

Molecular Analysis of *Trypanosoma cruzi* Isolates Obtained from Raccoons in South Central Kentucky

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Trypanosoma cruzi

- Protozoan parasite and etiologic agent of Chagas' Disease.
- Sylvatic and domestic mammals serve as vertebrate hosts, blood-sucking bugs in the family Reduviidae serve as vectors.



Blood-form Trypomastigote



Triatoma sanguisuga

Transmission of *T. cruzi*

- Triatomine vectors
- Congenital transmission
- Transfusions, organ/tissue donations
- Laboratory Accidents
- Foodborne



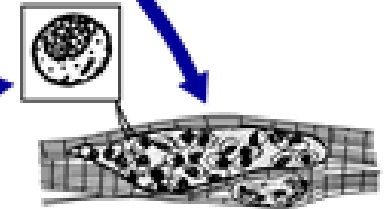
Triatomine Bug Stages

- 1** Triatomine bug takes a blood meal (passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)



Human Stages

- 2** Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.



- 3** Amastigotes multiply by binary fission in cells of infected tissues.

Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.

- 4** Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.

i = Infective Stage
d = Diagnostic Stage



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Chagas' Disease

- Causes a greater disease burden than any other parasitic disease in the Western Hemisphere (WHO, 2004).
- Estimated 8-10 million people infected in endemic Latin America (Bern and Montgomery, 2009).
- Historically, human disease was limited primarily to rural Latin America, however, migration has taken infected individuals to urban areas in Latin America as well as into the United States, Europe, and Japan.
- In the southern U.S.A., enzootic transmission of *T. cruzi* occurs involving several triatomine species and a variety of mammalian hosts including raccoons, opossums, and domestic dogs (Bern and Montgomery, 2009).

Chagas' Disease in the U.S.

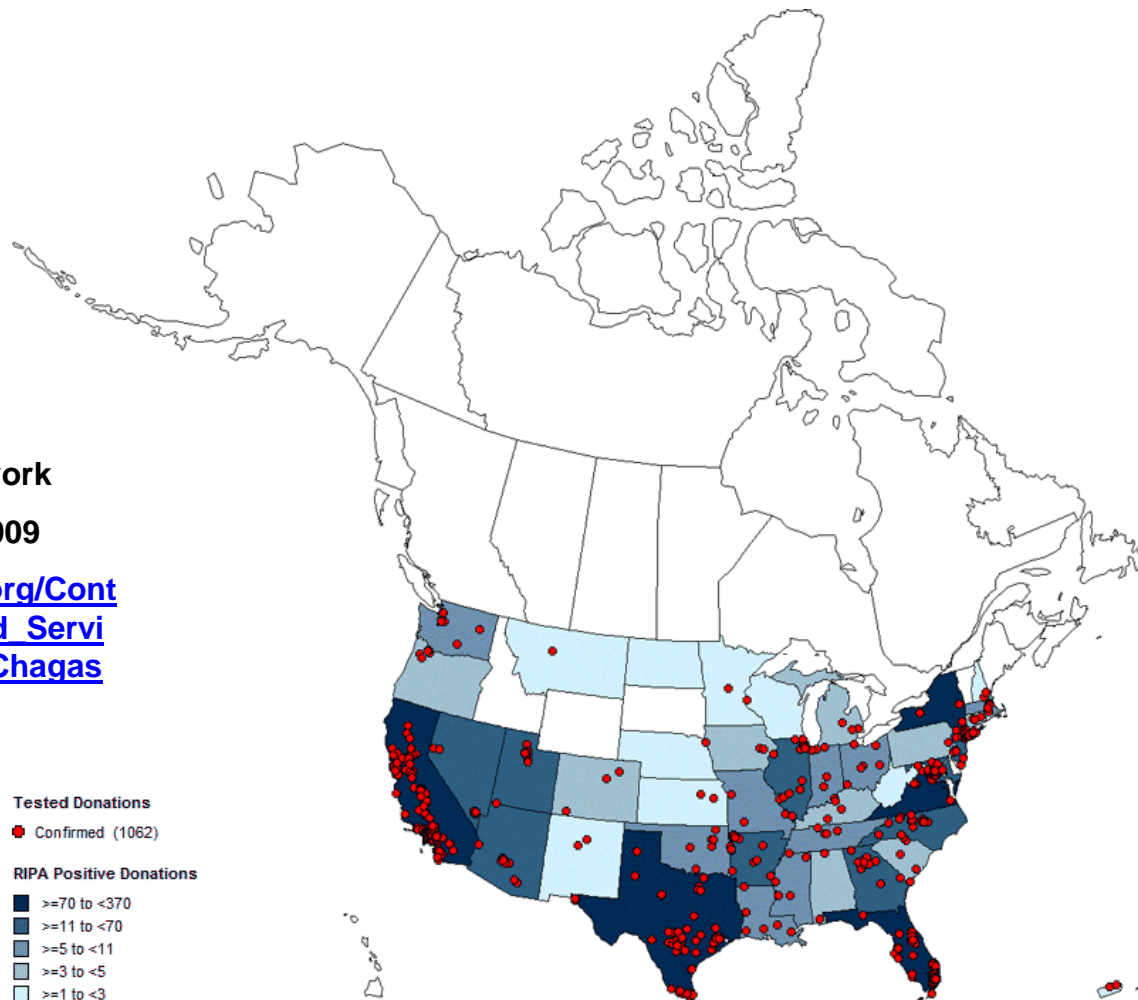
- Only 7 confirmed cases of vector-borne autochthonous infection with *T. cruzi* in the U.S.A. (Bern and Montgomery, 2009).
- Four cases occurred in Texas, and one case each occurred in California, Tennessee, and Louisiana.
- However, it has been estimated that over 300,000 people infected with *T. cruzi* living in U.S.A. This is approximately six times the estimated prevalence in Spain (second highest number of immigrants from Latin America; Bern and Montgomery, 2009).
- These same authors also conservatively estimate that up to 315 babies per year may be born with Chagas' disease and > 45,000 cases of heart disease each year could be attributed to Chagas' disease in the U.S.A.
- Chagas' disease is clearly under-diagnosed in this country, and when patients are diagnosed, health care providers are typically not familiar with the disease or with treatment protocols.
- Blood banks in the U.S.A. only began routine screening in Jan 2007.

