Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.015

RESEARCH ARTICLE

# Molecular Landscapes of Deoxyuridine 5'-Triphosphatase (dUTPase) as a Drug Target against Camel *Trypanosoma evansi*

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ARTICLE HISTORY (20-562)

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|---|---|
| Received: | October 29, 2020 |
| Revised: | December 18, 2020 |
| Accepted: | December 19, 2020 |
| Published online: | January 24, 2021 |

**Key words:**
Camel
drug discovery
dUTP
dUTPase
Pyrimidine
*Trypanosoma evansi*

**ABSTRACT**

The decoding of genome sequences of camel and its pathogens will help in speeding up the discovery of new drug targets of pathogens. In this context, *Trypanosoma evansi* (*T. evansi*) constitutes the major health hazard with confirmed broad host range and zoonotic infections of humans. Discovery of a drug target in *T. evansi* deoxyuridine 5'- triphosphate *(dUTP)* pathways by comparing *dUTP* metabolizing enzymes in dromedary camels and the parasite. The pyrimidine pathways were investigated and the enzymes involved in the metabolism of deoxyuridine 5'-triphosphate *(dUTP)* in camel and *T. evansi* were investigated by bioinformatics tools. *T. evansi* was devoid of deoxycytidine triphosphate deaminase rendering the source of dUTP under metabolic stress. There were interesting differences in predicted structure and function of dUTPase between the camel and *T. evansi*. The camel dUTPase is a trimeric enzyme of 165 amino acids highly similar to other vertebrates' enzyme, mw of 18 kDa and form trimers without multiple domain constituents. In comparison, *T. evansi* dUTPase is a dimeric enzyme with a higher mw of 32 kDa resembling bacterial and some protozoal enzyme and bearing multiple domain content of dUTPase-2 family, as well as a broad-spectrum nucleotide-binding domain. Additionally, both enzymes have different catalytic attacks at the nucleotide phosphates. Owing to the described structural and catalytic differences, dUTPase could be a useful target for anti-Trypanosoma drug discovery research.

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**To Cite This Article:** Kandeel M and Al-Taher A, 2021. Molecular landscapes of deoxyuridine 5'-triphosphatase (dUTPase) as a drug target against camel *Trypanosoma evansi*. Pak Vet J, 41(2): 235-241. http://dx.doi.org/10.29261/pakvetj/2021.015

## INTRODUCTION

Surra or camel trypanosomiasis is a fatal systemic protozoal disease caused by *T. evansi*. Among all trypanosomes, *T. evansi* has the largest range of potential hosts as well as the widest worldwide distribution (Desquesnes *et al.*, 2013). Recent concern about the zoonotic importance of *T. evansi* is evidenced by infection of humans with the animal parasite, probably by contamination of a wound with infected animal blood and mutations in the human genome leading to loss of trypanolytic factor in the human blood (Vanhollebeke *et al.*, 2006; Fong, 2017; Ragab and ElSanousi 2017). Furthermore, frequent infection of humans with *T. evansi* was concluded after finding of a 22% positive reaction to *T. evansi* test (Shegokar *et al.,* 2006).

Chemotherapy is the only useful tool in case of *T. evansi* infection since the vaccination is hindered by the expression of variant surface glycoproteins and control of vector flies is always nonsustainable (Witola *et al.,* 2005). Therefore, the finding of new drugs is the only reliable tool for treating *T. evansi* infections. Few drugs are available for treating trypanosomiasis. Suramin, diamidines and quinapyramine are extensively used as the sole treatment tool. Recent studies showed resistance to quinapyramine (Liao and Shen 2010), suramin (Wiedemar, 2019) and diamidines (Munday *et al.,* 2013) treatment. Recently, we provided several analytical studies for the potential structural and metabolic differences between the camel and *T. evansi* pyrimidine pathways, which can be targeted in drug discovery process (Kandeel and Al-Taher 2020a, b, c; Kandeel *et al.*, 2020a; Kandeel *et al.,* 2020b).

The sequence of the camel genome was recently published (Jirimutu *et al.,* 2012). This work was aiming to get benefit from the known camel genome sequences to gain insights into the secrets of camel life, metabolic aspects and drug discovery studies against camel pathogens. In this study, the pyrimidine metabolic pathways were investigated in both camels and the blood protozoan, *T. evansi*. During this investigation, the enzymes metabolizing deoxyuridine 5'- triphosphate (dUTP) were evaluated and compared. *d*UTP metabolism was traced by the KEGG maps (Kanehisa *et al*., 2017).

In this study, the enzymes involved in dUTP metabolic pathways in camels and *T. evansi* were investigated. Comparisons were made between dromedary camel and human, wild camel, and eukaryotic UTP metabolizing enzymes. The comparisons include homology rate, conserved domain composition, functional motifs and signatures, phylogenetic relationships and genetic composition.

In this work, we show the suitability of dUTPase to be a new drug target against *T. evansi*. Bioinformatics studies revealed a low homology rate (15.28%), different domain content and significant structural and functional differences between the camel and *T. evansi* dUTPase.

## MATERIALS AND METHODS

**Retrieval of genomic data:** The collection of genomic data was carried out by extracting the information from the Kinetoplastom genome resources, gene database (http://www.genedb.org) and the genes, genomes and protein databases at NCBI. The retrieved data comprises protein sequences, signal peptides and cell membrane domains, annotations, homologs, metabolic processes shared by the protein, expression schemes, domains families and motifs.

