaluated information should be combined together for a comprehensive assessment.

**FUTURE RESEARCH**

New diagnostic techniques for the discrimination of immune responses due to vaccination and to natural infection should be potentiated:

- New serological tests to distinguish between naturally-occurring antibodies and antibodies elicited by vaccination.
- New cellular immunity tests to distinguish between vaccination and natural infection.
- Development of less tedious cellular immunity techniques to evaluate response to vaccination and natural infection.

**REFERENCES**


