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Co-infection of *Trichuris vulpis* and *Toxocara canis* in different aged dogs: Influence on the haematological indices


**Trichuris vulpis** and *Toxocara canis* are worldwide parasitic nematodes affecting dogs and mammals of the Canine family. Due to the special structure of the shells, the eggs of these geohelminths can maintain their viability in soil, sand, water, and the environment for a long time. The study involved young (6–12 months old) and adult (1.5–8.0 years old) dogs affected by co-infection of *T. vulpis* + *T. canis*, the control group consisted of dewormed healthy animals of the same age. Parasitological examination of dogs’ faeces was performed using a "Counting Chamber for Ovoscopic Researches", morphological parameters, indicators of nutrient metabolism, mineral metabolism and activity of enzymatic systems were determined in blood and serum. According to the results of parasitological research on the dogs, it was found that young animals are more prone to toxocarosis, and adults – trichurosis. The co-infection of nematodes *T. vulpis* + *T. canis* in dogs develops several changes in haematological parameters: a significant decrease in erythrocytes, haemoglobin, MCV, MCH, and haematocrit, leukocytosis with basophils and eosinophils in young infected animals; and eosinophilia and basophilia (15.9 times) in adults, compared with healthy dogs of the same age. Among the changes in serum biochemical parameters, young infected dogs showed a decrease in the concentration of total protein and albumin content, an increase in the content of "acute phase" proteins (α-1, α-2 and β-globulins), an increase in cholesterol and total bilirubin; in adult infected dogs, a decrease in albumin content, an increase in the content of α-1, α-2, and β-globulins, an increase in the concentration of cholesterol and total bilirubin, a decrease in the concentration of area in comparison with healthy animals were determined. Also, among the indicators of mineral metabolism, a decrease in the concentration of calcium and magnesium in the serum was found in young infected dogs. Among the changes in the enzyme metabolism in the serum of infected dogs, there was an increase in the activity of all studied enzymes in animals of both research groups: ALT, AST, α-amylase, GGT and ALP. Thus, co-infection with nematodes *T. vulpis* + *T. canis* has a complex pathogenic effect on the body of dogs of all ages, which manifested itself in multiple changes in haematological parameters. In the future, the authors’ team plan to develop comprehensive measures to combat nematode infections in different living conditions of dogs, taking into account the results of the current research.

Keywords: Nematoda; Trichuridae; Ascarididae; parasites; canine; blood parameters.

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**Introduction**

*Trichuris vulpis* (Froelich, 1789) and *Toxocara canis* (Werner, 1782) are parasitic nematodes, the definitive hosts of which are dogs and members of the Canine family. Both parasites are geohelminths, their eggs mature in the environment, maintaining viability for a long time due to the special structure of the shells (Abou-El-Naga, 2018; Boyko & Brygadyrenko, 2017; Zazharska et al., 2018; Feshchenko et al., 2019). At the same time, the areas of geohelminths distribution are more limited, tied to a special structure of the shells, the eggs of these geohelminths can maintain their viability in soil, sand, water and the environment for a long time. The study involved young (6–12 months old) and adult (1.5–8.0 years old) dogs affected by co-infection of *T. vulpis* + *T. canis*, the control group consisted of dewormed healthy animals of the same age. Parasitological examination of dogs’ faeces was performed using a "Counting Chamber for Ovoscopic Researches", morphological parameters, indicators of nutrient metabolism, mineral metabolism and activity of enzymatic systems were determined in blood and serum. According to the results of parasitological research on the dogs, it was found that young animals are more prone to toxocarosis, and adults – trichurosis. The co-infection of nematodes *T. vulpis* + *T. canis* in dogs develops several changes in haematological parameters: a significant decrease in erythrocytes, haemoglobin, MCV, MCH, and haematocrit, leukocytosis with basophils and eosinophils in young infected animals; and eosinophilia and basophilia (15.9 times) in adults, compared with healthy dogs of the same age. Among the changes in serum biochemical parameters, young infected dogs showed a decrease in the concentration of total protein and albumin content, an increase in the content of "acute phase" proteins (α-1, α-2 and β-globulins), an increase in cholesterol and total bilirubin; in adult infected dogs, a decrease in albumin content, an increase in the content of α-1, α-2, and β-globulins, an increase in the concentration of cholesterol and total bilirubin, a decrease in the concentration of area in comparison with healthy animals were determined. Also, among the indicators of mineral metabolism, a decrease in the concentration of calcium and magnesium in the serum was found in young infected dogs. Among the changes in the enzyme metabolism in the serum of infected dogs, there was an increase in the activity of all studied enzymes in animals of both research groups: ALT, AST, α-amylase, GGT and ALP. Thus, co-infection with nematodes *T. vulpis* + *T. canis* has a complex pathogenic effect on the body of dogs of all ages, which manifested itself in multiple changes in haematological parameters. In the future, the authors’ team plan to develop comprehensive measures to combat nematode infections in different living conditions of dogs, taking into account the results of the current research.

