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PREVALENCE OF ANTIBODIES TO SELECTED VIRUSES AND PARASITES IN INTRODUCED AND ENDEMIC CARNIVORES IN WESTERN MADAGASCAR

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ABSTRACT: Introduced animals impact endemic populations through predation, competition, and disease transmission. Populations of endemic carnivores in Madagascar are declining, and pathogens transmitted from introduced species may further endanger these unique species. We assessed the exposure of introduced and endemic carnivores to common viral and parasitic pathogens in two national parks of Madagascar (Kirindy Mitea National Park and Ankarafantsika National Park) and their neighboring villages. We also identified variables associated with the presence of antibodies to these pathogens in fosa (*Cryptoprocta ferox*). Introduced and endemic species were exposed to canine parvovirus, canine herpesvirus, feline calicivirus, and *Toxoplasma gondii*. Domestic dogs (*Canis familiaris*) and cats (*Felis catus*) may be sources of infection for these pathogens. Prevalence of antibodies to *Toxoplasma* in captured fosa was >93%, and adults were more likely to be exposed than immature individuals. Our data provide a basis upon which to evaluate and manage risks of pathogen transmission between species.

Key words: Carnivore, conservation medicine, *Cryptoprocta ferox*, fosa, serosurvey, *Toxoplasma*.

INTRODUCTION

Domestic dogs (*Canis familiaris*) and cats (*Felis catus*) have been introduced virtually on every landmass, reaching populations of 700 and 500 million, respectively (Serpell 2000; Driscoll et al. 2007; Hughes and Macdonald 2013). These domestic animals impact wildlife populations on continental landmasses and have contributed to wildlife extinctions on islands (Courchamp et al. 2003; Medina et al. 2011). Introduced animals can exclude endemic species by predation or by reducing available resources (Vanak and Gompper 2009, 2010). Additionally, domestic animals may transmit pathogens to endemic wildlife through direct contact or environmental contamination. This pathogen "spillover" is a particular threat to endangered species and may lead to local extinctions (Woodroffe 1999; Daszak et al. 2000). For example, rabies and canine distemper viruses, from dogs, have decimated populations of African wild dogs (*Lycaon pictus*), Ethiopian wolves (*Canis simensis*), and African lions (*Panthera leo*) (Gascoyne et al. 1993; Laurenson et al. 1998). Pathogens can also be an important factor determining the outcomes of trophic or competitive interactions through "apparent competition" (Holt 1977).

Madagascar harbors four introduced species of carnivores, the domestic dog, the domestic cat, the African wildcat (*Felis silvestris*), and the Indian civet (*Viverricula indica*), and 10 endemic carnivore species in the family Eupleridae (Yoder et al. 2003). Of the Euplerid species, all but the Malagasy ring-tailed mongoose (*Galidia elegans*) are listed in one of the threatened categories on the International Union for the Conservation of Nature red list (IUCN 2014). Malagasy carnivores are sensitive to habitat degradation

and are further endangered by bushmeat hunting (Golden 2009; Gerber et al. 2012). Furthermore, research on Eupleridae has shown a negative correlation between detection of endemic carnivores or primates and introduced animal activity at camera sites (Gerber et al. 2012; Farris et al. 2014). As in other ecosystems, the reason for the decline is likely a combination of factors including resource competition, predation, and disease (Vanak and Gompper 2009; Knobel et al. 2013; Belsare and Gompper 2015).

The fosa (also spelled fossa; *Cryptoprocta ferox*) is the largest extant native carnivore in Madagascar. It is listed as endangered on the IUCN red list and threatened by habitat loss and hunting (IUCN 2014). Fosa are solitary, cathemeral carnivores ranging throughout Madagascar. They are found at low densities mainly in forested areas but are also known to visit villages and prey on poultry (Hawkins and Racey 2005; Kotschwar Logan et al. 2015). These raids into villages may facilitate interactions with domestic animals and transmission of pathogens across species.

Despite the importance of the fosa as the top predator in the ecosystem and growing awareness of the risks of pathogen transmission between domestic and wild animals, little information is available about the pathogens affecting introduced and endemic carnivores in Madagascar. We assessed the exposure of introduced and endemic carnivores to selected viral and parasitic pathogens at two sites in Madagascar and identified variables associated with the presence of antibodies to these pathogens in fosa.

