Understanding the Epidemiology of *Trypanosoma Evansi* Infection in Dogs (*Canis lupus familiaris*) from Urban Areas of Colombia

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**Abstract:** *Trypanosoma evansi* is a protozoan parasite that is the causative agent of the animal disease known as "surra", which affects a wide variety of wild and domestic mammals including humans. This study aimed to evaluate the molecular point prevalence and clinic-epidemiological traits of *T. evansi* infection in dogs from municipalities of the Metropolitan Area of Bucaramanga (MAB), Santander, Colombia. From 2019 and 2020, a cross-sectional study in four municipalities of MAB was carried out to evaluate the above features in 213 healthy dogs from these cities. Molecular analysis using the primer ESAG/6 and ESAG/7, showed a *T. evansi* frequency of 1.8% (CI 95% = 0.04-3.68%), while Woo test didn't detect positive animals. A high prevalence of infection was detected in Piedecuesta (10.53%) and Bucaramanga (1.65%), apparently negative animals were detected in Giron and Florida Blanca. Clinical characteristics revealed that all positive animals (4/4) showed low Mean Corpuscular Hemoglobin (MCH) and (3/4) low Mean Corpuscular Volume (MCV), and one animal had a moderate fever with prolonged capillary refill time. The epidemiological characteristics showed that all animals received mixed food, (3/4) came from low socio-economic areas, and (2/4) from neighborhoods without weekly garbage collection. In conclusion, molecular methods detected a considerable prevalence of animals chronically infected by *T. evansi* in MAB, with more frequency in neighborhoods of a low socio-economic level where animals have more risk of oral transmission. These results should be considered during urban control programs of surra in MAB.

**Keywords:** Protozoan, Epidemiology, Pets, Disease Reservoirs, South America

**Introduction**

Canine trypanosomiasis is a disease complex caused by two main *Trypanosoma* species: *Trypanosoma (Schizotrypanum) cruzi* producing the Chagas disease (American form) and *Trypanosoma (Trypanozoon) evansi* producing the surra (African form) (Desquesnes et al., 2013). In America, dogs are considered the main reservoir for the transmission of *T. cruzi* infection in domestic areas, with most animals presenting a subclinical infection, with few cases developing hematological alterations and significant cardiac abnormalities that rarely cause fatality (Crisante et al., 2006; Jaimes-Dueñez et al., 2020). In contrast, dogs naturally infected with *T. evansi* show acute and severe clinical signs (case-fatality rate > 90%) (Echeverria et al., 2019; Filgueiras et al., 2019; Jaimes-Dueñez et al., 2017a), that discard the role of this host as an important reservoir of the parasite.

*T. evansi* infections in dogs exhibit acute manifestations such as apathy, weight loss, hyperthermia, corneal opacity, hepatosplenomegaly, anorexia, emesis, diarrhea, anemia, thrombocytopenia, liver damage, and death within two or three weeks, even after specific treatment with diminazene diaceturate (Echeverria et al., 2019; Filgueiras et al., 2019; Greif et al., 2018; Jaimes-Dueñez et al., 2017b). In South America, trypanosomiasis caused by *T. evansi* in dogs is often associated with rural areas where animals are exposed to vectors such as *Stomoxys calcitrans* and...
Tabanidae, and reservoirs such as capybaras (Hydrochoerus hydrochaeris) and rodents. Nevertheless, oral transmission through the consumption of domestic animals such as cattle, horses, and pigs, has also been associated with the infection (Desquesnes et al., 2013).

Although some studies have evaluated the dynamics of transmission of T. evansi in dogs from rural areas (Echeverría et al., 2019; Franke et al., 1994; Jaimes-Dueñez et al., 2017a), little is known about it in urban areas where sporadic cases are reported (Coelho et al., 2013; Filgueiras et al., 2019). In the last years an increase in canine trypanosomiasis cases has been recorded by parasitology methods in veterinary diagnostic centers of the Metropolitan Area of Bucaramanga Santander (2018, 0.059% (1/1689) of the diagnosis; 2019, 0.10% (2/1845) of the diagnosis; 2020 0.12% (2/1564) of the diagnosis); 2021, 0.0.21% (4/1875) of the diagnosis, ignoring the presence of T. evansi in these cases (Biovet, 2021). Considering the little knowledge about T. evansi infection in dogs from urban areas of Colombia, this study evaluated the molecular prevalence, and clinical and epidemiological traits associated with natural infection of T. evansi in dogs from the Metropolitan Area of Bucaramanga (AMB) Santander, Colombia.