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**Keywords**: Nematoda; Trichuridae; Ascarididae; parasites; canine; blood parameters.
A study conducted in 2020 at the Homeless Animal Welfare Centre “In Good Hands” (Vysneve town, Kyiv region), on the basis of the Scientific Research Institute of Internal Diseases of Animals and Laboratory of Department of Parasitology and Pharmacology, Bila Tserkva National Agrarian University. The research protocol of the current study was approved by the Ethics Committee of the Bila Tserkva National Agrarian University (Approval number: 21.05.2020 / No. 7, conclusion 3/1). Six weeks before the start of the study, we selected 25 young dogs aged 6–12 months and 20 adult dogs aged 1.5–8.0 years, spontaneously infected with *T. canis* and *T. vulpis* nematodes for inclusion in the study groups. At another site of the shelter, 20 and 10 animals were selected, respectively, and dewormed for further inclusion in control groups. Additionally, the enclosures of animals of this group were disinfected to prevent re-infection of dogs. The dogs of the experimental and control groups belonged to the German Shepherd, Labrador Retriever, Russian Spaniel, and Belgian Shepherd breeds and their crossbreeds. Animals of all groups were of both sexes and sterilized. At the end of the study, all dogs in the experimental groups underwent treatment for nematodes.

Infection of dogs with nematode agents before and during the study was established using the “Counting Chamber for Ovoscopy Research”. To do this, 1 g of faeces from the average sample was mixed with a flotation solution of granular ammonium nitrate (solution density – 1.300 g/L), stirring and bringing the volume to 30 mL. The resulting suspension was filtered through a metal sieve. To start working with the chamber, on its base, with the help of two pins, cover glass was fixed with the grating down. Using a pipette, the resulting suspension in a volume of 2 mL was introduced into one of the cells through the recess of the chamber base. The cell was considered filled when the suspension completely expelled air from under the cover glass. Thus, the surface of the suspension was in the same plane as the grid applied to the cover glass. Ovoscopy was performed 3 minutes after filling the cell (this time is required for flotation of eggs, as a result of which they are placed on the surface, in the same plane with the camera grid). In the field of view of the biological light microscope “Biomed +” XSM-20 (“BioMed”, China) at 4 × 10 we found a grid that serves as a guide for counting helminth eggs. After counting helminth eggs, their number was multiplied by 15 and a number obtained was taken as the number of eggs in 1 g of faeces of the test animal.

The intensity of nematode infection in a particular animal was considered the average number of parasite eggs in 1 g of its faeces according to the results of three trials. According to the results of parasitological research, the group of young dogs was characterized by average infection rates of 29.28 ± 3.76 *T. canis* eggs per 1 g of faeces and 5.5 ± 0.67 *T. vulpis* eggs per 1 g of faeces (Fig. 1). For animals of the adult dogs’ group, these values were 11.35 ± 3.29 and 29.55 ± 7.67 eggs per 1 g of faeces, respectively (Fig. 2).

