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Title: Clinical leishmaniasis in dogs living in the UK

Abstract:

Objective: To investigate the prevalence of leishmaniasis in a canine population in the UK and to describe clinical presentation, clinicopathological abnormalities, therapeutic protocols and outcome in this non-endemic country.

Materials and Methods: Medical records of dogs diagnosed with leishmaniasis at 7 referral centres in the UK were retrospectively reviewed.

Results: The prevalence was between 0.007 and 0.04% with a higher number of cases in southern England. All dogs had a history of travel to or from an endemic country. Lethargy, dermatological signs, decreased appetite and lameness were the most common reasons for presentation. Allopurinol was used alone in the majority of cases.

Clinical significance: Although rare, CanL in the UK should be considered in patients showing compatible clinical signs and with a history of travel to or from endemic areas.

Keywords: Canine, Infectious Disease, Leishmania, UK
INTRODUCTION

Canine leishmaniasis (CanL) is a systemic zoonotic disease caused by the protozoan *Leishmania infantum*. Infected dogs are the main reservoir of the parasite (Baneth *et al.* 2008) and play an important role in the epidemiology of human visceral (HVL) and cutaneous (HCL) leishmaniasis. Approximately 0.2 to 0.4 and 0.7 to 1.2 million HVL and HCL cases, respectively, occur each year. More than 90% of global HVL cases occur in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. HCL is more widely distributed with about one-third of cases occurring in each of three epidemiological regions, the Americas, the Mediterranean basin, and western Asia from the Middle East to Central Asia (Alavar *et al.* 2012). CanL is endemic in more than 70 countries worldwide (Solano-Gallego *et al.* 2011) and especially in the Mediterranean areas of Europe (Cyprus, Greece, Albania, Croatia, Italy, Malta, France, Spain and Portugal) (Maia & Cardoso 2015), the Middle East and many tropical and subtropical areas of the world. However, the infection is spreading to non-endemic areas with an increasing number of cases reported in dogs living in North America (Gaskin *et al.* 2002, Duprey *et al.* 2006) and Northern Europe (Shaw *et al.* 2009, Maia & Cardoso 2015). Recent studies have documented the presence of the disease in Germany (Geisweid *et al.* 2012), United Kingdom (Shaw *et al.* 2009) and Netherlands (Teske *et al.* 2002). This is probably due to a wider spread of the vector in some of the above areas and especially to a larger numbers of dogs being imported from, or having visited, endemic countries. Since the introduction of the United Kingdom Pet Travel Scheme (PETS) in 2000, the number of dogs travelling into the UK has increased year after year with a total of 411,582 dogs recorded between 2000 and January 2008 (Mencke *et al.* 2011). As a result, the disease has gained importance in the UK, albeit largely limited to the dogs that travel. It is likely that only very little natural transmission occurs in the UK because environmental conditions prevent the viability of the vector (Shaw *et al.* 2009). However, other mechanisms of transmission are possible, including blood transfusion (de Freitas *et al.* 2006), vertical (Rosypal *et al.* 2005, Pangrazio *et al.* 2009, Boggiatto *et al.* 2011, Naucke *et al.* 2012, Turchetti *et al.* 2014) and venereal transmission (Diniz *et al.* 2006). Despite the increase in awareness, the prevalence of infected dogs entering the UK is unknown, as no pre-or post-travel testing is required. Furthermore, clinically apparent cases represent the minority of infected dogs in endemic areas (Solano-Gallego *et al.* 2009; Schallig *et al.* 2013), so dogs with subclinical infection that appear healthy may unknowingly be imported.
Only one study (Shaw et al. 2009) has previously investigated dogs with positive diagnostic tests for CanL in the UK. The objectives of this study were, firstly, to investigate the prevalence of leishmaniasis in a canine population attending referral centres in the UK and, secondly, to describe clinical presentation, most frequent clinicopathological abnormalities, diagnostic investigations, different therapeutic protocols used and outcome of dogs diagnosed with CanL in this non-endemic country.

