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BIOINFORMATICS OF URIDINE/DEOXYURIDINE PATHS IN *Trypanosoma evansi* REVEALED TARGETING URIDINE PHOSPHORYLASE AND CYTIDINE DEAMINASE

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ABSTRACT

The differences between the host and parasite metabolic paths could be a hot spot for discovery of antiparasitic targets. In this work, the metabolic paths of uridine and deoxyuridine in camels and the blood protozoan *Trypanosoma evansi* (*T. evansi*) were investigated by bioinformatics tools. While a set of de novo and salvage enzymes of uridine were found in camels, *T. evansi* was lacking uridine kinase and the source of UMP comes through salvage of uridine conversion to uracil. Therefore, inhibition of uridine phosphorylase (UPase) or cytidine deaminase (CDa) will affect the downstream UMP, dUMP and thymidylate synthesis by lowering the levels of uracil and uridine inside the parasite, respectively. Given the presence of two UPases in camel, low sequence similarity between camel and *T. evansi* UPases and CDase, targeting these enzymes might be without deleterious effects on the host cells.

Key words: Camel, cytidine deaminase, deoxyuridine, pyrimidine, *Trypanosoma evansi*, uridine phosphorylase

The drug discovery process depends heavily on computational approaches, which help to edit the genome sequences. Sequences in a host and target parasite can be compared to find new parasite-specific pathways and proteins that are unique to parasite life. In the last few years, the research in camel was revolutionized by the decoding of camel genome sequence (Jirimutu *et al,* 2012). In this context, computational tools had been used in approval of drug targets and their validation (Kandeel *et al,* 2019a; Kandeel *et al,* 2019b).

In this article, the uridine and deoxyuridine metabolic pathways were compared in camels and the blood protozoan, *T. evansi*. The KEGG maps (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016) were used to explore the enzymes involved in uridine and deoxyuridine metabolism. Comparisons were made between dromedary camel and human, wild camel, and eukaryotic uridine metabolising enzymes.

In this work, by using bioinformatics tools, we show that uridine and deoxyuridine pathway is not well developed in *T. evansi* as in camels. The deficiency of uridine kinase renders this pathway interesting for further studies.

Materials and Methods

Retrieval of genomic data— Collection of genomic data was carried out by extracting the information from the gene database (http://www.genedb.org) (Hertz-Fowler *et al,* 2004), Kinetoplastom genome resources (http://tritrypdb.org/tritrypdb/) and protein and genome databases at (http://www.ncbi.nlm.nih.gov).

Searching homologues— Protein sequence homologues was searched using the NCBI BLAST (Basic Local Alignment Search Tool) (Madden, 2013) or PSI-BLAST (Position- Specific Iterated-BLAST) servers (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searching against the non redundant (nr) database, with filtering of low complexity regions.

Multiple sequence alignment program for proteins and construction of phylogenetic tree— It was carried out using the tools available at (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers and Higgins, 2014). Multiple sequence alignment program (ClustalW2) 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 Dendroscope phylogenic tree viewer

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(Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

Putative domains— It was searched by the domain prediction program available at (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer *et al,* 2005). The genomic and molecular information was obtained from Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/).

Proteomic and genomic tools— ExPASy Proteomics tools (http://us.expasy.org/tools/) (Gasteiger *et al,* 2003) and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al,* 2007) were used.

Results and Discussion

Uridine

Uridine production process was proposed by four routes 1) dephosphorylation of uridine 5′-monophosphate by the action of uridine 5′-nucleotidase 2) from uracil by the action of uridine phosphorylase 3) by deamination of cytidine by the action of cytidine deaminase 4) the 3′-nucleotidase uses 3′-UMP to yield uridine as shown in Fig 1. In the catabolic direction, uridine was used by uridine phosphorylase or uridine nucleotidase to give uracil (Fig 1). In the de novo pathway of pyrimidine synthesis, UMP was produced by the kinase activity of uridine kinase.

Uridine phosphorylase (EC 2.4.2.3; pyrimidine phosphorylase); UrdPase; UPH; UPase. It is a glycosyltransferase enzyme which participates in pyrimidine ribonucleosides catabolism and salvage pathways in which, it catalyses the reversible phosphorolysis of uridine to generate uracil and alpha-D-ribose 1-phosphate so that the nucleotide base may be recycled. In addition, mammalian UPase also receives 2′-deoxyuridine (Liu *et al,* 1998). Unfortunately, Information about de novo pyrimidine biosynthesis in parasitic protozoa is seriously restricted. Moreover, de novo pyrimidine biosynthesis vs. salvage differs from organism to organism and even from one growth stage to another. The first characterisation of UPase from *Trypanosoma brucei* viewed that it is a homodimeric (Larson *et al,* 2010). Additionally, *Trypanosoma cruzi* UPase reaction mechanism of is defined as steady state that revealed no rate-limiting step after formation of reaction products (Silva and Schramm, 2011). Furthermore, UPase has an expanded S(N)2 character transition state (Silva *et al,* 2012).

After bioinformatics investigations, both camels and *T. evansi* shared common features of the absence of 3′-nucleotidase and uridine nucleotidase. Uridine kinase and uridine 5′-nucleotidase were detected in camels but not in *T. evansi*. Therefore, cytidine deaminase and UPase are the only enzyme of uridine pathway that is present in both of camel and *T. evansi* (Fig 1-3 and Tables 1-3).

Table 1. Enzymes involved in metabolic pathways of uridine.

ID (E.C. number)	Definition (Enzyme name)
3.1.3.6	3′-Nucleotidase
3 2.4.2.3	Uridine phosphorylase
3.2.2.3	Uridine nucleosidase
3.5.4.5	Cytidine deaminase
3.1.3.5	Uridine 5′-nucleotidase
2.7.1.48	Uridine kinase

Table 2. The expected enzymes involved in metabolic pathways of uridine in camels.

