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Conventional and molecular diagnostic testing for the acute neurologic patient

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Abstract

Objective – The aim of this review is to describe and evaluate both conventional and molecular diagnostic testing utilized in dogs and cats with acute neurologic diseases. Various types of polymerase chain reaction (PCR) are explored along with novel molecular diagnostic testing that ultimately may prove useful in the critical care setting.

Data Sources – PUBMED was searched to obtain relevant references material using keywords: 'canine OR feline meningitis AND meningoencephalitis,' 'feline infectious peritonitis,' 'canine distemper,' 'canine OR feline AND toxoplasma,' 'canine neospora,' 'canine OR feline AND rickettsia,' 'granulomatous meningoencephalitis,' 'steroid responsive meningitis arteritis,' 'necrotizing encephalitis,' 'novel neurodiagnostics,' 'canine OR feline AND CNS borrelia,' 'canine OR feline AND CNS bartonella,' 'canine OR feline AND CNS fungal,' 'nested OR multiplex OR degenerate OR consensus OR CODEHOP AND PCR.' Research findings from the authors' laboratory and current veterinary textbooks also were utilized.

Human Data Synthesis – Molecular diagnostic testing including conventional, real-time, and consensus and degenerate PCR and microarray analysis are utilized routinely for the antemortem diagnosis of infectious meningoencephalitis (ME) in humans. Recently, PCR using consensus degenerate hybrid primers (CODEHOP) has been used to identify and characterize a number of novel human viruses.

Veterinary Data Synthesis – Molecular diagnostic testing such as conventional and real-time PCR aid in the diagnosis of several important central nervous system infectious agents including canine distemper virus, *Toxoplasma gondii*, *Neospora caninum*, rickettsial species, and others. Recently, broadly reactive consensus and degenerate PCR reactions have been applied to canine ME including assays for rickettsial organisms, *Borrelia spp.* and *Bartonella spp.*, and various viral families.

Conclusions – In the acute neurologic patient, there are several key infectious diseases that can be pursued by a combination of conventional and molecular diagnostic testing. It is important that the clinician understands the utility, as well as the limitations, of the various neurodiagnostic tests that are available.

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Keywords: CODEHOP, meningitis, meningoencephalitis, microarray, neurodiagnostics, PCR

Conventional Diagnostic Testing

Cerebrospinal fluid (CSF) analysis

CSF analysis is a key component of the neurodiagnostic work-up, and an invaluable resource in both the clinical and research setting. While abnormalities in CSF cytology and protein are relatively *sensitive* indicators of central nervous system (CNS) disease, they are rarely *specific* for individual disease processes. On occasion, bacteria, fungi, protozoa, parasites, or tumor cells may be identified on microscopic examination of CSF (Figure 1). However, this is extremely rare. The CSF profile helps the clinician to narrow the differential diagnosis (Table 1), but must be interpreted in the context of case signalment, history, clinical signs, and neuro-imaging. One must be especially cautious not to *overinterpret* the CSF profile. For example, in confirmed cases of CNS neoplasia or inflammation, CSF may be misleadingly normal. Conversely, although rare, a CSF pleocytosis may be present in cases with minimal or no histopathologic evidence of parenchymal or meningeal inflammation.[1] When these important caveats are considered, CSF may provide valuable ancillary data for clinicians to make sound decisions in the critical care setting.

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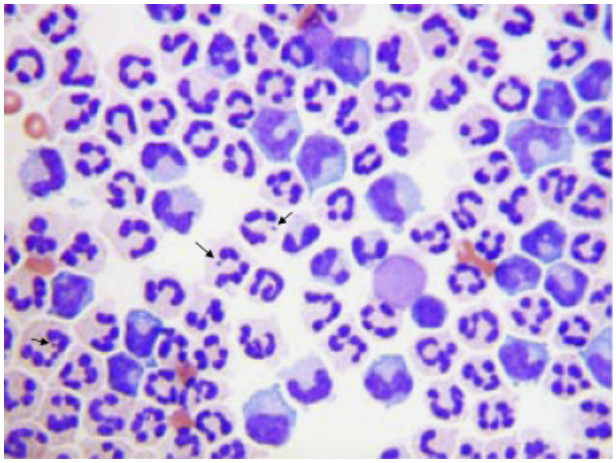

Figure 1: Cerebrospinal fluid from a 7-month-old Labrador Retriever with bacterial meningitis. Note the intracellular bacteria (arrows) within several neutrophils.

CSF color: Normal CSF is clear and colorless. The CSF may appear cloudy when a marked pleocytosis ($>500\,\text{WBC} \times 10^6/\text{L}$ [WBCs/μL]) is present.[2] Elevated protein levels may further increase CSF turbidity and viscosity. Red CSF indicates hemorrhage; typically this is iatrogenic due to penetration of radicular or meningeal blood vessels. Confirmation of a traumatic tap can be determined via centrifugation, which clears iatrogenic hemorrhage. If red or yellow color persists, this typically indicates chronic hemorrhage. Yellow or straw-tinged CSF is referred to as xanthochromic, and it suggests prior subarachnoid hemorrhage (in the absence of hyperbilirubinemia). Xanthochromia is caused by an accumulation of blood pigments such as hemoglobin, and it may occur within several hours of an acute hemorrhagic insult (trauma, bleeding disorders and occasionally severe CNS inflammation).

CSF cell counts and cytology: The total number of cells present in CSF typically is determined by use of a cell counting chamber, such as a Fuchs-Rosenthal chamber. Ideally, the counting should be performed within 30 minutes to 1 hour of CSF collection, as cells may degrade in CSF with low protein content. Refrigerating helps to minimize cellular degeneration. In the CSF of normal dogs and cats, $0–5\,\text{WBC} \times 10^6/\text{L}$ (WBCs/μL) is considered to be normal.[2] A traumatic tap minimally affects the cell count.

In cases with CSF pleocytosis ($>5\,\text{WBC} \times 10^6/\text{L}$ [WBCs/μL]), the next step in the analysis is the determination of the differential cell count via cytospin.[2] After staining (eg, DiffQuick, Papanicolaou), the percentage of the different types of leukocytes should be counted, and the size and appearance of the cells should be evaluated. A close assessment for microorganisms, index of mitosis, and neoplasia should be completed. The utility of the cytospin is that the cytocentrifugation process concentrates all of the cells in a volume of 0.5–1.0 mL of CSF. In the case of a marked pleocytosis, 200 μL typically is sufficient for a differential cell count. If a cytospin is not available, a sedimentation chamber also provides reliable cell counts. Some

Table 1: Cerebrospinal spinal fluid characteristics of canine and feline CNS diseases

Disease	Total protein	Cell counts	Predominant cell type
Viral meningoencephalitis (CDV and other)	Normal – markedly elevated	Normal – moderate pleocytosis	Mononuclear
Bacterial meningoencephalitis	Mildly – markedly elevated	Moderate – marked pleocytosis	Predominantly neutrophilic
Protozoal meningoencephalitis	Mildly – markedly elevated	Moderate pleocytosis	Mixed, occasionally eosinophilic
Fungal meningoencephalitis	Markedly elevated	Moderate – marked pleocytosis	Mixed, occasionally eosinophilic
CNS Parasites	Mildly – markedly elevated	Mild – moderate pleocytosis	Mixed, often eosinophilic
Granulomatous meningoencephalomyelitis	Mildly – markedly elevated	Normal – marked pleocytosis	Variable: mononuclear, mixed, occasionally eosinophilic
Eosinophilic meningoencephalitis	Mildly – markedly elevated	Mild – marked pleocytosis	Eosinophils
Steroid-responsive meningitis-arteritis	Mildly – markedly elevated	Moderate – marked pleocytosis	Acute: neutrophilic; Chronic: mononuclear
Necrotizing meningoencephalitis/ leukoencephalitis	Mildly elevated	Mild – marked pleocytosis	Mononuclear
Feline infectious peritonitis infection	Markedly elevated	Moderate – marked pleocytosis	Mixed, occasionally eosinophilic
Neoplasia	Variable: normal – markedly elevated	Variable: normal – marked pleocytosis	Variable: mononuclear, neutrophilic (eg, meningioma), occasionally eosinophilic or neoplastic cells (eg, LSA)
Degenerative disorders	Normal – moderately elevated	Normal	–
Necrosis	Normal – markedly elevated	Variable: normal – marked pleocytosis	Mixed pleocytosis (often neutrophilic)

CDV, canine distemper virus; CNS, central nervous system; LSA, lymphosarcoma.

labs prefer that protein (fetal calf serum or hetastarch) is added to CSF samples to improve cytospin preparations.[3] This is not critical when CSF samples have a total protein (TP) elevation.