**MATERIALS AND METHODS**

**Patients and eligibility**

Medical records of dogs diagnosed of clinical leishmaniasis in seven different referral centres in the UK (University of Liverpool, University of Bristol, University of Edinburgh, University of Cambridge, Royal Veterinary College of London, Anderson Moores Veterinary Specialists, Animal Health Trust), between January 2005 and January 2014, were retrospectively reviewed. The database of each institution was searched by use of the following terms: leishmaniasis, leishmaniosis, allopurinol, N-methylglucamine antimoniate and miltefosine. Dogs on therapy with allopurinol for other diseases than CanL were excluded. In this way only patients with a final diagnosis of clinical leishmaniasis were selected and then included. The prevalence of the disease was calculated as the percentage of the ratio between the number of cases diagnosed of CanL in the study period at each referral centre and the total canine population that attended the respective centre in the same time period. The study was approved by the Veterinary Research Ethics Committee of the University of Liverpool.

**Data collection**

The diagnosis of CanL was made when there were compatible clinical signs and/or laboratory abnormalities together with detection of the parasites by polymerase chain reaction (PCR) or cytology (from lymph node, bone marrow, spleen or skin), and/or detection of antibodies using an immunofluorescence assay (IFAT) or an enzyme-linked immunosorbent assay (ELISA). Where available, information was reviewed regarding travel history (i.e. country to which the dog had travelled or from where it had been imported), reasons for presentation, physical examination findings, results of diagnostic investigations (e.g. haematology, biochemistry, urinalysis, cytology, serology, and PCR), therapeutic protocol used, and outcome. Dogs were
tested for other vector-borne diseases (*Ehrlichia canis, Babesia canis*) if a co-infection was suspected.

Survival time was defined as time (in days) from first presentation to last re-check or to time of death.

**Diagnostic investigations**

All routine clinicopathological analyses, serology, and real-time quantitative PCR (qPCR) assays were conducted at the respective university or by commercial laboratories. Clinicopathological abnormalities such as anaemia, azotaemia, hypoalbuminaemia were defined when results were outside the reference intervals established by each corresponding laboratory. Proteinuria was diagnosed by an elevated urine protein-to-creatinine ratio (UPCR >0.5) with inactive urinary sediment. Renal azotaemia was defined as increased creatinine with concurrent isosthenuria (1.008-1.012).

For serological investigations, the upper reference interval for IFAT was either 1:80 or 1:128, depending on the laboratory, whilst the positive threshold value for ELISA used by all laboratories was 35 ELISA Units (EU). Serological results were classified as low, medium or high positive if IFAT titres were <2-fold, 2- to 4-fold, or >4-fold greater than the threshold positive value indicated by the reference laboratory. ELISA results were classified as mild when <80 EU, moderate when between 80 and 150 EU, and high when >150 EU.

Detection and quantification of *Leishmania* kinetoplast DNA was performed on blood, bone marrow, and/or skin samples by qPCR as previously described (Caldin *et al.* 2004, Solano-Gallego *et al.* 2007, Maia *et al.* 2008).

**Classification of cases**

Dogs were classified at the time of diagnosis in different clinical stages according to the Canine Leishmaniasis Working Group (CLWG) guidelines (Paltrinieri *et al.* 2010). Dogs were also classified according to the International Renal Interest Society (IRIS) guidelines, based on measurement of serum creatinine concentration at the first two appointments.

**Statistical analysis:**

Data are reported as median or mean and range (minimum and maximum).
RESULTS:

**Patient population**

Thirty-eight dogs were included in the study: 14 were diagnosed at the Royal Veterinary College, 7 at the University of Liverpool, 7 at the University of Edinburgh, 3 at the University of Bristol, 3 at the University of Cambridge, 3 at the Animal Health Trust and 1 at Anderson Moores Veterinary Specialists. Median age was 4.8 years (range 1.11 years - 12.2 years) and median body weight was 26.3 kg (range 5.9 - 49 kg). The prevalence of the disease was between 0.007 and 0.04 per cent and higher incidences (0.04 per cent at the Royal Veterinary College of London, 0.03 per cent at the University of Bristol and 0.02 per cent at the Animal Health Trust) were found in southern England.

All dogs had a history of having travelled to or been imported from an endemic area for *Leishmania infantum*. No clinical or clinicopathological differences were noted between dogs imported from and dogs that have travelled to an endemic area. No autochthonous cases were found in this population. Details of patient and travel history are presented in Table 1.

