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SARCOCYSTIS SPECIES: A POSSIBLE THREAT TO CATTLE HEALTH AND FOOD SAFETY IN SOUTH SINAI GOVERNORATE, EGYPT

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Abstract

Sarcocystis is a zoonotic worldwide cyst-forming coccidian parasite. The study aimed to determine the frequency of *Sarcocystis* infection in ruminants in South Sinai, identify *Sarcocystis* spp. and genotypes that can infect these animals using molecular techniques.

A total of 353 blood samples were collected from ruminants to molecular screen for sarcocystosis using two PCR assays targeting *Sarc-cattle* and *Sarc-sheep* genes amplified 600bp & 1100bp, respectively. The results showed that goats and sheep didn't have *Sarcocystis* infection, whereas *Sarcocystis* infection was in cattle with its risk pathogenesis. The demonstrated cattle sarcocystosis was in 44/113(38.94%), but neither among 172 sheep nor 68 goats. The recovered sequences were deposited in the GenBank under the accession number MZ197780 as *S. fusiformis* and MZ197784, MZ197785, MZ197786, and MZ197787 as *S. cruzi*.

Keywords: Egypt, South Sinai, *Sarcocystis cruzi*, *Sarcocystis fusiformis*, Cattle, Genotypes.

Introduction

Sarcocystis is an apicomplexan protozoan intracellular parasite that can infect several livestock species (Taylor *et al,* 2007). It is a worldwide cyst-forming coccidian parasite that can represent zoonosis threats to animal health and food safety because of its high transmission. Besides, it leads to great economic losses caused the clinical and subclinical disease (Radostits *et al,* 2008). It has a specific intermediate host, but herbivore can serve as an intermediate host for several *Sarcocystis* species, which names were related to the hosts (Dahlgren and Gjerde, 2007).

Sarcocystis requires two separate hosts for life cycle completion: a definitive host (in which sexual stage develops, usually a carnivorous predator) and an intermediate host (often herbivorous prey), begins with ingestion of infectious sporocysts or oocysts (Dong *et al,* 2018). Sporulated oocysts (2 sporocysts) and separate sporocysts pass in stool, sporocysts with 4 sporozoites & a refractile residual body. Sporocysts ingested by intermediate host (edible animals) rupture to sporozoites, which enter endothelial cells of blood vessels and undergo schizogony, resulting in first-generation schizonts. Merozoites invade small capillaries and blood vessels, becoming second-generation schizonts. The second generation merozoites invade muscle cells and develop into sarcocysts containing bradyzoites, the infective stage for definitive host. Man becomes infected by eating undercooked meat with sarcocysts. Bradyzoites are released from ruptured cysts in small intestine and invade the lamina propria of intestinal epithelium, differentiated into macro - & microgametocytes. Male and female gametes fusion gave oocysts that sporulate in the intestinal epithelium and shed in host feces (CDC, 2017). Most of the animals are asymptomatic, but in cattle acutely affected with *S cruzi* showed fever, anorexia, cachexia, less milk yield, diarrhea, muscle spasms, anemia, tail hair loss, hyper-excitability, weakness, prostration, and death, but after recovery from acute illness, calves failed to grow well and eventually died in a cachectic state (Dubey *et al,* 2016).

Sarcocystosis was detected macroscopically and muscle squash with visible *Sarcocystis*, which may give false-result, and thus molecular evidence is required for proper diagnosis (Poulsen and Stensvold, 2014). GenBank contained many 18S rRNA gene sequences of genus *Sarcocystis* for species identification (Pritt *et al*, 2008). There are numerous *Sarcocystis* species usually affect animals (cattle, sheep, goats, pigs, horses

and even birds) but also can also cause disease in humans. Two types of human disease can occur; one type causes diarrhea, mild fever, and vomiting by intestinal type; *S. hominis* and *S. suihominis* (Dubey *et al,* 2016*).* Some highly pathogenic species are; *S. cruzi*, *S. hirsuta*, *S. hominis*, *S. rommeli*, *S. heydorni*, *S. ovicanis*, *S. medusiformis*, *S. capracanis*, *S. hircicanis*, and *S. moulei* (Yang *et al,* 2018). In Egypt, *Sarcocystis spp*. infecting dromedary camels and bovine (cows and water buffaloes) were identified and molecularly characterized in Nile-Delta governorates (Sayed *et al,* 2008; Hilali *et al,* 2011; El-Seify *et al,* 2014; Nahed *et al,* 2014; Ahmed *et al,* 2016; Gareh *et al,* 2020).