CSF TP: In the dog and cat, normal TP content evaluated from CSF collected from the cerebellomedullary cistern typically is <250 mg/L (25 mg/dL), and it should be <450 mg/L (45 mg/dL) when collected from the lumbar subarachnoid space.[2] Elevated TP serves as a *nonspecific* indicator of CNS disease and it may be caused by either a damaged blood-brain barrier (BBB) or increased local (intrathecal) IgG production. Elevated CSF TP may be present in degenerative, anomalous, metabolic, neoplastic, infectious/inflammatory, traumatic, vascular, and toxic disorders.

Cross-sectional imaging: Although it may be necessary to delay cross-sectional imaging until neurologic signs stabilize, magnetic resonance imaging (MRI) and computed tomography (CT) scans may be valuable in the critical patient. Both imaging modalities typically require general anesthesia, so the decision to perform MRI or CT scan is made on a case-by-case basis. MRI is the gold-standard imaging modality for intracranial disease, as it provides excellent soft-tissue resolution, at times strongly reflective of the gross histopathology associated with specific encephalopathy. Although there are overlapping imaging features among intracranial disorders, MRI often directs the presumptive antemortem diagnosis. The major drawback of MRI, particularly in the critical patient, is that it is a slow imaging modality, especially with low field strength magnets. Conversely, CT scan is a quick imaging tool and may be particularly useful in the acute neurologic patient (eg, head trauma). Despite the limited soft tissue detail provided by CT scan, when coupled with CSF analysis, it may help to provide evidence of ME. The imaging features for several inflammatory brain diseases have been described[4,5]; however, the CT appearance of ME is variable and nonspecific. The presence or absence of contrast enhancement with inflammatory brain disease depends upon the degree of BBB breakdown.[5,6] Despite the fact that it cannot definitively differentiate among disease processes, CT scan may be especially useful for localizing lesions before brain biopsy. An important limitation of CT scan is that it produces a beam hardening artifact (due to preferential absorption of low energy x-ray beams), most notably adjacent to the petrous parts of the temporal bones. This artifact may obscure the clinician's ability to interpret brainstem and cerebellar lesions.

Microbial culture: Infectious diseases should be given consideration when a CSF pleocytosis is present in a dog or cat. Bacterial and fungal culture of CSF typically is reserved for cases in which the index of suspicion is high for infectious disease, including the presence of systemic signs and blood count/biochemical abnormalities (fever, leukocytosis, etc.). In addition to CSF culture, other biological samples such as urine and blood can be cultured to help pursue the diagnosis. Culture of numerous sites such as blood and urine should be considered due to the low diagnostic yield of CSF culture alone.[7]

Serology: Serologic testing also should be considered in acute neurologic patients where there is a high index of suspicion for infectious disease. Serologic testing may be evaluated using serum or CSF, or both. Typical antibody titers evaluated in canine and feline CNS diseases include: *Toxoplasma gondii*, *Neospora caninum*, *Ehrlichia spp.*, *Anaplasma spp.*, *Rickettsia rickettsii*, and *Coccidiodes immitis*. Antigen testing, when available (eg, *Cryptococcus* antigen testing), may circumvent problems associated with the interpretation of antibody testing. Antigen testing, however, may be insensitive because it requires the presence of the organisms in the biological sample under evaluation. In the acute neurologic patient, the authors recommend evaluating antibody titers for regional infectious diseases and also for pathogens to which animals may have been exposed during travel (Figure 2). Although antibody titers reflect direct exposure to the organism, a positive titer does *not* confirm active infection; titers must be evaluated in the context of the patient's signalment, history, clinical signs, and neuro-imaging results. The clinician should recall that IgM and IgG antibodies reflect acute and chronic infections, respectively. As such, a mildly elevated IgM titer in an acute neurologic patient (with no previous neurologic or systemic disease) may support an infectious etiology, whereas elevated IgG titers may be indicative of previous exposure to a pathogen or vaccination, rather than active disease.

Although rarely performed in veterinary practice, serial antibody titers or antibody indices may be helpful for the identification of causative agents as in human neurology.[8] An IgG antibody index may be calculated as a quotient, using the IgG and albumin content of CSF and serum, to assess for intrathecal IgG synthesis:

$$\frac{\text{IgG CSF/IgG serum}}{\text{Albumin CSF/albumin serum}}$$

It has been suggested that the IgG index may distinguish between inflammatory and other disorders of the CNS.[9] Although inflammatory disorders commonly are associated with an elevated (>1.3) IgG index,[10] the IgG index may be normal in certain inflammatory condi-