Blood samples from dogs of all groups were performed in the morning before feeding from the *Vena cephalica antebrachii*. Blood samples were taken in vacuum tubes 10 mL volume (Vacutest, Italy). An anticoagulant ethylenediaminetetraacetic acid (EDTA) was used. In the dogs’ blood, the total number of erythrocytes, haemoglobin content, haematocrit, average erythrocyte volume (MCV), the content of leukocytes (including the ratio of their fractions) and platelets were determined by an automatic haematological analyzer Mythic 18 (Orphée SA, Switzerland) and reagents by PZ Cormay SA (Poland). Haemoglobin content in erythrocyte (MSN) was calculated mathematically. Biochemical parameters of dogs’ serum were determined using HTI BioChem SA Semi-Auto Chemistry Analyzer (High Technology, Inc., USA), using appropriate reagents from the same manufacturer.

The datasets were expressed as mean (x) ± standard error of the mean (SE). Mathematical analysis of the study results was conducted in Statistica 13.3 IT Application (StatSoft Inc., USA). Differences between average values were considered statistically significant at P < 0.05 (ANOVA).
Results

According to haematological studies, co-infection of nematodes caused many significant changes in morphological parameters of blood and biochemical parameters of blood serum. In young infected animals (Table 1), a significant decrease in the number of erythrocytes (by 19.3%) and haemoglobin concentration (by 31.2%) was found in comparison with healthy animals of the same age range (Table 2). Thus, young animals were characterized by a decrease in the concentration of total protein (by 11.3%), and in the structure of protein fractions there was a decrease in albumin content (by 19.6%), and an increase in α-1 (by 52.0%), α-2 (by 29.2%) and β-globulins (by 44.2%). There was also an increase in the concentration of cholesterol (by 82.5%) and total bilirubin (by 28.8%) in the serum of dogs in this group.

Adult infected dogs were also characterized by changes in the ratio of serum protein fractions (decrease in albumin content by 24.4%, increase in α-1 by 36.7%, α-2 by 25.9% and β-globulins by 45.4%), however, without significant changes in the concentration of total protein. There was also a sharp increase in the concentration of cholesterol (2.35 times), total bilirubin (2.04 times) and a decrease in the concentration of urea (by 23.4%) in the blood serum of animals in this group. Mean creatinine levels in dogs of both groups and urea in young animals had no significant changes, but individual values of individual animals differed significantly within the group, which was manifested in large values of standard error (SE).

Speaking of the indicators of mineral metabolism in the serum of dogs infected with co-invasion of *T. vulpis* + *T. canis*, no significant changes were found (Table 3). However, young dogs were characterized by a decrease in the concentration of calcium (by 12.8%) and magnesium (by 11.5%) in the serum.

Examining the indicators of enzyme metabolism in the serum of infected dogs, we found an increase in the activity of all studied enzymes in animals of both experimental groups (Table 4). Thus, among young dogs the activity of ALT was increased 3.22 times, AST – 2.00 times, α-aminotransferase – 70.2%, GGT – 2.94 times, and ALP – 4.24 times, compared with healthy animals of the same age. For the group of adult infected dogs, these indicators were 5.10 times higher, 4.75 times higher, 29.6% higher, 3.58 times higher, and 5.94 times higher, respectively.

Thus, co-infection was found to be predominance of parasitization by *T. canis* in young dogs and *T. vulpis* in adults. The results of haematological examination revealed significant deviations in infected animals of both age groups.

Discussion

Examining the prevalence of *T. canis* infection, the researchers found that this rate was significantly higher in young dogs, while there was no significant difference in the prevalence of *T. vulpis* by age (Savilla et al., 2011). Similar results were obtained by other scientists (Gebremedhin et al., 2020), who found that the probability of infection with *T. canis* is 4.5 times higher in young dogs than in adults. Motta et al. (2019) and Stafford et al. (2020), like previous authors, indicate that the age of dogs increases the risk of infection with the nematode *T. canis* and do not consider the spread of *T. vulpis* related to the age of the owner. However, some publications describe an increased risk of *T. vulpis* infection in older dogs (Symeonidou et al., 2017).