**Searching homologues** Protein sequence homologues was searched against the non-redundant (nr) database using the NCBI Basic Local Alignment Search Tool (BLAST).

**Multiple sequence alignment for proteins and construction of phylogenetic tree** was carried out using the tools available at the European Bioinformatics Institute (Sievers and Higgins 2014). Clustal omega was used to calculate the best match of the selected sequences. The resultant alignment was used to generate a phylogenic tree, which is visualized by CLC genomics workbench (Qiagen, Denmark).

**Putative domains were searched** requests for domain content were submitted to either to the NCBI domain search tool, the motif search tool at the Kyoto Encyclopedia of Genes and Genomes (KEGG), or ExPASy tools (http://prosite.expasy.org/).

**Proteomic and genomic tools** ExPASy Proteomics tools and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al.,* 2007) was also used for the analysis of nucleotide and gene sequences. Protein sequences of target genes was analyzed for data such as pI, extinction coefficient and MW for the tagged protein sequence by PROTParam.

**Secondary structure prediction, protein modeling and model analysis** Requests for protein structure homology modeling was either submitted to the SWISS-MODEL server or to Protein Homology Analogy Recognition Engine (PHYRE). The quality of the produced models was checked with VADAR (Volume, Area, Dihedral Angle Reporter). The compatibility of an atomic model (3D) with its own amino acid sequence (1D) was verified by using Verify3D program. The MOLprobity server was used to analyze model geometry, including rotamer outliers and residues of bad bonds and angles.

## RESULTS

**Deoxyuridine-5'-diphosphate pathways:** dUTP can be synthesized from uridine-5'-diphosphate (UDP) and deoxycytidine-5'-triphosphate (CTP) by the actions of nucleoside-diphosphate kinase, deoxycytidine triphosphate (dCTP) deaminase, respectively. In the catabolic pathway, the phosphorylation of dUTP by thymidine triphosphatase or by nucleoside-diphosphate kinase can yield dUDP. Besides, pyrophosphates can be removed from dUTP by the action of deoxyuridine diphosphatase or dUTPase to produce deoxyuridine-5'-monophosphate (dUMP) (Fig. 1 and Table 1).

After bioinformatics investigations as described in materials and methods, two enzymes were predicted to be encoded in camel and *T. evansi* genomes, nucleoside diphosphate kinase and dUTPase. Summary of results of dUTP metabolizing enzymes is shown in Tables 1 and 2. In Table 1, the dUTP metabolizing enzymes are listed. In Table 2, the proposed enzymes encoded in camel and *T. evansi* genomes are listed. Graphical representation of dUTP metabolizing enzymes is shown in Fig. 1 and the proposed enzymes encoded in camel and *T. evansi* genomes are shown in Fig. 2.

**Comparison of camel and *T. evansi* with eukaryotic dUTPases:** Camel and human dUTPase shares high identity of 88.1% with 20 amino acid differences (Fig. 3). Besides, all three camels. Dromedary, Bactrian and feral camels, dUTPases showed zero differences or 100% identities (Fig. 4). Multiple alignments and sequence comparison with a wide range of prokaryotic and eukaryotic organisms revealed interesting differences. Camel dUTPase showed 79.8 to 92.8% similarity with other domestic animals. In comparison with some bacterial and protozoal dUTPase (Fig. 5), the homology rate drops to 23-29%. The most significant difference in homology rate was with *T. evansi*, which was highly related to prokaryotic enzymes (Fig. 5).

**Table 1:** Enzymes involved in metabolic pathways of deoxythymidine 5'-diphosphate

| ID (E.C. number) | Definition (Enzyme name) |
|---|---|
| 2.7.4.6 | Nucleoside diphosphate kinase |
| 3.6.1.39 | thymidine-triphosphatase<br>thymidine triphosphate nucleotidohydrolase |
| 3.5.4.13 | dCTP deaminase; deoxycytidine triphosphate deaminase |
| 1.17.4.2 | ribonucleoside-triphosphate reductase |
| 3.6.1.23 | dUTP diphosphatase; deoxyuridine-triphosphatase<br>dUTPase |