In Egypt, little is known about *Sarcocystis* species infect small ruminants and their potential impact on meat condemnation due to its infective stages, mainly in Sinai (Abdel-Rahman and El Manyawe, 2010; El-Morsey *et al,* 2019, 2021). Thus, this study aimed to identify and characterizes *Sarcocystis* genotypes and discussed its role in cattle epidemiology.

Materials and Methods

Study area: South Sinai Governorate occupies the southern triangle of Sinai Peninsula, Egypt, between Suez Gulf and Aqaba Gulf (total area of 31, 272 km2). Two cities were selected for collection, their coordinates located from (ElTur: 28°14′30″N 33°37′20″E to Ras Sudr: 29°35′30″N 32°42′20″E), with many farm animals. Fresh blood samples were collected from live animals in tubes containing EDTA. Samples were obtained from private farms in Ras Sudr, affiliated Desert Research Center in Cairo from March to November 2020.

Sample collection: Overall, of 353 blood samples were collected from ruminants to be screened for sarcocystosis. Of which 113 cattle, 172 sheep, and 68 goats were selected from 18 herds. Sheep and goats are constricted together, while cattle breed in separate farms with intensive care. Of those examined animals, 13 aborted, diarrheic, and anaemic (cattle), 15 aborted goats, 12 subclinical sheep with bloody feces were symptomatic, and 313 were healthy.

DNA recovery and selected primers: Genomic DNA was extracted from whole blood samples using the QIAamp® DNA easy Kit (Qiagen, Hilden, Germany) after manufacturer's instructions. The DNA samples were kept frozen at -20 °C until used as templates for PCR amplification. Two species-specific pairs of primers were conducted in the present study; one targeted a part of the 18S ribosomal RNA gene for Sarc-sheep at 1100 bp length, according to Pritt *et al*. (2008). Their sequence: F5/GGA TAA CCG TGG TAA TTC TAT G3/ and R: 5/TCC TAT GTC TGG ACC TGG TGAG3/. The second pair primer was designed by Wong and Pathmanathan (1994) to detect Sarc-cattle by amplifying a 600 bp fragment. The sequence of the primer was Sar F 5/ GCA CTT GAT GAA TTC TGG CA 3/ and Sar R 5/ CAC CAC CCA TAG AAT CAA G 3/.

PCR amplification: The PCR amplifications were carried out in two separate reactions. For Sarc-sheep, each sample was run in PCR reaction 25µL contained 5µL of the sample DNA, 20 pmol of each primer, 12.5µL of PCR Master Mix (TaKaRa, Japan), and 5.5µL distilled water. Cycling was performed 5 min. primary denaturation at 94 °C followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 90 s, as well as a final elongation of 72 °C for 10 min (Rahdar and Salehi, 2011). It contained 3µL of the sample DNA, 20 pmol of each primer, 12.5µL of PCR Master Mix and 7.5µL of distilled water for Sarc-cattle, according to Rahdar and Kardoon (2017). Thermal cycling started with 94 °C for 5 min. followed by 40 cycles of 94 °C for 2 min, 55 °C for 1 min, and 72 °C for 90 s, followed by a final elongation step at 72 °C for 5 min. Ten-µl of each PCR product was electrophoresis analyzed in 1.5 % agarose gel, stained with ethidium bromide, and gels were photographed with UV transillumination.

Identification and genotyping of products:

Sarcocystis spp. was identified by sequence analysis of purified PCR fragments of SarCF /SarCR primer pair was performed. Generated fragments were subjected to a 2-way sequence analysis using ABI 3130 automated DNA Sequencer (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA, USA) using the same forward and reverse primers for PCR. Sequences were submitted to Gen Bank & BLASTn with various *Sarcocystis* spp. They sorted using the Clustal Walgorithm. Sequence similarities compared to former sequences closely related species. Phylogenetic tree was reconstructed by Neighbor-joining model & Boot-strap tests (1000 re-peats), and similarity between isolates was determined using maximum Likelihood test in MEGA6 software (Tamura *et al*, 2013).