MATERIALS AND METHODS

Study sites

This study was conducted in two protected areas in Madagascar, Ankarafantsika National Park (ANP) and Kirindy Mitea National Park (KMNP), and at villages near these protected areas. The ANP covers 65,520 ha of dry deciduous forest in northwestern Madagascar. Animals were trapped in the Ampijoroa area at 16°15′S, 46°48′E. Within the national park, altitude varies between 80 m and 370 m. Rainfall is 100–1,500 mm/yr, 95% of this falling November–April. The mean annual temperature is 26 C (11.4–39.3 C).

The KMNP covers 152,000 ha of dry deciduous forest in western Madagascar. Animals were trapped around the research station at 20°47′17″S, 44°10′08″E. Altitude in this area varies between 50 m and 100 m. Mean annual rainfall is approximately 700 mm. The KMNP has a dry season from April to early November and a hotter, rainy season during the remainder of the year. The temperatures vary 7–40 C, with an annual mean of 24 C.

Sample collection

Activities in this project were approved by the Institutional Animal Care and Use Committee of the Duke University and adhered to all research requirements in Madagascar. Samples from introduced and endemic carnivores were collected as part of an ongoing ecologic study of carnivores in 2000, 2001, 2003–06, 2008, 2012, and 2013 in ANP and in 2005–06 in KMNP. Samples were collected mainly during the dry season (April–November) at both sites.

Free-ranging carnivores were trapped in cage traps (Tomahawk Live Trap, Tomahawk, Wisconsin, USA) and anesthetized by intramuscular injection of tiletamine-zolazepam hydrochloride (Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) dosed at approximately 10 mg/kg or a combination of ketamine (Ketaset, Fort Dodge Animal Health) dosed at approximately 4 mg/kg plus medetomidine (Domitor, Pfizer, New York, New York, USA) dosed at approximately 0.1 mg/kg. The injection was administered to the trapped animal via short blowpipe and dart (Pneu-Dart, Inc., Williamsport, Pennsylvania, USA, or Telinject dart, Telinject USA Inc., Agua Dulce, California, USA).

Once anesthetized, each animal underwent an external physical examination, and a passive radiofrequency identification tag was inserted subcutaneously between the shoulder blades for future identification. The sex and age category of the animal were recorded. Animals were classified as immature or adult based on body size, dentition, and allometric measurements. Blood (not exceeding 1% of the animal body weight) was collected by venipuncture of the lateral saphenous vein and immediately placed in serum separator tubes (Corvac Sherwood Medical, Saint Louis, Missouri, USA). Animals were left to recover in the trap and released at the capture site.

Domestic dogs and cats from villages near the national parks were also sampled. Dogs and cats in this region are owned and unowned animals that are all free-ranging. None of the dogs or cats had a previous history of vaccination. Samples from owned dogs and cats were collected with the

TABLE 1. Selected pathogens tested in endemic and introduced carnivores of western Madagascar between 2002 and 2013, their methods of transmission, serologic diagnostic method, and positive cutoff values.

Pathogen	Method of transmission	Serologic diagnostic method[a]	Cutoff value	
			Suspected	Positive
Canine adenovirus	Exposure to body fluids; in utero	SN	8	>16
Canine distemper virus	Exposure to aerosolized body fluids	SN	8	>16
Canine herpesvirus	Exposure to body fluids; in utero	SN	8	>16
Canine parvovirus	Exposure to feces	HAI	10	>20
Feline calicivirus	Inhalation of aerosolized oronasal and ocular secretions	SN	8	>16
Feline herpesvirus	Inhalation of aerosolized oronasal and ocular secretions	SN	8	>16
Feline coronavirus	Exposure to feces, body fluids; in utero	SN	8	>16
Feline immunodeficiency virus	Bite wounds	ELISA	Positive/Negative	
Toxoplasma gondii	Ingestion of infected tissues or fecal oocysts; in utero	ELISA		64

[a] SN = serum neutralization; HAI = hemagglutination inhibition; ELISA = enzyme-linked immunosorbent assay.

owners' consent. Animals were classified as immature or adult based on body size, dentition, allometric measurements, and behavior. Only dogs and cats ≥5 mo old were sampled to avoid detection of maternal antibodies. Dogs and cats were manually restrained or anesthetized with tiletamine-zolazepam (as described previously for fosa) and blood (not exceeding 1% of body weight) was collected from the cephalic, medial/lateral saphenous, or jugular vein and placed in serum separator tubes.

