



Control of *Giardia* infections with ronidazole and intensive hygiene management in a dog kennel

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ABSTRACT

Infections with the intestinal protozoan parasite *Giardia* in dogs and cats are common. Clinical signs vary from asymptomatic to small bowel diarrhea and associated discomfort. The control of infections in dogs is frequently a frustrating issue for animal owners and veterinarians. Drugs with antiprotozoal activity such as fenbendazole and metronidazole are recommended, however, they do not show 100% efficacy and superinfections occur regularly. Ronidazole is currently the drug of choice for the treatment of *Tritrichomonas foetus* in cats and there is now limited information available about its efficacy against *Giardia* spp. In the kennel investigated, dogs regularly showed loose feces and the presence of *Giardia* (assemblage C, renamed as *G. canis*) cysts. An elimination strategy of this parasite involving strict hygiene management and disinfection of the enclosures with 4-chlorine-M-cresol, oral treatment with ronidazole (30–50 mg/kg BW bid for 7 days) and two shampoos (containing chlorhexidine) at the beginning and the end of the treatments was implemented for a group of 6 dogs. As a control another group of 7 dogs was transferred to the disinfected enclosures and shampooed, but left untreated. Dog feces were tested for the presence of *Giardia* cysts (SAF concentration technique) or *Giardia* antigen with a commercial ELISA (NOVITEC®) and a quick immunochromatography-based test (SensPERT®) before and between 5 and 40 days after the last treatment. All ronidazole-treated dogs were negative for *Giardia* cysts and antigen up to 26 days after the last treatment, while between 1 and 5 of the control animals tested positive in each of the test series. At this point, also dogs of the control group were again moved into clean enclosures, shampooed twice and treated with ronidazole. Five, 12 and 19 days after the last treatment, the dogs in the control group tested negative for *Giardia* cysts and antigen. However, all animals had again positive results at later time points in at least one of the three applied diagnostic techniques within 33–61 days after treatment. Furthermore, all dogs had episodes of diarrhea (for 1–4 days) within 14–31 days after treatment and unformed feces during the whole experiment. The positive effect of ronidazole against *Giardia* infections in dogs could be confirmed in this study. In particular, the combination of ronidazole treatment combined with the disinfection of the environment and shampooing of the dogs was highly effective in reducing *Giardia* cyst excretion and may therefore constitute an alternative control strategy for canine giardiasis.

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1. Introduction

Giardia is an intestinal protozoan with a broad host range in wild and domestic mammals. Although the adverse consequences of *Giardia* infection and its pathogenic potential are best recognized in humans

(Thompson, 2004), it is also a well known causative agent of diarrhea in dogs and cats. Diarrhea is common in both animal species, with many possible causes: non-infectious (stress, disturbances in water balance, nutritional and immune status, malnutrition, neoplasia, inflammatory disease) and infectious (bacterial, parasitic, or viral infections) causes, but also any combination of the above (Payne and Artzer, 2009). Since stress has an effect on the function and the immunological reactions in the gut, it is not surprising that high *Giardia* prevalences were identified among animals housed in stressful situations such as dog rescue shelters (Upjohn et al., 2010) or kennels (Scaramozzino et al., 2009).

Molecular tools are commonly used for the genetic characterization of *Giardia* isolates. Currently, seven *Giardia* genotypes, designated assemblages and in some cases assigned distinct species names, are recognized. Dogs are infected by parasites of four assemblages (A, B, C, D), of which assemblages C and D (also defined as *G. canis*) are found exclusively in dogs, while parasites of assemblages A and B (also defined as *G. enterica*) are zoonotic (Covacin et al., 2011; Thompson, 2004; Thompson and Monis, 2011).

Giardia cysts are therefore frequently found in routine diagnostic examination of dog feces, also from asymptomatic dogs (Covacin et al., 2011). In a recent study performed with 878 shelter dogs (Upjohn et al., 2010), the apparent prevalence of *Giardia* was 9.9% and the true prevalence, based on the known sensitivity and specificity of the ELISA test, was 21.0%, which is in the same range as found in previous studies. Since stress has an effect on the function and the immunological reactions in the gut, it is not surprising that high *Giardia* prevalences were identified among animals housed in stressful situations such as dog rescue shelters (Upjohn et al., 2010) or kennels (Scaramozzino et al., 2009). In addition, fecal samples that were graded concerning their consistency, confirmed previous results, i.e. a weak association between fecal consistency and infection with *Giardia* in dogs.