Results

Infections showed the 2 PCR assays targeting Sarc-cattle & Sarc-sheep. *Sarcocystis* was not detected in all by Sarc-sheep PCR, but only cattle harbored infected Sarc-cattle-PCR. It produced sharp and obvious fragments confirmed the parasite to *Sarcocystis*. Of 353 animals, Sarc-cattle–PCR amplified 600bp fragments from 44(12.46%), cattle samples 44(38.94%) collected from ElTur, but, none in sheep and goats. PCR followed by sequencing not only detected *Sarcocystis* in blood but also identified genotypes as *S. cruzi* and *S. fusiform*. The isolates were registered to GenBank with access the numbers MZ197780 as *S. fusiformis*, & MZ197784, MZ197785, MZ197786, & MZ197787 as *S. cruzi*. Regardless gene analyzed, the present isolates were clustered in two different clusters. One contained *S. fusiformis* isolates and second *S. cruzi*. Closely related ones were MZ197780 *S. fusiformis* KR186119, KR18-6121, KR186117, & KR1-86123 reference *S. fusiformis* with 100% identity and *S. cruzi* accession no. were MZ197784, MZ197785, MZ197786, & MZ197787 related to gene of LC171830, AF176933, AF176934, AF176-935, & KT901167.

Difference was between 4 *S. cruzi* and 1 *S. fusiformis* in 6 nucleotides position. Four matched to isolates showed polymorphisms with *S. fusiformis* were TG, GG replaced with AA, TT and have 2 unique nucleotides in *S. cruzi* as CT was not in *S. fusiformis*. Difference in identity between the 4 isolates ranged from 98.4 to 100% (*S. fusiformis*), and from 94.7 to 100% (*S. cruzi*). Genetic distance was from 0.0 to 0.6 in present ones, and from 0.0 to 4.6 with other global isolates. Details were given in tables (1, 2, & 3), as well as in figures (1, 2, 3, 4, & 5)

Table 1: Prevalence of *Sarcocystis* in ruminants by the two PCR assays

Animal	No.	*Sarc-cattle* (600bp)		*Sarc-sheep* (1100bp)	
		Infected	Non-infected	Infected	Non-infected
Cattle	113	44(38.94%)	69(61.06%)	0.0(0.00%)	113(100%)
Goats	68	0.0(0.00%)	68(100%)	0.0(0.00%)	68(100%)
Sheep	172	0.0(0.00%)	172(100%)	0.0(0.00%)	172(100%)
Total	353	44(12.46%)	309(87.54%)	0.0(0.00%)	353(100%)

Table 2: GenBank database of *Sarcocystis* isolated from cattle in South Sinai, Egypt

No.	Host	Place	Date	*Sarcocystis* species	Accession No. and ID
1	Cattle	El-Tur	11-2020	*S. cruzi*	MZ197780 Sc1-DRC-Eg
2	Cattle	El-Tur	11-2020	*S. fusiformis*	MZ197784 Sc2-DRC-Eg
3	Cattle	El-Tur	11-2020	*S. cruzi*	MZ197785 Sc3-DRC-Eg
4	Cattle	El-Tur	11-2020	*S. cruzi*	MZ197786 Sc4-DRC-Eg
5	Cattle	El-Tur	11-2020	*S. cruzi*	MZ197787 Sc5-DRC-Eg

Discussion

Sarcocystis is one of the zoonotic protozoan parasites in striated muscles of livestock meat (Tappe *et al,* 2013). They are foodborne parasites with a massive impact on public health, for they can be global spread. It is a zoonotic parasite that causes different symptoms in humans. Egyptian government refuge to importation of water buffalo/cows meat and also lives animals from abroad because of the increased demands on protein foods with the sharp decrease in the meat industry (Hussein *et al,* 2017; Barghash *et al,* 2021).