ID (E.C. number)	Definition (Enzyme name)
3 2.4.2.3	Uridine phosphorylase
2.7.1.48	Uridine kinase
3.5.4.5	Cytidine deaminase

Table 3. The expected enzymes involved in metabolic pathways of uridine in *Trypanosoma evansi*.

ID (E.C. number)	Definition (Enzyme name)
3 2.4.2.3	Uridine phosphorylase
3.5.4.5	Cytidine deaminase

T. brucei was found to be devoid of uridine kinase activity (Hammond and Gutteridge, 1982). In this study, there was no route of converting uridine to UMP in *T. evansi* (Fig 3). In addition, the sole route of generating uridine was by deamination of cytidine. Therefore, the expected route of UMP biosynthesis is through the salvage of uridine to the direction of uracil by the action of uridine phosphorylase, which is then converted to UMP by the action of uracil phosphoribosyl transferase.

Uridine phosphorylase

Two uridine phosphorylases were found in camels, UPase 1 and UPase 2. BLAST search of these 2 enzymes against *T. evansi* database did not get any significant hits. A previous study of crystal structure of a putative protein in *T. brucei* revealed its content of uridine phosphorylase domain and activity. Despite sequence search and analysis revealed it is a nucleoside phosphorylase, the determined structure and biochemical activity revealed that

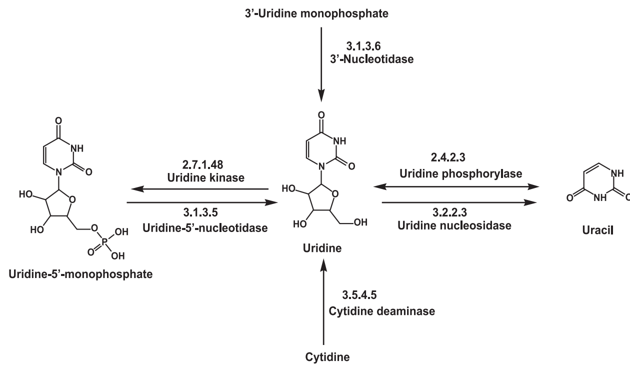


Fig 1. The proposed metabolic pathways of uridine.

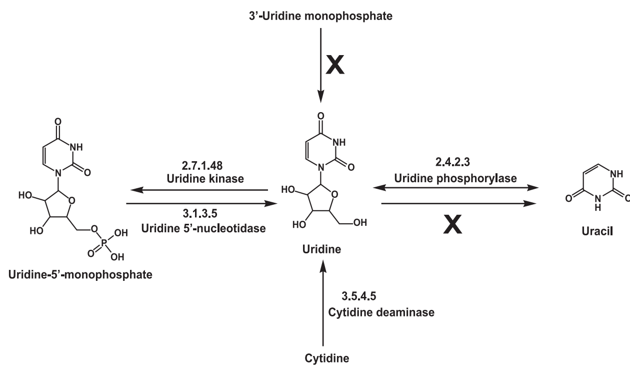


Fig 2. The proposed metabolic pathways of uridine in camel.

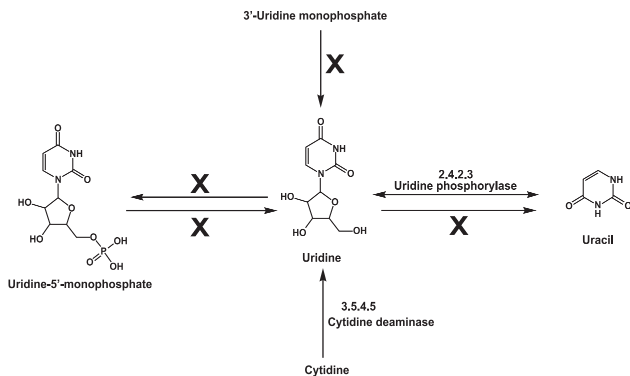


Fig 3. The proposed metabolic pathways of uridine *Trypanosoma evansi*.

it's a purine nucleosidase as it catalysed reaction involving uridine only and was nonspecific for other nucleosides (Larson *et al*, 2010). BLAST search of *T. evansi* database using the characterised enzyme from *T. brucei* retrieved a gene with 100% similarity to the *T. brucei* enzyme (Fig 4).

Comparison of human and camel uridine phosphorylases 1 and 2 is given in Fig 5. There was 49% similarity between camel uridine phosphorylases 1 and 2. While the similarity between human and camels was 65.1% for uridine phosphorylases 1 and 73.74% for uridine phosphorylases 2 and 78-100% similarity for uridine phosphorylases 1. This