According to the results of current studies, the same distribution of both studied nematodes was found among both young and adult dogs. However, there was a clear predominance in the intensity of *T. canis* infection among dogs aged 6–12 months, and *T. vulpis* in adult animals. Such indicators indicate the stationary focal contamination of the territory and premises of the studied nursery with eggs of parasitic nematodes and require comprehensive measures to combat these infections.

Table 1

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Young dogs (6–12 months)</th>
<th>Adult dogs (1.5–8.0 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>healthy, n = 15</td>
<td>infected, n = 22</td>
</tr>
<tr>
<td>Erythrocytes, ×10⁵/L</td>
<td>5.64 ± 0.39</td>
<td>5.21 ± 0.18***</td>
</tr>
<tr>
<td>Haemoglobin, g/L</td>
<td>158.9 ± 2.8</td>
<td>109.3 ± 3.3***</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV), fl.</td>
<td>67.6 ± 1.7</td>
<td>55.1 ± 1.3***</td>
</tr>
<tr>
<td>Mean content of haemoglobin (MCH), flm/cell</td>
<td>1.52 ± 0.06</td>
<td>1.30 ± 0.07***</td>
</tr>
<tr>
<td>Haematocrit, %</td>
<td>44.0 ± 1.1</td>
<td>23.7 ± 0.03***</td>
</tr>
<tr>
<td>Leukocytes, ×10⁹/L</td>
<td>10.36 ± 0.25</td>
<td>17.64 ± 0.63***</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>1.94 ± 0.07</td>
<td>18.56 ± 0.67***</td>
</tr>
<tr>
<td>Basophil, %</td>
<td>0</td>
<td>3.18 ± 0.11</td>
</tr>
<tr>
<td>Platelets, ×10⁹/L</td>
<td>305.3 ± 15.9</td>
<td>320.9 ± 18.2</td>
</tr>
</tbody>
</table>

Note: *P < 0.05, **P < 0.01, ***P < 0.001 compared to healthy young dogs; °P < 0.05, °°P < 0.01, °°°P < 0.001 compared to healthy adult dogs.
Studying the pathogenesis of helminthiasis in general and nematodes in dogs in particular, scientists have concluded that these pathologies are accompanied by various changes in haematological parameters. Thus, a study of the blood of dogs subclinically affected by Ancylostoma and other parasites (Shamsi et al., 2018; Miglio et al., 2020). However, the appearance of basophilic shifts in young and severe basophilia in adult dogs was specific, which may be justified by chronic inflammatory condition of the gastrointestinal tract (due to mechanical intervention of adult nematodes in the intestinal wall) and the same allergic reactions to helminths (Reck et al., 2011; Abbott & Allen, 2020).

As can be seen from the results of the study of nutrient metabolism in the serum of dogs with co-infection T. vulpis + T. canis (Table 2), the infection did not have a significant effect on hydrocarbon metabolism. But the metabolism of protein (and its fractions), as well as fats, has undergone significant changes, which, in turn, differed significantly between the age groups of the studied animals. Thus, a probable decrease in total protein concentration in young dogs and albumin content in both age groups indicate both protein deficiency and liver dysfunction (Zhang et al., 2021).

Such a nutritional deficiency can be the result not only of protein deficiency in the diet but also to a greater extent, impaired digestion and absorption of nutrients due to mechanical, trophic and toxic effects of parasites. The same types of negative effects of parasites on the body of the host can explain liver dysfunction (Cavalcanti et al., 2019), which is also confirmed by a significant increase in cholesterol and total bilirubin in the serum of dogs of both experimental age groups.