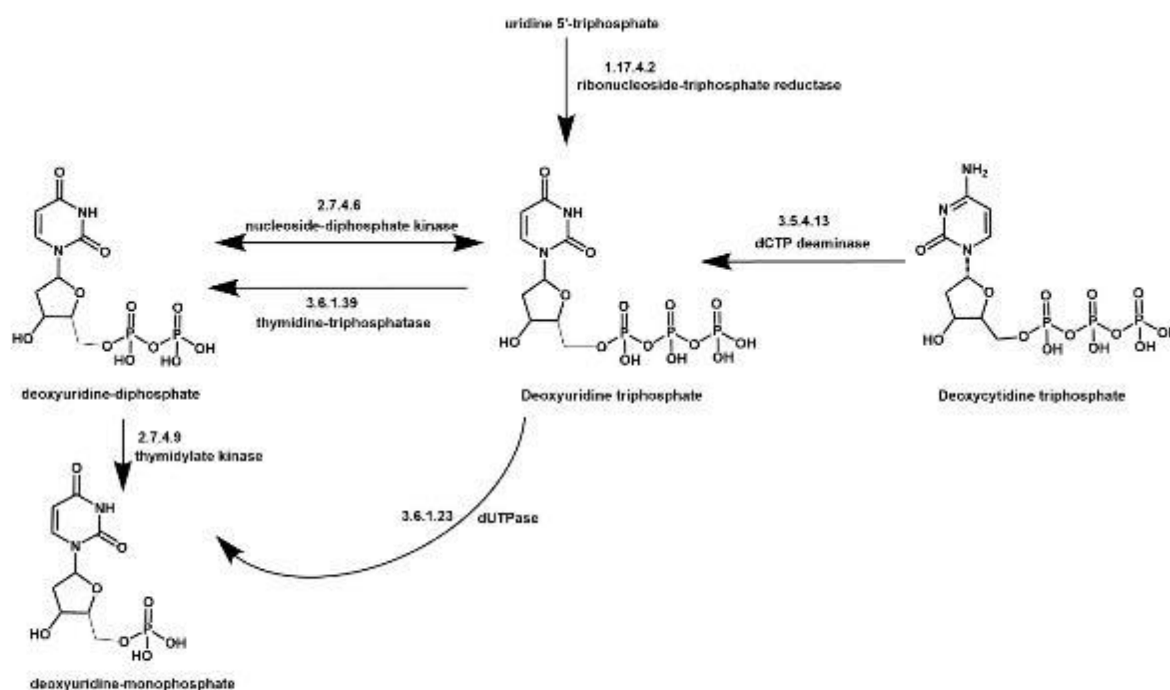


**Fig. 1:** The proposed metabolic pathways of deoxyuridine 5'-diphosphate.

**Table 2:** The expected enzymes involved in metabolic pathways of deoxyuridine 5'-diphosphate in camels and *T. evansi*

| ID (E.C. number) | Definition (Enzyme name) | Comment |
|---|---|---|
| 2.7.4.6 | Nucleoside diphosphate kinase | in camel and trypanosoma |
| 3.6.1.23 | dUTP diphosphatase; deoxyuridine-triphosphatase dUTPase | Significant structure difference. Dimeric enzyme in Trypanosoma and trimeric enzyme in camels. |

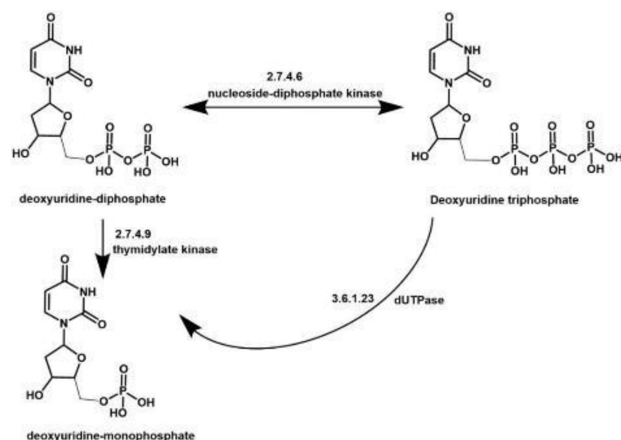


**Fig. 2:** The proposed metabolic pathways of deoxyuridine 5'-diphosphate in camels and *T. evansi*.

**Table 3:** Brief comparison between the *T. evansi* and camel dUTPases

| | Trypanosoma | Camel |
|---|---|---|
| Oligomeric status | Dimeric | trimeric |
| Amino acids length | 290 aminoacid | 165 aminoacid |
| Molecular weight (kD, monomer) | 32 | 18 |
| Protein domains | NTP-PPAse and dUTPase2 | Trimeric dUTPase |
| Multidomain available? | Yes | No |
| Difference in catalytic mechanism | Yes | |
| Design of specific inhibitor | Possible | |

**Comparison of camel and *T. evansi* dUTPases:** Pairwise comparison of camel and *T. evansi* dUTPase revealed a low homology rate of 15.28% with 255 differences (Fig. 6). The camel protein was 168 amino acids, while the protozoal enzyme was 287 amino acids in length.

**Phylogenetic comparisons:** Phylogenetic analysis (Fig. 7) showed that *T. evansi* is highly related to bacterial dUTPase. This was highly distant from the camel enzyme, which was highly developed eukaryotic enzyme.

**Motif and domain analysis:** Motif and domain analysis of camel and *T. evansi* dUTPase is provided in Fig. 8. A single domain constituent was predicted in camel comprising trimeric dUTPase superfamily, while in *T. evansi* multiple domains were predicted including the dimeric dUTPase and a general nucleotide triphosphatase superfamily. This implies that a strict single function was expected for the camel dUTPase including phosphorylation of dUTP. In contrast, the *T. evansi* dUTPase bears unique composition of multiple domains including the classical dUTPase and the potential of binding of all nucleotide triphosphates.

**Comparative structure models:** The differences between the camel and *T. evansi* dUTPase is summarized in Table 3. The camel dUTPase is a trimeric enzyme of 165 amino acids, mw of 18 kDa and form trimers without multiple domain constituents. In comparison, *T. evansi* dUTPase is a dimeric enzyme with a higher mw of 32 kDa and bearing multiple domain content.