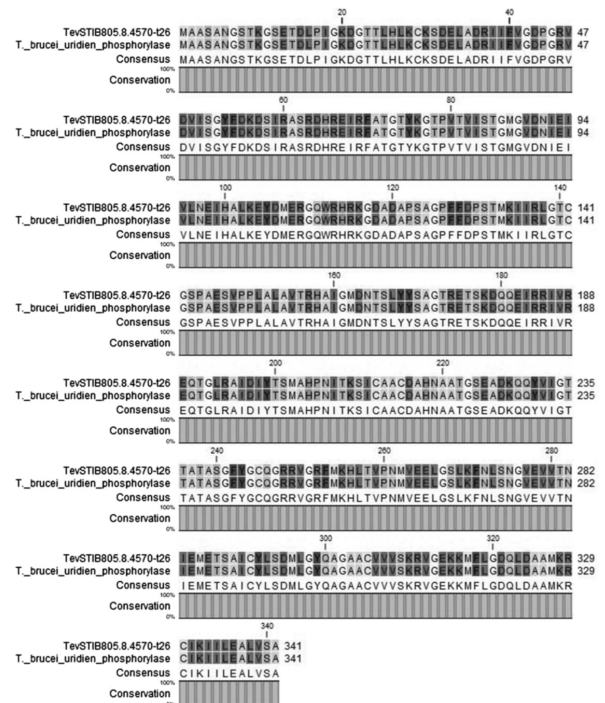
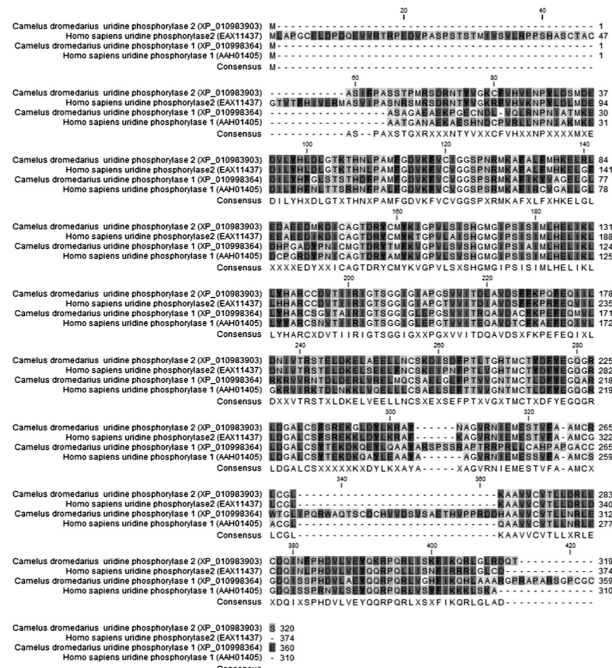


Fig 4. Pairwise sequence alignment of *T. evansi* and *T. brucei* uridine phosphorylase.



		1	2	3	4
Camelus dromedarius uridine phosphorylase 2 (XP_010983903)	1		99	186	125
Homo sapiens uridine phosphorylase2 (EAX11437)	2	73.74		246	183
Camelus dromedarius uridine phosphorylase 1 (XP_010998364)	3	49.32	41.98		126
Homo sapiens uridine phosphorylase 1 (AAH01405)	4	61.06	51.20	65.10	

Fig 5. Multiple sequence alignment of dromedary camel and human uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

		1	2	3	4	5	6
Camelus bactrianus uridine phosphorylase 2 (XP_010969642)	1		0	70	136	171	183
Camelus dromedarius uridine phosphorylase 2 (XP_010983903)	2	100.00		70	136	171	183
Camelus ferus uridine phosphorylase 2 (EQB77342)	3	78.12	78.12		163	198	209
Camelus bactrianus uridine phosphorylase1 (XP_010948509)	4	59.40	59.40	50.00		48	55
Camelus ferus uridine phosphorylase 1 (XP_006178697)	5	53.66	53.66	45.00	86.63		103
Camelus dromedarius uridine phosphorylase 1 (XP_010998364)	6	50.54	50.54	42.11	84.72	73.92	