Continental U.S. Map: Cumulative *T. cruzi* Positive Blood Donations (January 2007 to present)

AABB Chagas' Biovigilance Network

Updated Nov 5, 2009

[http://www.aabb.org/Content/Programs and Services/Data Center/Chagas](http://www.aabb.org/Content/Programs%20and%20Services/Data%20Center/Chagas)



- 75-90% of blood donations currently screened.
- Over 1000 confirmed seropositive blood donations (ELISA and Radioimmune precipitation assay).
- In preliminary analysis reported by Bern and Montgomery, 25% of seropositive donors identified in their study were born in the U.S.A. and autochthonous infection deemed likely.

- Recent studies in our laboratory documented the presence of the sylvatic cycle of *Trypanosoma cruzi* infection in raccoons from Warren and Barren counties of Kentucky.
(Groce, 2008- MS thesis)




Trapping Results

June 2007-June 2008

 Warren County

 25 raccoons

 Barren County

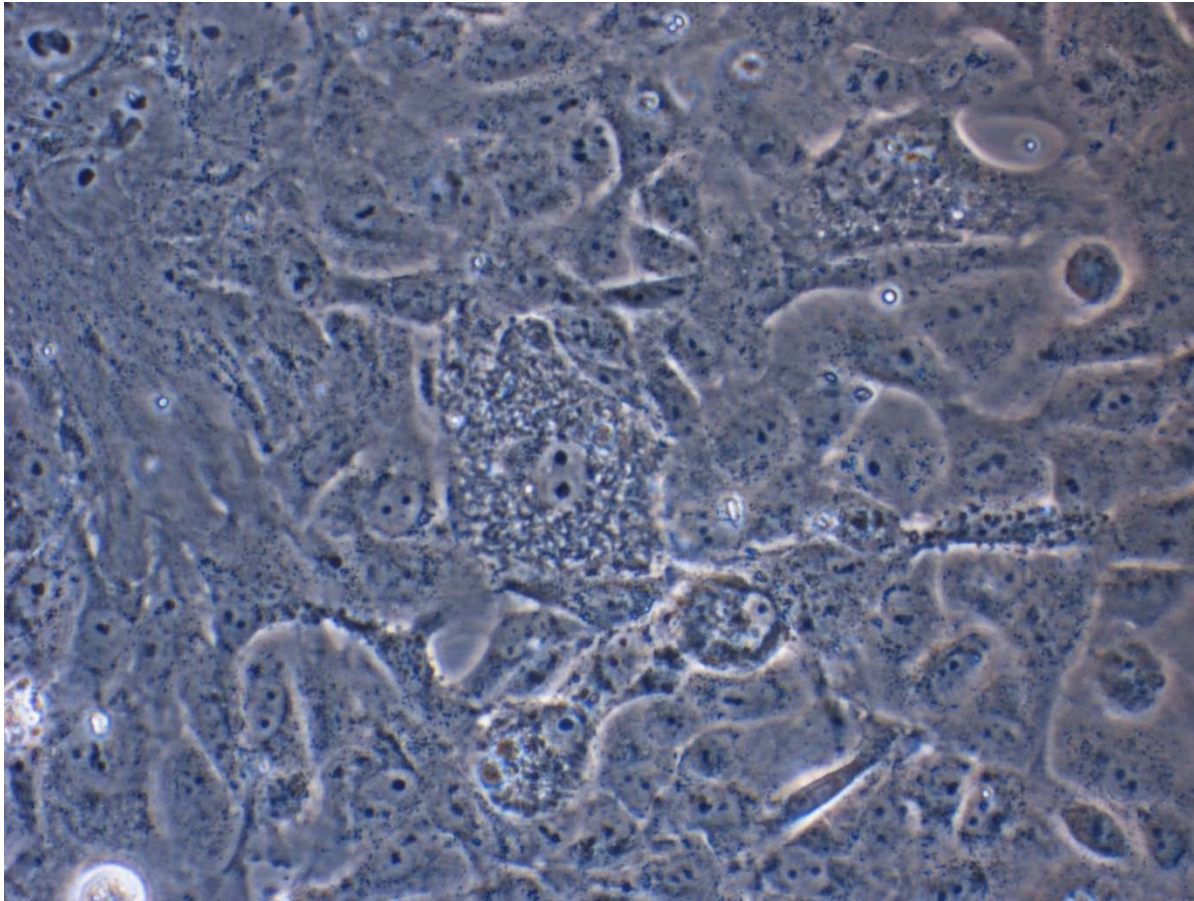
 19 raccoons



Raccoon Results

	Positive Hemoculture
Warren County	15/25
Barren County	3/19