## DISCUSSION

Deoxyutpase catalyzes the Mg2+-dependent hydrolysis (dephosphorization) of deoxyuridine-triphosphatase (dUTP) generating deoxyuridine-diphosphate (dUMP), supplying the substrate for EC 2.1.1.45 (thymidylate synthase) and provoking the formation of thymidine

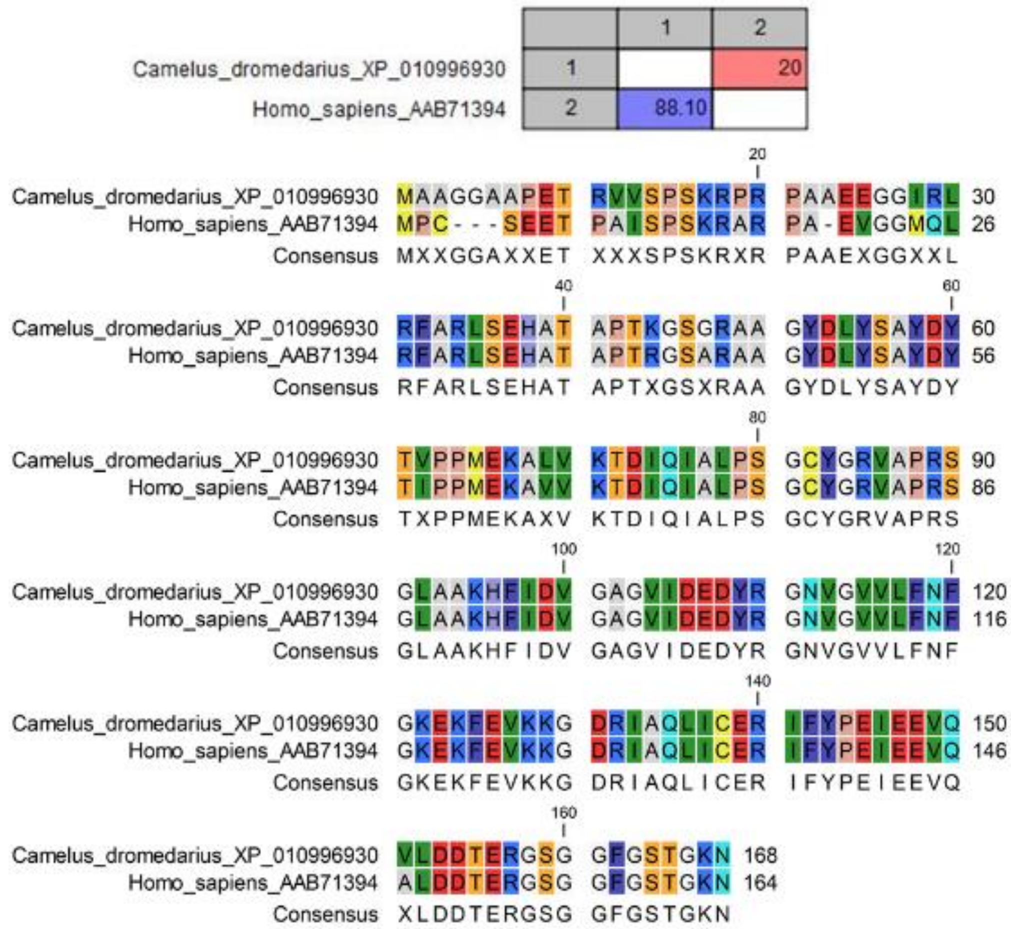


Fig. 3: Multiple sequence alignment of dromedary camel and human dUTPase. The upper panel represents sequence comparison statistics. The upper right diagonal region explains the number of differences between two sequences, while the lower left diagonal region explains the % of identity between two sequences.

