J Parasit Dis (Oct–Dec 2022) 46(4):1021–1027
https://doi.org/10.1007/s12639-022-01525-y

ORIGINAL ARTICLE

Prevalence of *Toxocara canis* infection in dogs and *Toxocara* egg environmental contamination in Baybay City, Leyte, Philippines

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Received: 5 December 2021 / Accepted: 2 August 2022 / Published online: 9 August 2022

Abstract *Toxocara canis* is a parasitic gastrointestinal nematode of dogs causing toxocariasis in humans, a neglected tropical disease. This study examined the burden of *T. canis* infection in dogs and egg contamination in school playgrounds and public parks in Baybay City, Leyte, Philippines. Fecalysis and egg detection in soils were performed to determine the parasite prevalence in dogs and the environment. In addition, pet owner's survey and GIS mapping were employed to identify the risk factors of infection and map the parasite distribution. Fecalysis revealed a *T. canis* prevalence of 64.44% (95% CI: 48.78–78.13) at the barangay level and 17.96% (95% CI: 14.12–21.80) at the animal level. Rural areas showed a much higher *T. canis* prevalence than urban. Factors associated with high *T. canis* infection included dog's age, specifically puppy (2–8 mo) relative to young (> 8–24 mo) [Adjusted Odds Ratio (AOR): 2.282; 95% CI: 1.137–4.579; $p = 0.020$] and adult (> 24 mo) (AOR: 3.542; 95% CI: 1.714–7.319; $p = 0.001$), access to dirty water (AOR: 2.749; 95% CI: 1.575–4.798; $p < 0.001$), and non-deworming (AOR: 2.961; 95%: CI 1.009–8.684; $p = 0.048$). Furthermore, we observed high *Toxocara* egg contamination in school playgrounds and public parks, with a prevalence of 51.11% (95% CI: 35.77–66.3) at the barangay level and 15.45% (95% CI: 10.67–20.23) in soil samples. This epidemiological study is the first in the central Philippines to report a high burden of *T. canis* infection in dogs and the environment, thus providing crucial data that will help understand the epidemiology of toxocariasis.

Keywords Toxocara canis · Soil-transmitted helminth · Prevalence · Risk factors · Spatial distribution · Philippines

Introduction

Toxocara canis is a neglected zoonotic disease in humans and a ubiquitous intestinal nematode in dogs, the parasite's definitive host. While *T. canis* matures in the small intestine of dogs, its larvae cannot develop further inside the human body but can survive in tissues for at least five years (Bradbury and Hobbs 2020). Hence, human infections of *T. canis* manifest as aberrant migrations of larvae, of which four clinical forms have been identified depending on the organs affected: visceral, ocular, neural, and covert toxocariasis (Chen et al. 2018).

T. canis infects humans when embryonated eggs are ingested from dirt or soils contaminated with dog's feces or when encapsulated larvae are eaten from raw and/or uncooked meat and liver of paratenic hosts, such as chicken and cattle. After ingestion, the infective larvae migrate from the intestinal lumen to the neighboring organs, where the parasite causes inflammatory reactions that lead to symptoms of visceral larva migrans, characterized by hepatic, gastrointestinal, and respiratory symptoms (Wu and Bowman 2020). Moreover, *T. canis* larvae traveling through the blood circulation may enter the human eye, causing intraocular inflammatory reactions that usually result in visual impairment (Magnaval et al. 2001; Waindok et al. 2021). In addition, when the circulating larvae lodge in the brain and spinal cord, severe inflammation occurs, resulting in cerebral and spinal lesions, thus causing neural toxocariasis. Finally,

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12639-022-01525-y.

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a covert form of the infection could occur as a general manifestation of toxocariasis, covering a variety of symptoms in patients with high levels of anti-*Toxocara* antibodies (Chen et al. 2018).

With the ability of *T. canis* to lay ~200 thousand eggs per day, it is expected that a single dog carrying at least six female and one male adult nematode may shed millions of eggs that can heavily contaminate the environment (Nicoletti 2013). Hence, access of *T. canis*-infected dogs to parks, playgrounds, and recreational spaces has been identified as the main factor influencing the global prevalence of human toxocariasis (Embil et al. 1988; Amaral et al. 2010; Bradbury and Hobbs 2020).