Raccoon isolates were found to be infective for mammalian cells in vitro.



Present study:

The primary goal of the present study was to use a molecular typing approach to determine the genotype (Type I, or Types IIa-IIe) of the hemoculture isolates.



Humans and marsupials commonly infected with *T. cruzi* Type I



Placental mammals such as raccoons, dogs, and skunks reported to be most commonly infected with Type IIa (Roellig et al., 2008)



A variety of primer sequences have been reported to be effective in detecting *T. cruzi* and classifying it as Type I or Type II (Virreira et al., 2003; Brisse et al., 2003; Roellig et al., 2008).

Material and Methods

- DNA samples were prepared from 15 of the isolates using a Qiagen mini kit.
- DNA concentrations were determined spectrophotometrically.

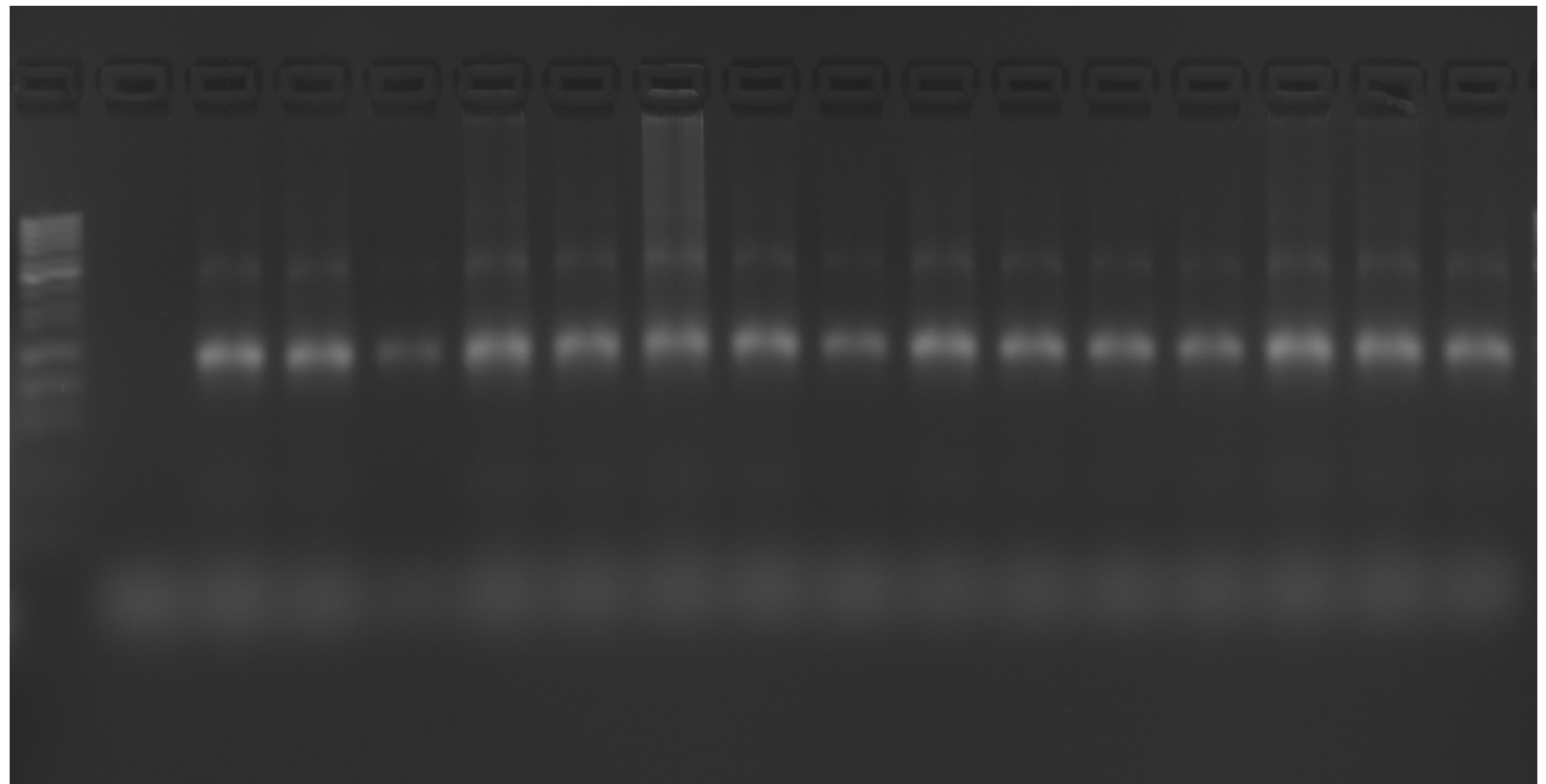
PCR Analysis

- To further confirm that all isolates were *T. cruzi*, DNA samples were first amplified using TCZ1 and TCZ2 primers that amplify a 188-base pair segment of the repetitive 195-bp nuclear DNA sequence of *T. cruzi*.

