13th Symposium on Wild Fassa. Vibo-Settimo (Italy), September 25-28, 2019

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Abstracts of the 13th Symposium of the Italian Society for Veterinary Molecular Biology on wild fauna.

1. RESULTS

The technique presented a limit of detection of 26 parasites for each buccal swab, regardless of the number of wolf cells that were collected in the sample. The specificity of the technique was determined by checking that there were no cross-reactions with other wolf cells or DNA from animals infected with L. canis. The qPCR was set up to express the results as numbers of parasites per 50000 wolf cells. For the interpretation of parasite loads, it must be borne in mind that a result between 1 and 100 parasita/50000 dog cells represents a very low load, probably due to contact with the vector, although without the development of disease. However, from 50000 parasita/50000 dog cells in some study clinical signs related to the disease have been observed (Table 1).

Table 1: qPCR results of Leishmania donovani infections in wolf samples used in the present study expressed as number of parasites/50000 wolf cells.

<table>
<thead>
<tr>
<th>ID</th>
<th>Geographic origin</th>
<th>Sample date</th>
<th>qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Vibo-Settimo</td>
<td>18/08/2018</td>
<td>Positive</td>
</tr>
<tr>
<td>119</td>
<td>Vibo-Settimo</td>
<td>18/08/2018</td>
<td>Negative</td>
</tr>
<tr>
<td>118</td>
<td>Vibo-Settimo</td>
<td>23/08/2018</td>
<td>Negative</td>
</tr>
<tr>
<td>117</td>
<td>Vibo-Settimo</td>
<td>23/08/2018</td>
<td>Negative</td>
</tr>
<tr>
<td>215</td>
<td>Vibo-Settimo</td>
<td>18/01/2013</td>
<td>Diluted (1:3)</td>
</tr>
<tr>
<td>227</td>
<td>Vibo-Settimo</td>
<td>21/01/2013</td>
<td>Negative</td>
</tr>
<tr>
<td>226</td>
<td>Vibo-Settimo</td>
<td>21/01/2013</td>
<td>Negative</td>
</tr>
<tr>
<td>225</td>
<td>Vibo-Settimo</td>
<td>21/01/2013</td>
<td>Negative</td>
</tr>
<tr>
<td>224</td>
<td>Vibo-Settimo</td>
<td>21/01/2013</td>
<td>Negative</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Across the area of Mediterranean influence canine Leishmaniosis represents a current issue. Not only because it is an endemic area, but also to the possible contagion to the man from the disease, since it is a zoonosis. The method that is usually used in veterinary clinics is rapid kits by immunochromatography, or the titration of the sample is carried out by means of IFI or ELISA. In all cases, the diagnosis is made by detecting antibodies in the blood. However, immunological assays can lead to false negatives in the first months after infection, because until the seroconversion takes place, the antibodies have not risen sufficiently for detection; or they can also give rise to false positives, when the antibody titre is maintained for a long time after a successful treatment. To avoid this problem, qualitative and quantitative PCRs have been developed that determine the presence of the parasite in different organic fluids. In the present work we give results from the development of a non-specific method for the detection of "canine Leishmania" in wolf samples.
Distantem in otter (Lutra lutra), a case report

Marina-Gonzalez E.D.1, Martinez-Ferrero N.2, Fernandez N.3, Espina F.4, Perez Y.5,5, Ferroton M.C.6

1Instituto de Salud Animal, Facultad de Veterinaria, Universidad de Castilla-La Mancha.
2Veterinary Medicine Faculty, University of La Rioja, Spain.
3Instituto de Salud Animal, Facultad de Veterinaria, Universidad de Castilla-La Mancha, Luzen, Spain.
4Veterinary Medicine Faculty, University of La Rioja, Spain.
5University of La Rioja, Spain.

Abstract: We describe a clinical case of distantem in an Otter. Viral disease is increasingly present in wild and domestic mustelids. This diagnosis has served to know a new weakness of wildlife. The otter is an unusual considered biomarker of the good state of our rivers. The work of the Wildlife Reception Centers (CRF) and the Wild Animal Recovery Centers (CRAS) is essential for knowledge of the Environment and the health of the ecosystems that surround us.

