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Prevalence of Gastrointestinal Parasites in Pet and Stray Dogs Found in Dir Lower, Pakistan

Javid Khan* and Akram Shah

Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology, Street 4, Sector K-1, Phase 3 Hayatabad, Peshawar, Khyber Pakhtunkhwa, Pakistan

ABSTRACT

The fecal material of dogs containing infective stages (eggs, larvae, cysts, and oocytes) of the parasite contaminates the soil and represents a great risk for the human health. Regardless of investigations, there is still a deficiency of information concerning the prevalence and seasonality of the intestinal parasites in pet and street dogs found in district Dir Lower. Therefore, this study has accomplished prevalence of parasites in domestic and street dogs found in Dir Lower, Pakistan and will provide reference point for forthcoming research. During this study fecal samples from 76 domestic and 124 stray dogs (n=200) were collected from different areas of Dir Lower and were examined for the presence of intestinal parasitic infections. Eight helminth species were demonstrated during this study from all dogs. These eight species included six species of nematodes and two species of cestodes. As trematodes parasitic infection in dogs is relatively uncommon therefore no flukes were found. During this study 44 dogs were infected with intestinal parasites showing the overall prevalence of 22%. The most common observed helminths parasites was *Toxocara canis* which was found in the fecal samples of 14 stray dogs and 4 domestic dogs 18 (9%). Other observed helminths parasitic species were *Trichuris* species 09 (4.5%), *Dipylidium caninum* 05 (2.5%), *Strongyloides stercoralis* 05 (2.5%), *Capillaria* spp., 04 (2%), *Taenia* spp., 04 (2%), *Ancylostoma caninum,* 03 (1.5%), and *Toxascaris leonina* 01 (0.5%) respectively. The study highlights the critical need to educate and inform local communities about the significant zoonotic risks posed by parasites. Additionally, the research aims to assess the extent of environmental contamination by dogs and the associated risks to human health.

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INTRODUCTION

In dogs the most frequent problem is the presence of intestinal parasites such as nematodes predominantly; hookworms and whipworms. Dogs procure these parasites by ingesting parasitic eggs when came in contact with contaminated soil, water, feces, sand and food. Puppy dogs also acquire these worms when they are in the womb or during nursing. In dogs these intestinal parasitic infections mostly cause gastrointestinal disorders. The prevalence of these gastrointestinal parasitic disorders is most common in developing countries (Daryani *et al*., 2009). Dogs have achieved a state of ubiquity, establishing their presence in nearly every corner of the globe where humans reside and, interestingly, in numerous locations where human presence is imprecise. For half of the countries in the world, estimates on the prevalence of dogs are available. It is noteworthy that the global per-capita average of 130 dogs for every 1000 people is a significant measurement, yet it's important to recognize the substantial variations exist in our neighboring countries (Sykes *et al*., 2020).

Various parasitic developmental stages such as ova, larval stages and cysts expelled through dog feces can persist infectious in the atmosphere over an extended period, depending on different conditions. Mostly when the infection is light the intestinal parasites do not show any symptoms. Therefor it is necessary to collect fecal samples after regular intervals and to study these for parasitic infection. When the dogs show clinical signs like weight loss, diarrhea with blood or mucus, swollen abdomen, stunted growth and vomiting then fecal examination is highly recommended. They are a risk factor mutually for humans and animal infections, especially for expecting females, kids, and immunocompromised individuals (Traversa *et al*., 2014). Domestic dogs in Pakistan have

* Corresponding author: akram_shah@uop.edu.pk
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acknowledged expressively more consideration for deworming than stray dogs. Due to this carelessness, stray dogs are more likely to contract parasites that are extremely harmful to human health. Research on dogs is typically navigated on pet ones, and the outcomes are partial because stray dogs are not included. Insufficient reports are available on the parasitic infection risk transmission from dogs to people living in villages and residential areas in Pakistan (Powell *et al*., 2019). Dogs spread infections between pets and people because they serve as a reservoir host for a variety of parasites. Many parasites have dogs as their definitive hosts, while for some parasites dogs act as intermediate hosts like *Giardia lamblia, T. canis,* hook worms*, E. granulosus, D. caninum,* and protozoan borne toxoplasmosis (Khalifa *et al*., 2023). Humans can become infected by ingesting contaminated food and water having eggs and cysts, touching polluted items, inhaling contaminated dust, or through larvae penetrating their skin. These dogs have frequent contact with other animals, their feces, and a variety of food-stuff contaminated with animal feces and subsequent human exposure. Therefore, to reduce the danger of human infection, revising the epidemiology of the parasites in the canine population is necessary (Yagoob and Bahman, 2014; Khalifa *et al*., 2023).