Materials and Methods

Animal Enrollment

Blood samples derived from 213 dogs originating from the AMB and collected under the framework of previous studies carried out in our research group were screened for the presence of T. evansi DNA (Jaimes-Dueñez et al., 2020). The inclusion criteria for pets, epidemiological information, and collection and processing of samples were carried out following Jaimes-Dueñez et al., (2020). AMB is a Colombian conurbation, located in the department of Santander, in the Río de Oro valley. Its main nucleus is Bucaramanga, and its satellite municipalities are Girón, Piedecuesta, and Floridablanca (DANE, 2018). Its human population is around 1,341,694 inhabitants, and the proportion of dogs corresponds to a dog by every 4.7 people (Florez and Solano, 2019). The study area consists of tropical dry forest covered by crops, and patches of native forest, with an average annual temperature of 23.4°C and a rainfall average of 1159 mm/year (IDEAM, 2021). In the area, high infestation rates by Ctenocephalides canis, Rhizophalus sanguineus, Stomoxys calcitrans, Culex spp., Aedes spp., and other arthropods were recorded in pets throughout the year (Reyes, 1938).

Parasitological Diagnosis

A total of 213 EDTA blood samples were used for parasitological diagnosis of Trypanosoma spp. using the Woo test. The procedures were performed according to Jaimes-Dueñez et al., (2020). The motility of trypanosomes was evaluated following the procedures described by Woo (1970).

DNA-Based Tests

T. evansi infection was evaluated using a conventional PCR based on the surface proteins (ESAG) of species using the primers ESAG/6ACATTCCAGGAGGTGGAG, and ESAG/7CACGTGAATCCTCAATTTTGT (Holland et al., 2001), which has an analytical sensitivity of 0.000001 ng T. evansi DNA, and a specificity of 100% for subgenus Trypanozoon (Fernández et al., 2009). All PCR reactions were performed in a final volume of 25 μL containing 1 X reaction buffer (100 mm Tris-HCl, 50 mm KCl, pH 8.8), 1.5 mm of MgCl₂, 0.2 mm dNTP, 0.4 μm of each primer, 0.625 UI of Taq polymerase (Corpo Gen, Bogota, Colombia), and 5 μL (50 ng) of DNA sample. Thermal conditions were used as reported elsewhere (Jaimes-Dueñez et al., 2017b). PCR products were separated on a 2% agarose gel for 45 min, stained with EZ-vision 10,000X (Amresco, Solon, United States), and visualized under UV light (Spectroline, NY, United States). Blood samples were considered positive for T. evansi when a PCR product of ~237 bp was observed in the gel (Holland et al., 2001). PCR controls were obtained from canine blood samples previously diagnosed as positive and negative for T. evansi by (Jaimes-Dueñez et al., 2017a). Finally, PCR inhibitors were evaluated as previously reported (Jaimes-Dueñez et al., 2020). Samples in which this housekeeping gene could not be amplified were excluded from the analysis.

Clinical Information

Before blood sampling, animals were evaluated in a clinical examination, which considered body condition (scale 1 to 5), respiratory rates, heart rate, body temperature, and capillary refill time. Some hematological parameters were measured like Packed Cell Volume (PCV), total Red Blood Cells (RBCs), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), MCH Concentration (MCHC), Platelet Counts (PLT), White Blood Cells (WBCs), and leukocyte differential counts using an ABC VET® animal blood counter (Sci1 Animal Care Company, Gurnee, USA). Finally, Creatine Kinase Myocardial Band (CK-MB) and Aspartate Aminotransferase (ASAT) were estimated using the commercial kits (Spinreact, Girona, Spain), in a spectrophotometer GENESYS 10S (Thermo Scientific, Madrid, Spain).

Statistical Analysis

Molecular prevalence and 95% IC were calculated using SPSS v.18.0 statistical software (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc; 2009). To identify, epidemiological variables associated with the T. evansi infection, a total of seven independent
variables (age, sex, breed, city, socioeconomic level, frequency of garbage collection, and access to rural areas) were analyzed with the result of the PCR (positive or negative) using binomial logistic regression. The level of statistical significance was set at P<0.05. Finally, quantitative parameters in the positive samples were shown as the median and Interquartile Range (IQR), whereas qualitative variables were described through frequency analysis.

