Examining the Genetic Role in *Cytauxzoon felis* Survival and Mortality

Veterinary Research Scholars Program
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Introduction

Cytauxzoon felis is a protozoan parasite that causes an infectious disease in cats with 97% mortality in untreated cats and 40% mortality in treated cats. Lonestar ticks (*Amblyomma americanum*) transmit *C. felis* from the natural reservoirs of bobcats (*Lynx rufus*) and pumas *(Puma concolor)* as a sporozoite **(Figure 1)**. Sporozoites replicate in phagocytes forming schizonts. Schizonts cause clinical disease in domestic cats because the enlarged phagocytes occlude the vasculature.[1] Schizonts will release merozoites into the blood to establish parasitemia **(Figure 2)**. After the infection, symptoms, such as icterus, fever, lethargy, and anorexia, will appear in 5-14 days. *Cytauxzoonosis* is diagnosed by a CBC with a microscopic observation of piroplasms or schizonts, identification of schizonts by fine-needle aspiration of peripheral tissue, or a diagnostic PCR assay.[1] Treatment with a combination of atovaquone and azithromycin should be started as soon as possible.[1] Why most bobcats, pumas, and some domestic cats survive a *C. felis* infection without treatment, while other cats do not, is unknown. *C. felis* resistance in wild felids and susceptibility in domestic cats is likely due to genetic differences within these species.

Figure 1: *Cytauxzoon felis* natural reservoirs. Bobcats and pumas are natural reservoirs for *C. felis*. Domestic cats previously infected may serve as a reservoir.[3]

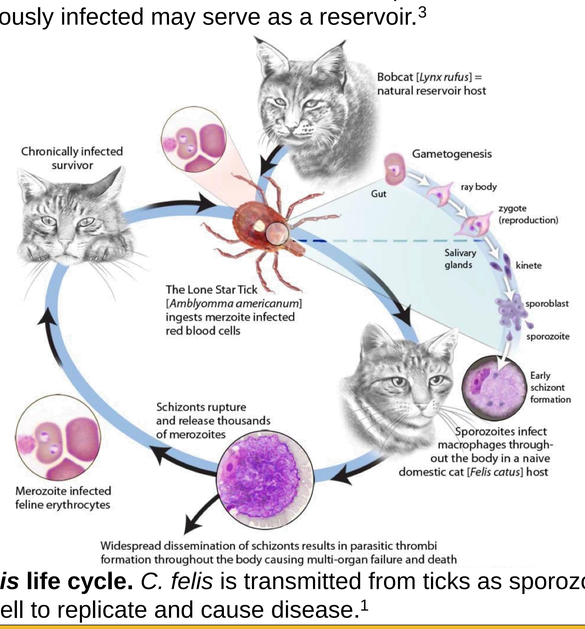

Figure 2: *Cytauxzoon felis* life cycle. *C. felis* is transmitted from ticks as sporozoites into hosts phagocytic mononuclear cell to replicate and cause disease.[1]

Objectives

- Identify genetic variation in domestic cats, bobcats, and pumas to determine a candidate gene and variants responsible for susceptibility or resistance to *Cytauxzoonosis.*
- Examine literature for candidate genes based on similar types of infection and diseases in other species – comparative medicine and comparative genetics.

Methods

- DNA from 3 ml of EDTA anti-coagulated whole blood or tissue from both a survivor and non-survivor domestic cat has been submitted for whole genome sequencing.
- Candidate genes involved in parasitic infections caused by parasites similar to *Cytauxzoon felis*, such as *Theileria*, *Babesia*, and *Plasmodium species,* have been identified with literature searches **(Table 1)**.
- Genes involved in intracellular parasitic infections and other diseases involving macrophages have been identified with literature searches.
- Candidate genes underwent protein multiple sequence alignment using COBALT to determine the genes homology between species.
- In the future, DNA variants will be examined between bobcats, pumas, and domestic cats to determine if these candidate gene variants influence the survival or fatality of domestic cats infected with *C. felis*.
- Once candidate gene variants are determined, examination of a cohort of cats infected with *C. felis* will determine their frequencies in survivors and non-survivors.