One published report by Khan *et al*. (2019) is available about the prevalence of gastrointestinal parasites in dogs in Dir Lower in which only simple microscopic examination was used for parasitic infection detection and no other techniques were used. In order to correctly investigate the prevalence of gastrointestinal parasites in pets and stray dogs we used the sedimentation procedure and zinc floatation technique. A comprehensive study was conducted with the objective of to explore the Prevalence and seasonality of intestinal parasitic infections in domesticated and stray dogs found in Dir Lower. Other objective of the study was to identify potential risk factors for transmission of gastrointestinal parasites to human population.

MATERIALS AND METHODS

Background of the study area

Present study was carried out in rural and urban areas of district Dir Lower. Geographically district Dir Lower is situated at 34.9161° N, 71.8097° E, lies to the North of Khyber Pakhtunkhwa (KP), Province in Pakistan. Numerous agroecological zones experience a variety of temperatures, with the maximum temperature of 40 °C in Timergara and the lowest temperature of 1°C in Shahi. The district has a total area of 1583 km^2.

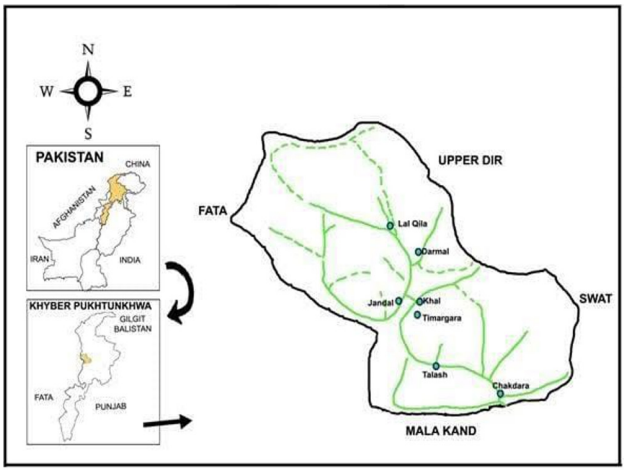

Fig. 1. Map of Dir lower.

Sampling sites

Pets and stray dogs are present in large numbers in Lower Dir. Sampling was done from most villages in Lower Dir. The area is mostly covered with mountain ranges, and the majority of the population lives in hilly areas, where people keep pet dogs for hunting and security purposes. Additionally, some people live in plain areas bordering districts Upper Dir, Bajaur, Swat, and Malakand, where the stray dog population is more prevalent.

Sampling method

Fecal samples from 76 domestic and 124 stray dogs (n= 200) were collected from different areas of Dir Lower from July 2023 to March 2024 and maintained in sterilized disposable labeled plastic bottles. The name and date of collection of the respective sample were written on each plastic bottle and immersed in 10% formalin. Important information was collected from all dog's owners for pet dogs in various villages of Dir Lower through pre-designed study questionnaire. The questionnaire was constructed on information associated to dog's gender, age, variety, anthelmintic practices and feeding procedures.

Parasites isolation and identification techniques

Sedimentation technique

10g of fecal sample was placed in a sterile Petri dish and diluted with 10 mL of distilled water. In order to remove extra plant materials and large debris using sterilized mesh the sample was strained. The filtrate was then poured into a centrifuge tube. The wash sample was centrifuged at a speed of 3000 revolution per minute (rpm) for 5 minutes. After centrifugation, the final supernatant was then discarded and the residue was placed on a clean

glass slide. For each tube, six slides (three direct and three Lugol's iodine smears) were prepared to increase the chance of parasite detection. The slide was then covered with cover slip and examined under the microscope at 100x and 400x objectives of compound microscope for detection and identification of possible parasitic ova, cysts and larvae.