TCZ1 and TCZ2 (repetitive 195-bp nuclear DNA sequence of *T. cruzi*)

Cont

15 Isolates



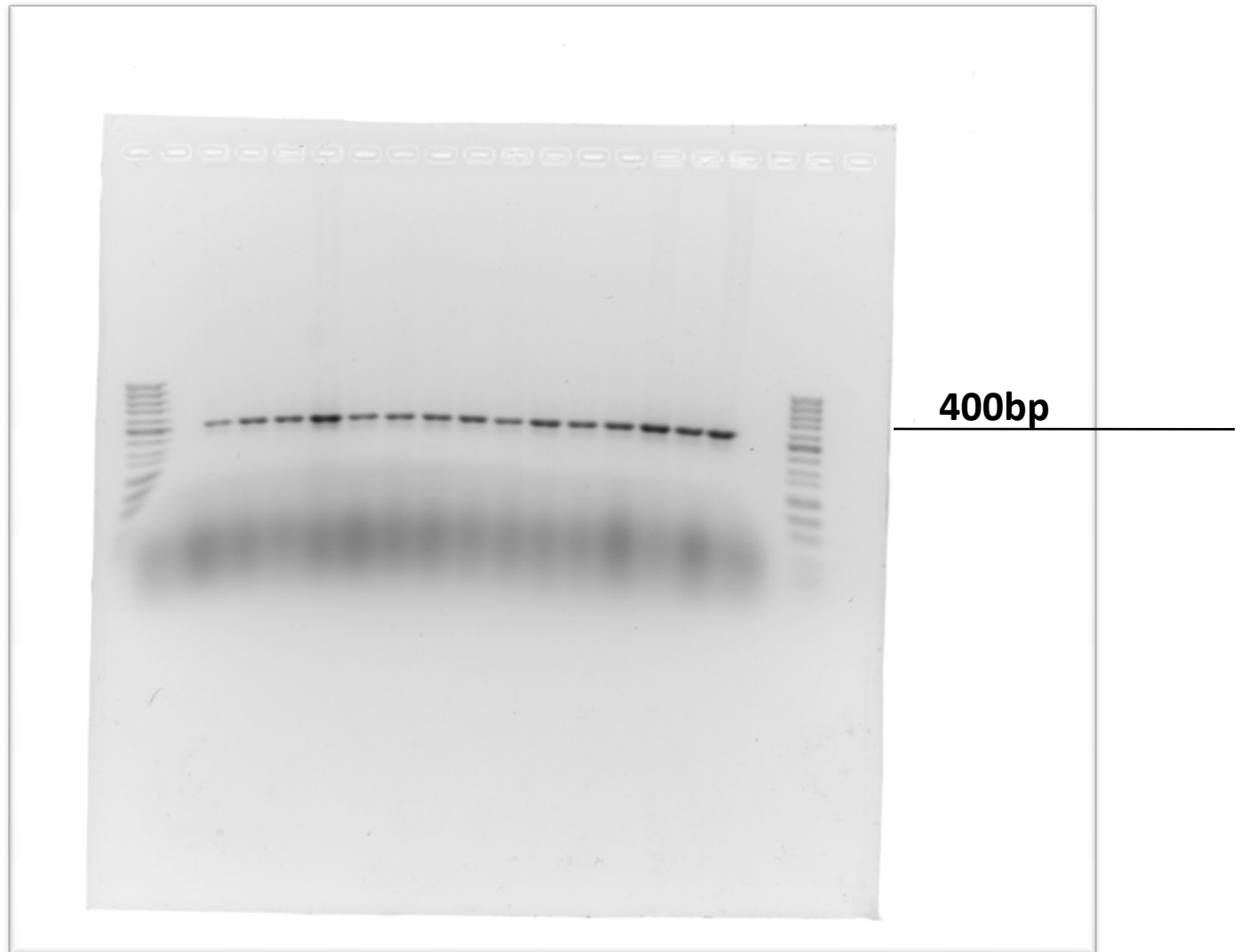
Additional PCR Analyses

- PCR amplification of the D7 divergent domain of the 24S α rRNA: D71 (5'-AAGGTG CGT CGA CAG TGT GG) and D72 (5'-TTT TCAGAA TGG CCG AAC AGT).
- PCR amplification of the non-transcribed spacer of the mini-exon gene: TC (5'-CCCCCC TCC CAG GCC ACA CTG), TC1 (5'-GTG TCC GCCACC TCC TTC GGG CC), and TC2 (5'-CCT GCA GGCACA CGT GTG TGT G)
- PCR amplification of the size-variable domain of the 18S rRNA sequences: V1 (5'-CAA GCG GCT GGG TGG TTA TTCCA) and V2 (5'-TTG AGG GAA GGC ATG ACA CATGT)