**Detection of Leishmania infantum and concurrent vector-borne diseases**

*Leishmania infantum* infection was demonstrated by serology and/or PCR and/or cytology. Details of the diagnostic tests are shown in Table 2. Only three dogs were tested for other vector-borne diseases, including two dogs tested by serology for *Ehrlichia canis* and one for *Babesia canis*. All three dogs were negative.

**Clinical signs**

All dogs had at least one clinical sign compatible with leishmaniasis. The most frequent reasons for presentation were lethargy (20/38, 53%), dermatological manifestations (17/38, 45%), decreased appetite and lameness (8/38, 21%). On physical examination the most common signs observed were dermatological signs (24/38, 63%, including localised or multifocal alopecia [10], and crusting dermatitis [8]) and systemic lymphadenopathy (22/38, 58%). Twenty-four per cent (9/38) of dogs were diagnosed with polyarthritis.

**Clinicopathological investigations**
Table 3 shows the main clinicopathological findings. All dogs had at least one laboratory abnormality compatible with leishmaniasis. In total, 19/32 dogs (60%) were anaemic, with the anaemia being classified as mild (haematocrit [HCT] 30-36%) and moderate (HCT 18-29%) in 11 (58%) and 8 dogs (42%), respectively, and also classified as non-regenerative (reticulocytes < 60 x 10^9/L) in 4 of the 6 cases where reticulocyte count was available. Eight dogs (8/23, 35%) had thrombocytopenia (median: 94 x 10^9/L, range: 30-150; laboratory reference interval: 155-400) and two (2/22, 9%) were pancytopenic. Renal azotaemia was detected in 6 dogs (6/25, 24%) and 20 dogs (20/30, 67%) were classified as being in IRIS stage 1 CKD (creatinine < 125 µmol/l), 4 (4/30, 13%) in IRIS stage 2 (creatinine between 125-180 µmol/l) and 6 (6/30, 20%) in IRIS stage 3 (creatinine between 181-440 µmol/l). None of the dogs were classified as being in IRIS stage 4 CKD (creatinine > 440 µmol/l). Nineteen (19/28, 78%) of dogs were proteinuric based on increased UPCR (median: 5.6, range: 0.7-18.8; normal values < 0.5). Finally, 28 dogs (28/30, 93%) had hypoalbuminaemia (median: 16 g/l, range: 11-20, laboratory reference interval: 23-31), hyperglobulinaemia (median: 58 g/l, range: 52.1-70; laboratory reference interval: 25-45) and a low (<0.6) albumin/globulin ratio. Serum protein electrophoresis was rarely used in the diagnostic work-up and/or in the follow-up rechecks.

**Treatment**

Of the 38 cases, 35 (92%) were given a specific treatment for CanL. In the majority of cases (17/35, 48%) allopurinol was used alone, followed by a combination of allopurinol and miltefosine (15/35, 43%) or allopurinol and N-methylglucamine antimoniate (3/35, 9%). A variety of other drugs were used in addition to the anti- \textit{Leishmania} therapy, depending upon the specific case and attending clinician’s judgement. Treatments included ace-inhibitors (benazepril, enalapril) anti-hypertensive drugs (amlodipine), anti-thrombotics (clopidogrel, aspirin) analgesics and anti-inflammatory drugs (tramadol, meloxicam), gastro-protectants (sucralfate, famotidine), anti-emetics (maropitant, ondansetron, metoclopramide), immune-suppressive drugs (prednisolone, azathioprine), diuretics (spironolactone), antibiotics (doxycycline, amoxicillin-clavulanate, enrofloxacin and marbofloxacin).

**Staging and survival**
Based on CLWG clinical staging, 32 dogs (32/38, 84%) were classified as stage C (sick dogs with clinically evident leishmaniasis), and 6 (6/38, 16%) as stage D (severely sick dogs often unresponsive to repeated courses of anti-Leishmania drugs). Twenty-eight (28/38, 74%) dogs were alive at the end of the study period and ten (10/38, 26%) had died or had been euthanased. Six of the ten non-surviving dogs (60%) were classified in stage D and 4 (4/10, 40%) in stage C. Median survival time was 400 days (range 2-2160 days).