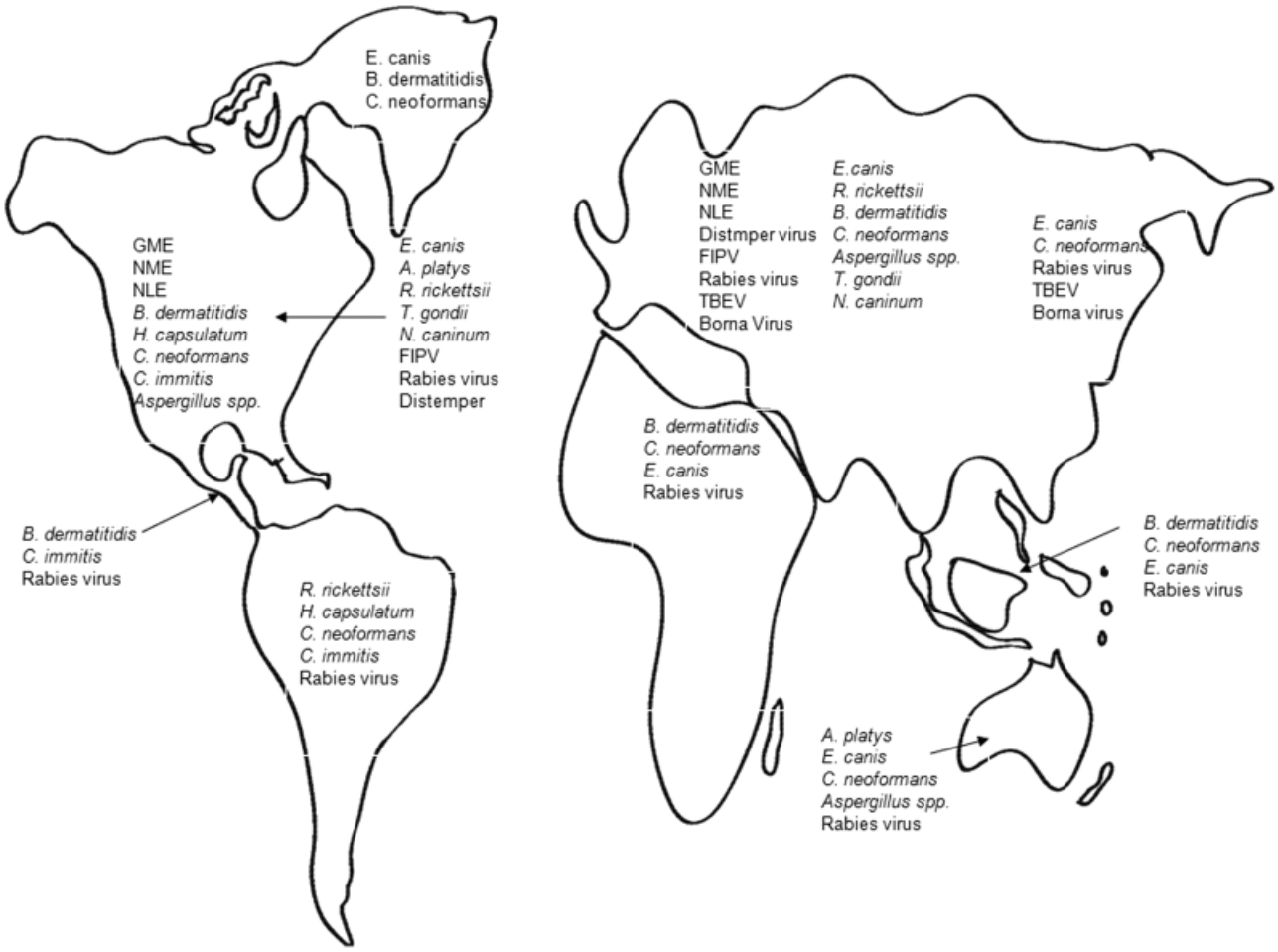

Figure 2: Geographic location of infectious and idiopathic meningoencephalomyelitides. In the United States, *B. dermatitidis* is predominantly located in the Ohio Valley River region, *C. immitis* in the southwestern USA, and *H. capsulatum* in the regions of the Ohio, Missouri, and Mississippi Rivers. GME, granulomatous meningoencephalomyelitis; NME, necrotizing meningoencephalitis; NLE, necrotizing leukoencephalitis; *E. canis*, *Ehrlichia canis; A. platys*, *Anaplasma platys; R. rickettsii*, *Rickettsia rickettsii;* FIPV, Feline infectious peritonitis virus*; B. dermatitidis*, *Blastomyces dermatitidis; H. capsulatum*, *Histoplasma capsulatum; C. neoformans*, *Cryptococcus neoformans; C. immitis*, *Coccidiodes immitis;* TBEV, *Tick-borne encephalitis virus*.

tions (eg, the acute stage of canine distemper virus [CDV] encephalitis).[11] Conversely, neoplastic conditions (eg, lymphoid tumors, meningioma) may be associated with an elevated IgG index. A practical approach is to recognize that an elevated IgG index may be indicative of inflammatory disease; however, it cannot absolutely discriminate between inflammatory and neoplastic disorders.

Measurement of IgA in CSF and serum occasionally may be helpful. For example, a combined elevation of CSF and serum IgA levels is strongly suggestive for steroid responsive meningitis-arteritis (SRMA).[12] Elevation of IgA in CSF alone is less discriminatory and may indicate a primary (infectious/inflammatory disease) or a secondary immune response (eg, neoplasia).

Molecular Diagnostic Testing

Polymerase chain reaction (PCR)

Over the past decade, PCR assays have become utilized routinely in veterinary medicine, providing a mechanism to detect and exponentially amplify small amounts of a microbe's nucleic acids (DNA or RNA) in biological fluids or tissues.

The PCR, applied to CSF, has revolutionized the diagnosis of human CNS infections and has similar potential in veterinary medicine. PCR has shown that 50–70% of human meningoencephalitis (ME) cases have a viral origin.[13,14] Herpes viruses, (Herpes simplex 1, 2, 6; Cytomegalovirus; Varicella-zoster, Epstein Barr) in particular, play a major etiologic role.[15,16] Adeno-,

orthomyxo- (parainfluenza, influenza), picorna- (enterovirus, poliovirus), paramyxo- (measles, mumps, Bornavirus), polyoma- (BK virus, JC virus), flavi- (Japanese encephalitis, tick-borne encephalitis), bunya- (La Crosse), reo- (Rotavirus), toga- (Eastern, Western, Venezuelan encephalitis), retro- (HIV, human T lymphotrophic viruses), arena- (lymphocytic choriomeningitis), and human parvovirus B19 comprise additional viral causes.[13,14,17,18]

The sensitivity and specificity of PCR for the diagnosis of specific viral ME in humans may be >95% and >99%, respectively, when CSF is tested between 48 hours and 10 days after the onset of neurologic signs.[16,17] This allows for rapid implementation of targeted antiviral therapies and excellent survival rates in humans compared with the situation in critical veterinary patients with neurologic disease. In dogs and cats with acute meningoencephalitis of unknown etiology (MUE), PCR of CSF should be considered to test for regional pathogens. When combined with serologic testing, this should maximize the chances of identifying a causative agent.

While PCR may be extremely useful for the identification of minute amounts of DNA or RNA from an infectious agent, PCR is not without pitfalls and results must be interpreted carefully. For example, the tremendous sensitivity of PCR creates the potential for reamplification of previously positive PCR reactions (so called PCR contamination). To avoid false positives with diagnostic PCR, rigorous negative controls must be evaluated in parallel to clinical samples. It is equally important to run positive controls and to perform *housekeeping PCR* on a canine or feline gene from the case in question. Without positive controls, one cannot exclude the possibility of PCR inhibitors (eg, hemoglobin, IgG) or problems with the nucleic acid extraction procedures, creating the possibility of false negative results. Finally, it should be noted that a negative PCR result does not *definitively* rule out infectious ME for 3 important reasons: (1) nucleic acids may be present in CSF, but at undetectable levels; (2) nucleic acids from organisms may be present in the CNS parenchyma but not in the CSF; and (3) the disorder may have been triggered by a pathogen that is no longer present. There are several permutations of PCR biotechnology used in the veterinary and human medicine including conventional PCR, multiplex PCR (mPCR), reverse transcriptase (RT)-PCR, quantitative PCR (qPCR), and consensus and degenerate PCR.

Conventional PCR

Conventional PCR can be utilized to test for the presence of DNA from infectious agents. To perform PCR, DNA must first be extracted from a biological sample (eg, CSF) along with the DNA of the patient. After extraction, DNA is combined with various reagents including 2 oligonucleotide primers (short DNA sequences complementary to flanking ends of a target DNA sequence), a DNA polymerase and individual nucleotides (adenine, guanine, cytosine, and thymine). Initially, the mixture is heated to a high temperature to separate the DNA double helix into individual strands. Next, the temperature is lowered to promote the annealing of the PCR primers to the target DNA, and then raised slightly, to promote the addition of nucleotides to a growing strand of DNA (a so-called amplicon). This process is repeated 25–45 times, exponentially amplifying the DNA sequence of interest. Conventional PCR is utilized routinely to test for DNA from *Rickettsia spp.*, *Ehrlichia spp.*, *Anaplasma spp.*, *Bartonella spp.*, *Borrelia spp.*, *T. gondii*, *N. caninum*, and many bacteria and fungi.[19–23]

Nested PCR

A variant of classical PCR termed nested PCR is utilized for analysis of specimens in which very few pathogen particles are presumed to be present (such as CSF), with the goal of substantially increasing the sensitivity and specificity of the PCR.[24] In nested PCR, the first PCR is followed by an additional amplification with a second set of primers, which are complementary to sequences internal to the sequence targeted by the first set of primers.