Laboratory analysis

Blood was allowed to clot before being centrifuged for 15 min at 400 × G. Serum was pipetted into cryotubes (cryovials, Nalgene Company, Rochester, New York) and immediately frozen in liquid nitrogen for storage and transport. Serum samples were submitted to the New York State Animal Health Diagnostic Laboratory (Ithaca, New York, USA) for analysis. Depending on volume of serum available from each animal, analyses were conducted to detect antibodies to a combination of the following pathogens: canine adenovirus (CAV), canine coronavirus, canine distemper virus (CDV), canine herpesvirus (CHV), and feline calicivirus (FCV) by serum neutralization (SN) assays; canine parvovirus (CPV-2) by hemagglutination inhibition test (HI); and feline herpes virus (FHV), feline coronavirus, feline immunodeficiency virus, feline leukemia virus, and *Toxoplasma gondii* by enzyme-linked immunosorbent assay. Table 1 presents the viruses and parasites for which exposure of introduced and endemic carnivores was tested along with their mode of transmission and the cutoff values for each serologic test. Levels of antibody titers, indicative of prior exposure in euplerids are unknown; therefore, we used a conservative approach and considered low positive titers as "suspect."

Statistical analysis

Prevalence of antibodies were estimated for each pathogen and exact binomial methods were used to calculate 95% confidence intervals (CIs). When animals were captured and sampled more than once (four individuals), only data from the first capture were included in statistical analyses.

As host biology, habitat, and behavior may influence exposure to pathogens, associations between demographic and temporal variables and antibody status of selected diseases were evaluated in fosa using logistic regression. We also assessed the association between presence of antibodies to multiple pathogens as a marker of potential coinfection. Univariable logistic regression models were constructed to investigate, separately, the association between putative risk factors and the presence of antibodies to the pathogens. The following variables were evaluated: age category (juvenile, subadult, adult), sex, location (ANP, KMNP), year of sampling, and presence of antibodies to another virus or parasite. Only variables that showed an association ($P<0.2$) were included in a multivariable model. Multivariable logistic regression models were built employing a backward elimination approach ($P>0.05$ as rejection criteria). The fit of the logistic regression model was assessed using

TABLE 2. Number of endemic and introduced carnivores captured by site and species in western Madagascar between 2002 and 2013.

Common name	Species	ANP[a]	KMNP[b]
Domestic dog	*Canis familiaris*	58	2
Fosa	*Cryptoprocta ferox*	22	32
African wildcat	*Felis silvestris*	27	6
Domestic cat	*Felis catus*	80	0
Narrow-striped mongoose	*Mungotictis decimlineata*	0	6
Indian civet	*Viverricula indica*	7	0

[a] ANP = Ankarafantsika National Park.
[b] KMNP = Kirindy Mitea National Park.

Hosmer-Lemeshow goodness of fit test (Hosmer and Lemeshow 2000). From the final models, the odds ratios (OR) and 95% CI were calculated for each predictor variable to estimate the magnitude of its association with pathogen exposure. Statistical analyses were conducted using the base package of R version 3.0.3 (R-Core Team 2014).

RESULTS

Animals sampled

We evaluated and sampled 240 animals from six species: domestic dog, domestic cat, African wildcat, fosa, narrow-striped mongoose (*Mungotictis decemlineata*), and Indian civets (Table 2). All animals appeared healthy upon physical examination. Domestic cats and Indian civets were sampled only in ANP, and narrow striped mongooses were trapped only in KMNP. Not every animal was tested for antibodies to all pathogens due to limited serum availability.

Antibody prevalences

Antibodies to *T. gondii* were detected in all species but the Indian civet, and the overall prevalence was 67%. Dogs were also positive for antibodies to CAV (14%), CDV (45%), CPV (67%), and CHV (20%). A large proportion of fosa (42/45) ha detectable antibodies to *Toxoplasma*, of which 33 had high titers (>1,024) suggestive of a recent or active infection. In addition, fosa were antibody positive to FCV (23%), CHV (9%), and CAV (4%) with maximum titers of 640, 60, and 48, respectively. Fosa also had low titers (suspected positive) to CDV and CPV. One narrow striped mongoose was positive for canine parvovirus antibody.

