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BROADLY REACTIVE PAN-VIRAL PCR OF CEREBROSPINAL FLUID IN CANINE MENINGOENCEPHALITIS OF UNKNOWN ETIOLOGY
The canine meningoencephalitides of unknown etiology (MUE)

• GME, NME, NLE
• Histopathologic lesions are similar to those present in human viral meningoencephalitis
• PCR method has demonstrated that 50-70% of human meningoencephalitides are caused by CNS viral infections.
• We hypothesize that a subset of canine MUE results from aberrant immune responses following infection of the CNS.

Objective:

• **Objective**: *To determine whether or not nucleic acids from infectious agents can be identified in cerebrospinal fluid (CSF) by applying degenerate viral PCR to 146 CSF samples, collected pre- and/or postmortem from dogs with MUE and control dog.*
Consensus Degenerate Primers (CODEHOP)

Motif: S I I Q A H N L C

CODEHOP: 5’ TCC ATC ATC CAG GCC C T C T C T A C G T

5’ Consensus Clamp
3’ Degenerate Core

Rose et al., 1998, Nucl Acids Res 26:1628
• **Viral Families:** Herpesviridae, Adenoviridae, Alphaviridae, Picornaviridae, Paramyxoviridae, Polyomaviridae, Flaviviridae, Bunyaviridae, Bornaviridae, Rhabdoviridae, Coronaviridae
Pan-Viral PCR Positive Controls
Methods

• DNA and RNA extracted from 146 CSF samples and non-neurological controls by standard methods (Qiagen and Invitrogen)
• Housekeeping PCR and RT PCR for GAPDH (DNA) and beta-actin to confirm DNA and RNA integrity
• PCR and RT PCR on ~ 5 µl of each sample in 20 various CODEHOP reactions
Broadly reactive pan-viral PCR on MUE CSF (146 cases)
Pan-bunyavirus PCR – LaCrosse virus

- CSF from 6/60 (10%) MUE cases positive on pan-bunyavirus PCR
- Sequences analysis disclosed 99% homology to LAC
- Specific LAC PCR underway
- Developing an Ab to LaCrosse to perform serology on CSF and serum in dog

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex</th>
<th>Color</th>
<th>Weight</th>
<th>CSF</th>
<th>MRI</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shih Tzu</td>
<td>M/N</td>
<td>Wh/Tan</td>
<td>7.4kg</td>
<td>RBC=1963 WBC=12 TP=23.8 Mixed pleocytosis</td>
<td>normal brain</td>
<td>MUE (brain)</td>
</tr>
<tr>
<td>Boxer</td>
<td>M</td>
<td>Fawn</td>
<td>37kg</td>
<td>RBC=87 WBC=9 TP=17.0 Macro56%,Neut18%, Lymph16%, Eos10%</td>
<td>T2W hyperintensity cingulate gyrus</td>
<td>brain tumor +/- meningitis</td>
</tr>
<tr>
<td>Pug</td>
<td>F/S</td>
<td>Black</td>
<td>8.25kg</td>
<td>RBC=0 WBC=3 TP=38.7</td>
<td>T2W IM hyperintensity thoracic cord</td>
<td>MUE (T/L)</td>
</tr>
<tr>
<td>Weimaraner</td>
<td>F/S</td>
<td>Gray</td>
<td>25.8kg</td>
<td>RBC=38 WBC=26 TP=96.1 Lymph85%, Macro13%, neut2%</td>
<td>N/D</td>
<td>MUE (brain)</td>
</tr>
<tr>
<td>Boston Terrier</td>
<td>M</td>
<td>Blk/Wh</td>
<td>9.3kg</td>
<td>RBC 69 WBC 20 TP 16.5; 2% nondeg neut 19% lg mono 79% sm lysphs</td>
<td>normal brain</td>
<td>MUE (brain)</td>
</tr>
<tr>
<td>Chihuahua</td>
<td>F/S</td>
<td>Wh</td>
<td>3.8kg</td>
<td>WBC 1 TP 19.5 ;1% nondeg neut 25% lg mono 73% sm lysphs</td>
<td>T2W multifocal hyperintense lesions throughout brain</td>
<td>MUE (brain)</td>
</tr>
</tbody>
</table>
Pan-polyomavirus PCR – Merkel Cell Polyomavirus

- CSF from 3/60 (5%) MUE cases positive on pan-polyomavirus PCR
- Sequencing (320 bp) shows 98% homology to Merkel Cell Polyomavirus (MCV)
- IHC on one case negative with human MCV Ab
- Specific MCV PCR underway
- CSF antigen testing

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sex</th>
<th>Color</th>
<th>Weight</th>
<th>CBC Results</th>
<th>MUE</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labrador Retriever</td>
<td>F/I</td>
<td>Chocol</td>
<td>26 kg</td>
<td>RBC = 1263 WBC = 11 TP = 19 16% Lymphs 10% Monos 73% Neuts 1% Eos</td>
<td>Normal cervical spine</td>
<td>MUE (cervical)</td>
</tr>
<tr>
<td>Border Collie</td>
<td>M/N</td>
<td>Blk/Wht</td>
<td>18.2 kg</td>
<td>RBC = 30, WBC = 139 TP 28 mixed pleocytosis</td>
<td>Hydrocephalus and syringohydromelia</td>
<td>MUE</td>
</tr>
<tr>
<td>Great Dane</td>
<td>M/N</td>
<td></td>
<td></td>
<td>RBC 4 WBC 53 TP 56 17% monos 52% eos 31% lymphs</td>
<td>Syringohydromelia</td>
<td>MUE</td>
</tr>
</tbody>
</table>
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