Fig 6. Multiple sequence alignment of dromedary, Bactrian and feral camels uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Camelus bactrianus uridine phosphorylase1 (XP_010948509)	1		48	55	54	93	58	79	79	75	140	82	101	110	145	148	145	145	144	207	144	169	147	151	197	240	186	276	277	278	287	293	326	326
Camelus ferus uridine phosphorylase 1 (XP_006178697)	2	86.63		103	89	128	94	111	117	113	177	117	127	147	176	179	176	176	176	238	175	200	177	181	219	266	221	312	313	314	322	328	361	361
Camelus dromedarius uridine phosphorylase 1 (XP_010998364)	3	84.72	73.92		104	147	110	131	129	125	189	128	151	155	191	194	192	192	187	250	187	215	190	195	242	280	229	317	318	319	324	330	361	361
Bos taurus uridine phosphorylase 1 (NP_001092446)	4	83.38	75.21	71.10		54	44	65	65	63	123	61	93	93	130	132	131	131	124	187	127	154	127	133	181	230	177	264	264	265	278	277	308	308
Capra hircus uridine phosphorylase 1 (XP_017902936)	5	74.69	68.00	63.52	84.62		90	83	106	103	115	104	127	136	166	168	165	165	162	186	165	195	167	171	191	245	219	303	303	306	319	320	352	352
Sus scrofa uridine phosphorylase 1 (XP_020934449)	6	82.15	73.82	69.53	85.76	77.21		58	64	59	120	65	83	86	122	127	121	121	124	180	119	147	121	127	171	229	174	260	260	265	275	277	311	311
Equus caballus uridine phosphorylase 1 (XP_005609190)	7	76.83	70.16	65.25	80.00	75.92	82.15		75	72	120	74	102	101	133	138	130	130	134	186	131	163	132	136	180	228	192	277	277	280	291	293	328	328
Mus musculus uridine phosphorylase 1 (NP_001152974)	8	76.77	67.59	64.36	79.17	70.06	79.49	77.06		14	126	67	101	96	133	136	126	126	135	188	127	155	134	137	184	235	175	267	267	269	282	281	313	313
Rattus norvegicus uridine phosphorylase 1 (NP_001025196)	9	77.06	68.78	65.56	79.87	70.99	81.15	78.05	95.51		119	63	97	98	129	133	122	122	131	184	123	152	130	134	180	233	175	268	268	270	283	281	316	316
Oryctolagus cuniculus uridine phosphorylase 1 (XP_008245640)	10	64.19	58.45	55.74	67.37	69.50	68.17	68.42	66.49	68.44		126	165	152	187	191	185	185	186	177	181	217	187	192	186	246	239	329	329	329	341	344	372	372
Homo sapiens uridine phosphorylase 1 (AAH01405)	11	74.85	67.50	64.64	80.32	70.45	79.03	77.23	78.53	79.87	66.58		107	96	128	131	125	125	128	184	123	150	128	133	183	229	169	266	266	264	281	282	308	308
Felis catus uridine phosphorylase ISOFORM1 (XP_011278568)	12	69.67	64.62	59.08	70.66	64.43	73.82	69.09	68.44	69.78	57.03	66.35		124	140	142	140	140	140	200	139	134	142	148	185	239	196	244	244	247	269	262	314	314
Gallus gallus uridine phosphorylase 1 (XP_025003495)	13	66.67	59.73	57.53	70.57	61.80	72.78	69.30	69.52	68.89	59.79	69.62	61.61		124	129	125	125	119	183	120	152	119	123	172	222	182	264	265	271	278	282	311	311
Bos taurus uridine phosphorylase 2 (NP_001069511)	14	56.97	52.04	48.66	59.50	53.76	61.99	59.70	58.70	59.94	51.17	60.12	57.19	61.61		14	25	25	27	94	34	87	48	59	151	173	190	273	274	273	286	289	318	318
Capra hircus uridine phosphorylase 2 (AEJ84348)	15	56.08	51.23	47.85	58.88	53.20	60.44	58.18	57.76	58.70	50.13	59.19	56.57	60.06	95.62		31	31	34	104	41	94	56	67	157	176	192	272	273	273	286	288	318	318
Camelus bactrianus uridine phosphorylase 2 (XP_010969642)	16	56.97	52.04	48.39	59.19	54.04	62.31	60.61	60.87	62.11	51.70	61.06	57.19	61.30	92.19	90.31		0	32	99	35	70	54	63	145	175	190	272	273	273	285	290	320	320
Camelus dromedarius uridine phosphorylase 2 (XP_010983903)	17	56.97	52.04	48.39	59.19	54.04	62.31	60.61	60.87	62.11	51.70	61.06	57.19	61.30	92.19	90.31	100.00		32	99	35	70	54	63	145	175	190	272	273	273	285	290	320	320
Sus scrofa uridine phosphorylase 2 (XP_020932122)	18	57.14	52.04	49.60	61.25	54.49	61.25	59.27	57.94	59.19	51.31	60.00	56.79	63.04	91.56	89.38	90.00	90.00		99	38	93	54	64	148	174	191	270	271	271	284	288	318	318
Homo sapiens uridine phosphorylase2 (EAX11437)	19	47.33	43.87	41.59	50.40	50.92	52.25	51.31	50.26	51.32	53.66	51.19	47.51	51.46	75.07	72.41	73.74	73.74	73.53		86	159	99	107	148	182	245	323	324	327	339	345	374	374
Oryctolagus cuniculus uridine phosphorylase 2 (XP_008256826)	20	57.27	52.32	49.73	60.44	54.04	62.93	60.30	60.56	61.80	52.74	61.68	57.49	62.85	89.38	87.19	89.06	89.06	88.12	77.19		93	38	46	137	169	185	271	272	274	283	289	318	318
Camelus ferus uridine phosphorylase 2 (EQB77342)	21	48.79	44.44	41.10	50.96	45.22	53.18	50.30	51.10	52.20	43.04	52.38	54.27	52.20	72.81	70.62	78.12	78.12	70.94	57.82	70.94		108	109	187	204	210	238	239	237	256	255	305	305
Mus musculus uridine phosphorylase 2 (EDL26945)	22	56.38	51.77	48.92	60.44	53.48	62.31	60.00	58.39	59.63	51.17	60.12	56.57	63.16	85.00	82.50	83.12	83.12	83.12	73.74	88.12	66.25		23	140	178	190	272	273	272	283	287	321	321
Rattus norvegicus uridine phosphorylase 2 (NP_001099951)	23	55.19	50.68	47.58	58.57	52.37	60.44	58.79	57.45	58.39	49.87	58.57	54.74	61.92	81.56	79.06	80.31	80.31	80.00	71.62	85.62	65.72	92.81		148	187	197	273	274	275	286	291	324	324
Gallus gallus uridine phosphorylase 2 ISOFORM1(XP_015145676)	24	49.49	46.97	43.06	51.60	50.90	54.28	52.63	50.93	52.00	52.19	51.07	50.27	54.01	59.52	57.91	61.13	61.13	60.32	61.46	63.27	49.60	62.47	60.32		210	243	322	322	326	333	343	375	375
Equus caballus uridine phosphorylase2 (XP_023478732)	25	38.30	35.44	33.96	39.47	37.82	39.74	40.00	38.16	38.68	38.04	39.74	37.11	41.42	54.59	53.81	54.07	54.07	54.21	54.16	55.64	46.17	53.28	50.92	46.15		268	337	338	340	344	350	391	391
Ascaris suum uridine phosphorylase (ADY47114)	26	43.47	39.29	36.91	43.99	38.83	44.94	41.99	44.44	44.44	37.11	46.52	39.51	42.41	41.18	40.56	41.18	41.18	40.68	35.36	42.72	34.17	41.18	39.01	35.37	29.47		273	273	275	274	284	322	322
Escherichia coli uridine phosphorylase (QAY46840)	27	18.82	16.80	15.69	19.02	17.21	20.25	19.01	18.60	18.54	16.07	18.65	21.29	20.00	18.99	19.29	19.29	19.29	19.16	17.39	19.58	19.87	19.29	18.99	17.01	14.25	17.02		8	76	164	196	282	282
Salmonella enterica uridine phosphorylase (QBA02662)	28	18.53	16.53	15.43	18.77	16.99	20.00	18.77	18.35	18.29	15.86	18.40	21.04	19.45	18.45	18.75	18.75	18.75	18.62	16.92	19.05	19.26	18.75	18.45	16.80	13.99	16.77	96.85		74	166	194	283	283
Pasteurella multocida uridine phosphorylase (ATN17377)	29	18.24	16.27	15.16	18.46	16.16	18.46	17.89	17.74	17.68	15.86	19.02	19.54	17.63	18.75	18.75	18.75	18.75	18.62	16.15	18.45	19.39	19.05	18.15	15.76	13.49	16.41	70.20	70.87		175	191	284	284
Streptococcus pyogenes uridine phosphorylase (QAZ37618)	30	15.09	13.67	13.37	14.98	13.55	15.90	15.16	14.02	13.98	13.01	14.33	15.14	15.76	15.38	15.38	15.68	15.68	15.73	13.96	16.27	14.95	16.27	15.38	14.40	12.02	16.72	38.11	37.36	33.96		193	289	289
Plasmodium falciparum (IGH-CR14) uridine phosphorylase (KNG74330)	31	12.54	11.35	11.05	13.98	12.09	13.98	13.31	13.00	13.27	11.11	12.69	14.38	13.23	13.21	13.51	12.91	12.91	13.25	11.31	13.21	12.07	13.81	12.61	10.68	9.79	12.35	26.04	26.52	27.38	27.17		296	296
TevSTIB805.8.4570-t26	32	15.76	14.45	13.43	17.20	14.77	16.40	15.46	16.31	15.73	15.07	17.43	15.14	17.51	16.97	16.97	16.45	16.45	16.54	14.61	16.97	15.04	16.19	15.40	13.59	11.34	14.59	19.89	19.37	19.09	18.36	14.94		0
T._brucei_uridien_phosphorylase	33	15.76	14.45	13.43	17.20	14.77	16.40	15.46	16.31	15.73	15.07	17.43	15.14	17.51	16.97	16.97	16.45	16.45	16.54	14.61	16.97	15.04	16.19	15.40	13.59	11.34	14.59	19.89	19.37	19.09	18.36	14.94	100.00	