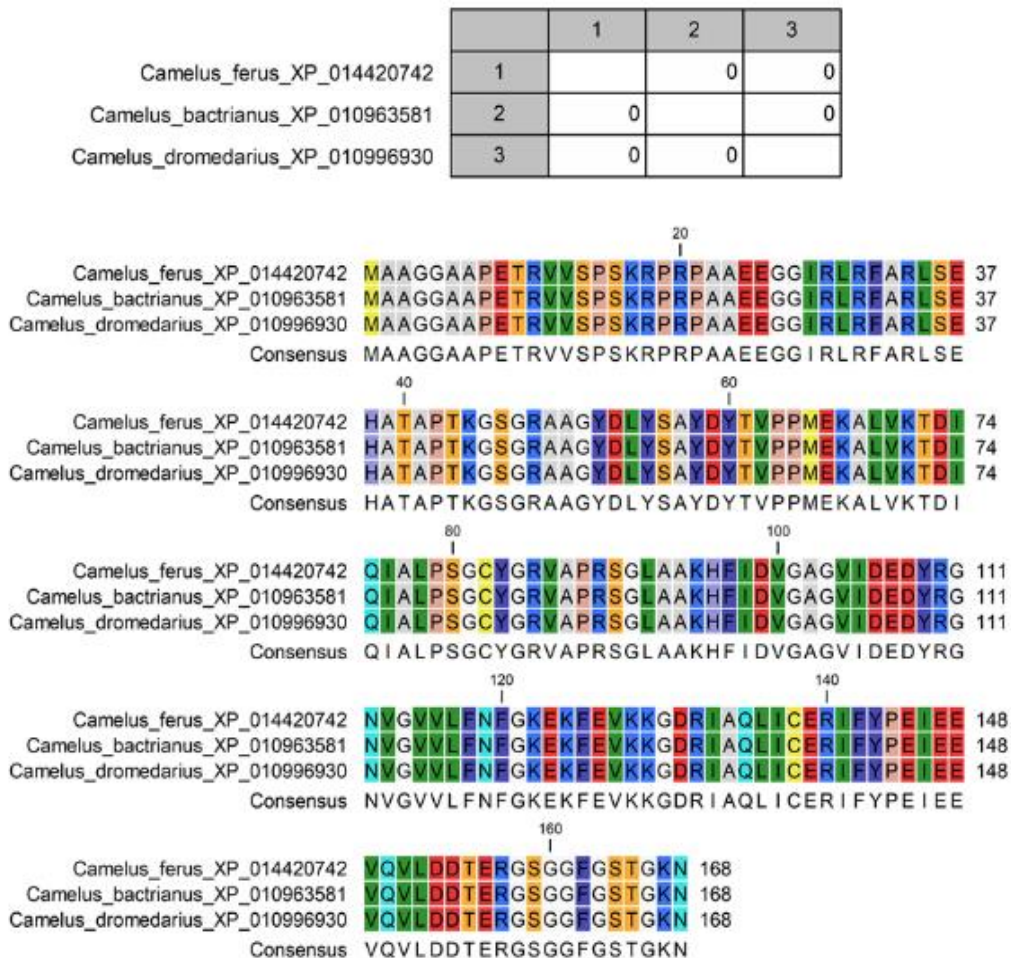


Fig. 4: Multiple sequence alignment of dromedary, feral and Bactrian camels dUTPase. The upper panel represents sequence comparison statistics. The upper right diagonal region explains the number of differences between two sequences, while the lower left diagonal region explains the % of identity between two sequences.

**Fig. 5:** Multiple sequence alignment of dromedary camel and other eukaryotes dUTPase. The upper panel represents sequence comparison statistics. The upper right diagonal region explains the number of differences between two sequences, while the lower left diagonal region explains the % of identity between two sequences.

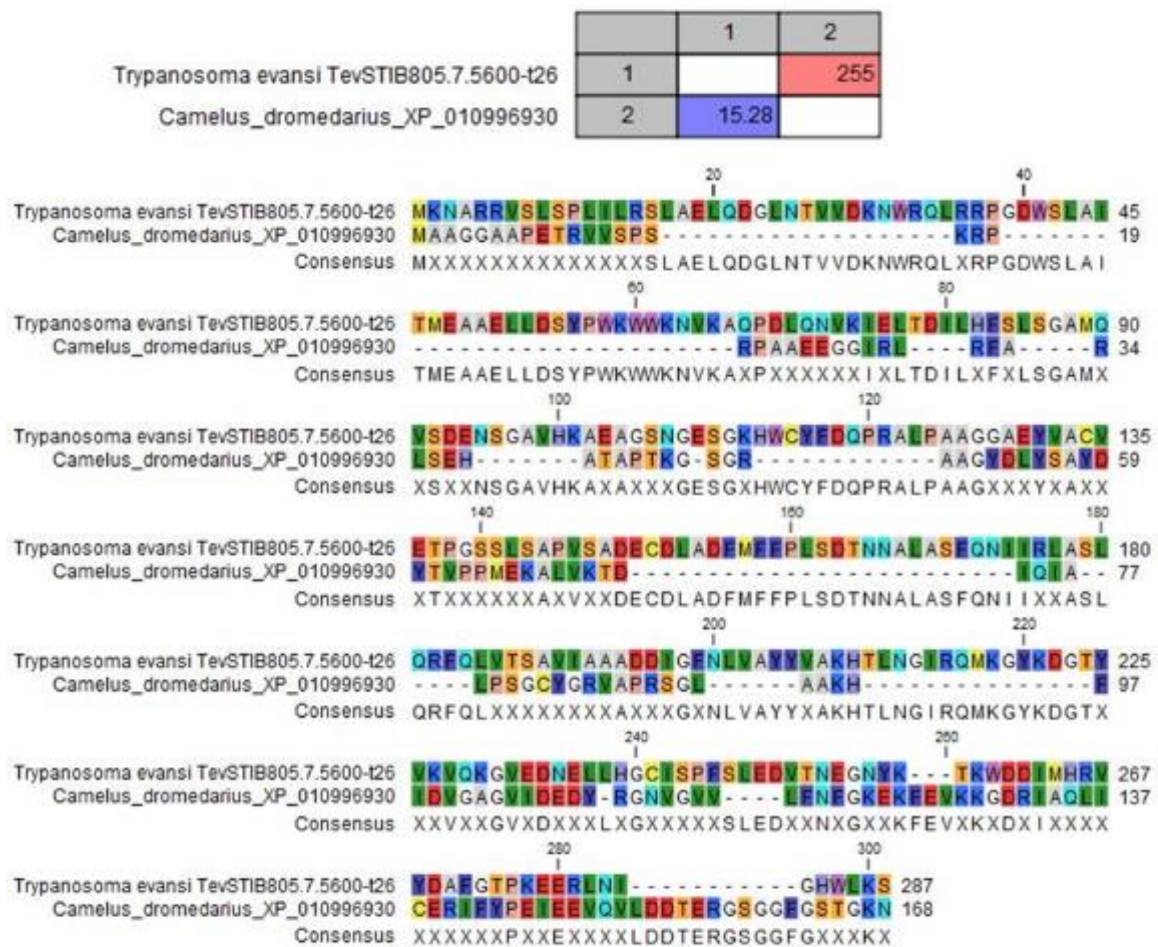


**Fig. 6:** Multiple sequence alignment of camel and *T. evansi* dUTPase. The upper panel represents sequence comparison statistics. The upper right diagonal region explains the number of differences between two sequences, while the lower left diagonal region explains the % of identity between two sequences.