Electrophoretic Analysis of PCR Products

- 1.5% or 3% Agarose Gels
- To achieve accurate estimates of band sizes, an *exACTGene** DNA Ladder > 50bp Mini Ladder (Fisher Scientific) and a 10bp DNA Ladder (Invitrogen Corporation) were used.
- Inclusion of Negative Control (no DNA template but water)

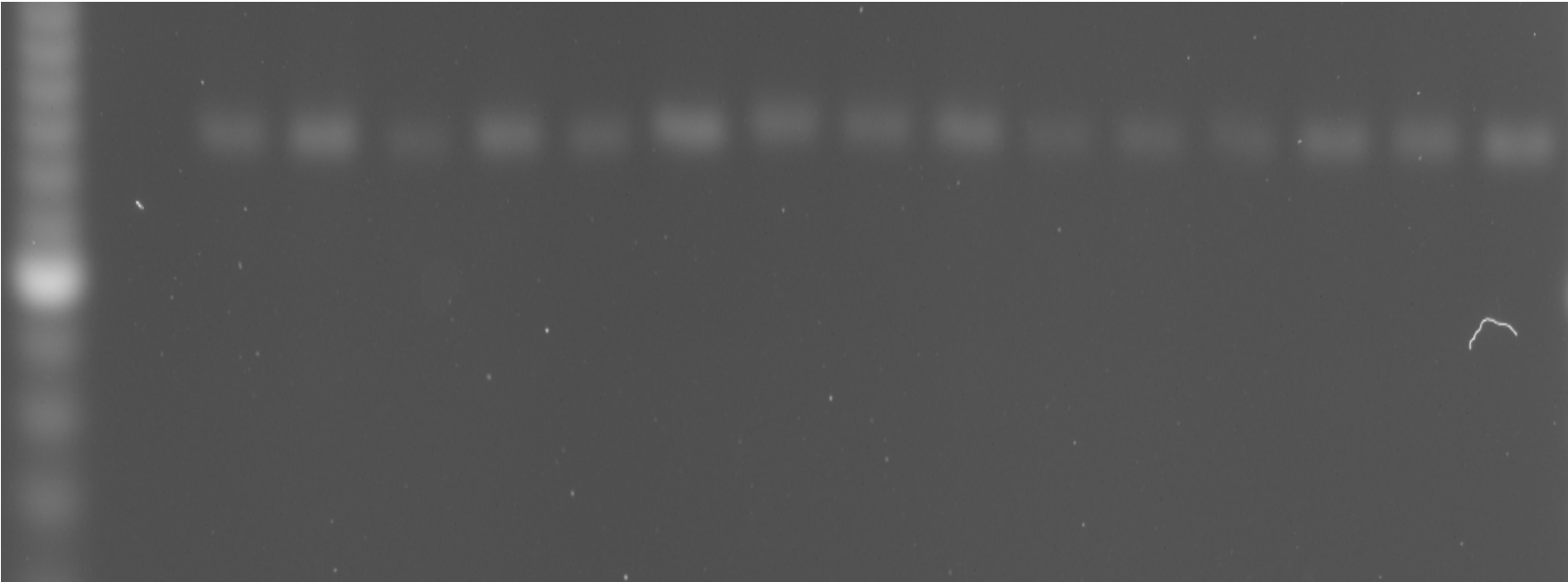
PCR amplification of the non-transcribed spacer of the mini-exon gene (TC series)



