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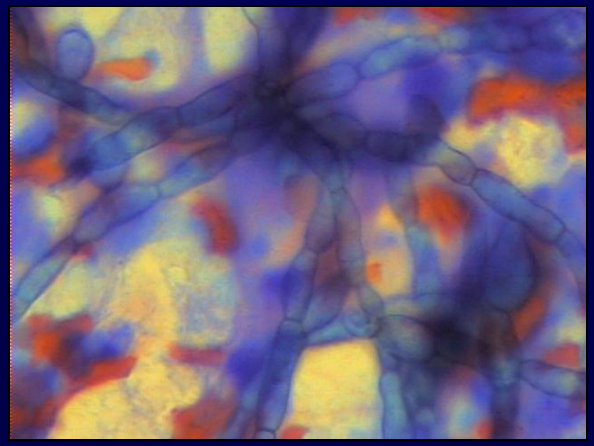
BROADLY REACTIVE PAN-VIRAL PCR OF CEREBROSPINAL FLUID IN CANINE MENINGOENCEPHALITIS OF UNKNOWN ETIOLOGY

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.



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- **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.*

AMERICAN KENNEL CLUB
CANINE HEALTH FOUNDATION

Consensus Degenerate Primers (CODEHOP)

Motif: S I I Q A H N L C

CODEHOP: 5′ TCC ATC ATC CAG GCC CA [C T] AA [C T] [C T] T [A C G T] TG

5’ Consensus Clamp

3’ Degenerate Core

Rose et al., 1998, Nucl Acids Res 26:1628

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Research to **protect, treat and cure** animals.

- **Viral Families:** Herpesviridae, Adenoviridae, Alphaviridae, Picornaviridae, Paramyxoviridae, Polyomaviridae, Flaviviridae, Bunyaviridae, Bornaviridae, Rhabdoviridae, Coronaviridae

AMERICAN KENNEL CLUB
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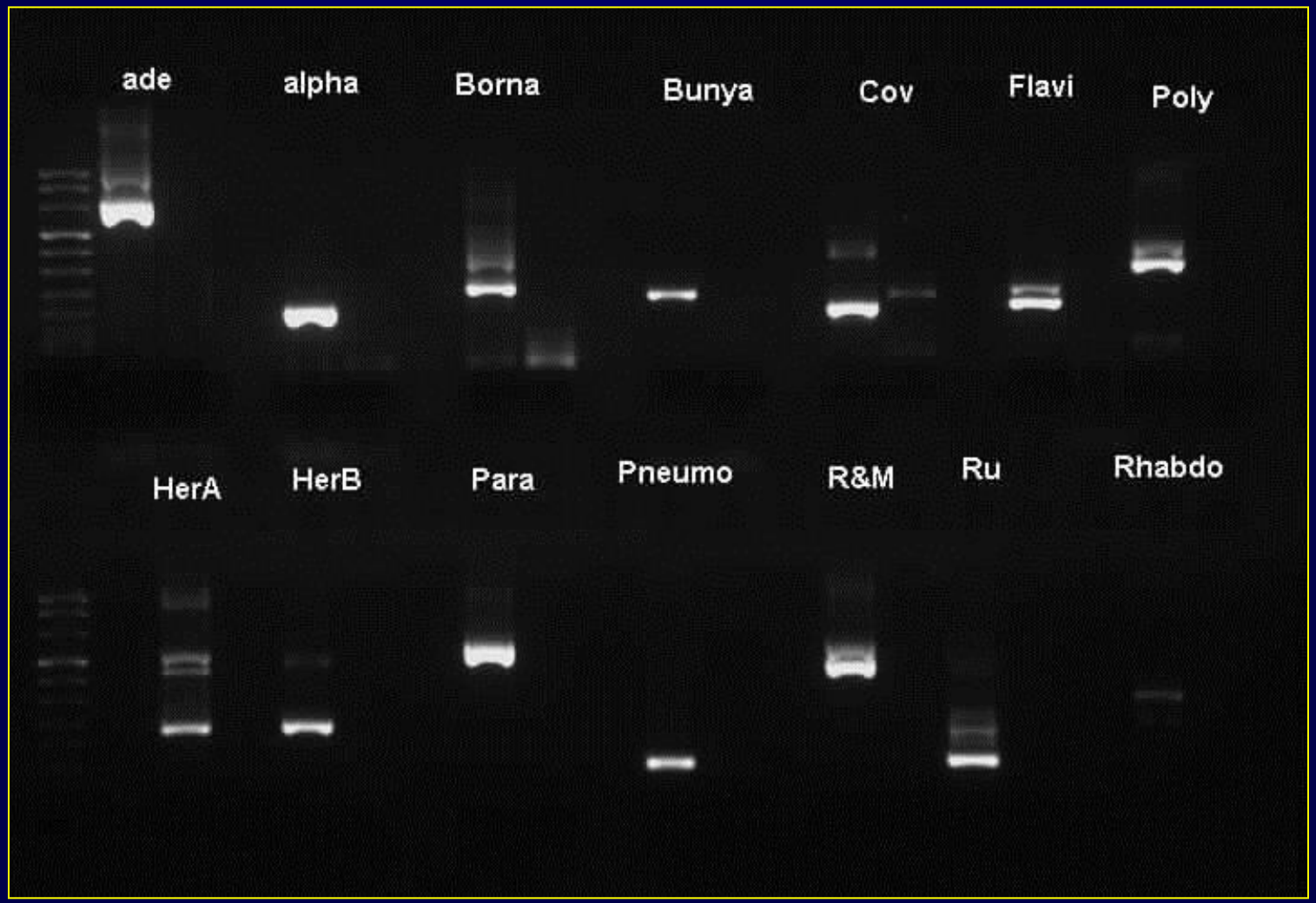
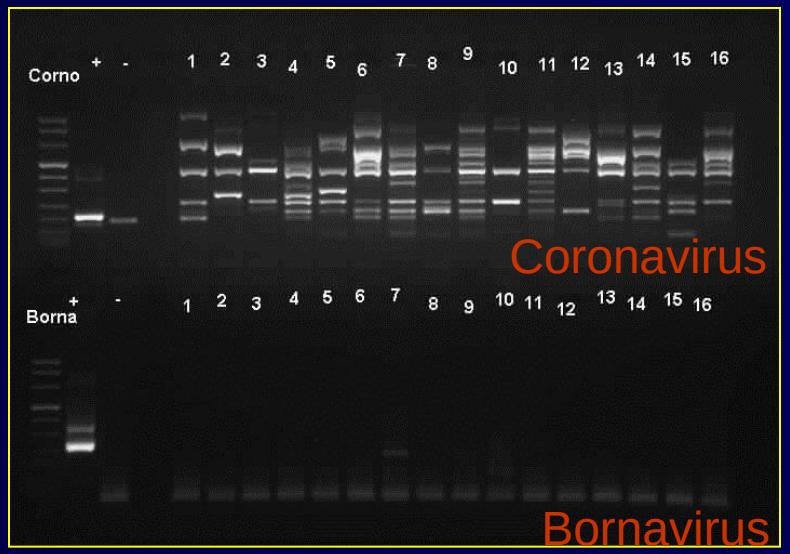
Pan-Viral PCR Positive Controls