Reasons for death and/or euthanasia included worsening of kidney disease (3/10, 30%), lack of response to therapy (3/10, 30%), acute thrombo-embolism (1/10, 10%), neurological signs due to myelomalacia likely secondary to severe systemic vasculitis (1/10, 10%) and developing of lymphoma (1/10, 10%) and osteosarcoma (1/10, 10%).

**DISCUSSION:**

In this study data from dogs diagnosed with leishmaniasis in seven different referral centres across the UK are reported. This is the first time that clinical CanL has been described in all its aspects in a population living in the UK. The prevalence of the disease in this study was low demonstrating that leishmaniasis is relatively uncommon in dogs living in the UK. However, the real prevalence of the disease is likely higher than the current report suggests since no cases from primary practices were included. Furthermore, only dogs with clinical leishmaniasis were considered, with either exposed or infected animals (those having positive results to the diagnostic tests but not showing any clinical and clinicopathological abnormalities of the disease) not being considered. It is unpredictable whether those dogs will develop clinical signs in the future. Moreover, due to the low familiarity of the veterinary surgeons in the UK with this disease, it is possible that some cases have been missed because CanL was not considered among the possible differentials. In addition, some clients could have declined serology testing.

Unfortunately, in many cases the time-frame between the travel from/to endemic areas and the development of clinical signs was not available. Anyway, it is well known that the time between the infection and the development of the clinical signs (incubation period) can be very variable and mainly dependent to the host’s immunologic response (Fisa et al. 1999, Cardoso et al. 2007).

Similar to previous reports (Shaw et al. 2009), most cases were found in southern England. However, caution should be exercised when interpreting this because no all geographical regions across the UK were included in
the present study. If cases from the south are genuinely overrepresented, it might be due to easier connections
to Europe and warmer weather. With regard to the latter, the climate has recently changed enough to support
the transmission and diffusion in these areas of other vector borne diseases (Medlock et al. 2007; Wilson et al.
2013). However, to date a vector of *L. infantum* has not been found in the UK and sand flies that are
introduced in the country by car or plane likely die soon after arrival due to a marked intolerance to climate
changes. In fact, the sand fly’s range of activity is between 15°C and 28°C in association with high relative
humidity and absence of strong rain and winds (Bogdan et al. 2001, Killick-Kendrick, 1999, Maroli et al.
2013). This does not rule out possible future epidemiological changes due to the ongoing global warming. To
date, there is little published information regarding the distribution of the competent sand fly in Northern
Europe and in the UK and how or if it is changing due to the warmer climate. Furthermore, other modes of
transmission have been described including blood transfusion (de Freitas et al. 2006), vertical transmission
from bitches to puppies (Rosypal et al. 2005, Pangrazio et al. 2009, Boggiatto et al. 2011, Naucke et al. 2012,
Turchetti et al. 2014) and venereal transmission (Diniz et al. 2006). Dog-to-dog mechanisms have been also
hypothesised to explain leishmaniasis outbreaks among foxhounds in the United States and Canada (Duprey et
2010).

All dogs included in the present study had a history of having travelled to or been imported from a region
endemic for *Leishmania infantum*. The majority of dogs were imported (32/38, 84%) versus a minority that
has travelled to an endemic country (6/38, 16%). This would suggest a higher risk in adopting a dog from an
endemic area respect travelling with the dog to those countries. Travelling dogs usually stay for only a short
period time and the overall risk they get infected with *L. infantum* is likely low (Hamel et al. 2011). However,
veterinarians in non-endemic regions should be aware of CanL, including its non-vectorial transmission
modes, and should advise dog owners on preventive measures (Shaw et al. 2009, Menn et al. 2010). The
majority of dogs in the present study had been in Spain, which is compatible with the high prevalence of
leishmaniasis in this country (Mattin et al. 2014) and its popularity as a destination for holidays. Imported
shelter and stray dogs have higher risk to be infected because of decreased preventive measures and greater
exposure to sand flies during the period of peak of activity (evening) (Manzillo et al. 2006). No
autochthonous cases were recognised in this study, which contrasts the findings of Shaw et al. (2009) who
identified 3 positive dogs obtained from UK re-homing centres with no history of travel abroad. It remains questionable if transmission was due to vectors, transplacental or even by direct contact.