No animal had detectable antibody to FHV or feline coronavirus. The proportions of animals positive or suspected positive for antibody to each pathogen are presented in Table 3.

Risk factors for exposure to viral and parasitic diseases

Presence of antibodies to another pathogen as well as temporal, environmental, and demographic variables were considered as putative risk factors for the presence of antibodies (high positive titers) to CAV, CHV, FCV, and *Toxoplasma* in fosa. Using univariable models, presence of antibodies to *Toxoplasma* was associated with age (P=0.03) and presence of antibodies to FCV (P=0.17). Presence of antibodies to CHV was associated with the exposure to FCV (P=0.07) and location (P=0.18). No variables were associated with the presence of antibodies to CAV.

Using multivariable logistic regression models, only age was retained as a risk factor for the exposure to *Toxoplasma* (P=0.03). Adults were significantly more likely to be exposed to *Toxoplasma* than immature individuals (OR=17.5; 95% CI: 1.4–432.6). Prevalence of antibodies to *Toxoplasma* in immature and adult fosa are presented in Table 4. Prevalence of antibodies to *Toxoplasma* in both study sites are presented in Table 5.

No variables were significantly associated with exposure to FCV and CHV.

DISCUSSION

We evaluated the prevalence of antibodies to eight pathogens of introduced and endemic carnivores living in or around two protected areas of Madagascar. These animals were exposed to a large array of introduced pathogens. Regardless of species, age category, sampling location, and year, these carnivores presented antibodies to *Toxoplasma*

TABLE 3. Proportion of samples with positive or suspected titers to selected pathogens in different species of domestic and endemic carnivores in Western Madagascar. CI = confidence interval. Dashes indicate that none were tested in that category.

	CAV[b]			CDV[b]			CHV[b]	
Species[a]	*n*	Suspect (95% CI)	Positive (95% CI)	*n*	Suspect (95% CI)	Positive (95% CI)	*n*	Suspect (95% CI)
C. familiaris	51	0	0.14 (0.06–0.27)	49	0.14 (0.06–0.28)	0.45 (0.31–0.6)	50	0.06 (0.02–0.18)
Cr. ferox	44	0.02 (0–0.12)	0.04 (0.01–0.15)	43	0.07 (0.02–0.2)	0	43	0.16 (0.07–0.3)
F. silvestris	2	0	0	2	0	0	2	0
F. catus	—	—	—	—	—	—	—	—
M. decimlineata	5	0	0	5	0	0	6	0
V. indica	1	0	0	1	0	0	—	—
	103	0.01 (0–0.06)	0.08 (0.04–0.16)	98	0.1 (0.05–0.18)	0.22 (0.14–0.32)	104	0.1 (0.05–0.17)

[a] *C. familiaris* = *Canis familiaris*; *Cr. ferox* = *Cryptoprocta ferox*; *F. silvestris* = *Felis silvestris*; *F. catus* = *Felis catus*; *M. decimlineata* = *Mungotictis decimlineata*; *V. indica* = *Viverricula indica*.

[b] CAV = canine adenovirus; CDV = canine distemper virus; CHV = canine herpesvirus; CPV = canine parvovirus; FCV = feline calicivirus; *Toxoplasma* = *Toxoplasma gondii*.

(68%), canine parvovirus (34%), canine distemper virus (22%), feline calicivirus (21%), canine herpesvirus (13%), and canine adenovirus (8%). These pathogens are potential threats to the conservation of endemic carnivores and may contribute to wild carnivore population declines. Dogs and cats are often considered the primary reservoir of these pathogens and a source of infection for wild animals. As such, monitoring domestic dogs' and cats' exposure to these viruses and parasites is an important first step to evaluate and manage the risks of disease transmission across species (Cleaveland et al. 2001; Slater 2001; Belsare and Gompper 2015). Second, identifying risk factors in fosa can guide allocation of limited resources for effective management and the conservation of this endangered species.

