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Author(s): Emily L. Blizzard, Cheryl D. Davis, Scott Henke, David B. Long, Christopher A. Hall, and Michael J. Yabsley

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DISTRIBUTION, PREVALENCE, AND GENETIC CHARACTERIZATION OF *BAYLISASCARIS PROCYONIS* IN SELECTED AREAS OF GEORGIA

Emily L. Blizzard*, **Cheryl D. Davis†**, **Scott Henke‡**, **David B. Long§**, **Christopher A. Hall‖**, **and Michael J. Yabsley***
Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia 30602. *e-mail: emily.blizzard@gmail.com, myabsley@uga.edu*

ABSTRACT: *Baylisascaris procyonis* is an intestinal nematode of raccoons (*Procyon lotor*) that can cause fatal larval migrans in numerous species of birds and mammals, including humans. Although this parasite has historically been absent in the southeastern United States, it has been found in isolated regions in the Appalachian Mountains and was recently documented in DeKalb County, Georgia. The first objective of the current study was to investigate the distribution and prevalence of *B. procyonis* in selected populations of raccoons in Georgia. Intestinal tracts of 312 raccoons from 25 Georgia counties were examined for *B. procyonis*. The only county where *B. procyonis* was detected was Clarke County, where 12 of 116 (10.3%) raccoons were infected. In Clarke County, significantly more juveniles ($P = 0.049$) were infected compared with adults, and no differences in prevalence were noted by sex, season of capture, or land use (rural vs. urban); however, significantly ($P = 0.0370$) higher worm burdens were found in infected raccoons from urban/suburban locations compared with rural areas. In addition, *Toxascaris leonina*, a morphologically similar ascarid, was found in 3 raccoons from Clarke County (n = 2) and Morgan County (n = 1). A second objective was to determine if sequence polymorphisms were associated with *B. procyonis* from different geographic regions. Because sequences from a single worm from Japan had been entered into GenBank, we obtained nematodes from Kentucky and Texas for comparison with our samples from Georgia. Sequence analysis of the 18S and 5.8S rRNA genes and the internal transcribed spacer (ITS) -1 and ITS-2 regions confirmed Georgia samples were *B. procyonis*. Although several polymorphic bases were observed within both ITS regions, none was associated with a particular geographic location. These data indicate that the distribution of *B. procyonis* within Georgia is increasing and only limited genetic variation is present in the rRNA and ITS gene regions among *B. procyonis* from the southern United States and introduced populations in Japan.

Increased anthropogenic changes to natural landscapes during recent decades has led to substantial increases in raccoon (*Procyon lotor*) population densities in some areas (Page et al., 2008). These heterogeneous landscapes, which are closely associated with humans, provide readily available food sources that raccoons commonly exploit (Lotze and Anderson, 1979; Riley et al., 1998; Gehrt, 2003). Raccoon densities in such urbanized areas can range from 37 to 333/km^2 compared to typical rural population densities of 2 to 20/km^2 (Lotze and Anderson, 1979; Riley et al., 1998; Gehrt, 2003; Prange et al., 2003). As anthropogenic change increases, more frequent interactions between animals and humans will occur. Combined with increasing raccoon populations, the risk for the transfer of zoonotic diseases could increase significantly. Increased density and interactions with other animals can result in increased transmission of numerous pathogens, including *B. procyonis* (Wright and Gomper, 2005; Page et al., 2009). Additionally, the illegal transport of raccoons can result in the introduction of novel diseases into new geographic areas (Nettles et al., 1979).