Table 3: GenBank database of detected *Sarcocystis* species

Accession no.	Host	Country	Sarcocystis	Reference
LC171830	Water buffalo	Japan	*S. fusiformis*	Murata *et al,* 2018
AF176934	Water buffalo	Japan	*S. fusiformis*	Yang *et al,* 2001
AF176935	Water buffalo	Japan	*S. fusiformis*	
KT901167	Water buffalo	Egypt	*S. fusiformis*	Gjerde, 2016
KR186117	Water buffalo	Egypt	*S. fusiformis*	
KR186123	Water buffalo	Egypt	*S. fusiformis*	
MK420018	Sheep	Spain	*S. cruzi*	Gjerde *et al,* 2020
LC364052	Sheep	Iraq	*S. cruzi*	Safa and Elham, 2018
MZ197780 Sc1-DRC-Eg	Cattle	Egypt	*S. cruzi*	The present study
MZ197784 Sc2-DRC-Eg	Cattle	Egypt	*S. fusiformis*	The present study
MZ197785 Sc3-DRC-Eg	Cattle	Egypt	*S. cruzi*	The present study
MZ197786 Sc4-DRC-Eg	Cattle	Egypt	*S. cruzi*	The present study
MZ197787 Sc5-DRC-Eg	Cattle	Egypt	*S. cruzi*	The present study

However, there was transportation of cattle to Sinai, from the Nile-Delta Governorates, with an introduction of *Sarcocystis* cysts

Diagnosis of muscular sarcocystosis happens in tissue samples from infected hosts, from the skeletal muscle, tongue, heart, diaphragm, and esophagus. When infection is very heavy in intermediate hosts, the clinical signs and histological evidence of schizont in the blood vessels of organs were alternative tools for detection (Urquhart *et al,* 1987). Most cysts are shown in feces, but it is insensitive and cannot differentiate between species because sporocysts lack specific staining criteria (ElSheikha *et al,* 2006; Verweij and Stensvold, 2014).

In the present work, local farmers detected *Sarcocystis* on some slaughtered cattle, besides diarrhea and abortion live cattle. With the availability of PCR techniques (Radostits *et al,* 2008), the molecular characteristics of *Sarcocystis spp*. in ruminants in the blood of live animals were done.

In the present study, despite *Sarcocystis* infection reported in Egyptian sheep and goats, none was PCR detected among them. but, examined cattle showed more or less infections compared to previous Egyptian studies. Difference might be due to ecological factors, samplings, and used technique. El-Seify *et al*. (2014) in Kafr-Elsheik found that 68.2% of old-aged animals and 13.2% of younger ones were infected with *S. fusiformis* (17.2%) and *S. buffalonis* (10.2%), respectively, as compared to locally GenBank. Metwally *et al.* (2014) in Assiut Governorate macroscopically identified *S. fusiformis* in buffaloes, and microscopically identified three species (*S. cruzi*, *S. levinei*, & *S. hominis)*. Abu-Elwafa *et al.* (2015) in Dakahlia Governorate reported 58.72% *S. fusiformis* cysts among slaughtered water buffaloes. El-Bahy *et al*. (2019) in Cairo reported *S. fusiformis* cysts as (0.1%) in cattle and (85.96%) in buffalo carcasses, but none in camels, sheep, nor goats. Gareh *et al*. (2020) in Gharbia Governorate reported *Sarcocystis* in 75% of camels and added that both aged and male ones were risky with rates of 87.7% and 81.4%, respectively. They added that the esophagus was the most affected organ (49%) and incriminated camels in the epidemiology of Egyptian sarcocystosis.

In the present study, DNA was extracted from the whole blood and 18S ribosomal RNA gene for Sarc-sheep and Sarc-cattle was used. Rahdar and Salei (2011) reported that cox1 was good target to taxonomic *Sarcocystis spp*. differentiation among edible intermediate hosts. Murata *et al*. (2018); Hoeve-Bakker *et al*. (2019); Rubiola *et al*. (2019) and Ras *et al.* (2021) preferred molecularly characterized *Sarcocystis* using different genes; 18S rRNA, & 28S rRNA, and nuclear rDNA internal transcribed spacer 1 (ITS1).

In the present study, cattle harbored *S. cruzi* and *S. fusiformis*, with sequence significant data identities (>90%) compared to archived genes. Most of them were in healthy cattle without clinical signs, others showed fever, anemia, weight loss, abortion, and diarrhea. This agreed with Taylor *et al*. (2007) and Radostits *et al*. (2008). Besides,

neurologic signs were in four herds of cattle with typical *S. cruzi* with accession no. of LC171830 from Japan (Murata *et al,* 2018), AF176934, and AF176935 (Yang *et al,* 2001), and KT901167 (Gjerde, 2016).