Antibodies to *Toxoplasma* were detected in 68% of the sera, including 93% of fosa. *Toxoplasma gondii* is a zoonotic, globally distributed protozoan parasite capable of infecting a wide range of animals including mammals and birds (Tenter et al. 2000; Vitaliano et al. 2014). The only recognized definitive hosts of *Toxoplasma* are domestic and wild felids, which shed the parasite in their feces. Humans and animals are then infected through ingestion of infective forms of the parasite from contaminated environment or through eating an infected prey item. Alternatively, vertical transmission of the parasite can also occur (Miller et al. 2008; Calero-Bernal et al. 2013). Madagascar has no endemic felid, and the presence of *Toxoplasma* in free-ranging fosa indicates parasite spillover from an introduced cat species,

TABLE 4. Prevalence of antibodies to *Toxoplasma* and odds ratio in different age categories of fosa (*Cryptoprocta ferox*) in western Madagascar. CI = confidence interval.

Age category	*n*	Prevalence (95% CI)	Odds ratio (95% CI)
Immature	6	0.67 (0.25–0.94)	Ref
Adult	38	0.97 (0.84–0.99)	17.5 (1.4–432.6)
Total	42	0.93 (0.79–0.98)	

TABLE 5. Prevalence of antibodies to *Toxoplasma* of fosa (*Cryptoprocta ferox*) in two National Parks of Madagascar. CI = confidence interval.

Location[a]	*n*	Prevalence (95% CI)
ANP	10	0.9 (0.54–0.99)
KMNP	32	0.94 (0.78–0.99)
Total	42	0.93 (0.79–0.98)

[a] ANP = Ankarafantsika National Park; KMNP = Kirindy Mitea National Park.

TABLE 3. Extended.

CHV[b]	CPV[b]			FCV[b]			*Toxoplasma*[b]	
Positive (95% CI)	*n*	Suspect (95% CI)	Positive (95% CI)	*n*	Suspect (95% CI)	Positive (95% CI)	*n*	Positive (95% CI)
0.2 (0.1–0.34)	49	0	0.67 (0.52–0.8)	2	0	0	16	0.88 (0.6-0.98)
0.09 (0.03–0.23)	44	0.09 (0.03–0.21)	0	44	0.21 (0.11-0.36)	0.23 (0.13-0.38)	42	0.93 (0.79-0.98)
0	2	0	0	20	0.15 (0.04-0.39)	0.35 (0.16-0.59)	8	0.38 (0.1-0.74)
—	—	—	—	61	0.21 (0.12-0.34)	0.11 (0.05-0.23)	18	0.22 (0.07-0.48)
0	5	0.6 (0.17–0.93)	0.2 (0.01–0.7)	5	0	0.4 (0.07-0.83)	6	0.17 (0.01-0.64)
—	1	0	0	0	—	—	1	0
0.13 (0.08–0.22)	101	0.07 (0.03–0.13)	0.34 (0.25–0.44)	131	0.19 (0.13-0.27)	0.21 (0.14-0.29)	91	0.67 (0.56-0.76)

either *Felis catus* or *Felis silvestris*. In a captive fosa, *T. gondii* caused encephalomyelitis resulting in ataxia, muscular atrophy, and eventually death (Corpa et al. 2013). However, the high prevalence of antibodies to *Toxoplasma* detected in free-ranging fosa at both sites of this study suggests that *Toxoplasma* infection may not be universally lethal in fosa.

Presence of antibodies to *Toxoplasma* in fosa was significantly associated with age. Adults (OR=17.5; *P*=0.03) were more likely to have antibodies to *Toxoplasma* than immature individuals (juveniles and subadults). This finding is consistent with other studies of the effect of age on presence of antibodies to *Toxoplasma* in wild animal species (Åkerstedt et al. 2010; Garcıa-Bocanegra et al. 2010). This suggests horizontal transmission of the parasite, which may occur through ingestion of an intermediate host. The diet of free-ranging fosa consists of a wide range of animal species including the black rat (*Rattus rattus*; Dollar et al. 2007), a potential intermediate host of the parasite. Similar prevalence of antibodies in fosa at both sites may result from comparable environmental exposure due to similar cat populations in the villages (L.D. pers. obs).

