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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					

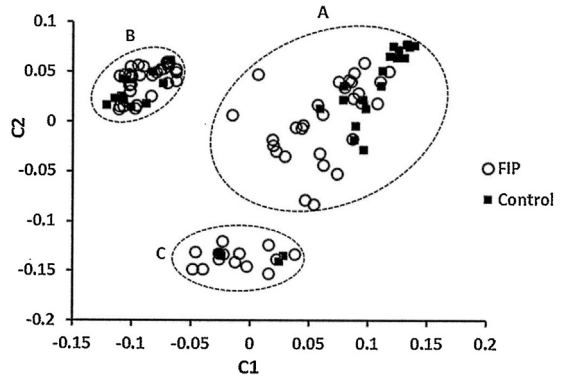

Fig. 1. Two dimensional (C1 and C2) MDS plot based on data from GWAS of 107/111 random bred specific pathogen free cats that had been experimentally infected with FIPV as documented in a previous study (Pedersen et al., 2014).

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