Abroad, Latif *et al*. (2015) in Malaysia found 86% in sheep as *S. ovicanis*, 61.8% in goat as *S. capracanis*, and 28.6% in cattle as *S. bovicanis*. Daptardar *et al*. (2016) in India found *Sarcocystis* cysts in 68% bovines by conventional PCR, and with more than I *Sarcocystis* species circulated. Dong *et al.* (2018) in China found 335/638 (52.51%) in sheep. Safa and Elham (2018) in Iraq reported *S. tenella* & *S. arieticanis* in sheep and *S. cruzi* & *S. bovifelis* (or *S. hominis*) in cattle, with possible mixed infection, and level of genetic variability depended on species, and geographical location. Prakas *et al.* (2020) in Lithuania foundin cattle, *S. cruzi* (96.1%), *S. bovifelis* (71.6%), *S. hirsuta* (30.4%), and *S. hominis* (13.7%), with mixed infection of 2 species (44.1%), 3 species (26.5%), 1 species (24.5%), and 4 species (4.9%) based on sequence analysis of cox1. Rubiola *et al*. (2021) in Italy reported *S. cruzi*, *S. hominis*, & *S. bovifelis* in 67.8% in slaughtered cattle and 90.7% in condemned carcasses, with cattle *S. cruzi* (61%), followed by *S. bovifelis* (10.2%), *S. hominis* (8.5%), and *S. hirsuta* (1.7%). Whereas Zeng *et al*. (2021) in Belgium found *Sarcocystis spp*. in 64% in carcasses, and female dairy cattle with high rate (91%) and species diversity compared to female & male. *S. cruzi* was in 56.5% carcasses, followed by *S. hominis* (21.0%), *S. bovifelis* (12.5%), *S. bovini* (2.0%), *S. hirsuta* (1.5%), & *S. heydorni* (0.5%).

Conclusion

This is the first molecular detection of *Sarcocystis* in South Sinai Governorate. Moderate parasite prevalence was only in cattle from El-Tur City *Sarcocystis* caused economic cattle risk to human welfare. The sequenced isolates were assigned in the GenBank under accession were MZ197780 as *S. fusiformis*, MZ197784, MZ197785, MZ197786, & MZ197787 as *S. cruzi* proved by GenBank. The outcome data showed genetic diversity within *Sarcocystis* species between two

species, homogeneity in *S. cruzi* isolates was very high bootstrap similarity value.

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Conflict of Interest: The author didn't have any conflict of interest.

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Explanation of figures

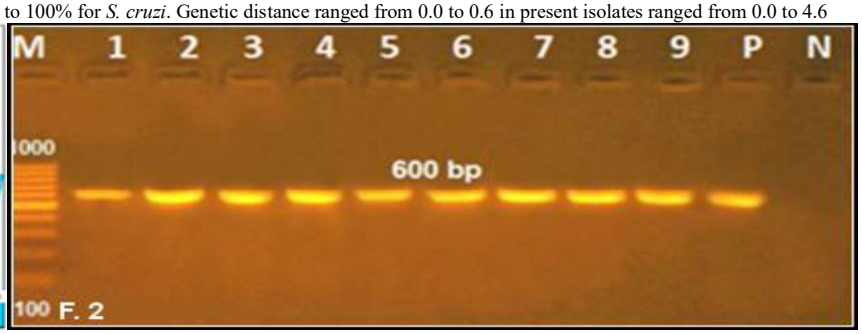
Fig. 1: Map of Sinai Peninsula shows Cities of Ras Sudr and ElTur .