Table 1: Comparison of gene predictions of the *Cytauxzoon felis* genome with related apicomplexans[2]

	C. felis		*T. parva*	*B. bovis*	*P. falciparum*
	GeneMark*	Glimmer*			
Genome Size (Mbp)	9.1	9.1	8.3	8.3	22.8
G + C Composition (%)	31.8	31.8	34.1	41.8	19.4
Protein Coding Genes	4,314	4,373	4,035	3,671	5,268
Average Protein (aa)	466	409	469	505	761
% Genes with Introns	68.7	61.7	73.6	61.5	53.9

*GeneMark is a gene prediction program, which utilizes Gibbs sampling algorithm.
*Glimmer uses interpolated Markov models to identify coding regions and distinguish them from noncoding DNA.

Results

- *SIRPB1* has differential expression causing *Bos taurus* to have severe inflammatory responses to *Theileria annulata* infections, while *Bos indicus* are resistant.
  - *SIRPB1* protein sequence is not highly conserved between cows, mice, humans, and cats with ~30% - 52% homology.
- Decreased *BDKRB2* expression in mice increases susceptibility to *Leishmaniasis*, a parasitic disease infecting macrophages.
  - *BDKRB2* protein sequence is highly conserved between species with ~80% - 83% homology.
- *NRAMP1* has been acquired by species for intracellular parasite resistance.
  - *NRAMP1* between cattle, mice, humans, and cats is highly conserved with ~86% - 88%. homology **(Figure 3)**.

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Bos taurus    1   MSGDTGPPKQGGTRYGSISSPPSPE---PQQAPPGGTYLSEKIPIPDTESGTFSLRKLWAFTGPGFLMSIAFLDPGNIESDLQAGAVAGFKLLWVLLWATVLG 100
Mus musculus  1   MISDKSPPRLSRPSYGSISSLPGPA---PQPAPCRETYLSEKIPIPSADQGTFSLRKLWAFTGPGFLMSIAFLDPGNIESDLQAGAVAGFKLLWVLLWATVLG 100
Homo sapiens  1   MTGDKGPQRLSGSSYGSISSPTSPTSPGPQQAPPRETYLSEKIPIPDTKPGTFSLRKLWAFTGPGFLMSIAFLDPGNIESDLQAGAVAGFKLLWVLLWATVLG 103
Felis catus   1   MTGDSSPQSLSRSSYGSISSPPSSE---PQKEPLRATYLSEKILIPDTEPGTFSLRKLWAFTGPGFLMSIAFLDPGNIESDLQAGAVAGFKLLWVLLWATVLG 100

Bos taurus    101  LLCQRLAARLGVVTGKDLGEVCHLYYPKVPRILLWLTIELAIVGSDMQEVIGTAIAFSLLSAGRIPLWGGVLITVVDTFFFLFLDNYGLRKLEAFFGFLI 201
Mus musculus  101  LLCQRLAARLGVVTGKDLGEVCHLYYPKVPRILLWLTIELAIVGSDMQEVIGTAISFNLLSAGRIPLWDGVLITIVDTFFFLFLDNYGLRKLEAFFGLLI 201
Homo sapiens  104  LLCQRLAARLGVVTGKDLGEVCHLYYPKVPRTVLWLTIELAIVGSDMQEVIGTAIAFNLLSAGRIPLWGGVLITIVDTFFFLFLDNYGLRKLEAFFGLLI 204
Felis catus   101  LLCQRLAARLGVVTGKDLGEVCHLYYHKAPRTLLWLTIELAIVGSDMQEVIGTAIAFNLLSAGRIPLWGGVLITIVDTFFFLFLDNYGLRKLEAFFGFLI 201

Bos taurus    202  TIMALTFGYEYVVAQPAQGALLQGLFLPSCPGCGQPELLQAVGIIGAIIMPHNIYLHSSLVKSREVDRSRRADIREANMYFLIEATIALSVSFLINLFVM 302
Mus musculus  202  TIMALTFGYEYVVAHPSQGALLKGLVLPTCPGCGQPELLQAVGIVGAIIMPHNIYLHSALVKSREVDRTRRVDVREANMYFLIEATIALSVSFIINLFVM 302
Homo sapiens  205  TIMALTFGYEYVVARPEQGALLRGLFLPSCPGCGHPELLQAVGIVGAIIMPHNIYLHSALVKSREIDRARRADIREANMYFLIEATIALSVSFIINLFVM 305
Felis catus   202  TVMALTFGYEYVVARPAQVALLRDLLLPSCSGCGSPELLQAVGIVGAIIMPHNIYLHSALVKSREIDRSRRADIREANMYFLIEATIALSVSFFINLFVV 302