RT-PCR

RT-PCR is used to identify nucleic acids from viruses with an RNA genome. Because the initial targeted nucleic acid is RNA, the enzyme RT must be utilized to first create a complimentary strand of DNA (cDNA) from the original RNA. Subsequently, the cDNA is amplified as per conventional PCR above. RT-PCR has been utilized to aid in the diagnosis of various CNS viral infections including feline infectious peritonitis virus (FIPV), CDV, Borna virus, and tick-borne encephalitis virus.[25–27]

mPCR

mPCR is a permutation of conventional PCR that allows for the simultaneous amplification of 2 or more DNA targets. This requires a PCR primer pair for each microorganism or host gene under investigation. While the technique has the potential to amplify several pathogens simultaneously, multiplex reactions may have lower sensitivities than standard singleplex reactions due to competition of multiple primer pairs and PCR reagents or preferential amplification of one amplicon over the other.[28] We have used mPCR successfully to amplify both *T. gondii* and *N. caninum* from cases of canine ME.[29]

qPCR

qPCR allows for objective quantification of a target nucleic acid in a given sample. With this technique, a fluorescent probe binds the target DNA sequence (or a fluorescent marker is intercalated into the amplicon), and the PCR product can be quantified by a specialized thermocycler. This method has several advantages over conventional PCR, including the elimination of post-amplification steps and the potential for increased sensitivity due to the use of fluorescent markers.[30] Quantitative real-time PCR allows one to observe the quantification cycles of the PCR amplicon simultaneously while the reaction is being performed.[31] qPCR assays are available for CDV, *T. gondii*, *Bartonella spp.*, and canine rickettsial organisms (*R. rickettsii*, *E. canis*).[32–34]

Consensus and degenerate PCR

Genome-based assays, including PCR-based assays, are powerful tools to detect pathogens, but are limited to individual pathogen species. Broadly reactive methods including consensus and degenerate PCR have been developed to identify novel or unsuspected pathogens in idiopathic diseases. Consensus PCR targets highly conserved genomic regions that are present in all members of a pathogen family or genera. With consensus PCR, amplicons must be subject to sequence analysis to determine their identity. An example of consensus PCR is eubacterial, or so-called universal bacterial PCR, which typically targets the highly conserved bacterial 16S ribosomal RNA (rRNA) gene. In human neurology, eubacterial PCR on CSF has been shown to be more sensitive than CSF culture for the diagnosis of a bacterial ME,[35] but contamination and the potential for false positive results present a major challenge with this diagnostic test.

Degenerate PCR is similar to consensus PCR and also targets conserved genomic regions that are present within all pathogens in a family or genera. However, with degenerate PCR a mixture of PCR primers is utilized to account for minor variations in one or more nucleotide positions in the targeted DNA region. As for consensus PCR, positive PCR products must be sequenced to determine their identity. This methodology has demonstrated the presence of herpes viruses in several animal diseases previously considered idiopathic or thought to have autoimmune pathogenesis, including Kaposi's sarcoma-associated herpes virus-like herpes viruses in chimps and gorillas,[36] a novel γ-2-herpes virus in chimpanzees,[37] and a novel herpes virus in tortoises.[38] Degenerate PCR also was utilized in landmark investigations for the identification of *Bartonella henselae* as the elusive pathogen responsible for bacillary angiomatosis in 1990,[39] and *Tropheryma whippelii* as the cause for Whipple's disease in 1991/1992.[40]

A third, emerging PCR technique is a combination of consensus and degenerate PCR that utilizes consensus degenerate hybrid oligonucleotide (CODEHOP) primers. This methodology not only amplifies nucleic acids from known members of a microbial family or genera, but is optimized to detect novel microbes due to the evolutionary relationships of the organisms. CODEHOP PCR has been used successfully to identify and characterize a number of novel human viruses including Severe Acute Respiratory Syndrome coronavirus,[41] hepatitis G,[42] Sin Nombre virus,[43] Human retrovirus-5,[44] and novel animal viruses such as the macaque γ-herpes virus and pig endogenous retrovirus.[44,45] Recently, we have utilized pan-viral CODEHOP PCR screening strategies in collaboration with the Centers for Disease Control (CDC) in ongoing investigations of canine MUE.[46]

Microarray analysis

Microarray analysis has emerged as a leading technology in molecular biology over the past decade. With this technology, hundreds to thousands of DNA sequences are arranged on a microchip for subsequent DNA or RNA hybridization studies. There are several diagnostic techniques and applications that utilize microarray technology including gene expression profiling, single nucleotide polymorphism detection, comparative genomic hybridization, chromatin immunoprecipitation on chip, alternative splicing detection, tiling arrays, and infectious disease diagnostic testing.[47]

In human neurology, microarray analysis has been utilized to detect the presence of infectious agents in cases of MUE.[48,49] Some labs report that microarray analysis has similar sensitivity and specificity to conventional PCR.[50] The advantage of this technique is that it allows for screening of multiple pathogens simultaneously; in theory, hundreds of microorganisms. Moreover, the technique is not biased toward specific or regional pathogens; therefore, a priori knowledge of potential infectious organisms is not necessary. The disadvantage is that microarray studies require sophisticated and expensive instrumentation. The potential role of this biotechnology in the veterinary setting remains to be determined.

Conventional and Molecular Diagnostic Testing in the Acute Neurologic Patient

Overview

The clinical presentation of the acute neurologic patient is variable and typically reflects the arrangement and location of the CNS lesions. Although the spinal cord

may be affected in acute neurologic patients, the clinical signs associated with encephalopathies are primarily considered here (Table 2). The differential diagnosis for dogs or cats that are presented for an acute neurologic disease includes congenital abnormalities, metabolic derangements, infectious and idiopathic ME, neoplasia, trauma, and toxin exposure (Table 3). In the remainder of this manuscript we focus on ME and the accompanying conventional and molecular diagnostic testing that may direct a presumptive or definitive diagnosis. A discussion of the current treatment recommendations for the various canine and feline infectious meningoencephalitides is beyond the scope of this review.

Table 3: Differential diagnoses for acute neurological signs

Meningoencephalomyelitis (infectious and idiopathic)
Metabolic derangements
Congenital anomalies (decompensating hydrocephalus, chiari-like malformation)
Tumors of the meninges (histiocytosis, lymphoma, meningioma)
Intervertebral disc disease
Atlantoaxial subluxation
Cerebrovascular accident
Head trauma
Mycotoxin and neurotoxic ingestion

SRMA, steroid-responsive meningitis-arteritis; GME, granulomatous meningocencephalomyelitis.

Meningoencephalomyelitis (MEM): definition and clinical signs

MEM is defined as inflammation of the meninges (dura, arachnoid, and pia mater) and the neuroparenchyma (ie, cereberal hemispheres, thalamus, brainstem, cerebellum, and spinal cord) and can be associated with various infectious and idiopathic CNS diseases (Table 4).

Table 2: Potential regions of brain and associated signs in dogs and cats presented for acute onset encephalopathies

Region of brain affected	Potential neurologic signs
Cerebrum/Thalamus	Seizures; changes in sensorium or behavior; circling and other propulsive activity; contralateral postural reaction deficits; contralateral visual impairment with normal pupillary light responses (rarely anisocoria); contralateral hypalgesia (especially nasal).
Midbrain	Depressed sensorium; postural reaction deficits; opisthotonus; mydriasis (+/− anisocoria), abnormal pupillary light responses, normal vision.
Pons/Medulla	Depressed sensorium; gait abnormalities ranging from paresis through recumbency; ipsilateral postural reaction deficits; opisthotonus; multiple cranial nerve deficits including: atrophy of muscles of mastication (V), facial hypalgesia (V), head tilt (VIII), resting or positional nystagmus (VIII), abnormal physiologic nystagmus (III, IV, VI, VIII), resting or positional strabismus (III, IV, VI, VIII), facial paresis or paralysis (VII), dysphagia (IX, X), tongue paresis or paralysis (XII); respiratory or cardiac abnormalities.
Cerebellum	Hypermetric/spastic gait with strength preserved; loss of balance truncal sway; intention tremor of head, neck or eyes; opsithotonus and extensor rigidity of all limbs with hips flexed; menace deficit with normal vision and normal pupillary light responses.

