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Technical report

Natural resistance to experimental feline infectious peritonitis virus infection is decreased rather than increased by positive genetic selection

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ABSTRACT

A previous study demonstrated the existence of a natural resistance to feline infectious peritonitis virus (FIPV) among 36% of randomly bred laboratory cats. A genome wide association study (GWAS) on this population suggested that resistance was polygenic but failed to identify any strong specific associations. In order to enhance the power of GWAS or whole genome sequencing to identify strong genetic associations, a decision was made to positively select for resistance over three generations. The inbreeding experiment began with a genetically related parental (P) population consisting of three toms and four queens identified from among the survivors of the earlier study and belonging to a closely related subgroup (B). The subsequent effects of inbreeding were measured using 42 genome-wide STR markers. P generation cats produced 57 first filial (F1) kittens, only five of which (9.0%) demonstrated a natural resistance to FIPV infection. One of these five F1 survivors was then used to produce six F1/P-backcrosses kittens, only one of which proved resistant to FIP. Six of eight of the F1 and F1/P survivors succumbed to a secondary exposure 4–12 months later. Therefore, survival after both primary and secondary infection was decreased rather than increased by positive selection for resistance. The common genetic factor associated with this diminished resistance was a loss of heterozygosity.

1. Introduction

Feline infectious peritonitis (FIP) is enzootic in virtually all multiple cat populations that involve either kitten production or housing (Pedersen, 2014). Eighty percent of FIP cases occur in cats younger than two years and 50% in kittens under 7 months of age (Worthing et al., 2012). The FIP virus (FIPV) is a result of a series of unique and common internally occurring mutations in the ubiquitous and largely non-pathogenic feline enteric coronavirus (FECV) (Pedersen, 2014; Pedersen et al., 2008). FIP cases almost always occur as mini-enzootics, with the incidence in some catteries varying from 0 to 10% over five years (Foley et al., 1997). Shelters suffer a similar pattern of disease. This variability in incidence reflects a complex web of potential risk factors. The strongest risk factors are: (1) severity of exposure to feline enteric coronavirus (FECV) (Foley et al., 1997); (2) the likelihood that FECV will undergo specific mutations that alter tropism from enterocytes to peritoneal macrophages (Pedersen, 2014); (3) maternal immunity to FECV infection (Pedersen et al., 2008); (4) the age at which a cat is confronted with FIPV (Pedersen et al., 2014); (5) the type of environment, husbandry procedures, and exposure to other infectious agents (Foley et al., 1997; Pedersen et al., 1977, 2004; Poland et al., 1996), and (6) heritable predisposition (Foley et al., 1997; Golovko et al., 2013).

The role of genetic factors in FIP resistance/susceptibility is based on both indirect and direct observations. Pedigreed cats are more likely to develop FIP than random-bred cats and certain breeds are also more severely affected than others (Bell et al., 2006; Norris et al., 2012; Pesteanu-Somogyi et al., 2006; Worthing et al., 2012). Heritability accounted for 50% of the incidence among Persian catteries that were studied over a five year period (Foley et al., 1997). Genome wide association studies (GWAS) confirmed that genetic susceptibility to FIP in Birman cats was highly polymorphic and genetic associations varied depending on the age of cats tested (Golovko et al., 2013). A recent study also found that 36% of laboratory cats from a specific breeding colony were also naturally resistant to FIPV infection, although GWAS was again

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Table 1
Parental relationships of cats used to create F1 and F1-backcross generations of kittens and the results of primary infection with FIPV.

Results of infection								
Cat-ID #'s	# of cats	FIP	No-FIP	Group	Sire-ID	Sire group	Dam-ID	Dam group
13P01-P06	5	5	0	BB	11-149	B	10-145	B
13P08-P09	2	1	1	BB	11-149	B	10-211	B
14P09-P10	2	2	0	BB	11-149	B	11-147	B
13P29-P33	5	5	0	BB	11-166	B	10-145	B
13P34-P37	4	4	0	BB	11-166	B	10-211	B
13P38-P41	4	4	0	BB	11-166	B	10-213	B
13P15-P20	6	5	1	BB	11-166	B	11-147	B
14P04-P08	5	5	0	BB	11-225	B	10-145	B
13P10-P14	5	5	0	BB	11-225	B	10-213	B
13-P07	1	0	1	BB	11-149	B	10-211	B
12A5-A8	4	4	0	BB	11-149	B	10-213	B
12-1A-4A	4	3	1	BB	11-166	B	10-145	B
14P18-P20	3	3	0	BB	11-166	B	10-211	B
14P13-P17	5	4	1	BB	11-166	B	10-213	B
12A9-A10	2	2	0	BB	11-225	B	11-147	B
14P21-P24	3	2	1	B/BB	11-166	B	12-4A	BB
14P01-P03	3	3	0	B/BB	11-225	B	12-4A	BB
Total	63	57	6					