Keywords: Distantem, Otter, Mustelid

1. INTRODUCTION

In the Iberian Peninsula its distribution is very irregular. It is relegated to mountainous areas frequently with heavy rains. The presence of the otter in the river is the best indication we have about the good state of a river. Un fortunately the pollution of the rivers, the uncontrolled garbage dumps, the cutting of forests and agro-irrigation of the riverbanks, the biocide and sulfur used by poachers in trout fishing, and other aggressions suffered by our rivers, are causing the decrease of its population. It is included in the List of Species included in the List of Wild Species under Special Protection Regime (R.D. 139/2011). This species is protected by the CITES International Convention, in which it is catalogued as of imminent extinction due to trade, which is explicitly prohibited. It is also included in Annex II of the Berne Convention, among animals whose danger of extinction is serious and requires special care for recovery.

The Moquillo, also known as Distantem or Carr's Disease, is a pathology that mainly affects domestic dogs. The disease is caused by a virus belonging to the genus Morbillivirus and family Paramyxoviridae, and it is a single-stranded RNA virus. Recently it has become a very common disease of mustelids, especially ferrets. Normally attends with respiratory, digestive and nervous symptoms.

2. MATERIALS AND METHODS

In October 2017, a specimen was collected by an Environmental Agent in Manganese de la Lamprosena, a small village in the region of Tierra del Pan, Zamora (Spain). The municipality belongs to the Natural Reserve of the Lagunas de Villafáfila. The otter was transferred to the Wild Animals Reception Center (CRF) of Villarubio where the action protocol for catalogued species was applied. Due to its general poor health condition, it was immediately explored.

Fig. 2: Reception the Otter (Lutra lutra) and take an extrusion line.
Clinical signs

Ataxia, incoordination, dehydration and extreme weakness were observed. The fur lacked without shine and was in poor condition. The animal had incipient oedema in various areas, without apparent injuries. Other symptoms were hypothermia, 37 ° C, slight tachypnoea and dyspnea, as well as the anal area spotted by watery diarrhea. The body weight was 3,150 gr. An intravenous line was placed to improve hydration and Ringer lactate (150 ml) was applied. Broad spectrum antibiotic treatment was also applied. Fig. 2. Blood and biochemical analysis did not show significant alteration of the parameters analyzed. The observed nervous signs were ataxia, incoordination, along with the lack of strength and weakness. All together the animal suffered of starvation and a pathological process that affected the central nervous system. Unfortunately, four days after admission and after a relative and apparent improvement, with food intake and improvement in the strength of the animal, appeared dead in its confinement. Regulated necropsy was performed and samples were sent to the Pathology Department of the University of León, where they make the diagnosis as Distemper.

Histological results

The lung presented areas with thickening of the interalveolar septa due to muscle fibre hypertrophy and the presence of lymphocytes and macrophages, alterations compatible with interstitial pneumonia, Fig 3. By means of the immunohistochemical technique with a monoclonal antibody against morbillivirus, intense positivity was detected against the viral antigen in bronchiolar and macrophage epithelial cells. For the diagnosis, a positive control of distemper (dog lung) was used. Other alterations observed were the following: lymphoid depletion and extracellular sphen hyalnosis, Fig 4, nephrosis and disseminated intravascular coagulation (DIC) Fig 5. In addition to the distemper related lesions, pathology results described in the kidney: Intense vacuaming of the cytoplasm of the epithelial cells of the proximal contoured tubules. In addition to the presence of eosinophilic material in tubular lights (hyaline cylinders), it was compatible with protein nephrosis. Fig 6. Neutrophil infiltrates in renal pelvis (acute pyelonephritis?). In the heart: An intracellular cyst with bradyarrhythmia compatible with Sarcozystis spp. Fig 7. In liver samples: Mild liver degeneration. Some lymphocytic portal infiltrates. The stomach, pancreas and intestine were not altered.

4. PHOTOGRAPHIC ANNEX

Fig. 3. Afflicted distemper lung. A characteristic lesion of the distemper is interstitial pneumonia, and the presence of viral antigen in macrophages.

Fig. 4. Lymphoid depletion and extracellular hyaline in sphen.

Fig. 5. Disseminated intravascular coagulation (DIC).

Fig. 6. Presence of eosinophilic material in tubular lights (hyaline cylinders), compatible with protein nephrosis.