Zinc sulphate floatation technique

For this technique, Zinc sulphate 331 g was dissolved in 900 mL warm distilled water and some additional distilled water was added until the entire solution weighed 1180 g (specific gravity of 1.18). The solution was stirred gently for mixing and then specific gravity was checked by using hydrometer. The residues in the above technique were mixed with zinc sulphate solution. Flotation procedure was used (as revealed previously) for the finding of helminth eggs, larvae and protozoa cysts. The sediments were mixed with zinc sulfate solution and poured into tubes and the solution was left overnight. Then, using a dropper, only the solution from the above the fluid (floatation fluid) was transferred to a clean glass slide. Lugol's iodine stain was used and unstained smears were examined for the parasite stages. For microscopy the mixture was applied onto a freshly clean slide, and a cover slip was gently being placed to evade air froths and overflowing.

The morphological keys currently in use were used to identify any parasite that is found. We used the morphological key reprinted by WHO (2019) with corrections. A dog was considered positive if parasite species was found in the fecal sample.

Data arrangement and analysis

Data regarding pets and stray dog's fecal samples was transferred to excel sheet and was arranged according to locality, host age, seasons and sex. The *p*-value was computed individually for each variable, and a significance level of 0.05 or less was employed to determine statistical significance.

RESULTS

A total of 200 fecal samples from two breeds of dogs (households=76 and stray=124) were examined for the presence of intestinal parasitic infections. Eight helminth species were demonstrated during this study from all dogs. During this study 44 dogs were infected with intestinal parasites showing the overall prevalence of 22%. The most common observed helminths parasites were *Toxocara canis* which were found in the fecal samples of 14 stray dogs and 4 domestic dogs, (n=18 9%). Other observed helminths parasitic species were *Trichuris* species 09 (4.5%), *Dipylidium caninum* 05 (2.5%), *Strongyloides stercoralis* 05 (2.5%), *Capillaria* spp., 04 (2%), *Taenia* spp., 04 (2%), *Ancylostoma caninum.,* 03 (1.5%), and *Toxascaris leonina* 01 (0.5%) respectively. The prevalence and intensity of different gastrointestinal parasites in two breeds of dogs is presented in Table I.

Table I. Overall prevalence of gastrointestinal parasites in pets and stray dog's fecal samples collected from district Dir lower, Pakistan.

Parameters	Stray dogs	Pet dogs	Total (%)
Total samples examined	124	76	200
Samples positive	31(25%)	13(17.1%)	44 (22%)
Total samples negative	93 (75%)	63(82.9%)	156 (78%)

Diversity of parasites

In total 200 fecal samples of stray and pet's dogs eight species of intestinal helminths were observed. These eight species included six species of nematodes and two species of cestodes. As trematodes parasitic infection in dogs is relatively uncommon therefore no flukes were found. The six species of nematodes included *Toxocara canis*, *Trichuris* spp., *Strongyloides stercoralis, Capillaria* spp*., Ancylostoma caninum,* and *Toxascaris leonina.* The two species of cestodes were *Dipylidium caninum* and *Taenia* spp. The prevalence of these zoonotic parasites is shown in Table II.

Table II. Parasite species found in fecal samples of pets and stray dogs collected from district Dir Lower, Pakistan.

Parasite	Prevalence in stray dogs	Prevalence in Pet dogs	Overall Prevalence (%)
Toxocara canis	14 (11.3%)	4 (5.26%)	18 (9%)
Trichuris spp.	05 (4.03%)	4 (5.26%)	09 (4.5%)
Dipylidium caninum	05 (4.03%)	0	05 (2.5%)
Strongyloides stercoralis	05 (4.03%)	0	05 (2.5%)
Capillaria spp.	02 (1.61%)	2 (2.63%)	04 (2%)
Taenia spp.	03 (2.41%)	1 (1.31%)	04 (2%)
Ancylostoma caninum	01 (0.80%)	2 (2.63%)	03 (1.5%)
Toxascaris leonina	01 (0.80%)	0	01 (0.5%)
Total parasites found	36	13	49 (24.5%)
Total dogs infected	31	13	44 (22%)
Total examined	124	76	200