The spectrum of clinical signs and laboratory abnormalities in the study group of dogs were similar to that reported in endemic areas (Ciaramella et al. 1997, Koutinas et al. 1999, Paltrinieri et al. 2010, Solano-Gallego et al. 2011). Dermatological signs and lymphadenopathy were the most frequent clinical findings. Polyarthritis was present in 9 dogs (24%), similarly to previously published work from the UK (Shaw et al. 2009) (17%). Polyarthritis should be then considered among common presenting signs of leishmaniasis in dogs diagnosed in UK referral centres. The most frequent clinico-pathological abnormalities found in the study group included mild-to-moderate anaemia, renal azotaemia, hyperglobulinaemia, hypoalbuminaemia, decreased albumin/globulin ratio and proteinuria. These are considered hallmarks of CanL also in endemic areas (Ciaramella et al. 1997, Koutinas et al. 1999, Paltrinieri et al. 2010, Solano-Gallego et al. 2011).

Given that non-pathognomonic clinical signs and laboratory abnormalities, as well as the low familiarity with the disease of the veterinary surgeons in the UK, more than one test was used to confirm the final diagnosis in the majority of cases. Serology and PCR on peripheral blood was the most common combination of diagnostic tests used in this population. However, PCR on peripheral blood lacks sensitivity and different tissues such as lymph nodes, spleen and/or bone marrow would harbour a higher number of Leishmania amastigotes (Caldin et al. 2004). Furthermore, serum protein electrophoresis was included in the initial diagnostic investigation and in follow-up rechecks only in a very low number of cases. However, this test can provide important information, especially during reassessment, because improvement or normalisation of the protein electrophoresis trace generally happens before a negative serology titre occurs (Torres et al. 2011). At time of diagnosis, the authors recommend the evaluation of haematology, biochemistry profile, urinalysis including UPCR, serology titre and serum protein electrophoresis. In case of peripheral lymphadenopathy and/or skin lesions, fine-needle aspiration for cytology and/or PCR can be also useful. After the first month of therapy, previous abnormal parameters can be re-checked together with serum protein electrophoresis: in fact, as stated before, this test will the first one to show an improvement or even a normalisation of previous hypoalbuminaemia and hyperglobulinaemia (usually polyclonal gammopathy). At this stage, a significant reduction of the serology titre is unlikely. The latter is generally re-evaluated at 3 and 6 months from
diagnosis together with quantitative PCR on lymph-nodes, spleen and/or bone marrow that can demonstrate a progressive reduction of the number of amastigotes.

The majority of dogs were treated with allopurinol alone, most likely because N-methylglucamine antimoniate is not available in the UK and must be imported and miltefosine requires a special treatment certificate. Where additional anti-\textit{Leishmania} drugs were used, miltefosine was more frequently used than N-methylglucamine antimoniate, probably because it is an oral solution and easier to administer. In contrast, N-methylglucamine antimoniate must be injected subcutaneously, and can often be associated with localised pain and inflammation. Anyway, both drugs have been previously shown to be similarly effective (Miró \textit{et al.} 2009). Currently, N-methylglucamine antimoniate or miltefosine in association with allopurinol are recommended as standard therapy for CanL (Oliva \textit{et al} 2010, Solano-Gallego \textit{et al} 2011, Roura \textit{et al} 2013, Noli \textit{et al} 2014).

Some dogs also received other drugs according to the attending clinician’s decision. The influence of these drugs on the anti-\textit{Leishmania} therapy and outcome is unknown.