Although large numbers of *B. procyonis* adults may be present in the small intestine of raccoons, little to no disease has been reported (Carlson and Nielsen, 1984). In addition, domestic dogs can serve as definitive hosts (Bowman et al., 2005). When paratenic hosts, including humans, ingest mature eggs, migrating larvae can cause severe visceral, ocular, and neural larval migrans. Over 90 species of birds and mammals are susceptible to infection, and such infection often results in high morbidity or mortality of certain species of rodents, lagomorphs, and birds (Kazacos, 2001). In raccoons, the highest prevalences of *B. procyonis* are found in the northeastern, midwestern, and mid-Atlantic states, and along coastal areas of Texas, California, Washington, and Oregon (Kazacos, 2001). In the Southeast, this parasite has historically been restricted to the Appalachian regions of West Virginia and Virginia and isolated regions of Kentucky and Tennessee and a single infected raccoon from a northeast Georgia mountainous county during 1976 (Harkema and Miller, 1962; Jacobson et al., 1976; Bafundo et al., 1980; Schaffer et al., 1981; Jones and McGinnes, 1983; Smith et al., 1985; Cole and Shoop, 1987; Kazacos, 2001; Owen et al., 2004; Souza et al., 2009). In 2002, *B. procyonis* was detected in 22% (11/50) of free-ranging raccoons from Dekalb County, Georgia (Eberhard et al., 2003). Concurrent with that finding, a wildlife rehabilitator in Clarke County, Georgia reported finding *B. procyonis* in a single raccoon, although the history of this animal is unknown, e.g., county of origin (Eberhard et al., 2003). Because of the zoonotic and wildlife health implications of this parasite, we initiated this study to determine the prevalence and distribution of *B. procyonis* in selected counties of Georgia. In addition, we analyzed samples of *B. procyonis* from Georgia, Texas, and Kentucky to determine if genetic polymorphisms were present in the 18S and 5.8S rRNA genes and internal transcribed spacer (ITS) -1 and ITS-2 regions.

MATERIALS AND METHODS

Sample collection

From February 2005 to September 2009, 228 raccoons were collected from 4 northeastern Georgia counties (Clarke, Morgan, Oglethorpe, and Oconee) and 4 southern or coastal counties (Baker, Camden, Chatham, and Long) (Table I). All animals were either captured in box traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin) baited with sardines or canned cat food, or were fortuitous road-kill collections.

All capture and handling procedures were approved by the UGA Institutional Animal Care and Use Committee. Captured raccoons were anesthetized with an intramuscular injection of ketamine hydrochloride

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* Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, Georgia 30602.
† Department of Biology, Western Kentucky University, Bowling Green, Kentucky 42101.
‡ Caesar Kleberg Wildlife Research Institute, Texas A&M University–Kingsville, Kingsville, Texas 78363.
§ United States Department of Agriculture, Animal Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Kingsville, Texas 78363.
‖ Berry College, Mt. Berry, Georgia 30149.
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(25 mg/kg body weight, Aveco Co., Fort Dodge, Iowa) plus xylazine (0.25 mg/body weight, Mobay Corp., Animal Health Division, Shawnee, Kansas) or tiletamine/zolazepam (Telazol®, 0.6 mg/kg body weight, Aveco Co.). Anesthetized animals were sexed and aged, and ectoparasites and a whole blood sample were collected for other projects (Yabsley et al., 2008; Brown et al., 2010). After blood collection, raccoons were killed with an intracardiac injection of sodium pentobarbital (Beuthanasia®-D Special, Schering-Plough Animal Health Corp., Omaha, Nebraska). Animals were necropsied and the intestinal tract was either examined immediately for *B. procyonis* or frozen for future analysis. Additional tissue samples were collected for other ongoing studies. If large nematodes were noted, they were collected and stored in 70% ethanol for identification based on morphologic characteristics (Sprent, 1968) and sequence analysis.

In addition to the raccoons sampled in the current study, we examined records from 84 clinical cases (1997–2009) from 17 other counties, submitted to the Southeastern Cooperative Wildlife Disease Study (College of Veterinary Medicine, Athens, Georgia). All of these raccoons were necropsied for routine diagnostic evaluations, which included screening of intestines for the presence of large nematodes such as *B. procyonis*.

Data analysis

Only animals caught in Clarke County, Georgia were used to assess effects of sex, age, season, and land use, i.e., animals captured from urban vs. rural locations, on the prevalence of *B. procyonis*. Fisher's exact test was used to determine if differences existed between prevalence and these variables. Trapping locations within Clarke County were classified as urban or rural based on data from the Georgia Land Use Trends Project (Natural Resources Spatial Analysis Laboratory, Odum School of Ecology, University of Georgia, Athens, Georgia). Raccoons were classified as juveniles ($<$1 yr) or adults based on weight, tooth wear, and development of reproductive organs (Grau et al., 1970). Seasons were classified as follows: winter (December–March), spring (April–May), summer (June–July), and fall (August–November). Analysis of variance was used to determine if significant differences in nematode intensity were present due to age, season, or land use.