Prevalence of parasites: alone or in combination

A total of 49 parasitic infections were detected in 44 dogs out of 200. In fecal samples of 27 stray dogs and 13 household dogs single parasitic infection was found (Table III). The single parasitic infection found in the fecal samples of stray dogs were *Toxocara canis, Trichuris vulpis, Capillaria* spp., *Strongyloides stercoralis, Ancylostoma caninum, Dipylidium caninum* and *Taenia* spp. Similarly, in pet dogs *Toxocara canis, Trichuris vulpis, Capillaria* spp., *Ancylostoma caninum* and *Taenia* spp. were found as monoparasites. In pets dogs no polyparasitism was observed. In fecal samples of 4 stray dog's polyparasitism was observed. In fecal sample of 2 stray dogs *Toxocara canis* and *Trichuris vulpis* were present together. Similarly, *Toxocara canis* was present along with *Toxascaris leonina* in the fecal sample of same stray dog. In the fecal sample of another stray dogs three parasitic species eggs were found *i.e., Toxocara canis, Capillaria* spp and *Trichuris vulpis* (Fig. 2). *Toxocara canis* was also found in the fecal sample of a stray dog (Fig. 3D).

Table III. Prevalence of parasites alone or in combination with pets and stray dogs of district Dir Lower.

Parasite	Species	Stray dogs	Pet dogs	Total
Parasites found alone				
	Toxocara canis,	10	4	14
	Trichuris vulpis	2	4	6
	Capillaria spp. *Strongy-*	1	2	3
	loides stercoralis	5	0	5
	Ancylostoma caninum	1	2	3
	Dipylidium caninum	5	0	5
	Taenia spp.	3	1	4
	Total cases	27	13	40
Parasites found in combinations				
2 species	*Toxocara canis*+ *Trichuris vulpis*	2	0	2
	Toxocara canis+ *Toxascaris leonina*	1	0	1
3 species	*Toxocara canis* + *Capillaria* spp + *Trichuris vulpis*	1	0	1
	Total	4	0	4

Relevance of gastrointestinal parasites to host age, season and sex

Fecal samples from domesticated and stray dogs (*Canis lupus familiaris*) were collected and examined for the presence of parasitic infections. Majority of owners are unaware of the use of anthelminthic drugs. Among 76 pet dogs only one owner gave Ivermectin to his two dogs. In the study area the stray dogs were more infected (25%) with gastrointestinal parasites than pet dogs (17.1%) (Table IV). The high prevalence of parasites in stray dogs is due to poor management and unhygienic habits of stray dogs. The adult dogs were more affected by the parasites than the puppies. The prevalence of gastrointestinal parasites in adult pet dogs were recorded as 18.96% and in puppies it was 11.11%. There is no significant variation in the prevalence of gastrointestinal parasites relative to the sex of host dogs (Table IV).

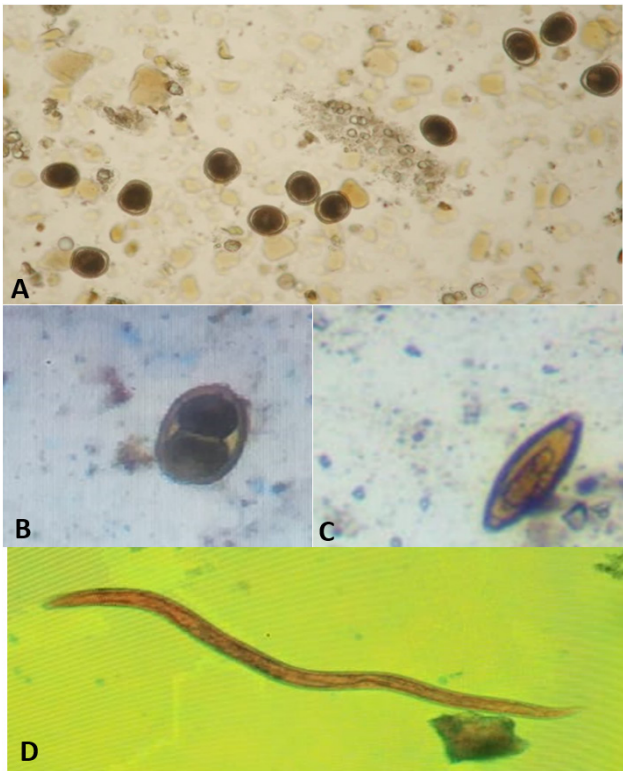

Fig. 2. Gastrointestinal parasitic eggs found in fecal samples of stray and households' dogs: A, *Toxocara canis;* B, *Strongyloides stercoralis;* C, *Trichuris vulpis*; D, *Toxacara canis*.