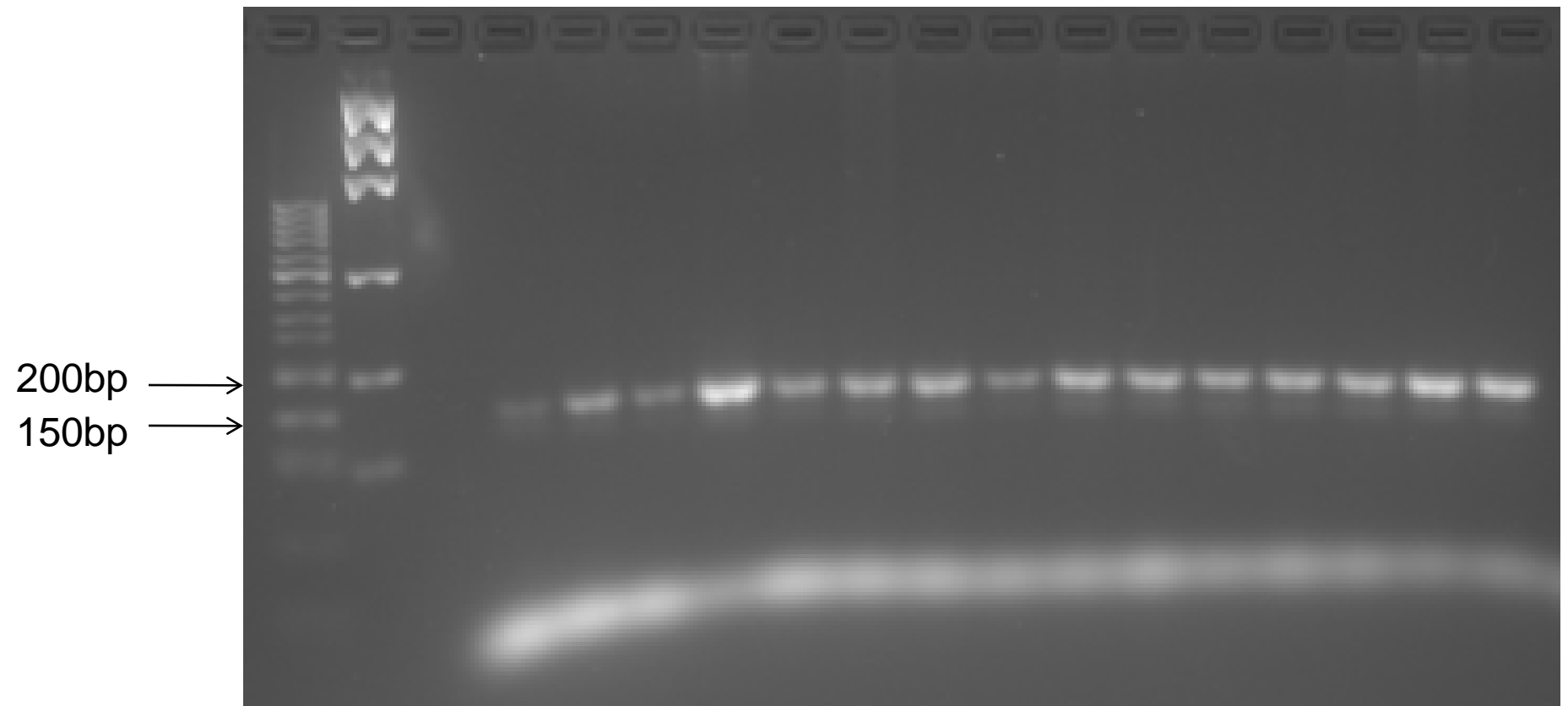
Amplification of divergent domain of the 24S α rRNA (D71 and D72)