In the Philippines, the *Toxocara* egg contamination rate in soils collected from playgrounds, backyards, and empty lots has been shown to correlate with the seroprevalence rate of anti-*Toxocara* antibodies in children, suggesting that infection might have been acquired through those spaces while playing (Fajutag and Paller 2013). However, the initial prevalence rate of *Toxocara canis* infection in dogs has not been estimated. Indeed, studies on the prevalence of canine *Toxocara* infection are surprisingly rare across the Philippines, although there are a few reports from local veterinary clinics (Urgel et al. 2019; Rostami et al. 2020). Nevertheless, prior studies in humans informing high seroprevalence rates of 43% in children and 67% in patients are reported in select Philippine locations (Auer et al. 1995; Fajutag and Paller 2013). In several provinces, the rate of *Toxocara* egg contamination in the soils ranged from 4 to 77%, with no data on *Toxocara* prevalence rates in dogs (Paller and de Chavez 2014; Paller and Babia-Abion 2019; Delaluna et al. 2020).

In this study, we determined the prevalence of *T. canis* in dogs and identified the risk factors associated with canine infection in Baybay City, Leyte, Philippines. We further assessed the contamination rate of *Toxocara* eggs from soils in schools and public spaces to assess the extent of contamination and potential soilborne transmission. Our data offer the first baseline information about *T. canis* in dogs and soils in one of the cities in the central Philippines, which are critical factors to understanding the epidemiology of toxocariasis in the country.

Methods

Description of the study area

This study was performed from May to July 2021 in Baybay City, Leyte, specifically on the western coast (coordinates 10°41′N, 124°48′E) of Leyte island in the central Philippines. Baybay City has 68 rural and 24 urban smallest administrative divisions, referred to as barangays. The area has a tropical climate with an average annual rainfall of 2421 mm and an ambient temperature of 27 °C (SD ± 19 °C). On average, most residents owned two dogs, approximately 72% of which were found in urban barangays (Lañada et al. 2019).

Sample size calculation

A total of 384 dogs' feces samples were collected based on a 50% expected *T. canis* prevalence, 5% margin of error, and 95% confidence level (Thrushfield 1986). A proportional allocation sampling was performed to determine the sampling weight (i.e., number of dogs), sampled randomly from each barangay (Stevenson 2012). Briefly, using the pre-survey data on dog population, the number of samples in each barangay (x) was calculated as the barangay dog population (n) multiplied by the estimated sample size (384 dogs) and divided by the total dog population in the city ($N = 3486$). For the environmental survey of *T. canis* eggs contamination in soils, a total of 220 soil samples from school playgrounds, parks, and main roads were randomly collected from five different locations in 44 barangays with the highest dog population (Supplementary Table 1).

Sample collection

For *T. canis* infection in dogs, fresh fecal samples (~5 g) were collected directly from the dog's rectum using lubricated hand gloves, transported using a cold container, and examined on the same day or stored at 4 °C for the next-day examination. For *T. canis* eggs contamination in public spaces, soil samples of 25 cm^3 (i.e., sampling area of 10 cm^2 and depth of 2.5 cm) were collected randomly from corners and centers of parks or playgrounds in schools. In barangays without playgrounds or parks, the main roads were sampled using the same protocol, except that the site was selected randomly every 100-m apart. In all cases, soil samples were transported in a cold container and immediately examined for *T. canis* eggs.

Fecal and soil *T. canis* egg examination

A modified McMaster technique was used to determine *T. canis* eggs from dog feces using Whitlock universal counting chamber. Briefly, 3 g of dog feces was homogenized with 27-ml flotation solution [i.e., saturated sodium chloride (NaCl) solution; 1.2 specific gravity] to yield a 30 ml total volume. While keeping the mixture in constant agitation, 0.5 ml of the solution was pipetted off into the Whitlock universal counting chambers. The slide was set aside for 5 min to allow nematode eggs to rise on top. Then, the slide was viewed under the microscope (100× magnification) for the presence of *T. canis* eggs. The technique has a sensitivity of 25 eggs per gram of feces (Zajac and Conboy 2021).