Fig 7. Dromedary camel, *T. evansi* and other prokaryotes and eukaryotes uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

was affected by the long sequence insertions in the dromedary camel sequence (Fig 6).

Multiple sequence alignment (Fig 7) and phylogenetic analysis (Fig 8) with a set of prokaryotes and eukaryotes uridine phosphorylases revealed close relation of the single bacterial and protozoal UPase with the eukaryotic UPase 1, which were distant from UPase 2. The similarity rate was 66-86% within UPase 1, which lowers to the range of 43-56% after comparison with uridine phosphorylase 2. The prokaryotic and Trypanosoma UPase showed very low similarity rates of 15.7-18.8% compared with the eukaryotic uridine phosphorylases.

Comparison between *T. evansi* and camel UPase showed that the trypanosomal enzyme shared 18.64 and 15.39% similarity with the camel UPase 2 and 1, respectively (Fig 9). The domain prediction tool at NCBI predicted that the retrieved sequence is similar to the confirm uridine phosphorylase from *T. brucei* (Fig 10).

Equus caballus uridine phosphorylase2 (XP_023478732)
Camelus ferus uridine phosphorylase 2 (EQB77342)
Camelus dromedarius uridine phosphorylase 2 (XP_010983903)
Camelus bactrianus uridine phosphorylase 2 (XP_010969642)
Sus scrofa uridine phosphorylase 2 (XP_020932122)
Bos taurus uridine phosphonlase 2 (NP_001069511)
Capra hircus uridine phosphorylase 2 (AEJ84348)
Oryctolagus cuniculus uridine phosphorylase 2 (XP_008256826)
Homo sapiens uridine phosphorylase2 (EAX11437)
Mus musculus uridine phosphorylase 2 (EDL26945)
Rattus norvegicus uridine phosphorylase 2 (NP_001099951)
Gallus gallus uridine phosphorylase 2 ISOFORM1(XP_015145676)
Gallus gallus uridine phosphorylase 1 (XP_025003495)
Felis catus uridine phosphorylase ISOFORM1 (XP_011278568)
Homo sapiens uridine phosphorylase 1 (AAH01405)
Sus scrofa uridine phosphorylase 1 (XP_020934449)
Equus caballus uridine phosphorylase 1 (XP_005609190)
Bos taurus uridine phosphorylase 1 (NP_001092446)
Capra hircus uridine phosphorylase 1 (XP_017902936)
Camelus ferus uridine phosphorylase 1 (XP_006178697)
Camelus bactrianus uridine phosphorylase1 (XP_010948509)
Camelus dromedarius uridine phosphorylase 1 (XP_010998364)
Rattus norvegicus uridine phosphorylase 1 (NP_001025196)
Mus musculusuridine phosphorylase 1 (NP_001152874)
Oryctolagus cuniculus uridine phosphorylase 1 (XP_008246640)
Ascaris suum uridine phosphorylase (ADY47114)
Escherichia coli uridine phosphorylase (QAY46840)
Salmonella enterica uridine phosphorylase (QBA02662)
Pasteurella multocida uridine phosphorylase (ATN17377)
Streptococcus pyogenes uridine phosphorylase (QAZ37618)
Plasmodium falciparum (IGH-CR14) uridine phosphorylase (KNG74330)
0.250
T._brucei_uridien_phosphorlase
TevSTIB805.8.4570-t26

Fig 8. Phylogram of camel and *Trypanosoma evansi* uridine phosphorylase in relation to a set of eukaryotic and prokaryotic organisms.