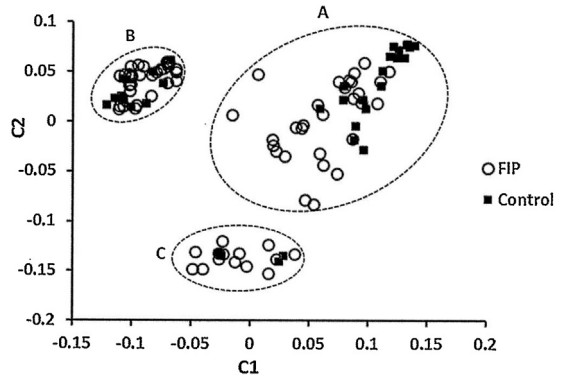

Fig. 1. Two dimensional (C1 and C2) MDS plot based on data from GWAS of 107/111 random bred specific pathogen free cats that had been experimentally infected with FIPV as documented in a previous study (Pedersen et al., 2014).

unable to identify strong genetic associations (Pedersen et al., 2014).

The present study was an offshoot of earlier experiments with FIPV infection among randomly bred laboratory cats (Pedersen et al., 2014). Because of difficulties in collecting sufficient DNA samples from the field, a decision was made to enhance the likelihood of identifying FIP protective genotypes by inbreeding FIPV resistant cats resulting from previous laboratory studies. Although immunity to infectious diseases in humans is polygenic, specific polymorphisms associated with risk have been identified for a number of important infections (Chapman and Hill, 2012). The basic premise was that if resistance traits were of limited number and of sufficient strength that it should be possible to concentrate these genotypes by positive selection, making it easier to define them by GWAS or whole genome sequencing. The expectation was that inbreeding FIPV resistant cats would further decrease mortality in their kittens.

2. Materials and methods

2.1. Experimental animals

Feline coronavirus free randomly bred, cats used for this study were obtained from the breeding colony of the Feline Nutrition and Pet Care Center and housed in their Feline Research Laboratory. All animal experiments were in compliance with relevant regulatory standards as documented in UC Davis IACUC protocols #16988 and #18215.

2.2. FIPV infection and disease monitoring

Sixty three kittens 6 months of age were experimentally infected with FIPV by the intraperitoneal route. The origins of Type I FIPV–m3c2 and the preparation of cell-free infectious inoculates have been published (Pedersen et al., 2012, 2014). Affected cats were either euthanized with an intravenous overdose of pentobarbital and phenytoin sodium or transferred to an antiviral drug treatment protocol (Kim et al., 2015) when clinical and laboratory signs indicated that their infection would be inevitably fatal, usually within 3–4 weeks of exposure (Pedersen et al., 2015).

2.3. Genetic testing

The genetic relationship of cats from the P, F1 and F1/P backcross generations was confirmed by using allele frequencies obtained from 42 microsatellites across the cat genome (Menotti-Raymond et al., 2003, 2009). Genotyping was conducted by the Veterinary Genetics Laboratory, UC Davis, and data were analyzed using STR and analysis software (Toonen and Hughes, 2002). Population genetic and principal coordinate analyses (PCoA) were conducted using GenAIEX 6.5 (Peakall and Smouse, 2012). The results of genome wide association studies (GWAS) and multi-dimensional scaling (MDS) were described in an earlier publication (Pedersen et al., 2014).

3. Results

3.1. Breeding scheme for P, F1, and F2 generation cats

Seventy seven of 111 cats described in an earlier study were susceptible to FIPV infection and 34 were resistant; DNA from 107 of these cats was assessed by GWAS and MDS as previously reported (Pedersen et al., 2014). The cats were differentiated into three genetically distinct subpopulations labeled A–C when examined by MDS (Fig. 1). The 34 cats that resisted FIPV challenge-exposure were randomly segregated among the three subpopulations (Fig. 1).

Population B formed the largest and tightest cluster and three toms and four queens were selected from among this group to create a parental (P) generation. These seven cats produced 57 F1 kittens between them (Table 1). One F1 female (12-4A) was bred

Table 2
Genetic assessment of P, F1 and F1-backcross cats using 42 genome-wideSTR loci. Genetic values include average alleles/locus (Aa), average effective alleles/locus (Ae), and observed heterozygosity (Ho).