Considering that the majority of dogs were treated only with allopurinol, it is noteworthy that the overall outcome was good with a reasonable survival time. Furthermore, it should be considered that only dogs with moderate-to-severe disease (stages C and D) were included in the study and that these animals generally have a guarded-to-poor prognosis in endemic areas (Solano-Gallego \textit{et al} 2011; Roura \textit{et al} 2013). This finding can, perhaps, be explained by the minimal chance of re-infection given the geographical location, and low risk of having a concurrent vector-borne disease (Shaw \textit{et al} 2009). The latter cannot be completely ruled out in this study population since only three dogs were tested for other vector-borne diseases. In this respect, response to CanL is known to be influenced by both concurrent disease and immunological stimulation or suppression by shifting the balance from a protective Th1 response to a Th2 immune response that favours the development of a non-protective and possibly detrimental humoral reaction (Koutinas \textit{et al} 2014).

Most non-surviving dogs experienced a worsening of kidney disease. It is recognised that advanced renal failure is the major cause of death and/or euthanasia in CanL (Panellas \textit{et al} 2009). Further studies evaluating IRIS staging in a bigger population and also in patients already on therapy could provide more useful information regarding its possible prognostic value.
Finally, all dogs in clinical stage D died or were euthanased. Currently clinical staging at time of diagnosis and periodic re-classification in line with disease progression and regression is considered a useful way to predict outcome (Solano-Gallego et al. 2009, Oliva et al. 2010, Paltrinieri et al. 2010).

In conclusion, although rare, veterinary surgeons in the UK should consider CanL in patients with a history of travel to or from endemic areas, where there are compatible clinical signs and clinicopathological abnormalities. An early diagnosis and appropriate therapy can be associated with a relatively good control of the disease (Roura et al. 2013; Torres et al. 2011). As Leishmania infection is known to have a long incubation period, practitioners should inform the owners of imported dogs to retest them for Leishmania for at least two years after importation or in case of a clinical suspicion (Paltrinieri et al. 2010). Moreover, veterinarians should be aware of non-vectorial transmission ways, and should advice clients on preventive measures before travelling to endemic countries.

REFERENCES:


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### Table 1: Patient population & travel history

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number of dogs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed breed</td>
<td>12 (31%)</td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Lurcher</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>Cocker spaniel</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Golden retriever</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Staff bull terrier</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Bassett hound</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Border collie</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Boxer</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>English pointer</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>English setter</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Greek hare hound</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Greyhound</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Siberian husky</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Labradoodle</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Miniature poodle</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Miniature schnauzer</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Rottweiler</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Spanish galgo</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Neutered males</td>
<td>18 (47%)</td>
</tr>
<tr>
<td>Neutered females</td>
<td>15 (40%)</td>
</tr>
<tr>
<td>Entire males</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>Entire female</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>

**Imported from**
- Spain: 32 (84%)
- Greece: 16 (42%)
- Cyprus: 7 (18%)
- Italy: 3 (8%)
- Portugal: 2 (5%)
- Hungary: 2 (5%)
- Brazil: 1 (3%)

**Traveled to**
- Spain: 6 (16%)
- France: 3 (8%)
- Germany: 2 (5%)
- Italy: 1 (3%)
Table 2: Diagnostic tests used to indentify *L. infantum* infection

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Number of dogs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serology + PCR</td>
<td>10 (26%)</td>
</tr>
<tr>
<td>Serology + PCR + Cytology</td>
<td>8 (21%)</td>
</tr>
<tr>
<td>Serology</td>
<td>7 (18%)</td>
</tr>
<tr>
<td>PCR</td>
<td>7 (18%)</td>
</tr>
<tr>
<td>Serology + Cytology</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>PCR + Cytology</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Cytology</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

- **Serology**
  - ELISA: 28 (74%)
  - IFAT: 9 (32%)

  - *Mild*: 7 (25%)
  - *Moderate*: 11 (39%)
  - *High*: 10 (36%)

- **PCR**
  - Blood: 12 (44%)
  - Spleen: 4 (15%)
  - Lymph node: 3 (11%)
  - Bone marrow: 2 (7%)
  - Blood + Bone marrow: 2 (7%)
  - Blood + Spleen: 1 (4%)
  - Spleen + Lymph node: 1 (4%)
  - Blood + Conjunctiva + Skin: 1 (4%)
  - Blood + Bone marrow + Joint fluid: 1 (4%)

- **Cytology**
  - Lymph node: 8 (57%)
  - Spleen: 3 (22%)
  - Bone marrow: 2 (14%)
  - Lymph node + Spleen: 1 (7%)