nucleotides. Interestingly, *T. brucei* lacking dUTPase was severely sensitive to thymidine deprivation and their growth was severely inhibited (Castillo-Acosta *et al.,* 2013). This highlights the critical rule of dUTPase in trypanosomes life.

The dimeric dUTPase was found in bacteria and some protozoa e.g. *Leishmania major* (Hidalgo-Zarco *et al.,* 2001), *Campylobacter jejuni* (Musso-Buendia *et al.,* 2009). Additionally, *Trypanosoma cruzi* dUTPase showed a new structure fold giving rise to the important new family of dUTPase (Harkiolaki *et al.,* 2004).

dUTPase is essential for maintaining the chromosome integrity by hydrolysis of dUTP, thus keeping the chromosome from mutations. Most dUTPases are trimeric composed of 3 subunits and hydrolyses dUTP to give dUMP. The dimeric nature of the *T. evansi* enzyme implies differences in structure and function. The camel dUTPase is predicted to form trimers (Fig. 9) on the right: each monomer subunit is given in a different colour, orange, green and blue). In contrast, the Trypanosoma dUTPase (on the left) is expected to form dimers (one given in surface green and the other monomer is represented as helices).

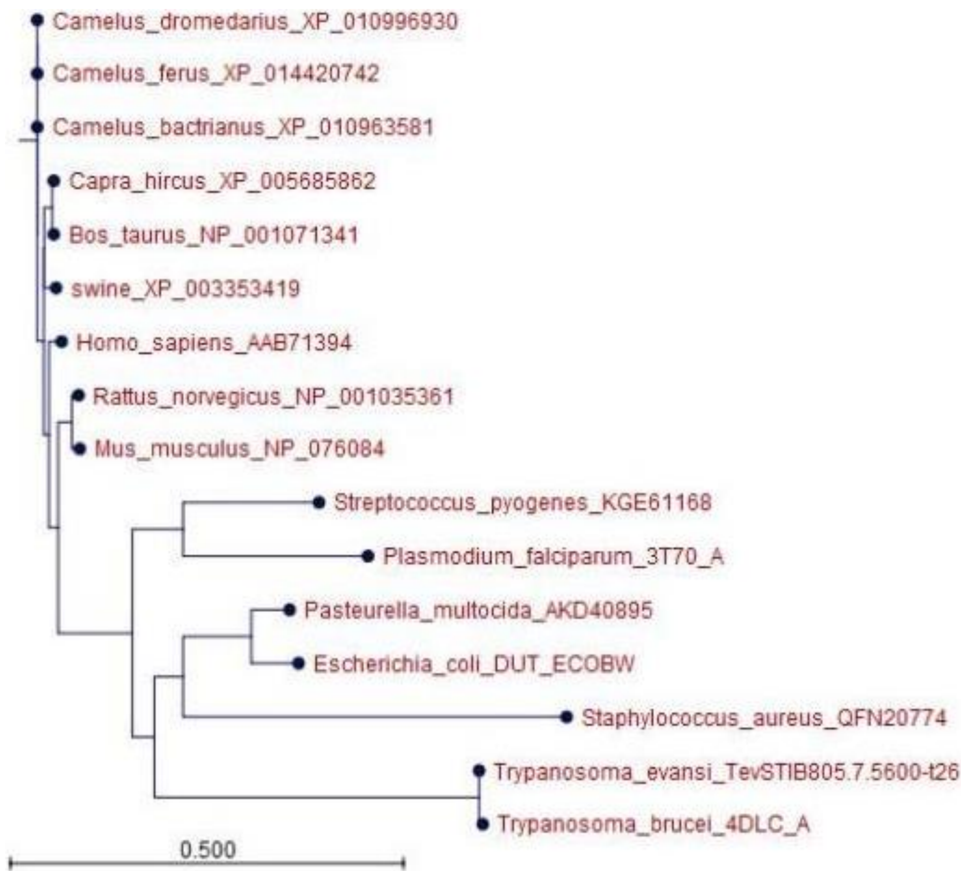


**Fig. 7:** Phylogram of camel and Trypanosoma dUTPase in relation to a set of prokaryotic and eukaryotic organisms.

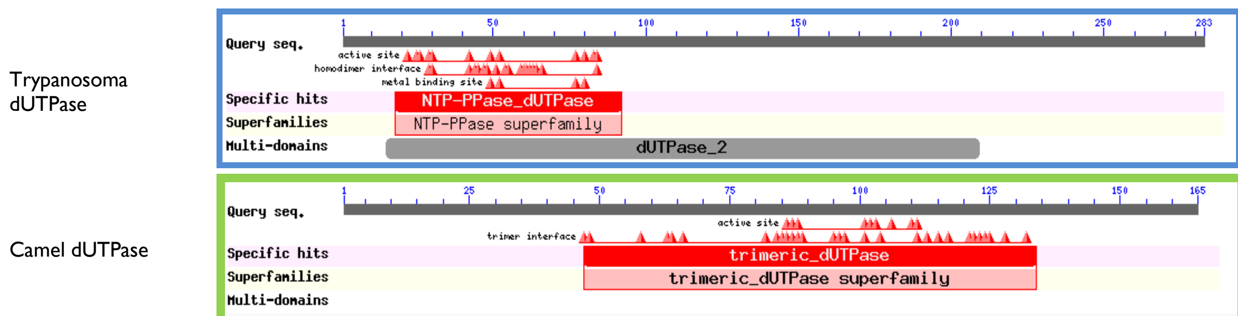


**Fig. 8:** Motif and domain search of *Trypanosoma evansi* and camel dUTPase using NCBI domain search tool.

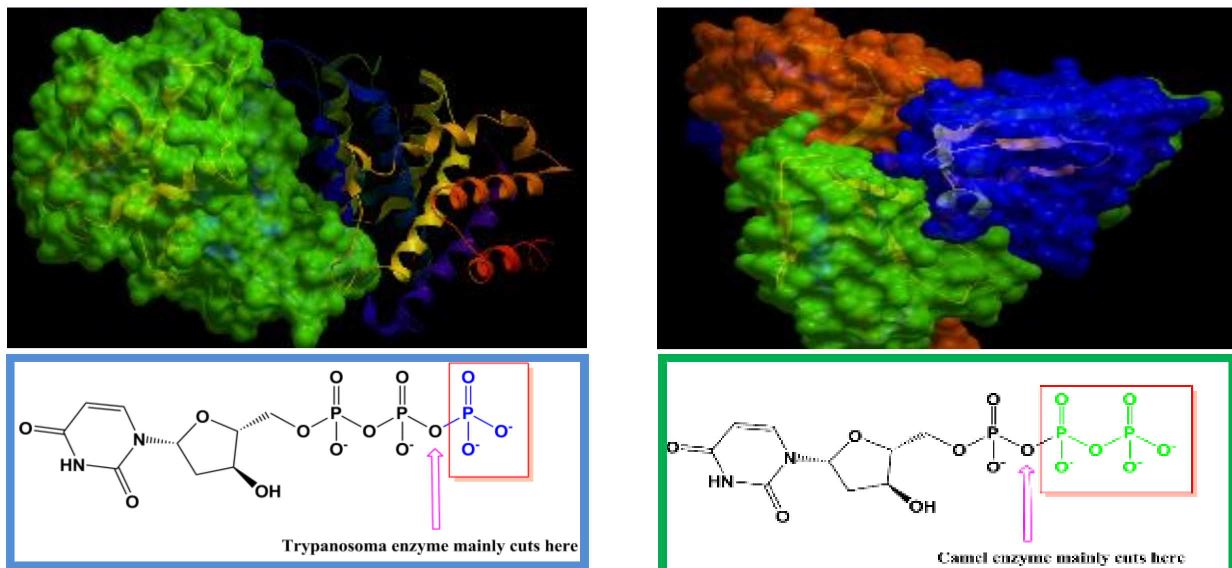