MEM may be focal, multifocal, or disseminated and clinical signs typically are asymmetric in nature. Clinical signs of encephalitis reflect the location of the lesion(s) within the prosencephalon (cerebral hemispheres and thalamus), brainstem, or cerebellum (Table 2). Clinical signs of myelitis reflect whether the spinal cord lesion(s) are within the upper motor neuron or lower motor neuron system, or both. Pain or hyperesthesia, or both, are common with meningitis (brain or spinal cord). Because of the typical *acute* and *progressive* nature of MEM, dogs and cats commonly are presented on an emergency basis.

Infectious CNS diseases of the dog and cat

Infectious causes for acute MEM are proven infrequently in veterinary medicine, but are important differentials nonetheless. Infectious differentials for MEM should be pursued vigorously to help differentiate infectious MEM from autoimmune and neoplastic disorders. Pathogens that may cause canine or feline MEM include bacteria, viruses, protozoa, fungi, and rarely rickettsia and parasites (Table 4). Infectious MEM occurs most commonly in young or immunocompromised animals; however, any dog or cat may develop MEM. Some infectious agents are capable of affecting multiple organ systems in addition to the CNS, which may prove helpful in distinguishing CNS infections from autoimmune/idiopathic meningoencephalitides (Figure 3). The severity of CNS infection is dependent on several factors including: the status of the animal's immune system at the time of inoculation, strength of the animal's immune response during active infection, nutritional status of the animal, strain and virulence factors of the infectious agent, and environmental factors. Here we review the most common infectious CNS diseases in the dog and cat and the conventional and novel molecular diagnostic tests used to detect them in CSF and other tissues.

Table 4: Infectious and idiopathic meningoencephalomyelitides

- Infectious MEM
  - Bacterial
    - Aerobic (c,d)
    - Anaerobic (c,d)
  - Viral
    - Rabies virus (d)
    - Canine distemper virus (c)
    - Feline infectious peritonitis virus (c) Feline leukemia virus (FeLV) (c)
    - Borna disease virus (c,d; Europe and Japan)
    - Tick-borne encephalitis virus (d; Europe and Asia)
    - West Nile virus (d; North America)
    - Eastern equine encephalitis virus (d; North America; rare)
  - Protozoal
    - *Neospora caninum* (d)
    - *Toxoplasma gondii* (c,d)
    - *Sarcocystis canis* (d; rare)
    - *Encephalitozoon cuniculi* (d; rare)
    - *Trypanosoma cruzi* (d; rare)
    - *Acanthamoeba spp.* (d; rare)
    - *Babesia spp.* (c, d; rare)
    - *Leishmania spp.* (c, d; North America, Mediterranean basin, and Portugal)
  - Rickettsial
    - *Rickettsia rickettsii* (d)
    - *Ehrlichia spp.* (d)
    - *Anaplasma spp.* (d)
  - Fungal
    - *Cryptococcus spp.* (c, d)
    - *Coccidiodes immitis* (c, d)
    - *Blastomyces dermatitidis* (c, d)
    - *Histoplasma capsulatum* (c, d; rare)
    - *Aspergillus spp.* (c, d; rare)
  - Parasitic
    - *Cuterebra* larva migration (c, d; rare)
    - *Dirofilaria immitis* – aberrant migration (c, d; rare)
- Idiopathic (autoimmune) MEM
  - Granulomatous meningoencephalomyelitis (GME)
  - Necrotizing encephalitis (NME)
  - Necrotizing leukoencephalitis (NLE)
  - Meningoencephalitis of unknown etiology (MUE)
  - Steroid-responsive meningitis-arteritis (SRMA)
  - Idiopathic tremor syndrome

Bacterial meningitis and CNS abscesses

Bacterial MEM is diagnosed infrequently in dogs and cats. Extraneural signs are present in approximately 40% of cases and include fever (most common), urinary tract infection, conjunctivitis, gastrointestinal signs, upper respiratory infection, sinusitis, retrobulbar disease, and vomiting.[7,51–55] Hematogenous spread from distant foci to the CNS (eg, ear and eye infections, bite wounds, vegetative endocarditis, urinary tract infections, lung abscesses) is a common route of CNS infection.[7] Penetrating foreign bodies occasionally are the source of infection. Interestingly, the majority of animals in case reports are neither febrile nor systemically ill. Clinical signs may include neck pain, hyperesthesia along the vertebral column, and potentially multifocal or focal neurologic signs.[7]

Diagnostic testing: With bacterial ME, CSF typically has a marked elevation in neutrophils and TP. CSF cultures may be positive but often show no growth (occasionally, even in cases where bacteria are visualized in the CSF). MRI may be normal or may disclose the presence of diffuse or focal meningeal enhancement, ventriculitis, and possibly brain edema. Alternatively, MRI may disclose the presence of a focal abscess. In the case of otitis media/interna, with central extension to the brainstem, a contiguous contrast-enhancing lesion may be present in the inner ear (and tympanic bullae) and the brainstem with adjacent meningeal enhancement.

Universal or consensus bacterial PCR of the 16S rRNA gene common to all bacteria has been utilized recently in a case of canine ME in which *Streptococcus* DNA was identified in CSF, despite negative urine, blood, and CSF cultures.[56] At the University of Georgia, College of Veterinary Medicine, we have developed similar methodologies for the detection of 16S bacterial sequences in veterinary patients. Although extremely sensitive, PCR contamination and the potential for false positive results present a considerable challenge with eubacterial PCR.

CDV

CDV (family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*) causes multisystemic disease in dogs. Infection is initiated in lymphoid tissue of the oropharynx with subsequent spread to the respiratory, alimentary, urogenital, and CNS.[57] CNS lesions generally consist of varying degrees of lymphoplasmacytic inflammation, demyelination, and necrosis. The clinical course and nature of the CDV CNS disease in dogs is complex and a detailed coverage is beyond the scope of this review. However, the acute encephalitis typically seen in young puppies 2–6 months of age is discussed below[57]:

1. Initial CNS infection: typically dogs are *asymptomatic* and have a mild nonsuppurative leptomeningitis and perivascular encephalitis.
2. Gray matter (GM) disease (approximately 1 w post-infection): neurologic signs due to a nonsuppurative ME. Dogs with GM disease often die within 2–3 weeks (commonly with seizures), may recover with a prompt immune response, or progress to white matter (WM) disease.
3. WM disease (approximately 3 w post-infection): Most common form of clinical CDV – likely follows a subclinical GM infection. The primary lesion is demyelination with relative axonal sparing.