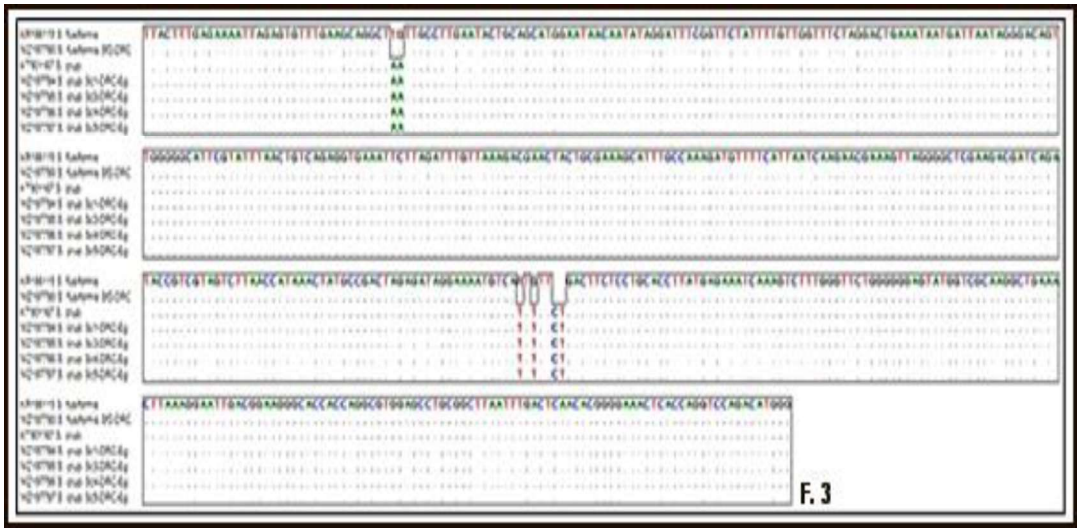
Fig. 2: Agarose gel electrophoresis of ethidium bromide stained *Sarc-cattle*-PCR products of 600 bp. Lane M: 100 bp DNA ladder. Lanes P & N positive and negative controls.

Fig. 3: Sequence alignments of targeted *Sarc-cattle* gene compared to other *Sarcocystis spp*. Six nucleotide polymorphisms with *S. fusiformis* as TG, GG replaced with AA, TT and two unique nucleotides in *S. cruzi* as CT not found in *S. fusiformis*.

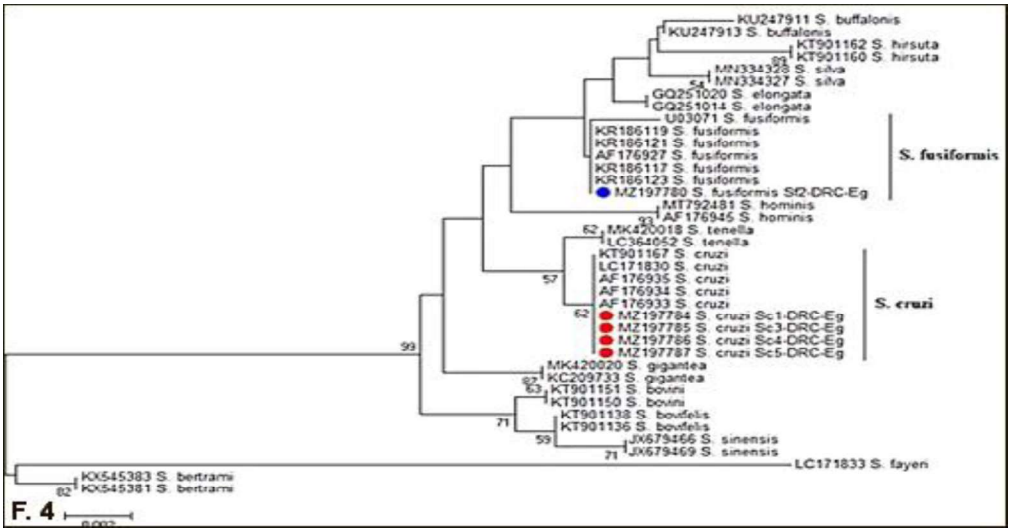
Fig. 4: Phylogenetic tree by comparing amplified and sequenced present isolates with GenBank references. Present sequences registered to the GenBank under accession no: MZ197780 as *S. fusiformis*, and MZ197784, MZ197785, MZ197786, and MZ197787 as *S. cruzi*.