Cont

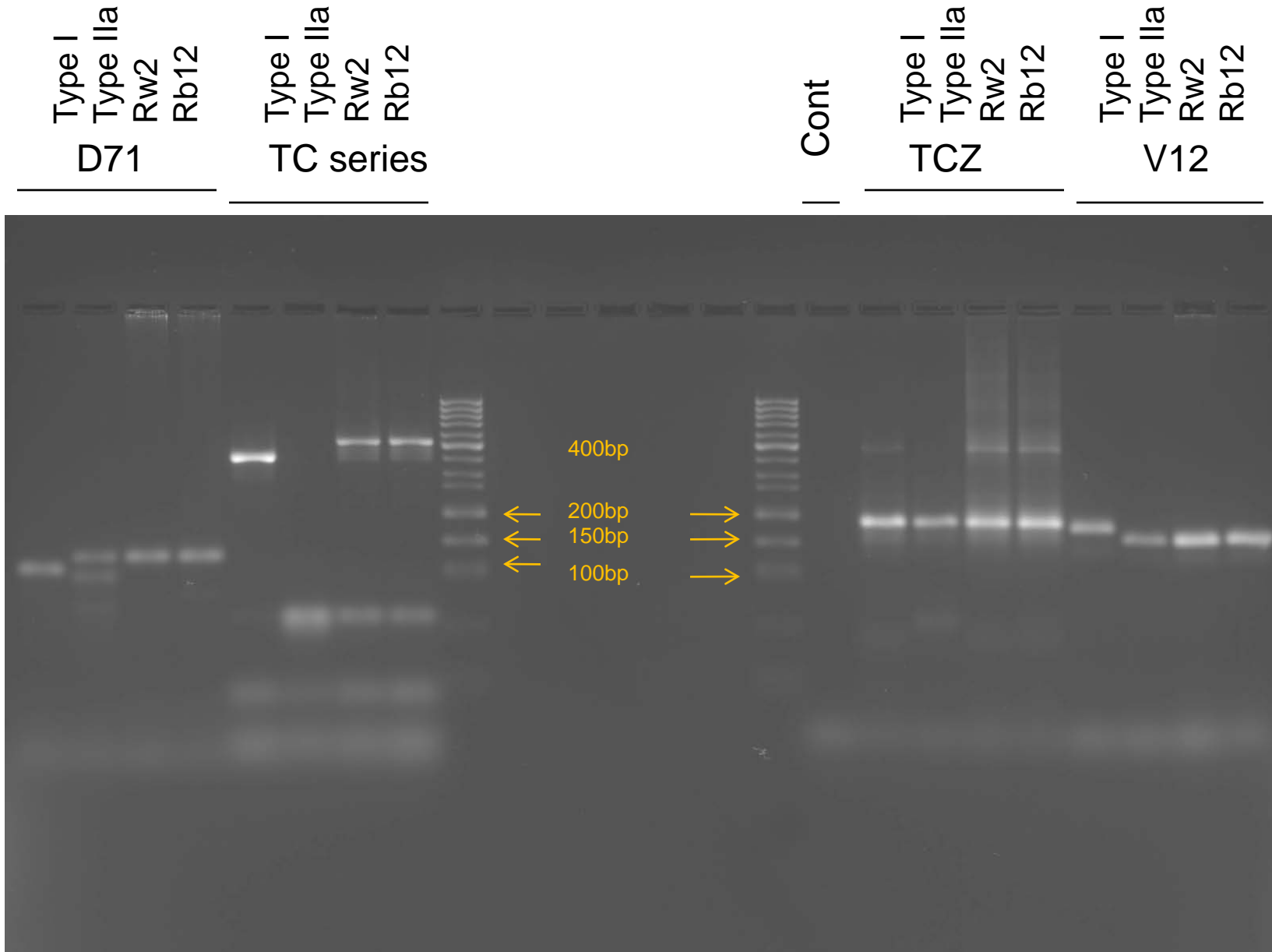
15 Isolates PCR products



Size-variable domain of the 18S rRNA sequences (V1 and V2)



Type I, Type II, RW2, RB12 with 50bp DNA ladder



D7-1 and D7-2 primers RB12

- *Trypanosoma cruzi* 92122102r 24S- α ribosomal RNA gene, partial sequence (Type IIa)

E-value = 2.21e-14, Score = 46, Bitscore = 86.0662, Identities = 53/56 (94%), Positives = 53/56 (94%), Gaps = 2/56 (3%)

Frame = +1

```
DRW5R      5 CACCCCCCCCCGT-AAGAGAAGGGAAAGAGAGCAAAAAAAAAAAGAACTCCCCC 59
           CACCCCCCCCCGT AAGAGAAGGGAAAGAGAGCAAAAAAAAAA GA ACTCCCCC
AY367114   73 CACCCCCCCCCGTAAAGAGAAGGGAAAGAGAGCAAAAAAAAAA-GAGACTCCCCC 19
```

TCZ 1 and TCZ 2 RB12

- *Trypanosoma cruzi* strain CanIII satellite sequence (Type IIa)

E-value = 1.74e-49, Score = 224, Bitscore = 203.264, Identities = 120/125 (96%), Positives = 120/125 (96%), Gaps = 0/125 (0%)
Frame = +1

```
TCZRB12      1 GTGACAGAGTGTGTCTCTGACTCCCACCATTTCATAATTCGCAACAAAAATTTGGACCACA 60
              GTGACAGAGTGTG CTCTGACTCCCACCATTTCATAATTCGCAACAAAAAT TGGACCACA
EU178923    125 GTGACAGAGTGTGCCTCTGACTCCCACCATTTCATAATTCGCAACAAAAATCTGGACCACA 66

TCZRB12      61 ACGTGTGATGCAGCAGCCGCTCGAAAACGATCCGCCGAGTGCAGCACCCGTGTGGGCAAG 120
              ACGTGTGATGCAGCAGCCGC CGAAAACGATCCGCCGA TGCAGCACCCGTGTGGGCA G
EU178923    65 ACGTGTGATGCAGCAGCCGCCCGAAAACGATCCGCCGACTGCAGCACCCGTGTGGGCAGG 6

TCZRB12      121 AGCTC 125
              AGCTC
EU178923     5 AGCTC 1
```

V1,V2 primers

RB12

- *Trypanosoma cruzi* isolate TryCC 778 18S ribosomal RNA gene, partial sequence (Type IIa)

E-value = 2.60e-46, Score = 212, Bitscore = 192.443, Identities = 108/109 (99%), Positives = 108/109 (99%), Gaps = 0/109 (0%)
Frame = +1

```
VRB12      1 TTGTGTGGCACTCGTCGCCTTTGTGGGAAATCCGTGTGGCACTGTTTGTGTTGTTGGCGG 60
            TTGTGTGGCACTCGTCGCCTTTGTGGGAAATCCGTGTGGCACTGTTTGTGTTGTTGGC G
EU755232  325 TTGTGTGGCACTCGTCGCCTTTGTGGGAAATCCGTGTGGCACTGTTTGTGTTGTTGGCAG 384

VRB12      61 ACTTCGGTCTTGCCTTCGCATATTTACATGTGTCATGCCTTCCCTCAA 109
            ACTTCGGTCTTGCCTTCGCATATTTACATGTGTCATGCCTTCCCTCAA
EU755232  385 ACTTCGGTCTTGCCTTCGCATATTTACATGTGTCATGCCTTCCCTCAA 433
```

Conclusions

The sylvatic cycle of *T. cruzi* infection is present in south central Kentucky.

High prevalence of *T. cruzi* infection in raccoons trapped from both rural and suburban areas.









All raccoon hemoculture isolates were found to be Type IIa, which is the most common genotype of *T. cruzi* found in raccoons in the southern U.S.A.

Recently, PCR analysis was also successfully performed on DNA isolated from non-coagulated blood samples drawn from raccoons.