**Fig. 9:** Molecular models of dUTpase. On the right: trimeric camel enzyme. Each monomer subunit is given in a different colour, orange, green and blue). In contrast, the Trypanosoma dUTPase (on the left) is expected to form dimers.

The camel dUTPase is predicted to be trimeric enzyme without multifunction domains. Thus, it is predicted that the camel enzyme will be highly specific, can bind with dUTP only and has pyrophosphorylase activity to give dUMP. The trypanosome enzyme is predicted to be a multifunctional enzyme containing interesting domain substituents (Fig. 9). The dUTPase_2 domain subfamily is binding with dUTP producing one cut at the beta phosphate yielding dUDP, which has a strict need for metal ion for proper functioning. In addition to the dUTPase domain, Trypanosoma dUTPase contains a proposed multifunctioning NTPase activity, in which nucleotides other than dUTP are probably binding with its active site. More importantly, is that cytidine nucleotides are predicted to bind with the parasite enzyme with high probability.

Based on the modeled structure, Trypanosoma dUTPase attacks dUTP yielding one cut of the terminal phosphate then proceed to yield dUMP in a second cut attack (Fig. 9). In contrast, the camel enzyme is proposed to cut the last terminal phosphates generating dUMP in one reactive event. These differences can be targeted to develop specific inhibitors against the parasite enzyme.

**Conclusions:** During the investigation of pyrimidine metabolic pathways in camel and *T. evansi*. dUTPase was raised as a promising drug target. There was low sequence homology, different molecular composition, variable domains content and low sequence homology. dUTPase is an essential source for dUMP, which is used in the salvage pathway of thymidylate synthesis. Given the absence of deoxycytidine deaminase in Trypanosoma, inhibition of dUTPase will be profoundly influencing the parasite life.