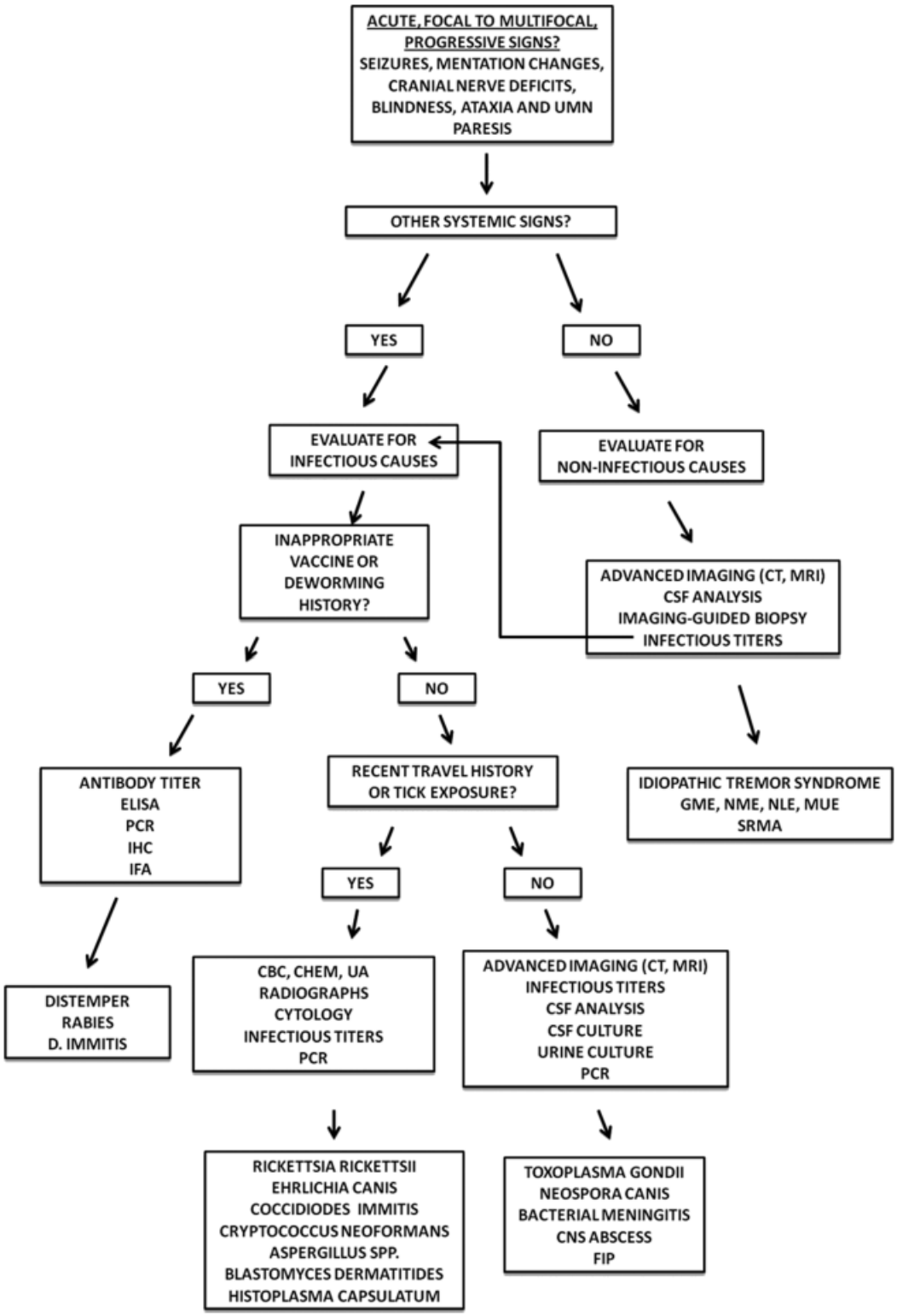

Figure 3: Diagnostic algorithm for infectious and idiopathic meningoencephalitis. UMN, upper motor neuron; CSF, cerebrospinal fluid; CT, computed tomography; MRI, magnetic resonance imaging; GME, granulomatous meningoencephalomyelitis; NME, necrotizing meningoencephalitis; NLE, necrotizing leukoencephalitis; MUE, meningoencephalitis of unknown etiology; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbant assay; IHC, immunohistochemistry; IFA, immunofluorescent antibody test; AGID, agar gel immunodiffusion; CBC, complete blood count; CHEM, serum chemistry panel; UA, complete urinalysis.

Dogs with WM disease may deteriorate and die within 4–5 weeks with *noninflammatory* demyelination, recover with minimal CNS injury, or may develop a persistent CNS infection that may progress to necrotizing encephalomyelitis.

4. Necrotizing encephalomyelitis (approximately 4–5 w post-infection): Nonsuppurative inflammation follows the initial primary demyelination phase of the disease. This is thought to be secondary to exuberant inflammatory WM lesions (exposure of previously hidden antigens). In addition to CNS lesions, chorioretinitis and uveitis may be seen. Some dogs will deteriorate and die with NE while others may slowly recover.

Diagnostic testing: While CNS histopathology and immunohistochemistry typically provide postmortem confirmation of CNS CDV infection,[58,59] antemortem diagnosis of CNS CDV infection is more challenging. Because CSF and MRI findings are highly variable with CDV infection,[60] diagnostic techniques with greater specificity are needed. Immunohistochemical testing for CDV antigen on biopsies of nasal mucosa, footpad epithelium, and the haired skin of the dorsal neck has been reported to detect CDV antigen in 88% (24/27 cases from epithelial cells of the nasal mucosa) to 96% (26/27 cases from a skin biopsy of the dorsal neck region), depending on the tissue sampled.[61] Similarly, RT PCR applied to RNA extracted from whole blood, urine, CSF, tonsilar, or conjunctival specimens may help with the diagnosis of CNS CDV infection.[32,62–64] Recently, the CDC has reported on a panel of 4 semi-nested RT-PCR assays that utilize CODEHOP primers for the diagnosis of paramyxovirus infections. We have utilized this pan-paramyxovirus PCR to determine CDV as the etiology in an unusual case of necrotizing meningoencephalitis (NME).[65]

FIPV

FIPV is a highly pathogenic mutant variant of the nonpathogenic feline enteric corona virus (FECV). Cats 6 months to 2 years of age are most commonly infected, followed by senior cats 15 years of age or older.[66] Depending on a cat's immunologic response to FIPV, either the effusive (wet) or noneffusive (dry) form may occur. MEM is most commonly associated with noneffusive FIP, with up to 30% of infected cats with clinical signs having neurologic involvement.[67] CNS FIP is characterized by a diffuse meningitis, ependymitis, and adjacent encephalomyelitis.[57]

Diagnostic testing: The CSF of cats with neurologic FIP commonly contains a mononuclear to neutrophilic pleocytosis and an elevated TP, although normal CSF does not rule out the disorder.[68] MRI and CT scan may show periventricular or meningeal contrast-enhancement or both, often with hydrocephalus.[68] Definitive diagnosis requires necropsy. Most cases have measurable serum titers to FECV/FIPV, but the immune responses to these 2 organisms are indistinguishable, so it is not possible to discriminate between the two. Occasionally, affected cats will have a negative coronavirus titer. Therefore, serum FIPV titers have a limited role in the diagnosis of FIP infection in cats. One study suggested that 15 of 16 cats had CSF FECV/FIV titers that were higher than expected (given the serum titers and the degree of blood-CSF barrier disturbance).[68] However, this should be interpreted cautiously. Positive RT-PCR of CSF is consistent with clinical FIP, but PCR has been shown to have limited sensitivity in one study (5/16 CSF samples and 10/15 brain samples were positive on confirmed cases).[68] Real time qPCR has not been evaluated rigorously in CNS FIP, but has the potential to improve both the specificity of sensitivity of FIPV detection. One RT PCR for FIPV RNA is available through Auburn University that shows promise for differentiating pathogenic from nonpathogenic FIPV infection. However, more studies are required for optimal validation.

Protozoal infections

Dogs and cats are definitive hosts for *N. caninum* and *T. gondii*, respectively. Transmission of *T. gondii* occurs by carnivorous ingestion (most common), orofecal contamination, or transplacentally. Clinical toxoplasmosis most commonly affects puppies and kittens and also immunocompromised dogs and cats. With CNS toxoplasmosis, granulomatous-like lesions or diffuse nonsuppurative MEM may be present. Consequently, the neurologic presentation may be that of a focal or multifocal neurolocalization.