Bos taurus    303  AVFGQAFYKQTNQAAFNICADSSLHDYAPIFPRNNLTVAVDIYQGGVILGCLFGPPALYIWAVGLLAAGQSSTMTGTYAGQFVMEGFLKLRWSRFARVLL 403
Mus musculus  303  AVFGQAFYQQTNEEAFNICANSSLQNYAKIFPRDNNTVSVDIYQGGVILGCLFGPAALYIWAVGLLAAGQSSTMTGTYAGQFVMEGFLKLRWSRFARVLL 403
Homo sapiens  306  AVFGQAFYQKTNQAAFNICANSSLHDYAKIFPMNNATVAVDIYQGGVILGCLFGPAALYIWAIGLLAAGQSSTMTGTYAGQFVMEGFLRLRWSRFARVLL 406
Felis catus   303  AVFGQAFYQQTNQAAFDVCANSSLHDYAKIFPKNNLTVEVDIYQGGVILGCLFGPAALYIWAVGLLAAGQSSTMTGTYAGQFVMEGFLKLRWSRFARVLL 403

Bos taurus    404  TRSCAILPTVLLAVFRDLRDLSGLNDLLNVLQSLLLPFAVLPILTFTSMPALMQEFANGLVSKVITSSIMVLVCAVNLYFVISYLPSLPHPAYFSLVALL 504
Mus musculus  404  TRSCAILPTVLVAVFRDLKDLSGLNDLLNVLQSLLLPFAVLPILTFTSMPAVMQEFANGRMSKAITSCIMALVCAINLYFVISYLPSLPHPAYFGLVALF 504
Homo sapiens  407  TRSCAILPTVLVAVFRDLRDLSGLNDLLNVLQSLLLPFAVLPILTFTSMPTLMQEFANGLLNKVVTSSIMVLVCAINLYFVVSYLPSLPHPAYFGLAALL 507
Felis catus   404  TRSCAILPTVLVAVFRDLKDLSGLNDLLNVLQSLLLPFAVLPILTFTSMPALMQEFANGRLSKAITSSIMALICAINLYFVIIYLPSLPHPAYFVLVALL 504

Bos taurus    505  AAAYLGLTTYLVWTCLITQGATLLAHSSHQRFLYGLPEEDQEKG-RTSG 548
Mus musculus  505  AIGYLGLTAYLAWTCCIAHGATFLTHSSHKHFLYGLPNEEQ-GGVQGSG 548
Homo sapiens  508  AAAYLGLSTYLVWTCCLAHGATFLAHSSSHHFLYGLLEEDQ-KG-ETSG 550
Felis catus   505  AAVYLGLTTYLVWTCFLAQGATVLAHSSHQHFLYGLPEEEE-KE-RISG 547
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Figure 3: Protein Alignment of *NRAMP1*. *NRAMP1* remains highly conserved between species. The dashes represent missing amino acids of the gene. Blue amino acids show where variation occurs in other species compared to a cat amino acid sequence.

Discussion

- *SIRP* family regulates inflammation by inhibiting phagocytosis and TNFα production in *Theileria species* infections. *SIRPA* is expressed in resistant species, but *SIRPB1* is expressed in species with severe inflammatory responses.
  - *SIRPB1* protein sequence in felines is incomplete compared to the other species, which needs to be examined to further determine the gene homology.
- Decreased *BDKRB2* expression causes non-functional G-coupled bradykinin receptors, which cannot reduce amastigote outgrowth in inflammatory macrophage.
- *NRAMP1* prevents intracellular parasitic infection by sequestering Fe^{2+} and Mn^{2+} and the pathogen cannot produce protective enzymes against reactive oxygen species.

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