Genetic characterization

Eighteen specimens of *B. procyonis* from Clarke County, Georgia (n = 9), Barren and Warren Counties, Kentucky (n = 4), and Duval County, Texas (n = 5) that were preserved in 70% ethanol were included in the genetic characterization. Nematodes were digested with the use of proteinase K digestion for 48 hr, and total genomic DNA was extracted with the use of DNAeasy extraction kit (Qiagen, Valencia, California). The internal transcribed spacer (ITS) -1 and ITS-2 regions were amplified by polymerase chain reaction (PCR) (Saiki et al., 1988; Zhu et al., 2001; Ishiwata et al., 2004) with the use of primers F2662 (ITS1 forward, 5′-GGCAAAAGTCGTAACAAGGT), R3214 (ITS1 reverse, 5′-CTGCAATTCGCACTATTTATCG), F3207 (ITS2 forward, 5′-CGAG-TATCGATGAAGAACGCAGC), and R3720 (ITS2 reverse, 5′-ATATGCTTAAGTTCAGCGGG). The 5.8S rRNA and ITS-1 and ITS-2 regions were amplified from some specimens with the use of primers NC5 5′-GTAGGTGAACCTGCGGAAGGATCATT-3′ (forward) and NC2 5′-TTAGTTTCTTTTCCTCCGCT-3′ (reverse) (Zhu et al., 1998, 2001). The 18S rRNA gene was amplified by using 18SascF 5′-CCCGATTGATTCTGTCGTCGGC (forward) and 18Sasc R 5′-CAA-CATACTTCCCCCGC (reverse), NC5 5′-GTAGGTGAACCTGCG-GAAGGATCATT-3′ (forward) (Nadler and Hudspeth, 1998). PCR was performed in 50-μl volumes with 100 pmol of each primer, 250 μM of each dNTP, 3.0 mM $MgCl_2$, and 2 U Taq polymerase (Promega, Madison, Wisconsin) under the following conditions: an initial denaturation at 94 C for 4 min followed by 44 cycles of 94 C for 30 sec, 52 C for 1 min, 72 C for 1 min, and a final step at 72 C for 5 min. PCR products were detected in a 1% agarose gel stained with ethidium bromide, purified with a Gel Extraction Kit (Qiagen), and independently bidirectionally sequenced at Georgia Genomics Facility (Athens, Georgia). Sequences of any of these genes or regions can be used to distinguish immature nematodes of these 2 morphologically similar ascarids. The sequences were obtained from the 18S and 5.8S rRNA genes from *B. procyonis* from Georgia, Kentucky, and Texas, and a sample from GenBank from Japan.

RESULTS

Survey

Baylisascaris procyonis was detected in 12 of 312 (3.8%) raccoons in Georgia; all of the positives were from Clarke County (n = 116, 10.3%) (Fig. 1, Table I). Identification of these adult and immature worms was confirmed based on morphologic characteristics, or sequence analysis, or both. Among the infected raccoons, a range of 1–22 worms (average intensity 6.9 ± 8.2) was detected. Significantly more juvenile raccoons ($P = 0.049$) and raccoons from nonrural locations ($P = 0.0370$) were infected (Table II) compared with adults and raccoons from rural locations. No difference in prevalence was noted between sexes ($P = 0.7601$) or between seasons (all P values >0.1). No differences in nematode intensity were observed between age, sex, or season classes; however, significantly ($F = 5.78$, $P = 0.0370$) higher worm burdens were found in infected raccoons from urban/suburban locations in Clarke County (Table II). Two raccoons from Clarke County and 1 from Morgan County were infected with another ascarid, *Toxascaris leonina*.