The method for *T. canis* egg analysis from soil samples was based on Motazedian et al. (2006), with modification. Briefly, 250 g of soil sample was washed with 400-ml water by passing through a 100 µm sieve, in which trapped large debris was discarded, and the filtered liquid was recovered. Next, parasite eggs were collected by passing the filtered liquid across a 30 µm sieve. Trapped eggs from the sieve were recovered by flushing with 40 ml of water. Then, four aliquots of a 5 ml sample with 5 ml flotation solution (saturated NaCl) were mixed thoroughly and centrifuged at 250 × g for 5 min. After centrifugation, the upper liquid at the test-tube brim was collected for parasite egg examination under a light microscope. The identification of *Toxocara canis* eggs was based on morphology and size described in previous studies (Paller and Babia-Abion 2019; Zajac and Conboy 2021).

Survey questionnaire

A survey questionnaire was designed to collect information about the sampled dogs, dog owners, and practices in raising dogs as potential risk factors associated with *T. canis* infection (Supplementary Figure 1). Data were derived through face-to-face interviews of dog owners and environmental observations during visits, in which timing usually corresponded with fecal sample collections. The data gathered were encoded and organized, checked for duplication, and re-examined by another researcher for accuracy using a Microsoft Excel document.

Geographic information system (GIS) mapping

For mapping *T. canis* infection and ova contamination, the coordinates of sampled animals and soils were collected using a handheld global positioning system (GPS) device (Garmin 010-00630-00 Etrex®). The coordinates were mapped using the ArcGIS software, detailing positive and negative locations where animals and soils were sampled.

Data analysis

Prevalence rates with 95% CI were calculated as the proportion of positive samples over the total samples, using the Epi-Info™ 7 software (CDC Atlanta). For the risk factor analysis, a univariate logistic regression for the age variable with three categories (i.e., puppy, young, and adult) and a chi-square test for other categorical binary variables were first performed to screen putative factors associated with *T. canis* infection using the threshold *p-value* of 0.20 (Supplementary Table 2). Next, a multivariable logistic regression analysis was conducted to build the best-fitting, parsimonious, and biologically sensible risk factor model using the list of explanatory variables with $p < 0.20$. Finally, a stepwise backward elimination approach was performed to build the multivariable logistic regression model by eliminating variables one at a time and keeping those significant variables in the model ($p < 0.05$). All analysis was performed using the Epi-Info™ 7 software (CDC Atlanta).

Results

General characteristics of the sample

Dogs from urban barangays contributed to the most sampled animals (65.89%; 95% CI: 60.90–70.62). The vast majority of dogs were mixed breed (98.95%; 95% CI: 97.35–99.72), and their age ranged from 2 to 180 months (18 months median age). Sampled dogs were relatively distributed between sexes (51% female and 49% male), while neutered animals accounted for a small proportion (2.6%; 95% CI: 1.26–4.73). Respondents acquired their dogs mainly as a gift (61.46%; 95% CI: 56.39–66.35), and many considered their animals as house guards (45.3%; 95% CI: 40.26–50.44) rather than pets (13.80%; 95% CI: 10.51–17.66).

Prevalence of *T. canis* infection and ova contamination

At the barangay level, the prevalence of canine toxocariasis was 64.44% (95% CI: 48.78–78.13), while the prevalence of *Toxocara* egg contamination was 51.11% (95% CI: 35.77–66.3) (Fig. 1a). However, *Toxocara* prevalence rates at the individual level were much lower than at the barangay level, with 17.96% (95% CI: 14.12–21.80) for dogs and 15.45% (95% CI: 10.67–20.23) for soils. Although *Toxocara* egg contamination in the environment is related to *Toxocara* infection in dogs, our data revealed a non-significant linear correlation between dog infection and soil contamination (Fig. 1b). In terms of location, the prevalence data between rural and urban barangays showed a significant difference in dog infection rates (Fig. 1c), revealing a much higher *T. canis* prevalence in rural compared to the urban setting. Indeed, our GIS maps demonstrated a clear *T. canis*-negative cluster in urban barangays, both with canine infection and ova contamination (Fig. 2).