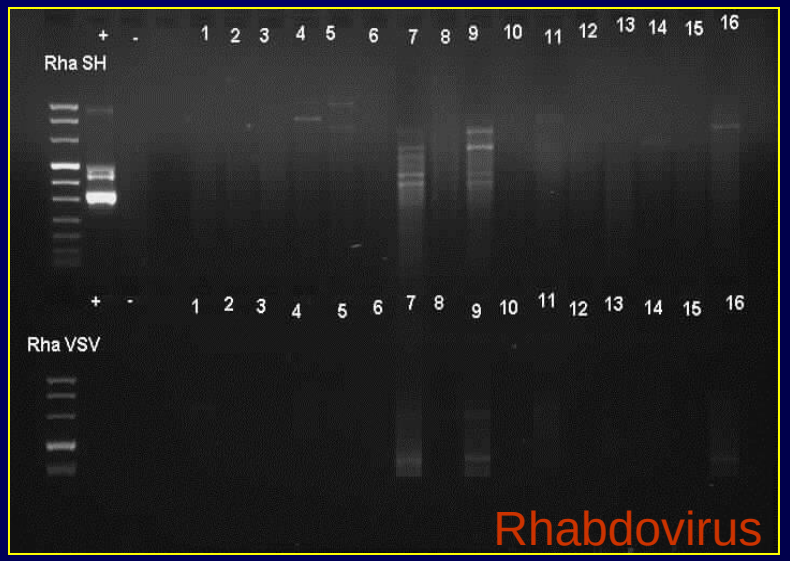


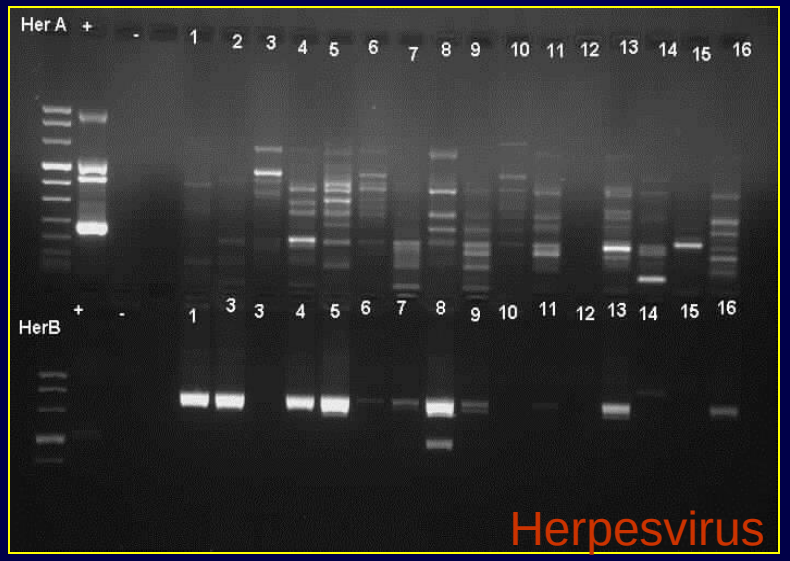
Methods

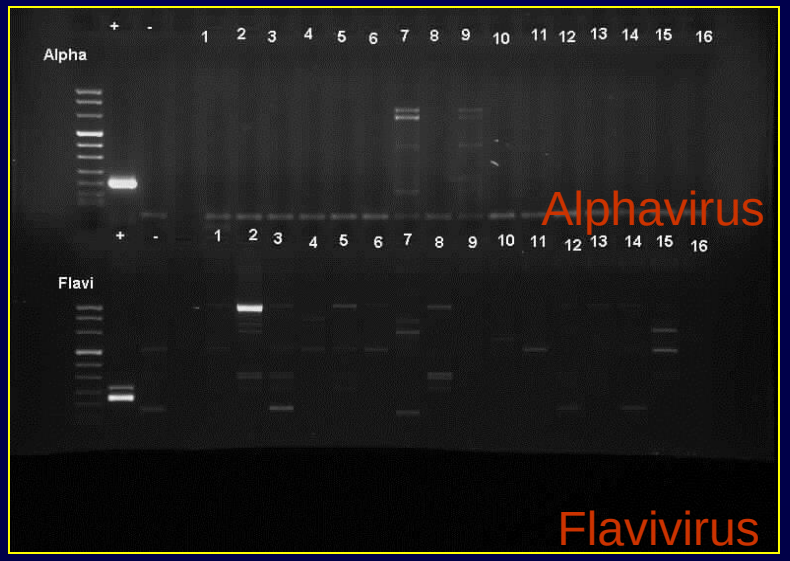
- DNA and RNA extracted from 146 CSF samples and non-neurological controls by standard methods (Qiagen and Invitrogen)
- Housekeeping PCR and RT PCR for GAPDH (DNA) and beta-actin to confirm DNA and RNA integrity
- PCR and RT PCR on ~ 5 µl of each sample in 20 various CODEHOP reactions

Broadly reactive pan-viral PCR on MUE CSF (146 cases)









Pan-bunyavirus PCR – LaCrosse virus

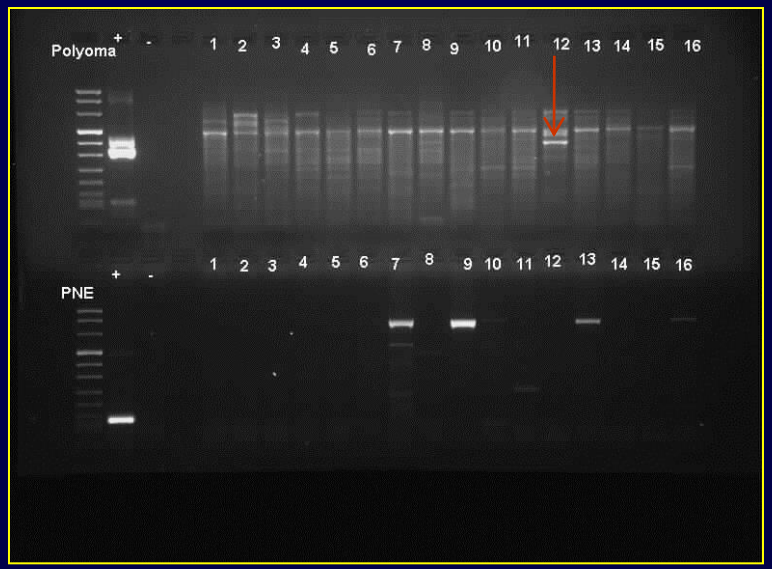
- CSF from 6/60 (10%) MUE cases positive on pan-bunyavirus PCR
- Sequences analysis disclosed 99% homology to LAC
- Black et al. J Vet Diagn Invest. 1994 Apr;6(2):250-4.
- Specific LAC PCR underway
- Developing an Ab to LaCrosse to perform serology on CSF and serum in dog

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

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)
- Feng et al. Science. 2008 Feb 22;319(5866): 1096-100.
- IHC on one case negative with human MCV Ab
- Specific MCV PCR underway
- CSF antigen testing

Labrador Retriever	F/I	Chocolate	26 kg	RBC=1263 **WBC=11** TP=19 16% Lymphs 10%Monos 73%Neuts 1%Eos	Normal cervical spine.	MUE (cervical)
Border Collie	M/N	Blk/Wht	18.2 kg	RBC = 30, **WBC = 139** TP 28 mixed pleocytosis	Hydrocephalus and syringohydromyelia	MUE
Great Dane	M/N			RBC 4 WBC 53 TP 56 17% monos 52% eos 31% lymphs	Syringohydromyelia	MUE



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