A large proportion of dogs (69%) were exposed to canine parvovirus. Canine parvovirus is transmitted indirectly from environmental contamination and by the fecal oral route. The virus is also highly resistant and can survive for months in the environment. These characteristics may facilitate persistence of the virus in the environment and its transmission to wild carnivore population (Fiorello 2004; Woodroffe and Donnelly 2011). Cross-species transmission of CPV-2 is well documented, and antibodies to CPV-2 were found in several species of *Felidae*, *Canidae*, *Procyonidae*, *Mustelidae*, *Ursidae*, and *Viverridae* (Steinel et al. 2001). Infection of free-ranging wildlife was attributed to increasing populations of domestic dogs and habitat overlap between species. The high prevalence of this pathogen in dogs in Madagascar could facilitate spillover from domestic to endemic carnivores.

One giant striped mongoose was positive for antibody to CPV-2 and 9% of fosa had low positive titers 10–20 anti-CPV-2. This shows that euplerids may be exposed to a parvovirus, but no clinical signs were detected in the captured endemic carnivores. Pathogenic effects of CPV-2 in euplerids are unknown, but in other wild carnivore species CPV may cause nonsuppurative myocarditis in pups (Hayes et al. 1979; McCandlish et al. 1981) or gastrointestinal signs in animals of all ages (Appel 1987). The absence of high positive titers in fosa may indicate that most of the infected fosa do not survive infection by CPV-2, an inadequate diagnostic method, or lack of exposure to feces from infected dogs.

Canine distemper is arguably one of the most important viral diseases affecting do-

mestic and wild carnivores worldwide (Deem et al. 2000; Terio and Craft 2013). It has caused morbidity and mortalities in a wide range of captive and free-ranging species including *Canidae*, *Felidae*, *Mustelidae*, *Ailuridae*, and *Ursidae* (Deem et al. 2000; Cottrell et al. 2013; Seimon et al. 2013), and domestic dogs were identified or suspected as the origin of infection. Studies suggest that tiger populations are 25 times more likely to go extinct if affected by canine distemper, and the most significant factors influencing infection were virus prevalence in the reservoir population (dogs) and its contact rate with wildlife (Gilbert et al. 2014).

Fosa only had low antibody titers (10) to CDV while 45% of the dogs tested had been exposed to CDV. The low titers detected in fosa may be due to the persistence of maternal antibodies, an early stage in seroconversion, waning titers from exposure to virus, cross reactivity, or toxicity of the sample for the test cells. However, the lack of positive titers (>16) to CDV in fosa may indicate 1) absence of exposure of fosa to this directly transmitted virus, 2) low survival rate of infected fosa, or 3) an inadequate diagnostic method for canine distemper in this endemic carnivore.

The prevalence of antibodies to FCV in fosa from this study is similar to the antibody prevalence in wildcats from Saudi Arabia and within the range of antibody prevalences found in hyenas from Tanzania (Ostrowski et al. 2003; Harrison et al. 2004). Feline *Calicivirus* is an RNA virus from the family *Caliciviridae*, causing oral ulcers and upper respiratory tract disease in affected cats, but clinical signs in other taxonomic groups are unknown. This virus has a short survival time in the environment and is transmitted through direct contact by saliva and nasal secretion (Radford et al. 2007). It is not known whether fosa become infected through contact with cats or if the virus may be transmitted directly between fosa. No fosa exhibited signs consistent with infection with FCV, and further studies are required to monitor the exposure of fosa to this virus and evaluate its ability to spread within the fosa population.

In conclusion, we report the prevalence of antibodies to common viral and parasitic pathogens in introduced and endemic carnivores in Madagascar. The use of serologic techniques in a cross-sectional manner limits us to discussing exposure to pathogens rather than current infection status. However, these data provide a basis for monitoring the exposure of carnivores to these pathogens and for assessing the risks of spillover among carnivores in this biodiverse area. Although we cannot rule out the possibility of false positives and cross-reaction, our results indicate that free-ranging fosa may be exposed to several common pathogens of dogs and cats. Further studies using metagenomic approaches, for example, are needed to identify the specific strains of viruses and parasites that are infecting domestic and endemic carnivores in Madagascar (Bodewes et al. 2014). Similarly, long-term health monitoring that may be based on "passive collection" coupled with population surveys are needed to evaluate the potential impact of diseases on wild fosa.

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