Also, a pronounced change in the serum biochemical parameters of infected dogs was an increase in the content of globulins α1 and α2 fractions, which are essential proteins of the so-called “acute phase” (Tothova et al., 2019). Such changes characterize the body’s fight against foreign agents in response to infection. Serum creatinine concentrations in infected dogs of both ages, as well as urea in young dogs, did not differ

Table 2
Changes in biochemical parameters of nutrient metabolism in the serum of dogs with co-infection T. vulpis + T. canis (x ± SE)

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Young dogs (6–12 months)</th>
<th>Adult dogs (15–80 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>healthy, n = 25</td>
<td>healthy, n = 10</td>
</tr>
<tr>
<td></td>
<td>infected, n = 25</td>
<td>infected, n = 20</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.93 ± 0.21</td>
<td>5.62 ± 0.31</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>62.3 ± 1.8</td>
<td>55.3 ± 1.5**</td>
</tr>
<tr>
<td>Albumin, %</td>
<td>51.2 ± 1.9</td>
<td>41.2 ± 1.4***</td>
</tr>
<tr>
<td>α-1 globulin, %</td>
<td>8.28 ± 0.39</td>
<td>12.59 ± 0.73***</td>
</tr>
<tr>
<td>α-2 globulin, %</td>
<td>8.57 ± 0.46</td>
<td>11.07 ± 0.31***</td>
</tr>
<tr>
<td>β- globulin, %</td>
<td>9.62 ± 0.32</td>
<td>13.87 ± 0.66***</td>
</tr>
<tr>
<td>γ globulin, %</td>
<td>22.31 ± 0.88</td>
<td>21.30 ± 0.79</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>84.2 ± 3.4</td>
<td>92.2 ± 4.6</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>6.70 ± 0.25</td>
<td>6.58 ± 0.40</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>3.15 ± 0.14</td>
<td>5.75 ± 0.24***</td>
</tr>
<tr>
<td>Total bilirubin, μmol/L</td>
<td>3.93 ± 0.22</td>
<td>5.06 ± 0.19***</td>
</tr>
</tbody>
</table>

Note: See Table 1.

Table 3
Changes in biochemical parameters of mineral metabolism in the serum of dogs with co-infection T. vulpis + T. canis (x ± SE)

<table>
<thead>
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<th>Indexes</th>
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<tr>
<td></td>
<td>healthy, n = 25</td>
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</tr>
<tr>
<td></td>
<td>infected, n = 25</td>
<td>infected, n = 20</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>1490 ± 4.3</td>
<td>1351 ± 6.3</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>5.72 ± 0.26</td>
<td>5.61 ± 0.23</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.82 ± 0.12</td>
<td>2.46 ± 0.10**</td>
</tr>
<tr>
<td>Phosphorus, mmol/L</td>
<td>2.49 ± 0.10</td>
<td>2.45 ± 0.11</td>
</tr>
<tr>
<td>Magnesium, mmol/L</td>
<td>0.96 ± 0.04</td>
<td>0.85 ± 0.03*</td>
</tr>
<tr>
<td>Chloride, mmol/L</td>
<td>107.8 ± 3.79</td>
<td>105.3 ± 4.1</td>
</tr>
</tbody>
</table>

Note: See Table 1.

Table 4
Changes in biochemical parameters of enzyme metabolism in the serum of dogs with co-infection T. vulpis + T. canis (x ± SE)

<table>
<thead>
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<th>Indexes</th>
<th>Young dogs (6–12 months)</th>
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</tr>
<tr>
<td></td>
<td>infected, n = 25</td>
<td>infected, n = 20</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>36.4 ± 1.9</td>
<td>117.5 ± 5.1***</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>40.1 ± 1.3</td>
<td>80.2 ± 3.7***</td>
</tr>
<tr>
<td>α-aminotransferase, U/L</td>
<td>898.8 ± 18.4</td>
<td>1529.3 ± 66.4***</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>6.32 ± 0.23</td>
<td>18.56 ± 0.92***</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>27.2 ± 0.7</td>
<td>115.3 ± 4.5***</td>
</tr>
</tbody>
</table>

Note: See Table 1.
significantly from those in healthy animals. However, the results obtained on these indicators differed significantly in individual animals within groups, which indicates different individual reactions of the organism to toxins of parasites Toxocara canis and T. vulpis. The result of the study of the concentration of urea in the serum of infected adult dogs, which was significantly lower than in healthy animals, was special. This phenomenon can be explained by the alimentary depletion of animals and severe liver pathology (Pizzi-matto et al., 2019).