Ethics Statement

All of the experimental procedures involving animals were conducted in accordance with the good animal practice as defined by the Colombian code of practice for the care and use of animals for scientific purposes, established by the law 84 of 1989. Informed consent about the risks and benefits of the study was completed by the owners. Ethical approval for analyzing animal specimens was obtained from the Animal Ethics Committee of the Cooperative University, Bucaramanga, Colombia (Act No. 043 of 2018).

Results

A total of 213 dogs were evaluated, of which 66.6% were females, 70.6% were adults, and 26.5% were puppies. According to the origin, 55.8, 20.1, 15, 9.1%, came from Bucaramanga, Floridablanca, Giron and Piedecuesta, respectively. Regarding the breed, 57.2% were Creole breed, and 12.5% were Labrador Retriever. Animals were clinically healthy during the clinical examination. No positive animals to Trypanosoma spp. were detected in the Woo test.

Molecular Detection of T. evansi Infection

Molecular analysis showed a total of 4 positive dogs to T. evansi (1.9, 95% CI = 0.04-3.68%). High frequencies were detected in Piedecuesta (10.5, 95% CI = -4.6-25.7%) and Bucaramanga (1.6, 95% CI = -0.65-3.96%), negative animals were observed in Giron and Floridablanca (Fig. 1).

Clinical and Epidemiological Variables Associated with T. evansi Infection

The clinical analyses revealed that all parameters were within normal values in all animals except for the heart rate, Hb, HCT, MCV, MCH, WBCs, neutrophils, lymphocytes, and eosinophils (Table 1). All animals showed low levels of MCH and MCV. No animals showed the appearance of hemotropic agents in the blood smear (Table 1).