Table IV. Prevalence of gastrointestinal parasites in domesticated dogs relative to host age and sex.

Category	Number examined	Number infected	%
Domestic dogs	76	13	17.1
Adults	58	11	18.96
Puppies	18	2	11.11
Male	40	7	17.5
Female	36	6	16.66

The prevalence of gastrointestinal parasites shows

great variation in different seasons. This study was carried out from July 2023 to March-2024. From July to September the weather was very hot in the study area and the prevalence of parasites was comparatively high in these months (29%). The prevalence was very low during October-March as compared to the previous three months. From this study it was concluded that the humidity, temperature and climatic conditions have great impact on the prevalence of gastrointestinal parasites in stray and pet dogs in district Dir lower (Table V).

Table V. Prevalence of gastrointestinal parasites in household and stray dogs relative to different months.

Months of survey	Number examined		Number infected		Prevalence (%)	
	Pet dogs	Stray dogs	Pet dogs	Stray dogs	Pet dogs	Stray dogs
Jul to Sep 2023	24	55	7	16	29.16	29.09
Oct to Dec 2023	27	40	3	9	11.11	22.50
Jan to Mar 2024	25	29	3	6	12.00	20.68
Total	76	124	13	31	17.10	25

DISCUSSION

This research holds significant value for veterinarians, parasitology researchers, and the general public, offering insights into the prevalence rates of parasites in dogs and aiding in parasite control measures. The findings also hold clinical relevance for human healthcare providers, as there are potentially dangerous zoonotic diseases linked with these parasites, posing a constant threat to human health. Insufficient awareness about these parasites exacerbates the risk, as individuals may inadvertently contract and transmit diseases. As data of this sort is limited in Pakistan and there is very little information available on the prevalence of gastrointestinal parasites in dogs with which to compare this data. It's important to note that relying solely on fecal examinations may underestimate parasite prevalence. Studies have demonstrated significant variations when combining fecal examinations with other techniques such as post-mortem examinations or more sensitive diagnostic methods (Barutzki and Schaper, 2011).

Gastrointestinal helminthiasis stands out as the most commonly encountered disease affecting dogs worldwide, including Pakistan. With its diverse climatic zones, ranging from cold alpine to wet tropical regions, Pakistan provides a fertile breeding ground for a diverse range of parasites and pathogens of both medical and veterinary significance. The transmission and distribution of these organisms are largely dictated by geographical, climatic, cultural, and socio-economic variables. Dogs, whether owned or stray, play a pivotal role in the transmission dynamics, representing a potential public health hazard due to their close association with humans. This symbiotic relationship, while offering numerous benefits, also presents a conduit for the transmission of parasitic infections from dogs to humans, either directly or indirectly through environmental avenues. While the specific contribution of each canine population segment to transmission dynamics remains somewhat nebulous, all types of dogs are implicated in this process (Dubná *et al*., 2007).

Questionnaire was used for the owners of pet dogs. The questionnaire contain questions about dogs name, breed, sex, age, approximate weight, number of dogs in home, history of dogs visit to pets clinic, symptoms of gastrointestinal parasites in dogs and treatment measures etc. The results of questionnaire show that in Dir Lower of total 76 pet dog owners no owner lick the face of their dogs. The dogs are not allowed the bedroom of the owners and also dogs have no access to reach the kitchen. Similarly no owner likes to sleep with their pet dogs. Pet's dogs in the study area were mostly stayed indoors. But the dogs defecate on open soil and no litter box was used for defecation. About 60% of the owners remove the feces daily. About 30% owners clean the dog territory twice a week and remaining owners never clean up feces of their dogs. Out of 76 pets dogs only a single owner dewormed his two dogs with Ivermectin. Other owners were mostly unaware from gastrointestinal parasites and deworming. But still the prevalence of gastrointestinal parasites in domesticated dogs is lower than stray dogs which may be due to geographic location and also stray dogs are roaming freely which have more chances of parasitic infection. In the present study the overall prevalence of helminths in both stray and household dogs was 22%. Similar finding was also obtained in various studies conducted in different countries of the world including Pakistan which range from 4.44% to 100% (Swai *et al*., 2010).