Risk factors of *T. canis* infection in dogs

We observed a threefold to fourfold lower prevalence of *T. canis* infection at the animal level than at the barangay level, suggesting that intrinsic and extrinsic dog-level factors are likely at play. In the final multivariable logistic regression model (Table 1), puppies aged 2–8 months showed the highest odds of *T. canis* infection compared to young dogs aged 8–24 months old (AOR: 2.28; 95% CI: 1.137–4.579; $p = 0.020$) and adult dogs beyond 24 months old (AOR: 3.542; 95% CI: 1.714–7.319; $p = 0.001$). Of

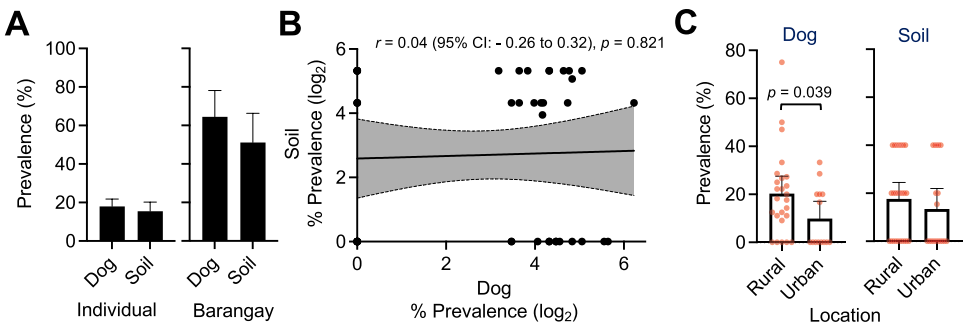

Fig. 1 Prevalence of *Toxocara canis* in Baybay City, Leyte, Philippines. **a** Prevalence with 95% CI of *T. canis* in dog and soil samples, determined at the animal and barangay level. **b** Pearson correlation between *T. canis* prevalence in soil and dog per barangay. **c** Prevalence with 95% CI of *T. canis* in dogs and soil categorized into urban and rural barangays. The red dot represents an individual barangay. *P* values were calculated using Welch's t-test (unequal variance assumption; two-tailed), with only those $p < 0.05$ shown in the figure