Genetic characterization

Sequences of the 18S rRNA (1,951 base pairs [bp]) and 5.8S rRNA (157 bp) genes from Georgia samples were identical to *B. procyonis* (GenBank U94368 and AJ001501, respectively). Only limited nucleotide polymorphisms were noted in the ITS-1 and ITS-2 regions (Tables III, IV). ITS-1 sequences from Georgia, Kentucky, and Texas were 99.3–98.8%, 99.3%, and 99.3–99.8%, respectively, identical to a *B. procyonis* sample from Japan (AB053230) (Table III). More sequence conservation was noted for the ITS-2 region with 10 samples from Georgia, Kentucky, and Texas being identical to a sample from Japan (AB051231) (Table IV). Single nucleotide polymorphisms (99.8% identity) were noted in 4 individual worms from Georgia (Table IV).

DISCUSSION

In Georgia, *B. procyonis* was first reported in a single raccoon (of 110, 0.9%) from a northeastern county in 1976 (Kazacos, 2001). Since that initial finding, additional surveys throughout Georgia failed to detect additional positives until 2002 when infected raccoons were detected in suburban Atlanta (DeKalb County) and a single raccoon with an unknown history admitted to a rehabilitation center in Clarke County (Jordan and Hayes, 1959; Harkema and Miller, 1962; Schaffer et al., 1981; Price and Haram, 1983; Eberhard et al., 2003). In the current study, we detected *B. procyonis*–infected raccoons only in Clarke County; however, low numbers of raccoons were surveyed in several of the tested counties. Currently, it is not known if the presence of *B. procyonis* in the Piedmont region of Georgia (DeKalb and Clarke Counties) is due to natural spread from endemic areas, e.g., from southern Tennessee (Souza et al., 2009), or possibly from translocation of infected raccoons from endemic areas. Historically, *B. procyonis*—infected raccoons in the southeastern United States were restricted to mountainous regions of West Virginia and Virginia and isolated regions of Kentucky and Tennessee (Harkema and Miller, 1962; Jacobson et al., 1976; Bafundo et al., 1980; Schaffer et al., 1981; Jones and McGinnes, 1983; Smith et al., 1985; Cole and Shoop, 1987; Owen et al., 2004; Souza et al.,

FIGURE 1. Adult *Baylisascaris procyonis* worms detected in the small intestine of a juvenile raccoon from Clarke County, Georgia, USA.

2009). A recent survey of raccoons from northwestern South Carolina failed to detect *B. procyonis* (Yabsley and Nobblet, 1999), so additional surveys in northern Georgia are needed to establish if *B. procyonis* is present in mountainous regions of Georgia. In addition, a recent finding of *B. procyonis* in northwestern Florida raises concerns that this parasite might be present in southern Georgia (Blizzard, 2010).

Similar to numerous previous studies, we found that the prevalence of *B. procyonis* in raccoons from Clarke County was highest in juveniles (Snyder and Fitzgerald, 1985; Ermer and Fodge, 1986; Robel et al., 1989; Evans, 2001; Kazacos, 2001; Yeitz et al., 2009). Although prevalence rates are expected to be highest in juveniles because juveniles are more susceptible to infection by ingestion of eggs compared with adults (Kazacos, 2001), some studies in coastal Texas (Kerr et al., 1997), eastern Tennessee (Souza et al., 2009), and in British Columbia, Canada (Ching et al., 2000) failed to detect differences in prevalence between juveniles and adults. We did not detect a difference in prevalence between sexes similar to several other studies (Ermer and Fodge, 1986; Ching et al., 2000; Souza et al., 2009; Yeitz et al., 2009), although some studies have found higher prevalences in males (Snyder and Fitzgerald, 1985; Cole and Shoop, 1987; Snyder and Fitzgerald, 1987; Kidder et al., 1989; Robel et al., 1989; Evans, 2001; Wirsing et al., 2007). In the northeastern and midwestern United States, the prevalence of *B. procyonis* peaks in late summer and autumn and decreases during the winter (Smith et al., 1985; Kidder et al., 1989; Evans, 2001; Kazacos, 2001; Evans, 2002; Page et al. 2005); however, our results were similar

TABLE I. Prevalence of *Baylisascaris procyonis* in raccoons captured from 1997 to 2009 from 25 counties in Georgia.