Khan *et al*. (2019) studied the prevalence of gastrointestinal parasites in stray and domesticated dogs of district Dir Lower and reported prevalence of gastrointestinal parasites as 26.8%. The most detected eggs were of *D. caninum* (11.8%), followed by *Toxocara canis* (10.5%), *Taenia* spp. (6.57%), *Ancylostoma caninum* (3.94%), *Capillaria* spp. and *Trichuris vulpis* (1.31% each) . The wandering dogs were infected (34.4%) than domesticated ones (16.1%). This study supports our data that after 5 years the situation is still unchanged.

The prevalence of gastrointestinal helminth parasites among dogs in this study (22%) was notably lower as compared to a previous study conducted by Olufemi and

Bobade (1979). over five decades ago, which reported a prevalence of 77.4% among dogs from veterinary clinics at the University of Ibadan and Oyo State Veterinary Clinic. Similar results were obtained by Onyenwe and Ikpegbu (2004), who reported a prevalence of 39.2% among 148 dogs presented to the University of Nigeria Veterinary Teaching Hospital (UNVTH), Nsukka Nigeria. This study also shows lower rate of prevalence than study conducted by Ramirez *et al*. (2004) who present 614 dogs to the veterinary clinic of the University of Zulia, Venezuela and obtained prevalence of 35.5%.

In neighboring Iran Beiromvand *et al*. (2013) conducted research on the prevalence of zoonotic intestinal parasites in domestic and stray dogs in the Chenaran County, Razavi Khorasan Province. From November 2009 to January 2010 they carried out random sampling from 17 villages and obtained the prevalence rate of 66%. This study showed much higher prevalence than our reported results.

In this study interestingly the prevalence of gastrointestinal parasites in pets and stray dogs is lower than some studies conducted in developed countries where the dogs are treated with special care and anti-parasitic programs are regularly carried out to deworm the dogs.

In this study the most common observed helminths parasites were *Toxocara canis* which are found in the fecal samples of 14 stray dogs and 4 household dogs. This prevalence of *T.canis* is less than study conducted by Schwartz *et al*. (2022) who studied 306 fecal samples in which 147 (48%) samples were positive for *T. canis* and reported prevalence of *T.canis* as 16.35%. Conde *et al*. (2022) reported 64.44% prevalence of *T. canis* in dogs and their environmental contamination in Baybay city, Leyte, Philippines. The prevalence of *T. canis* in this study was lower in Lahore Pakistan.

So all the above reports present higher prevalence from the present study. The observed disparities can be ascribed to differences in sampling size and type, geographic location, the rate of parasite infection of hosts in each area and sampling methodologies. Various laboratory techniques could also be involved in a role in retrieving different parasites since some procedures can either float or sediment the parasites. But still there are some studies in which the prevalence of *Toxocara canis* is less than the current study. Nijsse *et al*. (2015) reported 4.6% prevalence of *T.canis* in pet dogs. Another study was conducted in Northern Belgium by Claerebout *et al*. (2009) who tested 1159 fresh fecal samples and report prevalence of 4.4% for *T. canis.*

CONCLUSION

The current study aimed to assess the prevalence of intestinal parasites in dogs in district Dir Lower, Khyber Pakhtunkhwa Pakistan. Parasitic infections are still problem in the study area. Stray and domesticated dogs, are infected with different types of intestinal parasites that may root numerous common diseases to human being. The study highlights the critical need to educate and inform local communities about the significant zoonotic risks posed by parasites. Additionally, the research aims to assess the extent of environmental contamination by dogs and the associated risks to human health.

DECLARATIONS

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Ethical statement and IRB approval

Approval for collection of dogs faeces was obtained from dogs owners & Qurtuba University IRB and Ethical board.

Generative AI or AI-assisted technology statement

The data presented in this article are original and research work performs solely by the authors, without the use of AI or automated data generation tools.

Statement of conflict of interest

The authors have declared no conflict of interest.

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