Results Summary

	Raccoon Hemoculture Results	Raccoon Blood PCR Results (TCZ1/TCZ2)	Overall prevalence of <i>T. cruzi</i> in Raccoons
Warren County	52% +	62% +	62% +
Barren County	14% +	31% +	31% +

Acknowledgements

-  Chad Groce (M.S. student)
-  LiPeng Bi (current M.S. student)
-  Colin Moss & Matthew James (undergraduate students)
-  Dr. Michael Yabsley's Lab (UGA and SCWDS)
-  Department of Biology
-  Western Kentucky University Biotechnology Center
-  WKU Office of Graduate Studies
-  Administrative support and student research support from NIH Grant Number 2 P20 RR-16481 from the National Center for Research Resources is gratefully acknowledged.

Results obtained after 24Sα rRNA, mini-exon and 18S rRNA PCR characterisation of 50 the *Trypanosoma* stocks under study^{a,b}

Taxon	MLEE/RAPD lineage (clonal genotype) ^c	Stock	24Sα rRNA	Mini-exon	18S rRNA
<i>Trypanosoma cruzi</i>	I (20)	Cuica cl1 ^d	110	350	175
<i>T. cruzi</i>	I	P209 cl1 ^d	110	350	175
<i>T. cruzi</i>	I	SC13	110	350	175
<i>T. cruzi</i>	I (12)	Tehuentepec cl2	110	350	175
<i>T. cruzi</i>	I (13)	Davis2	110	350	165
<i>T. cruzi</i>	I (19)	133-79 cl7 ^d	110	350	170 and 180
<i>T. cruzi</i>	I (17, Z1)	X10 cl1 ^d	110	350	160
<i>T. cruzi</i>	Ila/SA (27, Z3)	CanIII cl1 ^d	120	None	155
<i>T. cruzi</i>	Ila/SA	Ep-255	120	None ^e	155
<i>T. cruzi</i>	Ila/SA	Ep-272	120	None ^e	155
<i>T. cruzi</i>	Ila/SA (29)	10 R26	120	None ^e	155
<i>T. cruzi</i>	Ila/SA	Saimiri 3	125	None	155
<i>T. cruzi</i>	Ila/NA	STC33R	130	None	155
<i>T. cruzi</i>	Ila/NA	STC5R	130	None	155
<i>T. cruzi</i>	Ila/NA	DogT	130	None	155
<i>T. cruzi</i>	Ilb	AAB2	125	300	165
<i>T. cruzi</i>	Ilb	CBB cl3 ^d	125	300	165
<i>T. cruzi</i>	Ilb	MSC2	125	300	165
<i>T. cruzi</i>	Ilb	SO50	125	300	165
<i>T. cruzi</i>	Ilb	VOV2	125	300	165
<i>T. cruzi</i>	Ilb (30, Z2)	Esmeraldo cl3 ^d	125	300	165
<i>T. cruzi</i>	Ilb (32)	TU18 cl2 ^d	125	300	ND
<i>T. cruzi</i>	Ilc	85/847	110	None	165
<i>T. cruzi</i>	Ilc	CM17	110	None	165
<i>T. cruzi</i>	Ilc	CM25	110	None	165
<i>T. cruzi</i>	Ilc	X109/2	110	None	165
<i>T. cruzi</i>	Ilc	X9/3	110	None	165
<i>T. cruzi</i>	Ilc (35)	M6241 cl6 ^d	110	None ^e	165
<i>T. cruzi</i>	Ilc (36)	M5631 cl5 ^d	110	None ^e	165
<i>T. cruzi</i>	Ild	BMS	110 ^f	300	165
<i>T. cruzi</i>	Ild	JSR6	110 ^f	300	165
<i>T. cruzi</i>	Ild	Kundera	110 ^f	300	165
<i>T. cruzi</i>	Ild	MN cl2 ^d	110 ^f	300	165
<i>T. cruzi</i>	Ild	P274	110 ^f	300	165
<i>T. cruzi</i>	Ild (39)	92.80 cl1 ^d	110	300	165
<i>T. cruzi</i>	Ild (39)	SC43 cl1 ^d	110	300	165
<i>T. cruzi</i>	Ile	50/1	125	300	None ^g
<i>T. cruzi</i>	Ile	CL Brener ^d	125	300	None
<i>T. cruzi</i>	Ile	Gusteque	125	300	None ^g
<i>T. cruzi</i>	Ile	P69/8	125	300	None ^g
<i>T. cruzi</i>	Ile	SABCHO 109 ^d	125	300	None ^g
<i>T. cruzi</i>	Ile	Tulahuen 1954	125	300	None
<i>T. cruzi</i>	Ile	X57/3	125	300	None
<i>T. cruzi</i>	Ile (43)	Tulahuen cl2	125	300	None
<i>T. cruzi</i> -like	None	A83	110	350	None
<i>T. cruzi</i> -like	None	A87	110	350	None
<i>Trypanosoma cruzi marinkellei</i>	None	B34	110	None	150
<i>T. cruzi marinkellei</i>	None	M1117	110	None	150
<i>Trypanosoma rangeli</i>	None	Ev26	135	None	None
<i>T. rangeli</i>	None	Xeno11	135	None	None