**Funding:** The authors acknowledge the financial support of this project by King Abdul-Aziz City for Science and Technology (KACST), Basic Research Programs, National Transformation Program, under Research and Development Grants Program for National Research Institutions and Centers (GPURC), Kingdom of Saudi Arabia (Grant No. 2-17-04-004-0001).

**Acknowledgements:** The authors acknowledge the financial support of this project by King Abdul-Aziz City for Science and Technology (KACST), Basic Research Programs, National Transformation Program, under Research and Development Grants Program for National Research Institutions and Centers (GPURC), Kingdom of Saudi Arabia (Grant No. 2-17-04-004-0001).

**Authors contribution:** MK and AA conceived and designed the study. MK and AA executed the experiment and analyzed the results. MK and AA analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

## REFERENCES


Castillo-Acosta VM, Aguilar-Pereyra F, Garcia-Caballero D, *et al.,*, 2013. Pyrimidine requirements in deoxyuridine triphosphate nucleotidohydrolase deficient Trypanosoma brucei mutants. Mol Biochem Parasitol 187:9-13.

Desquesnes M, Holzmuller P, Lai DH, *et al.,*, 2013. Trypanosoma evansi and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. BioMed Res Inter 2013:194176.

Fong I, 2017. New and emerging parasitic zoonoses. Emerging Zoonoses. Springer pp:211-39.

Harkiolaki M, Dodson EJ, Bernier-Villamor V, *et al.,*, 2004. The crystal structure of Trypanosoma cruzi dUTPase reveals a novel dUTP/dUDP binding fold. Structure 12:41-53.

Hidalgo-Zarco F, Camacho AG, Bernier-Villamor V, *et al.,*, 2001. Kinetic properties and inhibition of the dimeric dUTPase-dUDPase from Leishmania major. Protein Sci 10:1426-33.

Jirimutu, Wang Z, Ding G, *et al.,*, 2012. Genome sequences of wild and domestic bactrian camels. Nat Commun 3:1202.

Kandeel M and Al-Taher A, 2020a. Bioinformatics of uridine/ deoxyuridine paths in Trypanosoma evansi revealed targeting uridine phosphorylase and cytidine deaminase. J Camel Pract Res 27:111-9.

Kandeel M and Al-Taher A, 2020b. Metabolic drug targets of the cytosine metabolism pathways in the dromedary camel (Camelus dromedarius) and blood parasite Trypanosoma evansi. Trop Anim Health Prod:1-22.

Kandeel M and Al-Taher A, 2020c. Uridine 5'-monophosphate (UMP) metabolising enzymes uracil phosphoribosyltransferase and orotidine-5'-phosphate decarboxylase/ump synthase in camels and Trypanosoma evansi. J Camel Pract Res 27:81-8.

Kandeel M, Dalab A, Al-Shabebi A, *et al.,*, 2020a. Bioinformatics of pyrimidine metabolism in camels and trypanosoma evansi: uridine 5'-diphosphate (UDP) metabolic pathways and targeting atp diphosphatase. J Camel Pract Res:1.

Kandeel M, Dalab A, Al-Shabebi A, *et al.,*, 2020b. Uridine 5-triphosphate (UTP) metabolising enzymes nucleoside diphosphate kinase and cytidine triphosphate (CTP) synthase in camels and Trypanosoma evansi. J Camel Pract Res 27:61-8.

Kanehisa M, Furumichi M, Tanabe M, *et al.,*, 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 45:D353-61.

Labarga A, Valentin F, Anderson M, *et al.,*, 2007. Web services at the European bioinformatics institute. Nucleic Acids Res 35:W6-W11.

Liao D and Shen J, 2010. Studies of quinapyramine-resistance of Trypanosoma brucei evansi in China. Acta Trop 116:173-7.

Munday JC, Eze AA, Baker N, *et al.,*, 2013. Trypanosoma brucei aquaglyceroporin 2 is a high-affinity transporter for pentamidine and melaminophenyl arsenic drugs and the main genetic determinant of resistance to these drugs. The Journal of antimicrobial chemotherapy.

Musso-Buendia JA, Vidal AE, Kasinthan G, *et al.,*, 2009. Kinetic properties and inhibition of the dimeric dUTPase-dUDPase from Campylobacter jejuni. J Enzyme Inhib Med Chem 24:111-6.

Ragab SA and ElSanousi EM, 2017. Camel Trypanosomosis, A potential Emerging Zoonosis. Conference http://repository.sustech.edu/handle/123456789/19527.

Shegokar VR, Powar RM, Joshi PP, *et al.,* 2006. Short report: Human trypanosomiasis caused by Trypanosoma evansi in a village in India: preliminary serologic survey of the local population. Am J Trop Med Hyg 75:869-70.

Sievers F and Higgins DG, 2014. Clustal Omega, accurate alignment of very large numbers of sequences. Multiple sequence alignment methods. Springer pp:105-16.

Vanhollebeke B, Truc P, Poelvoorde P, *et al.,*, 2006. Human Trypanosoma evansi infection linked to a lack of apolipoprotein L-I. The New England Journal of Medicine 355:2752-6.

Wiedemar N, 2019. Suramin resistance in African Trypanosomes. University_of_Basel.

Witola WH, Tsuda A, Inoue N, *et al.,* 2005. Acquired resistance to berenil in a cloned isolate of Trypanosoma evansi is associated with upregulation of a novel gene, TeDR40. Parasitology 131:635-46.