Binomial logistic regression did not show epidemiological variables significantly associated with the infection; however, the frequentist distribution of the cases revealed that of the four positive dogs to T. evansi, all corresponded to adults, three (3/4) to males, and two (2/4) to creole breed. Likewise, half (2/4) was from Bucaramanga and the other from Piedecuesta. Three animals (3/4) lived in areas of low socioeconomic status and two (2/4) in areas without weekly garbage collection. All animals received mixed feed and a half (2/4) have access to the rural area.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Median (IQR)</th>
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<tbody>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body condition, 1 to 5</td>
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<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3 (0)</td>
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<tr>
<td>Temperature, °C</td>
<td>38-39.2</td>
<td>38</td>
<td>39.2</td>
<td>39.1</td>
<td>38.7</td>
<td>38.9 (1.0)</td>
</tr>
<tr>
<td>Capillary refill time, seconds</td>
<td>1-2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>60-180</td>
<td>98</td>
<td>74</td>
<td>64</td>
<td>56</td>
<td>69 (34)</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>10-30</td>
<td>24</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30 (5)</td>
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<tr>
<td>Hematological</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Red blood cells, 1 × 10^6/µL</td>
<td>4.8-9.3</td>
<td>6.75</td>
<td>7.50</td>
<td>9.15</td>
<td>8.90</td>
<td>8.2 (2.1)</td>
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<tr>
<td>Hemoglobin, g/dL</td>
<td>12.1-20.3</td>
<td>11.1</td>
<td>11</td>
<td>13</td>
<td>13</td>
<td>13 (3)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>36-60</td>
<td>33</td>
<td>39</td>
<td>45</td>
<td>40</td>
<td>39.5 (9.2)</td>
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<td>Mean corpuscular volume, fl</td>
<td>58-79</td>
<td>49.1</td>
<td>52.1</td>
<td>49.1</td>
<td>45.1</td>
<td>49.5 (5.2)</td>
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<tr>
<td>Mean corpuscular hemoglobin, p.g.</td>
<td>19-26</td>
<td>16.3</td>
<td>17.3</td>
<td>16.3</td>
<td>14.3</td>
<td>16.3 (1.9)</td>
</tr>
<tr>
<td>Platelets, 1 × 10^6/µL</td>
<td>170-400</td>
<td>320</td>
<td>256</td>
<td>254</td>
<td>233</td>
<td>255 (66)</td>
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<tr>
<td>White blood cells, 1 × 10^6/µL</td>
<td>4.000-15,500</td>
<td>13950</td>
<td>22450↑</td>
<td>11700</td>
<td>15500</td>
<td>14725 (8450)</td>
</tr>
<tr>
<td>Neutrophils, 1 × 10^3/µL</td>
<td>2.060-10,600</td>
<td>10044</td>
<td>11674↑</td>
<td>7722</td>
<td>7440</td>
<td>8883 (3756)</td>
</tr>
<tr>
<td>Lymphocytes, 1 × 10^3/µL</td>
<td>690-4,500</td>
<td>3208</td>
<td>8755↑</td>
<td>2457</td>
<td>5270↑</td>
<td>4239 (5239)</td>
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<td>Monocytes, 1 × 10³/µL</td>
<td>0-840</td>
<td>418</td>
<td>673</td>
<td>468</td>
<td>310</td>
<td>443 (284)</td>
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<tr>
<td>Eosinophils, 1 × 10³/µL</td>
<td>0-1,200</td>
<td>279</td>
<td>134↑</td>
<td>1053</td>
<td>2480↑</td>
<td>1200 (1725)</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>15-66</td>
<td>21.3</td>
<td>27.9</td>
<td>41.3</td>
<td>22.6</td>
<td>25.2 (16.3)</td>
</tr>
<tr>
<td>CK-MB U/L</td>
<td>26.7-75.0</td>
<td>14.7</td>
<td>21.6</td>
<td>45.2</td>
<td>28.4</td>
<td>25 (24.6)</td>
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<tr>
<td>Hemotropic agents in the blood smear</td>
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<td>Negative</td>
<td>Negative</td>
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<td></td>
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<td>Male</td>
<td>Male</td>
<td>Male</td>
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<tr>
<td>Age</td>
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<td>Adult</td>
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<td>Adult</td>
<td>Adult</td>
<td>-</td>
</tr>
<tr>
<td>Breed</td>
<td>-</td>
<td>Creole</td>
<td>Creole</td>
<td>German shepherd</td>
<td>Cocker springer</td>
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<td>Municipality</td>
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<td>Piedecuesta</td>
<td>Piedecuesta</td>
<td>Bucaramanga</td>
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<td>1</td>
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<td>Weekly garbage collection</td>
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<td>No</td>
<td>No</td>
<td>Yes</td>
<td>-</td>
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<tr>
<td>Number of pets</td>
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<td>4</td>
<td>3</td>
<td>2</td>
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<td>Access to the rural area</td>
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<td>yes</td>
<td>Yes</td>
<td>No</td>
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<td>Feed type</td>
<td>-</td>
<td>Concentrate and food</td>
<td>Concentrate and food</td>
<td>Concentrate and food</td>
<td>Concentrate and food</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: AST, Aspartate Aminotransferase; CK-MB, Creatine Kinase Myocardial Band. Values outside of reference value are shown in bold.
Fig. 1: Molecular prevalence of *Trypanosoma evansi* in dogs from the metropolitan area of bucaramanga, santander, Colombia. Buc, Flo, Gir and Pie, represent Bucaramanga, floridablanca, giron, and piedecuesta, respectively.

**Discussion**

In Colombia, the prevalence of *T. evansi* infection has been reported in wild and domestic animals (Jaimes-Dueñez et al., 2017a; Jaimes-Dueñez et al., 2018; Jaimes-Dueñez et al., 2019; Morales et al., 1976); however, the epidemiology of this parasite in Colombian dogs is little known, with few case reports suggesting a high susceptibility of the dogs to development fatal cases (Correa-Salgado et al., 2010; Jaimes-Dueñez et al., 2017b). Herein, we addressed a *T. evansi* epidemiological surveillance in dogs from MAB, showing a considerable prevalence of infection and animals that can keep chronic infections without complicated clinical signs (Fig. 1). These results show the ability of the parasite to maintain a life cycle in urban areas and suggest a zoonotic risk for the human population of the AMB.