Generation		# Cats	Aa	Ae	Ho
P	Mean	7	3.206	2.464	0.752
	SE		0.192	0.150	0.048
F1	Mean	57	3.206	2.443	0.538
	SE		0.192	0.151	0.033
F1/P	Mean	6	2.294	1.770	0.446
	SE		0.116	0.079	0.043

Table 3
The number and percent of genome wide STR loci with homozygous alleles.

Generation		# Homozygous loci	% Loci
P	Mean	8.43	0.25
	SD	1.62	0.05
BB	Mean	15.72	0.46
	SD	2.64	0.08
B/BB	Mean	18.83	0.55
	SD	6.59	0.19

once with her father (11-166) and once with a full brother of the father (11-225) to produced six F1/P-backcross kittens (Table 1). The breeding program was terminated at this point based on the results of subsequent FIPV challenge-exposures. Table 2 provides a genetic assessment of the P, F1 and F1/P backcross populations based on a panel of 42 STR loci. The average alleles/locus (Aa) and the average effective alleles/locus (Ae) remained the same in the P and F1 cats, but decreased in the F1/P backcrosses. Observed heterozygosity (Ho) decreased from the P to F1/P generations, reflecting the inbreeding. An increase in genome-wide homozygosity based on alleles at each of 42 genome-wide STR loci was also observed between the P and F1/P backcross generations (Table 3).

3.2. Resistance of F1 and F1/P backcross generation kittens to primary FIPV challenge-exposure

Fifty seven F1 kittens were produced and 52 (91%) developed terminal signs of FIP by 3–4 weeks post-infection. Five of six (83%) F1/P-backcross kittens also developed terminal FIP upon primary exposure (Table 2). The mortality among F1 and F1/P backcross cats to primary FIPV challenge exposure was significantly higher than the 21/34 (62%) mortality among B group cats that received a primary challenge-exposure at a similar age ($P = 0.0011$, two-tailed Fisher's exact test).

3.3. Resistance of F1 and F1/P backcross generation kittens to secondary FIPV challenge-exposure

Eight cats from the F1 and F1/P generations were re-challenged with FIPV 4–12 months after resisting primary infection and 6/8 (75%) succumbed to FIP. This mortality was higher than that observed following secondary challenge-exposure of group B cats that survived primary infection (4/13 = 31%), although the difference was significant only at $P = 0.08$ (two tailed Fisher's exact test).

4. Discussion

The present study was based on the assumption that resistance factors to FIPV infection in this particular colony of laboratory cats would be amenable to positive selection, thus yielding a small colony of inbred cats that would be much more resistant to FIPV than their parents and grandparents. These inbred cats would emphasize regions of the genome involved in FIPV resistance, a situation used to great advantage to identify both simple and polygenic traits in highly inbred breeds of dogs (Hedrick and Andersson, 2011; Ostrander et al., 2008). The outcome of the breeding study confounded these expectations and actually led to F1 and F1/P backcross kittens that appeared to be significantly more susceptible to experimental FIPV infection than their progenitors, both to primary and secondary challenge-exposures.

The only apparent genetic association with decreased resistance was a loss of heterozygosity. The effect of lost heterozygosity can be best explained by models of autoimmune and infectious diseases in humans (Barreiro and Quintana-Murci, 2010; Goris and Liston, 2012). These models implicate numerous genetic polymorphisms within the MHC and other parts of the genome. Each polymorphism contributes small degrees of genetic risk or protection against disease that is often below the level of detectability by GWAS, and with no single risk factor being essential. A number of different polymorphisms may also lead to the same clinical outcome. In this scenario, a loss of genetic diversity could actually decrease the number of resistance-associated options available to the host.

What are the observations among pedigreed cats that implicate a loss of heterozygosity in FIP resistance or susceptibility? Unfortunately, no one has directly studied the relationship between FIP incidence and inbreeding, although a number of cat breeds are known to be highly inbred (Lyons et al., 2008) and some breeds appear to be more susceptible to FIP than others (Bell et al., 2006; Norris et al., 2012; Pesteanu-Somogyi et al., 2006; Worthing et al., 2012). The use of certain sires has been linked to a higher incidence of FIP among their offspring (Foley and Pedersen, 1996), not because the susceptibility traits are carried only in toms, but because a single tom produces far more offspring than a single queen. Multiple cases of FIP within the same litter are frequently observed. Therefore, the results of this study confirm both the genetic complexity of FIPV resistance and the widely recommended practice of avoiding the use of cats with progeny dying of FIP for breeding programs in pedigreed catteries.

Conflict of interest

The authors declare no conflicts of interest.

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