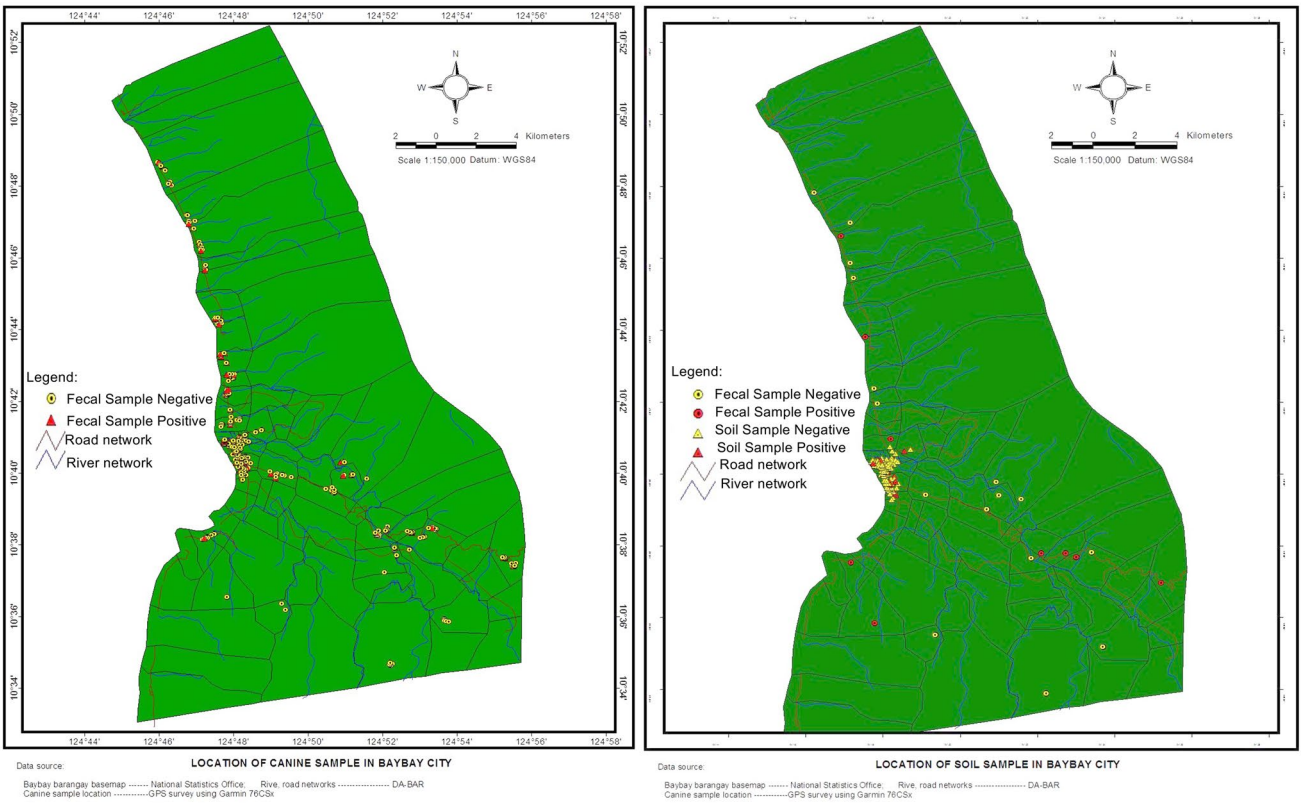

Fig. 2 Spatial distribution of *Toxocara* infection (left) and soil contamination (right). Red and yellow dots represent the coordinates where *T. canis* eggs were detected and not detected, respectively

note, we did not observe a significant difference in *T. canis* infection between young and adult dogs. Other intrinsic factors, such as dog sex and being neutered, did not significantly affect *T. canis* prevalence in the study site. Meanwhile, two extrinsic factors related to raising dogs were associated with *T. canis* infection in Baybay City. Firstly, non-dewormed dogs were almost three times (AOR: 2.961; 95%: CI 1.009–8.684; $p = 0.048$) more likely to carry *T. canis* eggs. Lastly, access to non-potable water, such as puddles and stagnant water in the ground or water from drainage or canals, increased the chance of canine *T. canis* infection by around three folds compared to those without access to dirty water (AOR: 2.749; 95% CI: 1.575–4.798; $p < 0.001$).

Table 1 Multivariable logistic regression model of the risk factors associated with canine *Toxocara* infection in Baybay City, Leyte

Variable	Adjusted odds ratio	95% CI	*p* value
Dog age			
*Puppy (2–8 mo)	3.542	1.714–7.319	$p=0.001$
Young (> 8–24 mo)	1.55	0.651–3.705	$p=0.322$
Adult (> 24 mo)	1 (Referent)		
Deworming			
Dewormed	1 (Referent)		
Not dewormed	2.961	1.009–8.684	$p=0.048$
Non-potable water			
No access	1 (Referent)		
Access	2.749	1.575–4.798	$p<0.001$

*The adjusted odds ratio for puppies is 2.28 (95% CI: 1.137–4.579; $p=0.020$) when the referent is young dogs

Number of samples = 384; LR chi^2 (4) = 39.100; Prob > chi^2 = < 0.001; Log likelihood = − 161.281; Pseudo R^2 = 0.108

Discussion

In the Philippines, epidemiological studies on *T. canis* infection in dogs are virtually lacking, while data on *Toxocara* egg contaminations are localized in Luzon, the northern part of the country. Here, we report the first epidemiological study on the burden of *T. canis* infection in dogs and *Toxocara* egg contamination in Baybay, one of the cities in the Leyte island, central Philippines. We observed a high prevalence of *T. canis* infection in dogs and identified which factors might have influenced its prevalence. Our data also reveal a high burden of *Toxocara* eggs in soils from school playgrounds, parks, or general public spaces, suggesting a potential source of human toxocariasis.