The life cycle of *N. caninum* is not completely understood, but infection during the neonatal period is suspected. In juvenile dogs <6 months of age, myositis, ascending polyradiculoneuritis, and encephalomyelitis predominate. Rigid limb contracture and arthrogryposis may occur as a result of neuritis and myositis. Severe MEM tends to be rare with *N. caninum* infection in young dogs. In dogs >1 year of age MEM (commonly cerebellitis) is the more typical presentation.[69]

Diagnostic testing: CSF analysis may reveal a mononuclear-polymorphonuclear pleocytosis and TP elevation. Serology may disclose an increase in *T. gondii*- or *N. caninuum*-induced antibodies. Serologic tests that are available for *T. gondii* are the indirect fluorescent antibody test (IFA), modified agglutination test, and the enzyme-linked immunosorbant assay (ELISA). An increase in *T. gondii* IgM antibody titers $>1:64$ is more

indicative of active infection than an increase in IgG antibody titers (which may indicate exposure only).[70] Serial increases in *T. gondii IgG* antibody titer >1:64 are needed to support an active infection. However, false positive antibody testing may occur because of the lack of specificity of anti-*Toxoplasma* immunoglobulins.[70]

Several serologic assays exist for *N. caninum* IgG antibody, including IFA, ELISA, and immunoprecipitation. IgG titers >1:64 are considered suspect.[70] Immunohistochemistry on muscle or nerve biopsies occasionally reveal organisms within these tissues.[71] mPCR has been shown to be useful for detection of CNS toxoplasmosis and neosporosis in the dog and cat.[29] The authors recommend serology (serum and CSF) and CSF PCR for the diagnosis of active *T. gondii* and *N. caninum* infection.

Rickettsial infections

Rickettsial organisms including *R. rickettsii*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* are uncommon etiologies for canine MEM.[57] They are transmitted to the dog via various tick vectors. Once rickettsial organisms infect a dog or cat, they enter endothelial cells, leading to a vasculitis of multiple organ systems including the CNS. Lymphoplasmacytic meningitis predominates; however, encephalomyelitis may occur when the underlying neuroparenchyma is affected. Although *Bartonella spp.* are not within the rickettsial family, they are considered here as uncommon causes of canine MEM. The etiopathogenesis of CNS *Bartonella* infection is not fully understood, but a suppurative to pyogranulomatous MEM predominates.[72–75] In a recent report of 3 dogs with *Bartonella* myelitis, all dogs had a concurrent pyogranulomatous dermatitis or panniculutis.[72]

Diagnostic testing: With CNS rickettsial infections, CSF analysis may reveal a mild to moderate mononuclear pleocytosis and TP elevations. Serologic testing, typically via IFA, can be used to help support a diagnosis of rickettsial infection. Recently, nested PCR was used to evaluate early infection of *E. canis* in dogs compared with the traditional IFA assay. The PCR assay proved to be a more sensitive test in the early stages of infection before *Ehrlichia*-induced antibodies were detectable in the serum.[76] We have recently utilized broadly reactive, consensus qPCR assays for *Rickettsia spp.*, *Ehrlichia spp.*, *Anaplasma spp.*, and *Borrelia spp.*, in a large cohort of cases of MEM and identified only a single case that was positive for *Bartonella* DNA.[34]

Mycotic infections

Mycotic agents occasionally infect the CNS of dogs and cats. *Cryptococcus neoformans* and *Cryptococcus gatii* are the most common CNS mycotic infection reported in these species.[77,78] *Blastomyces dermatitidis*, *Coccidiodes immitis*, *Histoplasma capsulatum*, and *Aspergillus spp.* comprise additional fungal causes of canine CNS infection.[70] The most common route of inoculation for a mycotic agent is inhalation. Morphologic conversion to the yeast form occurs in the animal and hematogenous or lymphatic spread to other organ systems may occur. Fungal organisms may cause an acute, focal, or multifocal-diffuse MEM. Clinical signs may be variable and often suggest multifocal disease.

Diagnostic testing: CSF analysis may reveal a mononuclear or polymorphonuclear pleocytosis and elevated TP. Occasionally, organisms may be identified within tissues and body fluids including CSF. Serologic assays including the latex agglutination (*C. neoformans*) and complement fixation or agar gel immunodiffusion (*C. immitis*) are sensitive and specific tests.[79,80] Serology also exists for *Aspergillus spp.* as well. With fungal MEM, MRI, or CT scan may disclose evidence of diffuse brain lesions, a fungal granuloma or pseudocyst (in the case of *Cryptococcus spp.*). If the patient is cytologically negative and serologically positive for a mycotic agent, fungal culture should be performed on CSF to help ascertain a definitive diagnosis.[70] Culture, cytology, and India ink testing also may disclose organisms if there is sufficient CSF. Biopsy, culture, or cytology of samples from extra-neural sites should be considered as well. A consensus pan-fungal PCR assay has been utilized recently in experimental *Zygomycetes* infection in rabbits; this method may prove useful to detect CNS mycotic infections in dogs and cats.[81]

SRMA

The numerous and sometimes colorful synonyms (eg, *necrotizing vasculitis*, *polyarteritis*, *panarteritis*, *juvenile polyarteritis syndrome*, *beagle pain syndrome*, *corticosteroid-responsive meningitis*, *aseptic suppurative meningitis*, *sterile meningitis*) for SRMA are reflective of both the clinical and histopathologic features associated with the syndrome. However, the diverse terminology for this disorder sometimes generates confusion among general practitioners and veterinary specialists. The name SRMA is well established in the veterinary literature and best describes the pathologic and clinical features of the disease, being a systemic immune disorder characterized by inflammatory lesions of the leptomeninges and the associated arteries that typically is responsive to corticosteroids.[1] The disorder may occur in any breed of dog, although Beagles, Boxers, Bernese Mountain Dogs, Weimaraners, and Nova Scotia Duck Tolling Retrievers are overrepresented. Age of onset is commonly between 6 and 18 months with a range from 4 months to 7 years.[12]

SRMA is a sporadic disorder characterized by episodes of profound cervical hyperesthesia, depression, and pyrexia.[1] Clinical signs result from a combined meningitis and arteritis of leptomeningeal vessels. The arteritis also may involve the vessels of the heart, mediastinum, and thyroid glands.[57] Occasionally, SRMA occurs concurrently with immune-mediated polyarthritis.[82] Two forms of SRMA exist including the classic, acute form and the chronic, protracted form. In acute SRMA, dogs most commonly present with hyperesthesia along the vertebral column, cervical rigidity, stiff gait, and fever.[83] Affected animals often manifest a hunched posture with profound guarding of the head and neck, sometimes mimicking a cervical intervertebral disc protrusion. Dogs may be so painful that *any* manipulation elicits a painful response. A second, more chronic form of SRMA may occur following relapses of acute disease or inadequate treatment.[83] In this form of disease, meningeal fibrosis secondary to the inflammatory process may obstruct CSF flow or occlude the vasculature, rarely causing secondary hydrocephalus or ischemia of the CNS parenchyma, respectively.[59] Involvement of the motor and proprioceptive systems may lead to variable degrees of paresis and ataxia; other neurologic signs such as a menace deficit, anisocoria, or vestibular signs may occur with severe disease.