Molecular tools are commonly used for the genetic characterization of *Giardia* isolates. Dogs are infected by parasites of four assemblages (A, B, C, D), of which assemblages C and D (also defined as *G. canis*) are found exclusively in dogs, while parasites of assemblages A and B (also defined as *G. enterica*) are zoonotic (Covacin et al., 2011; Thompson, 2004; Thompson and Monis, 2011). Several compounds have been tested for efficacy against *Giardia* infections in dogs, and some of them are frequently employed by veterinary practitioners. Several benzimidazoles (Barr et al., 1993; Villeneuve et al., 2000), in particular fenbendazole (Barr et al., 1994), or the combinations of febantel/fenbendazole (febantel is metabolized to fenbendazole) with other compounds proved to be effective (Barr et al., 1998). Furthermore, metronidazole, from the class of the nitroimidazoles, is used routinely to treat giardiasis in dogs and cats. It was argued that this compound is an effective therapy for diarrhea regardless of the cause, and may be used in combination with fenbendazole to relieve clinical signs and eliminate parasites (Payne and Artzer, 2009). However, metronidazole should not be used in doses above 60 mg/kg BW to avoid adverse side effects (Plumb, 1999). Nitazoxanide, a nitrothiazolyl-salicylamide, has been tested

in vitro (Cedillo-Rivera et al., 2002), while azythromycin, an azalide, has been used for the treatment of only one dog (Zygner et al., 2008). Therefore, further experiments are required to confirm the efficacy of these drugs against *Giardia* infection (Geurden and Olson, 2011). Ronidazole and tinidazole are also nitroimidazoles, and while the latter has recently been approved in the United States for the treatment of giardiasis in humans, ronidazole has been used for treatment of blackhead disease, caused by *Histomonas meleagridis* in turkeys. In addition, ronidazole is currently the drug of choice against *Tritrichomonas foetus* in cats (Gookin et al., 2006). A high antiprotozoic effect was demonstrated in vitro against *G. duodenalis* with an approximately fivefold higher activity than metronidazole (Boreham et al., 1985). The same authors also reported good efficacy of ronidazole against *Giardia* sp. in mice (Boreham et al., 1986).

Although several compounds are effective against *Giardia*, control programs combining drug treatment with cleaning and disinfection of the environment to reduce the environmental infection pressure are recommended (Geurden and Olson, 2011). Studies showed that calves as well as dogs re-excreted cysts shortly after the end of antiprotozoic treatment if no hygienic measures were implemented (Geurden et al., 2006; Villeneuve et al., 2000). In addition, thorough shampooing of companion animals is recommended after treatment to prevent reinfection through fecal material on the fur (Payne et al., 2002; Zajac et al., 1998). The aim of the present study was to assess the efficacy of ronidazole against *Giardia* infections in a dog kennel.

2. Materials and methods

2.1. Facility, animals, management

In the animal facilities of the Veterinary Faculty of the University of Zurich, beagle dogs are housed in groups of 2–4 in pens of 1.45 m × 4.5 m in size with access to an outside run of 1.45 m × 5.5 m. Some adjacent pens share a common outside run of 3 m × 11 m with a concrete floor. Pens are enriched by installations allowing dogs to jump and use the space tridimensional, as well as to rest and retreat.