Molecular prevalence observed here is higher than that recently reported in dogs from the Brazilian Amazon (1.2%) (Filgueiras et al., 2019), India, and Southeast Asia (1.5, 95% CI: 0.8-2.7%), using molecular methods (Nguyen et al., 2021). The high prevalence of *T. evansi* infection in dogs from MAB could be associated with the high density of dogs in AMB (Florez and Solano, 2019) as well as the high infestation rates by hematophagous ectoparasites as *C. canis, R. sanguineus, and S. calcitrans*, which have been increased in recent years, probably associated to global warming, landscape modification, and resistance to acaricides and insecticides (Alkishe et al., 2021; Barros et al., 2019; Dantas-Torres, 2010). Nevertheless, *T. evansi* infection through oral mucosa penetration of the parasite during the ingestion of contaminated food or consumption of infected rodents also be considered (Raina et al., 1985; van Bree et al., 2018). Although the statistical analysis did not show epidemiological variables associated with the infection, probably due to the few numbers of positive cases in the population, the infected dogs observed here received constant food waste, with most living in low-socioeconomic areas with a lack of weekly garbage collection. As shown in previous studies, these conditions favor the abundance of rodents and other synanthropic animals that are important reservoirs of *T. evansi* infection (Aregawi et al., 2019; Desquesnes et al., 2013; Echeverria et al., 2019), and that could transmit the infection to dogs through food contamination or direct consumption from these reservoirs. Further studies evaluating synanthropic reservoirs of *T. evansi* in the AMB are necessary to describe a complete transmission cycle in these urban zones.

In Colombia, *T. evansi* infection in dogs has been reported in three animals with fatal outcomes after experiencing clinical signs such as cachexia, anemia, thrombocytopenia, and hepatosplenomegaly (Correa-Salgado et al., 2010; Jaimes-Dueñez et al., 2017a). Interestingly, epidemiological surveillance performed here showed that positive animals did no evident clinical signs, however, hematological alterations were observed in this group. In this case, infected dogs showed a significant reduction of the MCH with a low MCV that suggests hypochromic microcytic anemia, the same as those observed during *T. evansi* infection in dogs from Brazil (Silva et al., 1995). Anemia is one of the most consistent findings in trypanosomiasis. Three phases of anemia have been reported in this pathology, phase I (acute crises), phase II (chronic), and phase III (recovery) (Mbaya et al., 2012). During the first one (acute crises), the parasitemia is usually high, fluctuating, and evident on most days. In this phase, the anemia is morphologically classified as macrocytic and normochromic, and the death commonly occurs due to
severe pancytopenia and other pathologies. The second is characterized by low levels of parasitemia with fluctuant erythrocyte values, in this point, the anemia could vary from normochromic and normocytic to hypochromic and microcytic. In the last phase, hematological parameters return to pre-infection values (Anosa, 1988). These postulates, plus the lack of positive animals in the Woo test, suggest that the positive dogs observed here correspond to chronic infections in which the parasitic load can only be detected by molecular methods. These findings show that although the mortality rate of *T. evansi* in dogs is high (>90%) (Echeverria *et al.*, 2019; Eloy and Lucheis, 2009), someone’s could be chronically infected and maintain low parasitic loads, which suggests they are not an effective source of infection to spread the disease. Further studies involving parasitological and immunological analyses are necessary to confirm these hypotheses.

**Conclusion**

An epidemiological survey of *T. evansi* in dogs from urban areas of Colombia allowed detected a considerable prevalence of animals chronically infected, with more frequency in the neighborhoods of a low socioeconomic level where animals have more risk of oral transmission; in addition, chronic infections were associated with signs of anemia, which considerably impairs the health and quality of life of these pets. This study provided relevant information about clinical and socioeconomic features associated with *T. evansi* infection in dogs from AMB, which must be taken into consideration when Surra control programs are implemented in these municipalities.

**Declaration of Interest**

All the authors have participated in the study and no conflicts of interest have been disclosed.

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**Author’s Contributions**

Jeiczon Elim Jaimes-Dueñez: Designed the study, conducted the analysis, and drafted the manuscript.  
Ángela Patricia Jiménez-Leaño: Designed the study and conducted the analysis.  
Daniela Montenegro-Ayala: Made experiments and provided critical revision of the manuscript.  
Maria Esteban-Mendoza: Designed the study, made experiments, and provided critical revision of the manuscript.

All authors contributed to and commented on subsequent revisions of the manuscript. All authors approved the final manuscript.

**Ethics**

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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