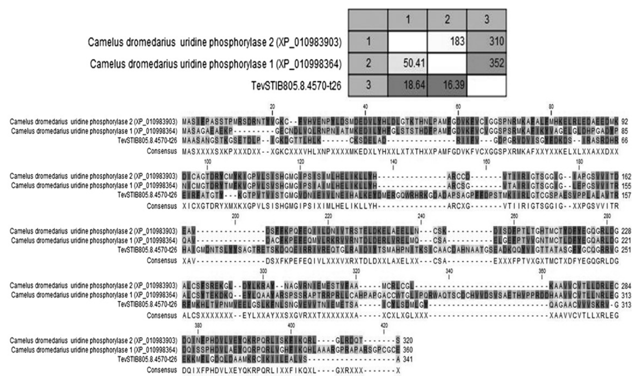


Fig 9. Pairwise sequence alignment of dromedary camel and *Trypanosoma evansi* uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Cytidine deaminase

Cytidine deaminase (EC 3.5.4.5, CDA). A hydrolase metalloenzyme (contains zinc), broadly disseminated amongst organisms, acting on carbon-nitrogen bonds, not peptide bonds. The enzyme implicated in salvage of both exogenous and endogenous cytidine and 2′ deoxycytidine for UMP synthesis. Likewise, it catalyses the deamination of cytidine and 2′-deoxycytidine with analogous effectiveness. Interestingly, the activity of the plant enzyme is almost very analogous to that of the human (Vincenzetti *et al,* 1999). *Crithidia fasciculata* (a mosquito parasite) and *Trypanosoma cruzi* (a human pathogen) have cytidine deaminase enzyme. The enzyme from *C. fasciculata* deaminated both cytidine and deoxycytidine, the affinity of CDA for cytidine being much lower than deoxycytidine. Meanwhile,

Fig 10. The expected uridine phosphorylase domain in *T. evansi*.

Fig 11. Pairwise sequence alignment of dromedary camel and *T. evansi* cytidine deaminase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Fig 12. Multiple sequence alignment of dromedary camel and human cytidine deaminase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

the *T. cruzi* enzyme has identical affinities for both substrates. Pyrimidine nucleosides as cytidine, uridine, 5-bromouridine, thymidine, and orotidine significantly triggers the production of the enzyme in *C. fasciculata*. Cytidine is the only nucleoside stimulated enzyme production in *T. cruzi* (Kidder, 1984).

It was previously reported that CDa is essential for the life of *T. brucei* (Moro-Bulnes *et al,* 2019). Additionally, CDa is the major source for uridine (Leija *et al,* 2016), which is important for dUMP and thymidylate synthesis. In *T. brucei*, CDa was found to be a tetrameric enzyme, which is similar to the

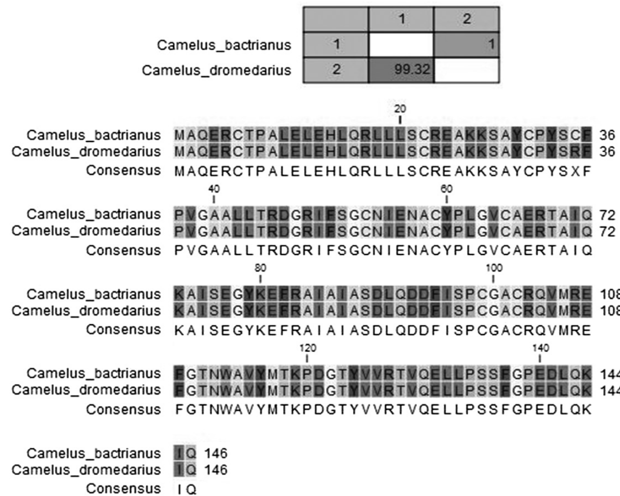


Fig 13. Multiple sequence alignment of dromedary and Bactrian camels cytidine deaminase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

eukaryotic and vertebrate CDa. In addition, CDa showed more important role in synthesis of dUMP and thymidylate synthesis than dUTPase (Moro-Bulnes *et al,* 2019).

Pairwise comparison of camel and Trypanosoma CDa showed that the protozoal enzyme is about 40 peptides longer than the camel enzyme with about 61% similarity (Fig 11). The camel and human CDa shared a high similarity rate, more than 82% Fig 12). Camel species showed very high similarity of 99.32% (Fig 13). Multiple sequence comparison and phylogenetic tree of camel and *T. evansi* cytidine deaminase in relation to a set of eukaryotic and prokaryotic organisms are provided in Fig 14 and 15. The *T. evansi* CDa was closely related to higher vertebrate CDas and distant from the prokaryotic and other protozoal enzymes. The prokaryotic and protozoal enzymes showed 5-13% similarity with the camel and higher vertebrates. While in some protozoa including *T. evansi* and helminths, the rate showed medium similarity values of 30-55%. In comparing the domain and motif content in camel and Trypanosoma, the tetrameric form was readily detectable with camel CDa (Fig 16) but not with *T. evansi*, which contained CDD cytidine deaminase superfamily nucleotide metabolism and transportation.