The total number of dogs in the facility varies between 12 and 42 with an age range between puppies and 6 years. Dogs are tested regularly (every 3 months) for the presence of parasites in their feces by sedimentation/flotation and by SAFC-technique (Eckert et al., 2008). For individual fecal samples, dogs are isolated overnight. Occasionally, some dogs presenting *Toxocara canis* infections are treated with an anthelmintic compound. Most of the dogs occasionally show loose feces but only in some of them *Giardia* cysts are detected. Prior to the start of this study, *Giardia* of the 'dog genotype' assemblage C was identified by PCR/sequencing of part of the 18s rRNA gene (Hopkins et al., 1997). Daily cleaning of the pens with a cleaning agent (Allzweckreiniger 681, Kärcher AG, CH-8108) is combined with the use of a disinfectant (Incidin® PLUS, Ecolab GmbH, 4132 Muttentz) twice a week in dosages according to the instructions of the manufacturers. The dogs are fed once

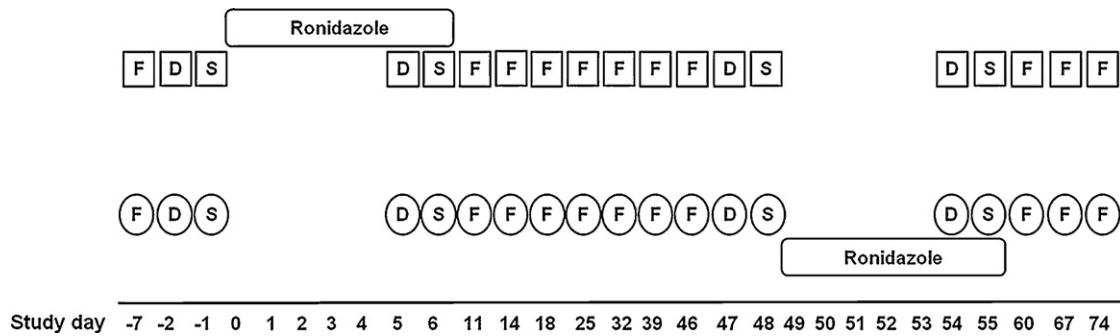


Fig. 1. Scheme of the experimental design with a therapeutic group (□, n=6) and a control group (○, n=7) of dogs infected with *Giardia duodenalis* and treated with ronidazole at different time points. F, fecal sample; D, disinfection; S, shampooing.

per day with standard commercial dog food at the recommended rates, while tap water is available in automatic drinking troughs.

3. Experimental design

In June 2010 a control strategy for *Giardia* infections, depicted in Fig. 1, was devised. Thirteen dogs (6 males and 7 females) aged between 13 and 19 months and excreting *Giardia* cysts, as confirmed by the SAFC (sodium acetate–acetic acid–formalin concentration) technique seven days before the start of the study (study day (SD) –7), were included. They were housed in groups and separated from the other dogs of the facility by leaving empty boxes in between. At SD –2, the pens of all dogs in the study were disinfected (including floors, walls and installations) with 4-chlorine-M-cresol (Neopredisan® 135-1, Vital AG, Oberentfelden, Switzerland) in a 3% dilution with water, applying approximately 0.4 L/m², as recommended for the elimination of coccidia and *Cryptosporidium*. A minimum of 2 h later, the disinfectant was removed with water at 80 °C with a high-pressure cleaner. During the following SD –1, the surfaces were allowed to dry completely. On the same day, all dogs were showered with warm water and shampooed with 4% chlorhexidine digluconate (Clorexyderm® 4%, ufamed AG, Sursee, Switzerland), which was left for 5–7 min as recommended. Afterwards, the solution was washed off the dog’s fur, and the animals were brought directly to the previously disinfected and dried enclosures. Drug treatment was initiated at SD 0 with six dogs (therapeutic group) selected on the basis of their previous housing (dogs housed together or housed close to each other): ronidazole (30–50 mg/kg BW bid for 7 days, SD 0–6) was orally administered in capsules, which were specially prepared by a pharmacist (Christoffel-Apotheke, Bern). The control group (7 dogs) was left untreated. On SD 6, all dogs were again washed and shampooed, and the enclosures were again cleaned, disinfected and dried the day before as described previously. All dogs were moved back to the cleaned enclosures on SD 6. Animal caretakers were instructed to change shoes every time they entered the disinfected sector of the facility.

Starting on SD 47 the same protocol was repeated with exception of treatment, which was implemented for the control dogs this time: disinfection of the facilities and

shampooing of all dogs twice (before and towards the end of the 7-day treatment of the control dogs with ronidazole, Fig. 1).