County(s)	Year(s)	n	No. positive (%)	Average intensity ± SD	Range
Baker/Thomas	2003–2008	35	0	na*	na
Barrow/Oconee/Jackson	1997–2009	11	0	na	na
Chatham/Long	2006–2008	57	0	na	na
Clarke	2007–2009	116	12 (10.3)	6.9 ± 8.2	1–22
Columbia/Lincoln	1999–2001	2	0	na	na
Coweta	2005–2006	7	0	na	na
Dade/Floyd	2001–2009	49	0	na	na
Forsyth/Hall/Lumpkin/White	2001–2005	6	0	na	na
Glynn/McIntosh/Camden	2003–2008	17	0	na	na
Greene/Morgan/Walton	1997–2008	6	0	na	na
Madison/Oglethorpe	1999–2008	6	0	na	na
Total		312	12 (3.8)		

* na, not applicable.

TABLE II. Relationships between *Baylisascaris procyonis* prevalence and age, sex, season, and land use in Clarke County, Georgia.

	n	No. positive (%)	Average intensity ± SD	Range
Age				
Juvenile	36	7 (19.4)	9.9 ± 9.5	1–22
Adult	78	5 (6.4)	2.8 ± 3.5	1–9
Sex				
Male	66	6 (9.1)	6.2 ± 6.3	1–17
Female	50	6 (12)	7.7 ± 10.3	1–22
Season				
Winter	15	1 (6.7)	9	9
Spring	14	3 (21.4)	6.7 ± 8.9	1–17
Summer	33	2 (6.1)	1	1
Fall	52	6 (11.5)	8.7 ± 9.9	1–22
Land use				
Rural	61	6 (9.8)	2.2 ± 2.4	1–7
Low- and high-intensity urban	50	6 (12)	11.7 ± 9.4	1–22

to other studies conducted in other southeastern states (Tennessee, Kentucky, and Texas) where no difference in prevalence was noted between seasons (Smith et al., 1985; Kerr et al., 1997).

Our marginally significant higher prevalence in juveniles and lack of seasonal differences could be due to the current low prevalence in Clarke County, low numbers of raccoons surveyed, or the presence of a large number of naïve adult raccoons that are susceptible to infection. Also, a lack of extreme seasonal temperature changes in the southeastern United States could increase the period of raccoon activity and exposure to *B. procyonis*, which may limit seasonal fluctuations in prevalence (Smith et al., 1985; Kidder, 1989; Kerr et al., 1997). It has also been suggested that prevalence decreases during the winter because of a reduction in food resources in northern temperate regions in which animals may lose up to 50% of their total body mass when they experience extreme weather conditions for extended periods of time, during which time self-cure may occur (Stuewer, 1943; Mech et al., 1968; Kidder et al., 1989; LoGiudice, 1995). Additionally, it is also plausible that raccoons throughout the southern United States may rely more heavily on berries and fruits and less on carnivorous diets than their northern counterparts, which has been suggested in a study in Alabama, where the diet of raccoons from April to November primarily consisted of fruits and plant material (~50 to 80%) and infrequently consisted of vertebrates (Johnson, 1970). Alternatively, increased heat could increase desiccation at latrine sites, which may decrease the risk of young raccoons acquiring infections at latrine sites. The thermal tolerance of *B. procyonis* eggs in water has been determined to be >47 C; however, the effects of heat, UV exposure, and desiccation have not been studied (Shafir et al., 2007). Collectively, these factors, i.e., less restrictive resource limitations, less substantial seasonal weight fluctuations during winter months, seasonal temperature differences, and decreased propensity to ingesting infected intermediate hosts, could decrease transmission of *B. procyonis*; however, additional work is needed to confirm this hypothesis (Goldman, 1950).

TABLE III. Nucleotide sequence variations within the internal transcribed spacer 1 region of *Baylisascaris procyonis* from Georgia (GA), Texas (TX), and Kentucky (KY).

	Nucleotide position*								
	6	20	48	57	67	276	282	324	392
AB053230†	C	T	C	A	G	G	T	G	T
Various‡	•§	–	•	G	•	•	K	•	•
GA308	G	•	G	•	T	R	•	C	•
GA267	•	•	•	•	•	•	•	•	•
TX3, TX11, TX12	•	•	•	•	•	•	•	•	G

* Numbering relative to AB053230.
† Raccoon from Hyogo Prefecture, Japan.
‡ Includes 6 samples from GA (197, 229, 259, 268, 270, and 279), 4 samples from KY (1, 2, 3, and 4), and 2 worms from TX (1 and 2).
§ •, same nucleotide as reference strain.