Deoxyuridine

Deoxyuridine production process is proposed by three routes (Fig 17) 1) dephosphorylation of

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
swine	1		11	12	12	10	11	22	19	25	88	96	130	130	130	270	270	280	288	163	175	190	169	210	177	324
Oryctolagus_cuniculus	2	92.47		16	16	14	15	25	22	26	87	95	129	129	129	270	270	279	288	164	175	191	169	209	178	323
Capra_hircus	3	91.78	89.04		0	13	14	23	20	28	91	95	131	131	131	267	267	278	284	164	175	191	169	209	178	324
Bos_taurus	4	91.78	89.04	100.00		13	14	23	20	28	91	95	131	131	131	267	267	278	284	164	175	191	169	209	178	324
Camelus_dromedarius	5	93.15	90.41	91.10	91.10		1	19	20	26	87	95	129	129	129	269	269	279	287	164	175	191	169	209	176	325
Camelus_bactrianus	6	92.47	89.73	90.41	90.41	99.32		19	20	26	88	95	129	129	129	269	269	279	287	164	175	191	169	209	176	325
Felis_catus	7	84.93	82.88	84.25	84.25	86.99	86.99		24	30	97	97	132	132	132	272	273	280	290	165	175	190	168	208	177	323
Equus_caballus	8	86.99	84.93	86.30	86.30	86.30	86.30	83.56		21	92	96	129	129	129	268	269	279	287	165	176	192	169	209	178	323
Homo_sapiens	9	82.88	82.19	80.82	80.82	82.19	82.19	79.45	85.62		91	95	130	130	130	269	270	279	289	163	176	192	169	209	177	324
Gallus_gallus	10	54.40	54.92	52.85	52.85	54.92	54.40	49.74	52.08	52.85		139	163	163	163	269	268	279	285	201	209	220	201	242	203	330
Ascaris_suum	11	37.25	37.91	37.91	37.91	37.91	37.91	36.60	37.25	37.91	30.50		131	131	131	268	264	277	285	159	169	185	160	201	177	334
T_brucei	12	30.48	31.02	29.95	29.95	31.02	31.02	29.41	31.02	30.48	27.23	29.57		0	0	287	284	291	295	183	190	203	181	214	191	340
T._evansi	13	30.48	31.02	29.95	29.95	31.02	31.02	29.41	31.02	30.48	27.23	29.57	100.00		0	287	284	291	295	183	190	203	181	214	191	340
Trypanosoma_brucei_brucei_TREU927	14	30.48	31.02	29.95	29.95	31.02	31.02	29.41	31.02	30.48	27.23	29.57	100.00	100.00		287	284	291	295	183	190	203	181	214	191	340
Salmonella_enterica	15	12.90	12.90	13.87	13.87	13.23	13.23	12.26	13.55	13.23	15.41	13.27	11.42	11.42	11.42		37	164	221	287	284	282	277	303	290	441
Escherichia_coli	16	12.90	12.90	13.87	13.87	13.23	13.23	11.94	13.23	12.90	15.72	14.56	12.35	12.35	12.35	87.41		164	223	290	286	285	278	303	292	445
Pasteurella_multocida	17	10.54	10.86	11.18	11.18	10.86	10.86	10.54	10.86	10.86	11.99	11.22	11.01	11.01	11.01	45.15	45.15		238	291	289	288	286	305	297	439
Entamoeba_histolytica	18	10.84	10.84	12.07	12.07	11.15	11.15	10.22	11.15	10.53	11.76	11.49	12.20	12.20	12.20	29.84	29.21	24.68		304	305	300	303	315	314	451
Clostridium_tetani	19	11.41	10.87	10.87	10.87	10.87	10.87	10.33	10.33	11.41	9.05	10.17	10.29	10.29	10.29	6.82	5.84	6.43	5.59		86	112	100	144	141	306
Staphylococcus_aureus	20	9.79	9.79	9.79	9.79	9.79	9.79	9.79	9.28	9.28	8.73	9.63	10.38	10.38	10.38	7.79	7.14	7.37	5.28	44.87		100	98	143	154	315
Streptococcus_pyogenes	21	9.09	8.61	8.61	8.61	8.61	8.61	9.09	8.13	8.13	8.33	8.42	8.97	8.97	8.97	8.44	7.47	7.99	6.83	34.50	41.52		113	144	159	321
Corynebacterium_pseudotuberculosis	22	10.11	10.11	10.11	10.11	10.11	10.11	10.64	10.11	10.11	9.46	11.60	11.27	11.27	11.27	10.06	9.74	8.04	5.90	34.64	39.51	34.68		148	149	310
Babesia_bovis_T2Bo	23	7.08	7.52	7.52	7.52	7.52	7.52	7.96	7.52	7.52	6.20	8.22	9.32	9.32	9.32	5.61	5.61	6.44	5.41	25.00	25.52	25.00	22.51		166	337
Mus_musculus	24	7.81	7.29	7.29	7.29	8.33	8.33	7.81	7.29	7.81	7.31	7.81	9.05	9.05	9.05	7.64	7.01	6.31	4.56	22.10	18.09	19.70	18.58	19.42		319
Plasmodium_falciparum_3D7	25	5.54	5.83	5.54	5.54	5.25	5.25	5.83	5.56	5.54	6.78	4.30	5.82	5.82	5.82	4.34	3.47	4.57	3.84	11.05	10.26	11.33	9.88	8.67	7.54	

Fig 14. Multiple sequence alignment camel and *Trypanosoma evansi* cytidine deaminase in relation to a set of eukaryotic and prokaryotic organisms. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two