In this study, the high prevalence of *T. canis* in dogs is prominent in rural areas and significantly associated with puppies, the absence of deworming, and access to dirty water. The lack of access to veterinary care in rural areas intertwines with the absence of deworming or appropriate parasite interventions, which likely explain the high burden of *T. canis* infection. Similar studies on the lack of deworming regimen and increased burden of parasites in other animals (livestock) have also been reported in the same region (Rupa and Portugaliza 2016; Portugaliza et al. 2019). As patent *T. canis* infection persists among the majority of dogs, the environmental contamination of parasite eggs becomes prevalent, thus explaining the risk of *T. canis* positivity when dogs have access to dirty water, a proxy variable for a contaminated environment. Also, this finding connects with the burden of parasite egg contamination in soils in the study site, which is relatively higher in rural than in urban areas. As previously described, the prevalence of *T. canis* in young dogs under six months of age is higher than in older dogs, possibly due to their exploratory eating behavior and the role of transplacental and lactational transmissions (Overgaauw and van Knapen 2013; Eslahi et al. 2020).

Our study also demonstrated the high prevalence of *Toxocara* eggs in school playgrounds and public parks. In several studies, the presence of stray dogs and improper disposal of dog feces significantly contributed to disseminating *Toxocara* eggs in the environment, highlighting the importance of responsible pet ownership in the community (Nijsse et al. 2015; Szwabe and Blaszkowska 2017; Rostami et al. 2020). However, in many settings, quantifiable contributions of infected dogs to *Toxocara* egg contamination in the environment and consequently to the prevalence of human toxocariasis remain poorly understood. Although the direct contribution of dogs to human toxocariasis remains largely unknown, an earlier modeling study in the Netherlands estimated that household dogs could contribute to 39% of *T. canis* eggs contamination. However, this direct contribution could drop significantly upon compliance with deworming regimen and dog feces clean-up (Nijsse et al. 2015). Of note, even direct contact with household dogs has been associated with larva migrans in children, explaining that dogs may also carry *Toxocara* eggs through their hairs (Amaral et al. 2010; Keegan and Holland 2010; Merigueti et al. 2017; Maurelli et al. 2019).

In the Philippines, *Toxocara* egg prevalence in soils and vegetables (e.g., lettuce) varied between 3 and 77%, suggesting the influence of geographical settings and pet ownerships (Fajutag and Paller 2013; Paller and de Chavez 2014; Ordoñez et al. 2018; Paller and Babia-Abion 2019; Delaluna et al. 2020). In addition, a report in the Philippines suggests that *Toxocara* egg contaminations in soils correlate with *Toxocara* seroprevalence rate in children (Fajutag and Paller 2013). The reported seroprevalence of human toxocariasis, ranging from 49 to 67%, may suggest high endemicity in selected parts of the country (Auer et al. 1995; Fajutag and Paller 2013). With these findings, future works should address whether *Toxocara* egg contamination correlates with anti-*Toxocara* antibodies in close residents, especially among children in the study site. In addition, our study has limitations inherent to the point prevalence design, which only give data to a specific time window. Therefore, future works should include frequent sampling across the year (e.g., period prevalence) to cover climatic and seasonal conditions, with extended time and more sampling coverage and numbers from the soil, dog, and human.

Conclusion

In conclusion, this study revealed a substantial burden of *T. canis* infection in dogs and *Toxocara* egg environmental contamination in Baybay City, Leyte, Philippines. The *T. canis* prevalence in dogs is much higher in rural areas than

in urban areas. Moreover, the parasite's high prevalence is associated with puppies, access to dirty water, and a lack of deworming. In addition, high *Toxocara* egg contamination in school playgrounds and public parks might have resulted from high levels of *T. canis* infection in dogs. Future studies should involve a one-health approach to holistically characterize the extent of *T. canis* infection in the central Philippines.

Acknowledgements The authors acknowledge the voluntary participation of pet owners in Baybay City, Leyte, Philippines. The authors are grateful for the support of the College of Veterinary Medicine, Visayas State University.

Author contributions MDPC collected the samples, performed laboratory analysis, organized the data, performed the initial data analysis, and contributed to the writing of the manuscript. HPP performed the data analysis, organized the data figures and tables, wrote the first draft, and contributed to the final writing of the manuscript. EBL designed the study, supervised the conduct of the study, analyzed the data, and contributed to the final draft of the manuscript.

Funding This project is partly funded by the Visayas State University.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This is an observational study. The CVM-VSU Research Ethics Committee has confirmed that no ethical approval is required. The handling of animals for fecal samples was conducted following the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 3rd Ed., 2010).

Informed consent Informed consent was obtained from respondents included in the study.

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