The decrease in the concentration of calcium and magnesium in the serum of young dogs (Table 3) was probably due to a nutritional deficiency of these trace elements in the body due to disruption of digestion and absorption of nutrients as a result of parasitism of adult nematodes in the intestine (Smith et al., 2005; Sweeny et al., 2021). Perhaps a deficiency of these micronutrients is one of the factors in the chain of the pathogenesis of seizures in puppies with a high intensity of gastrointestinal parasitic infection (Ryan, 2020). The absence of changes in the concentration of other macro-, and micronutrients in the serum of dogs with co-infection with T. vulpis + T. canis indicates the preservation of the functioning of the osmoregulatory system.

The most pronounced were the changes in the activity of the enzymes ALT, AST, α-amylase, GGT, and ALP, which were significantly higher in infected dogs of both ages. Significantly higher intensity of increase in ALT activity in comparison with AST, as well as other changes in enzyme activity, indicate the hepatic origin of the pathology (Kaushik et al., 1997; Mazzaro et al., 2019). Significant increase in α-amylase activity in the serum of young dogs, much higher than in adult animals may be due to the possibility of parasitism of T. canis in the ducts of the pancreas and in the gland itself at a high intensity of infection with a significant number of adult nematodes (Miller, 2020). This is exactly the situation we observed in the group of young experimental dogs, as described above. Significantly sharper changes in morphological and trophic blood parameters in young dogs, and metabolism in adults, should be explained by sensitivity to nutrient deficiencies in the former and age-related accumulation of chronic pathologies of vital organs in the latter.

Conclusions

According to the results of our parasitological study of dogs, it was found that the intensity of T. vulpis nematode infection among adult dogs (1.5–8.0 years) was 5.37 times higher than among young dogs (6–12 months). The intensity of T. canis infection, in contrast, was 2.58 times higher in young dogs than in adults.

Co-infection of T. vulpis + T. canis nematodes in young dogs showed signs of anaemia (erythrocytopenia, haemoglobinolisation, decreased MCV and MCH, macrocytosis), inflammation and hyperimmune reactions (leukocytosis, basophilia and eosinophilia, increased t-1, α-2 and β-globulins), nutritional deficiency of protein and minerals (proteinaemia, albuminuria, calcium and magnesium), liver dysfunction (increased cholesterol and total bilirubin, increased activity of ALT, AST, α-amylase, GGT and ALP).

According to the results of a haematological examination of adult nematode infection with T. vulpis + T. canis, there were signs of moderate sensitization and hyperimmune reaction (eosinophilia and basophilia, increased levels of α-1, α-2 and β-globulins), profound liver dysfunction (albuminuria, increased cholesterol and total bilirubin, decreased urea, increased activity of enzymes ALT, AST, α-amylase, GGT and ALP). Thus, co-infection with nematodes T. vulpis + T. canis has a complex pathogenic effect, which involves vital organs and body systems of dogs of all ages.

The current studies are the initiative of the authors and do not have any outside financial support. The research was carried out within the framework of the initiative topic for scientific work “Development of schemes for antiparasitic treatments of animals and methods for evaluating their effectiveness” (state registration No. 0160005326).

The authors are grateful to the leaders of the Homeless Animal Welfare Center “In Good Hands” – director Kateryna Zhukovska and veterinarian Stanislav Zaremychuk for their help and assistance in conducting research, as well as for their important work in helping homeless animals.

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