3.1. Diagnostic methods

Individual fecal samples were collected on SD 11, 14, 18, 25, 32, 39 and 46, corresponding to 5, 8, 12, 19, 26, 33 and 40 days after receiving the last dose of ronidazole treatment in the therapeutic group. Both groups were also followed-up by fecal examinations at SD 60, 67 and 74, corresponding to 5, 12 and 19 days after the last treatment in the control group.

Dog feces were tested at the diagnostics unit of the Institute of Parasitology, Zurich, for the presence of *Giardia* cysts (SAFC technique) and *Giardia* antigen with a commercial ELISA (NOVITEC®, Diagnostic GmbH, Freiburg, Germany). The laboratory and the method are accredited by norm ISO/IEC 17025 for human and animal samples. In addition, a quick test based on immunochromatography (SensPERT®, VetAll Laboratories, Kyunggi-Do, South Korea) was performed according to the manufacturers’ instructions. ELISA results were correspondingly graded from 0 (negative) to 4 (very high-grade positive) based on optical density values adjusted to the positive and negative controls, while the immunochromatographic test was subjectively graded by eye from 0 (nothing visible) to 4 (well visible).

4. Results

An overview of the coproscopic results is given in Table 1. All fecal samples of dogs in the treatment group were negative for *Giardia* cysts and coproantigens 11 days after the last treatment and remained negative on further four investigations up to SD 32. However, on SD 39 (33 days after the end of the therapy) one dog tested coproantigen positive and on SD 46 four dogs tested coproantigen positive. The first excretion of cysts was detected on SD 60 in one dog. In the following days (up to SD 74, i.e. 33–54 days after the last treatment) all dogs of this group tested positive in at least one of the tests applied.

In the control group, 1–5 dogs tested regularly positive in at least one of the three applied diagnostic techniques until SD 46. When these control dogs were moved into clean enclosures and treated with ronidazole (at SD 49–55)

Table 1

Coproscopic results of six dogs (therapeutic group) treated orally with ronidazole (30–50 mg/kg BW bid) at study day (SD) 0–6 and of seven dogs (control group) treated at SD 49–55. Additional measures including strict hygiene management, disinfection of the enclosures and shampooing at the beginning and the end of the treatments were applied in both groups. Test methods were: SAFC-technique (S), ELISA (NOVITEC®) (E), and a quick test based on immunochromatography (SensPERT®) (P). M, male dogs; F, female dogs.

Dog-ID	SD=11			SD=14			SD=18			SD=25			SD=32			SD=39			SD=46			SD=60			SD=67			SD=74					
	S ^a	E ^b	P ^c	S	E	P	S	E	P	S	E	P	S	E	P	S	E	P	S	E	P	S	E	P	S	E	P	S	E	P			
<i>Therapeutic group, treated on SD 0–6</i>																																	
5078, M	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	+	2	1	–	1	0	–	1	1	–	1	1
5085, M	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	1	0	–	1	0	+	3	3	–	3	3	–	1	0
5090, M	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	3	1	+	3	1	+	3	1
8095, F	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	3	0	–	1	0	–	0	0	–	0	0	–	0	0	–	2	2
8420, F	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	1	0	–	0	0	–	0	0	–	0	0	+	3	3
8492, F	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	1	0	–	0	0	+	3	4	+	3	4	+	3	2
No. of positive ^d	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	1	2	1	2	4	3	3	6	5			
<i>Control group, untreated</i>														<i>Control group, treated (SD=49–55)</i>																			
4266, F	–	0	0	–	1	0	–	0	0	–	1	1	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0
5082, F	+	3	2	+	3	3	+	3	2	+	2	2	+	1	0	+	0	0	+	1	1	–	0	0	–	0	0	–	0	0	–	0	0
5084, F	–	1	0	–	3	2	–	2	2	–	1	0	+	1	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0
5093, F	–	1	0	–	4	3	–	2	1	–	2	1	–	1	0	–	0	0	–	1	1	–	0	0	–	0	0	–	0	0	–	0	0
5527, M	–	1	1	+	3	1	+	3	3	–	0	0	–	0	0	–	0	ni	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0
6635, M	–	0	0	–	0	0	–	0	0	–	1	1	–	0	0	–	0	0	–	3	3	–	0	0	–	0	0	–	0	0	–	0	0
6775, M	–	0	ni	–	0	0	–	0	ni	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0	–	0	0
No. of positive ^d	1	4	2	2	5	4	2	4	4	1	5	4	2	3	0	1	0	0	1	3	3	0	0	0	0	0	0	0	0	0			