TABLE IV. Nucleotide sequence variations within the internal transcribed spacer 2 region of *Baylisascaris procyonis* from Georgia (GA), Texas (TX), and Kentucky (KY).

	Nucleotide position*			
	610	650	327	765
AB051231†	A	T	C	C
Various‡	•	•	•	•
GA 267	T	•	•	•
GA 268	•	G	•	•
GA 197	•	•	T	•
GA 229	•	•	•	G

* Numbering relative to AB051231.
† Raccoon from Hyogo Prefecture, Japan.
‡ Includes 4 samples from GA (259, 269, 270, and 308), 3 samples from KY (1, 2, and 3), and 3 worms from TX (1, 11, and 12).

In contrast to Page et al. (2008), we observed a trend toward higher prevalence and intensity of infection in suburban/urban areas compared with raccoons from nonurban areas of Clarke County, Georgia. Page et al. (2008) attributed the lower prevalence and lower nematode intensity in urban raccoons to behavioral factors, including change in foraging habits and differences in home range, which lead to a decreased exposure to *B. procyonis* eggs. Urbanized raccoons are more likely to forage in refuse and other human-provided food sources that would decrease the chances of a raccoon ingesting *B. procyonis*–infected paratenic hosts (Page et al., 2005, 2008). Furthermore, urbanized raccoons have been shown to have smaller home ranges, which limit contact and foraging in potentially contaminated areas (Gehrt, 2003; Prange et al., 2004; Page et al., 2008). In our current study, we classified raccoons as primarily low- and high-intensity urban or nonrural, based on dwelling or commercial property criteria. However, a high percentage of our nonurban lands (forest, sparse, pasture/crops) was in close proximity to urban areas, which suggests that raccoons in Clarke County likely forage across a range of habitat types. Furthermore, areas classified as urban or nonurban in Clarke County, Georgia are more similar to each other compared with the urban and nonurban areas examined by Page et al. (2008). Similar behavior and movement studies have not been conducted on raccoons from Clarke County, Georgia. Interestingly, we found significantly higher nematode intensity in infected raccoons from suburban/

urban areas compared with rural areas; however, only a few raccoons from the urban areas were infected with the highest worm burdens (n = 21 and 22 worms each), which may have influenced this observation.

In addition to *B. procyonis*, we detected *T. leonina*, which is a common parasite of domestic canids and felids, and several wild carnivore species, including raccoons in Texas and Saskatchewan, Canada (Wirsing et al., 2007; Kresta et al., 2009). Careful morphologic examination of adult male nematodes or eggs from mature females is needed to distinguish these species definitively. In the current study, we detected several singleton immature infections, which complicated identification because distinguishing characteristics (roughened perianal patch on male *B. procyonis* or small cervical alae on *T. leonina*) are difficult or impossible to discern on immature nematodes.

Molecular characterization of the ascarids in this study served 2 purposes. First, it allowed definitive diagnosis of the worms, especially those that were immature. Second, it provided information on the genetic variability among our selected gene targets and geographic regions. For this work, we selected the 18S or 5.8S rRNA genes and the ITS-1 or ITS-2 regions, which have been used in previous studies to distinguish among *Baylisascaris* spp. and related ascarids (Zhu et al., 1998, 2001). We confirmed that the sequences of any of these genes or regions can be used to distinguish immature nematodes of the 2 morphologically similar ascarids that we found in the current study. The sequences we obtained from the 18S and 5.8S rRNA genes from *B. procyonis* from Georgia, Kentucky, and Texas and a sample from GenBank from Japan were identical. Although limited genetic variability was noted in the ITS -1 and ITS-2 regions, these polymorphisms were not associated with the any of the 4 locations from which *B. procyonis* was available. It would be interesting to obtain sequences from additional samples or gene targets from other *B. procyonis* endemic sites, e.g., western, upper midwestern, or northeastern United States, to determine if additional variation is present in these regions where *B. procyonis* was historically common.

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