Diagnostic testing: Analysis of the CSF in acute disease reveals a marked polymorphonuclear pleocytosis in addition to an elevated protein and variable red blood cells.[9,83] Red blood cells may be present in CSF secondary to leakage from damaged vessels or contamination from peripheral blood. Typically, the CSF neutrophils have no toxic changes; however, in severe cases, both banded and segmented neutrophils may be observed. The CSF in the chronic form of SRMA may be variable, consisting of predominantly mononuclear cells or a mixed cell population with normal or mildly elevated TP.[9] Bacterial cultures are negative. Radiographs of the cervical vertebral column are normal. CT scan or MRI may demonstrate contrast enhancement of the meninges.[84] In some dogs, the inflammation also affects the meninges of the brain and the choroid plexus.[85]

In both forms of SRMA, diagnostic testing may show a neutrophilia with a left shift, an increased erythrocyte sedimentation rate, and an elevated α-2-globulin fraction.[12] The majority of affected dogs have elevated IgA levels in both the CSF and serum, a finding that is most likely secondary to dysregulation of the immune system.[9,86,87] Elevated serum and CSF IgA levels help differentiate SRMA from other idiopathic and infectious canine meningoencephalitides; however, elevated IgA levels may be associated with primary or secondary inflammation. Elevated IgM or IgG, or both, in the CSF also have been documented.[86] More recently, acute phase proteins (APPs), including C-reactive protein (CRP) and α-2-macroglobulin, have been shown to be elevated consistently in the serum of dogs with SRMA.[60] However, elevation of APPs is not pathognomonic for the disorder and other systemic inflammatory diseases should be included in the differential diagnosis when present. Once SRMA has been confirmed, elevated CRP serum concentrations may be used reliably to monitor response to therapy, rather than repeated CSF collection and analyses.[60] These results were confirmed recently by Lowrie et al.[88]

Granulomatous meningoencephalomyelitis (GME)

GME is difficult to distinguish from the various forms of MEM on clinical grounds, but may represent up to 25% of canine CNS disease.[86] Typically, GME presents as an acute onset, progressive, focal to multifocal neurologic disease that may be fatal if left untreated.[57,89] Females and Toy and Terrier breeds are overrepresented for GME; however, both sexes and all breeds may be affected. The mean age of onset of neurologic signs is 55 months (range, 6–144 months).[89] Clinical signs reflect focal or multifocal CNS disease and they are dependent on the lesion location within the neuraxis. Neurologic deficits referable to the caudal fossa (vestibulo-cerebellar signs) and cervical spinal cord, in addition to seizures and visual deficits, have been reported most frequently.[57]

Three forms of GME have been described based on both morphological and clinical neurologic abnormalities: disseminated, focal, and ocular.[1] The disseminated form is most common and it typically manifests as an acute onset of rapidly progressive, multifocal neurologic signs involving the cerebrum, caudal brainstem, cerebellum, or cervical or thoracolumbar spinal cord.[89] Neurologic signs associated with the uncommon, focal form of GME typically are slowly progressive and they are suggestive of a single space-occupying mass lesion.[89,90] In the focal form of GME, solitary granuloma-like lesions may form in the cerebrum, caudal fossa, or spinal cord.[57] Focal GME must be differentiated from CNS malignant histiocytosis and primary CNS lymphosarcoma. The ocular form of GME manifests with an acute onset of visual impairment, variable pupillary changes (commonly dilated and unresponsive), variable degrees of optic nerve swelling, and occasionally chorioretinitis, especially in the nontapetal fundus.[57,90,91] Dogs with ocular GME may concurrently have or progress to develop disseminated CNS lesions.

Diagnostic testing: The antemortem diagnosis of GME is challenging, because histopathologic confirmation is required for a definitive diagnosis. In most cases, a presumptive antemortem diagnosis is achieved via a multimodal approach that includes: assessment of case signalment, neurologic signs and neuroanatomic localization, CSF analysis, cross-sectional imaging, and infectious disease testing. The antemortem diagnosis often is complicated by an overlap in the neurodiagnostic profiles (especially between GME, infectious ME, and CNS neoplasia). Therefore, the terminology MUE may be preferable on an antemortem basis in cases of idiopathic ME where histopathologic testing is lacking.[92]

In all forms of GME, meningeal inflammation may result in mild to severe CSF mononuclear pleocytosis and a TP elevation; however, the CSF occasionally is normal. Although not specific for GME, the most common MRI findings for the disseminated form include multiple hyperintensities on T2-weighted or fluid-attenuated inversion recovery (FLAIR) sequences scattered throughout the CNS white matter.[93] These lesions typically assume an infiltrative appearance and have irregular margins. Despite the predilection of the GME for white matter, MRI lesions often are distributed throughout both gray and white matter. The lesions have variable intensity on T1-weighted images and have variable degrees of contrast enhancement.[93] Vasogenic edema in the white matter is commonly present on T2-weighted images and appears hyperintense to the neuroparenchyma. Although meningeal enhancement has been described,[84] it is not commonly apparent. Infectious MEM, CNS lymphosarcoma, and less commonly metastatic neoplasms may present with similar MRI findings to disseminated GME, and discriminating among these differentials may be challenging.

Necrotizing encephalitis (NE)

NME and necrotizing leukoencephalitis (NLE) are CNS inflammatory disorders with similarly elusive etiopathogeneses to that of GME. Historically referred to as Pug Dog Encephalitis and Necrotizing Encephalitis of Yorkshire Terriers, respectively, these idiopathic meningoencephalitides have now been reported in various Toy breeds including the Pug, Maltese, Chihuahua, Yorkshire Terrier, Pekingese, West Highland White Terrier, Boston Terrier, Japanese Spitz, and Miniature Pinscher.[59,94–98] To avoid confusion associated with the breed specific terminology, we have suggested that these inflammatory disorders are best described with neuropathologic nomenclature reflective of the topographies of the brain lesions associated with each (eg, NME and NLE).[92] Because of the overlap in clinical signs and neuropathology, the encompassing term NE may be preferable on an antemortem basis.

The onset of neurologic signs associated with NME varies from 6 months to 7 years of age, and most commonly occurs in young dogs, with a mean age of 29 months.[94,99] NLE typically manifests between 4 months and 10 years of age, with a mean age of onset of 4.5 years.[100] Dogs with both NME and NLE commonly manifest cerebro-thalamic signs due to the predominance of lesions in the prosencephalon; NLE also may cause mid to caudal brainstem signs.[1] However, due to the multifocal nature of inflammatory disease, variations may occur with either disorder and clinical signs are primarily reflective of the lesion locations. The signs associated with NE typically are rapidly progressive and most commonly include seizures, depression, circling, vestibulo-cerebellar signs, visual deficits, and ultimately death.

Diagnostic testing: The CSF profiles for NME and NLE overlap with a mononuclear (lymphocytes, monocytes) pleocytosis and TP elevations being most common. Typical MRI lesions associated with NME include asymmetric, multifocal prosencephalic lesions affecting the gray and white matter, with variable contrast enhancement on T1-weighted imaging. Loss of gray/white matter demarcation also may be discernible. Lesions appear hyperintense on T2-weighted images and isointense to slightly hypointense on T1-weighted images, with slight contrast enhancement. In NLE, multiple, asymmetric bilateral prosencephalic lesions mainly affecting the subcortical white matter have been described. The NLE lesions are hyperintense on T2-weighted and FLAIR images and often include multiple cystic areas of necrosis. These lesions are hypointense or isointense on T1-weighted images and contrast enhancement is variable.[101]

Conclusion

In the acute neurologic patient, there are several key infectious diseases that can be pursued by a combination of conventional and molecular diagnostic testing. It is important that the clinician understands the utility, as well as the limitations, of the various neurodiagnostic tests that are available in the critical care setting. When a multimodal neurodiagnostic approach is utilized, it is often possible to differentiate infectious ME from autoimmune and neoplastic disorders. In the future, novel molecular tests including CODEHOP PCR and microarray analysis may help to elucidate the etiologies of various idiopathic meningoencephalitides. Moreover, it is expected that such tests, along with other emerging biotechniques, will become standard diagnostic tools

available in the critical care setting, allowing clinicians to implement quicker, more targeted therapies, ultimately improving the prognosis for various canine and feline meningoencephalitides.

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