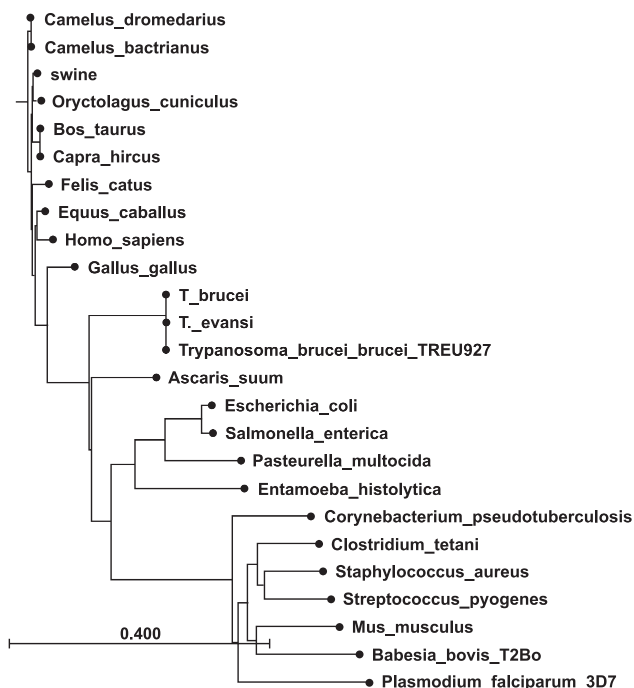


Fig 15. Phylogram of camel and *Trypanosoma evansi* cytidine deaminase in relation to a set of eukaryotic and prokaryotic organisms.

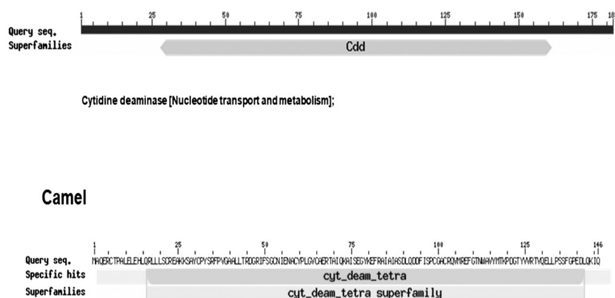


Fig 16. Motif and domain search of camel and *Trypanosoma evansi* cytidine deaminase.

uridine 5′-monophosphate by the action of uridine 5′-nucleotidase or 5′-deoxynucleotidase 2) from uracil by the action of uridine phosphorylase or pyrimidine nucleoside phosphorylase 3) by deamination of deoxycytidine by the action of cytidine deaminase. In the catabolic direction, deoxyuridine is used by uridine phosphorylase or nucleotide phosphorylases to give uracil (Fig 17). In the de novo pathway of pyrimidine synthesis, dUMP is produced from deoxyuridine by the kinase activity of thymidine kinase. Camels maps obeys the general described paths for deoxyuridine. In contrast, *T. evansi* showed similar profile with exception of the lack of dUMP degradation to give deoxyuridine (Fig 18, 19).

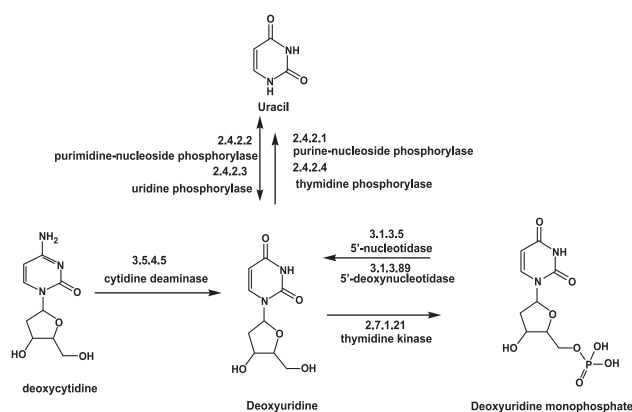


Fig 17. The proposed metabolic pathways of deoxyuridine (dU).

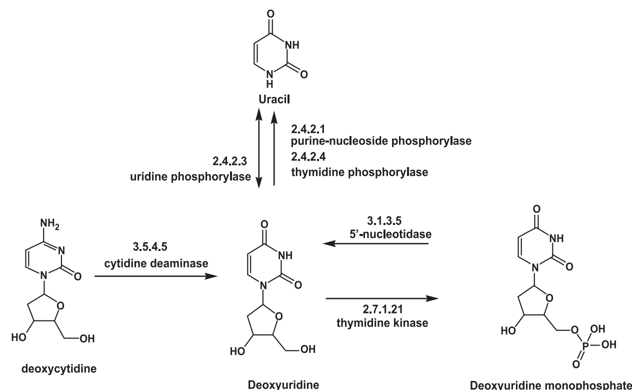


Fig 18. The proposed metabolic pathways of deoxyuridine (dU) in camels.

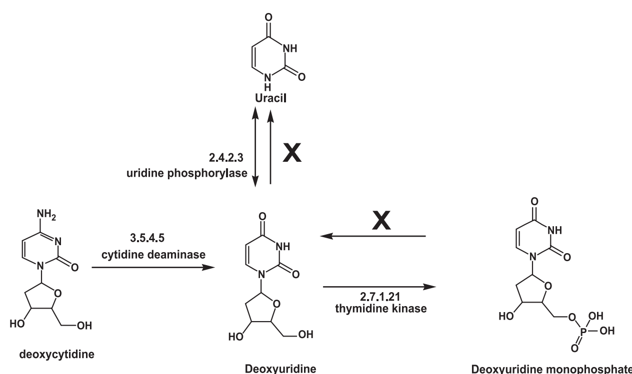


Fig 19. The proposed metabolic pathways of deoxyuridine (dU) in *Trypanosoma evansi*.

Conclusions

The uridine metabolic pathway in *T. evansi* is an important target in *T. evansi*. The lack of uridine kinase in *T. evansi* had led to dependence on conversion of uridine to uracil to help in getting UMP by the salvage of uracil by uracil phosphoribosyl transferase. The obtained results from bioinformatics investigations suggests targeting UPase and CDa as drug targets by affecting uridine and uracil paths.

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