^a SAFC-technique: “–” = negative; “+” = positive (cysts were detected).

^b NOVITEC®-ELISA: 0 = negative, 1 = slightly positive, 2 = moderately positive, 3 = high-grade positive, 4 = very high-grade positive.

^c SensPert®-Immunochromatography: 0 = negative, 1 = very slightly visible, 2 = slightly visible, 3 = visible, 4 = well visible, ni, not interpretable.

^d No. of positive dogs per each technique of totally 6 (therapeutic group) or 7 (control group) animals.

and shampooed twice, they were already negative for *Giardia* cysts and coproantigens 5 days after the last treatment. In the follow-up until SD 74 the dogs remained negative.

Several dogs had episodes of unformed feces and diarrhea (for 1–2 days) during the entire experiment albeit without a temporal correlation with ronidazole treatments.

5. Discussion

The results presented here confirmed a good antiprotozoic effect of ronidazole against *Giardia* in dogs as shown previously in vitro or in a mouse model (Boreham et al., 1985, 1986). As no information was available about the use of ronidazole in dogs, the same dose as for cats against *T. foetus* was adopted for this trial, i.e. 30–50 mg/kg BW bid for 7 days. The dosage of nitroimidazole compounds, especially metronidazole, needs particular consideration, since a considerable number of significant side-effects such as nausea, diarrhea, anorexia and neutropenia may occur when administered to humans or animals (Payne and Artzer, 2009; Plumb, 1999). In our study diarrheic episodes were monitored in treated and untreated dogs and can therefore not be correlated with ronidazole treatment. Stress, active *Giardia* infections and many other factors may be the cause for the observed fecal alterations.

Giardia has been described as “one of the most commonly misdiagnosed, underdiagnosed, and overdiagnosed parasites in veterinary practices today” (Payne and Artzer, 2009). Cysts are shed intermittently, and therefore repeated fecal analyses may be needed before cysts are recovered in a sample if the SAFC-technique is applied. This method can be considered as a gold standard technique and is being applied routinely for *Giardia* cyst detection in many diagnostic laboratories. However, the identification of the small cysts is challenging for personnel with little experience. In our study the NOVITEC® ELISA results were quantified as proposed by the manufacturer. In samples with high cyst abundance, positive results were also obtained with the other two techniques. This confirms the higher sensitivity of these methods if compared with the SAFC-technique. The additionally performed immunochromatographic test (SensPERT®) is a rapid test which can be used by veterinary practitioners. The results show that more positive results were obtained with this test compared with the SAFC-technique, but fewer than with the ELISA. In addition, some results were not interpretable because of the insufficient visibility of potential bands; possibly further development of this test may increase its usefulness in practice.

The adopted ELISA and other easy to use tests available to veterinarians (i.e. the SNAP Giardia Test for dogs and cats, IDEXX Laboratories, or SensPert®) reliably identify *Giardia* spp. cyst antigens (GSA 65 in the NOVITEC® and a 65 kDa cyst antigen in the SensPert® test) shed with the feces. Because of inconsistent results of several population and comparative studies, it was concluded that none of the methods was 100% reliable and therefore combined testing methods (Payne and Artzer, 2009) as well as multiple sampling over several days were suggested to identify true prevalences (Geurden et al., 2008; Thompson, 2004). The current study confirms the irregularity of cyst excretion

and differences in the sensitivity of tests. In concrete situations when diagnosing potentially affected dogs, additive costs have to be considered in case of combined testing methods.

The combination of ronidazole treatment conjoined with the disinfection of the pens and shampooing of the dogs had an impact on *Giardia* cyst excretion: the therapeutic group started to be positive again only 33 days after the last treatment, and also the control group remained negative after treatment until the end of the experiment (19 days after the last treatment).

Although chemotherapy may be highly effective in eliminating *Giardia* infection, there are many cases of humans and animals with persisting *Giardia* cyst excretion which do apparently not respond to treatment. It was suggested that reinfection is the most common cause for treatment failure (Payne and Artzer, 2009). Re-infections frequently occur if the sources of environmental contamination are not eliminated. This applies particularly to localized endemic foci where environmental infectious pressure is high, for instance in kennels and catteries (Thompson, 2004). Therefore, in addition to antiprotozoic treatment of all contact animals, accompanying measures such as bathing after treatment and sanitation of the environment were recommended before resistance to the medications should be considered (Payne and Artzer, 2009). Clearly, the high tenacity and the ubiquitous presence of *Giardia* cysts play an important role. *Giardia* cysts are described to survive particularly well in high humidity and water: for 11 weeks in water at 4 °C (Olson et al., 2004) and up to 84 days in cold river and lake water (deRegnier et al., 1989). Shampooing of the dogs before and after the treatment with ronidazole aimed at the elimination of *Giardia* cysts on the fur of affected animals. Contemporaneously implemented hygiene measures such as two disinfections of the pens (before and at the end of antiprotozoic treatment) and change of shoes of the animal caretaker had the aim to prevent reinfections with cysts from the environment. It was assumed that at the end of an efficacious seven-day antiprotozoic treatment no additional *Giardia* cysts should be excreted and therefore the remaining cysts from the environment had to be inactivated. However, neither chlorhexidine digluconate in the shampoo nor 4-chlorine-M-cresol in the disinfectant solution are specifically indicated for elimination of *Giardia* spp. cyst. As a matter of fact, to our knowledge none of the available products at the beginning of study was certified and therefore indicated for this purpose. Since regular use of detergents and hot, soapy water as recommended for the reduction of *Giardia* cysts (Payne and Artzer, 2009) were not able to eliminate the *Giardia* problem at the facility, additional measures were experienced. In particular the product Neopredisan® was chosen because of its indication for the elimination of infectious *Cryptosporidium* oocysts, which are regularly described to be even more difficult to inactivate than *Giardia* cysts (Betancourt and Rose, 2004; Korich et al., 1990). Similarly, chlorhexidine digluconate was the only available disinfectant contained in a dog shampoo and was applied to further increase the level of hygiene, a factor considered fundamental in *Giardia* elimination strategy (Payne and Artzer, 2009). However, since

none of the animals were tested with ronidazole treatment alone or with cleaning or disinfection alone, it cannot be determined which component was most important to suppress *Giardia* shedding. Such investigations would be particularly useful, considering that for most of the dog owners such a strict hygiene management is simply not feasible, that *Giardia* cysts are ubiquitous in the environment, and that most people and animals will be exposed to cysts without becoming ill. Furthermore, the question arises if it is necessary to treat asymptomatic animals excreting *Giardia* cysts. In the past, antiprotozoic treatment of *Giardia* in dogs and cats, ill or asymptomatic, has been strongly recommended because of the possible zoonotic risk (Thompson, 2004). The prevalence of zoonotic assemblages in dogs was recently shown to be subjected to high variations, depending on the analyzed countries and dog populations (Leonhard et al., 2007; Upjohn et al., 2010) and it was therefore suggested to not draw conclusions from one geographical region to another in terms of the prevalence or assemblage composition of *Giardia* infections in dogs (Covacin et al., 2011). In any case, the awareness about this potential zoonotic risk was recommended to be maintained for all people involved (Upjohn et al., 2010).

The combination of an efficient antiprotozoic treatment with accompanying hygienic measures was able to suppress *Giardia* excretion for sometime in a dog kennel with controlled management. However, even under restricted conditions in a professionally conducted dog kennel, reinfections occurred despite all applied hygienic measures. Whether such measures may be applicable with success by private animal owners remains open.

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