Fig. 5: Identities and divergence between the present isolates of *Sarcocystis spp.* compared to other isolates worldwide in GenBank based on *Sarc-cattle* gene. Accession number of submitted isolates is followed byrespective place of origin (DRC-Eg). Identity *S. fusiformis* ranged from 98.4 to 100% and from 94.7 to 100% for *S. cruzi*. Genetic distance ranged from 0.0 to 0.6 in present isolates ranged from 0.0 to 4.6





F. 3



F. 4

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1		99.8	99.6	98.2	98.6	96.5	98.2	96.2	99.0	98.0	98.2	98.4	98.4	98.4	97.8	98.6	97.6	93.9	98.4	100.0	98.4	98.4	98.4	1
2	0.0		99.8	98.4	98.8	96.8	98.0	96.0	99.2	97.8	98.2	98.4	98.4	98.4	97.6	98.8	97.6	93.7	98.4	99.8	98.4	98.4	98.4	2
3	0.2	0.2		98.2	98.6	96.6	97.8	95.7	99.0	97.6	98.0	98.2	98.2	98.2	97.4	98.6	97.4	93.5	98.2	99.6	98.2	98.2	98.2	3
4	0.4	0.4	0.6		98.8	95.7	96.4	95.5	98.0	96.2	96.6	96.8	96.8	96.8	96.0	98.2	96.0	93.1	96.8	98.2	96.8	96.8	96.8	4
5	0.6	0.6	0.8	0.6		96.6	95.8	96.2	98.4	96.6	97.0	97.2	97.2	97.2	96.4	98.6	96.6	93.3	97.2	98.6	97.2	97.2	97.2	5
6	0.8	0.8	1.0	1.5	1.3		97.0	95.7	96.6	96.6	96.8	96.6	96.6	96.6	96.4	97.0	97.0	93.1	96.6	96.6	96.6	96.6	96.6	6
7	0.8	0.8	1.1	1.3	1.5	0.6		97.2	98.2	99.6	98.6	98.4	98.4	98.4	99.4	97.8	97.8	94.9	98.4	98.2	98.4	98.4	98.4	7
8	2.3	2.3	2.6	2.8	2.8	2.1	2.3		96.6	97.2	97.0	97.0	97.0	97.0	97.0	96.4	96.6	97.2	97.0	96.2	97.0	97.0	97.0	8
9	0.4	0.4	0.6	0.4	0.6	1.5	1.1	3.2		97.8	97.8	98.0	98.0	98.0	97.6	99.2	98.0	93.3	98.0	99.0	98.0	98.0	98.0	9
10	1.1	1.1	1.3	1.5	1.7	1.1	0.4	2.3	1.5		98.8	98.6	98.6	98.6	99.8	97.4	97.8	94.9	98.6	98.0	98.6	98.6	98.6	10
11	0.8	1.1	1.3	1.5	1.7	1.3	1.1	2.1	1.9	0.8		99.8	99.8	99.8	98.6	97.4	98.0	94.7	99.8	98.2	99.8	99.8	99.8	11
12	0.6	0.8	1.1	1.3	1.5	1.5	1.3	2.1	1.7	1.0	0.2		100.0	100.0	98.4	97.6	98.2	94.7	100.0	98.4	100.0	100.0	100.0	12
13	0.6	0.8	1.1	1.3	1.5	1.5	1.3	2.1	1.7	1.0	0.2	0.0		100.0	98.4	97.6	98.2	94.7	100.0	98.4	100.0	100.0	100.0	13
14	0.6	0.8	1.1	1.3	1.5	1.5	1.3	2.1	1.7	1.0	0.2	0.0	0.0		98.4	97.6	98.2	94.7	100.0	98.4	100.0	100.0	100.0	14
15	1.3	1.3	1.5	1.7	1.9	1.3	0.6	2.5	1.7	0.2	1.0	1.3	1.3	1.3		97.2	97.6	94.7	98.4	97.8	98.4	98.4	98.4	15
16	0.2	0.2	0.4	0.8	1.0	1.3	0.8	3.0	0.2	1.3	1.7	1.5	1.5	1.5	1.5		97.6	93.9	97.6	98.6	97.6	97.6	97.6	16
17	0.8	1.1	1.3	1.5	1.5	1.7	1.3	3.0	1.0	1.3	1.5	1.3	1.3	1.3	1.5	0.8		93.1	98.2	97.6	98.2	98.2	98.2	17
18	4.5	4.6	4.8	5.2	5.6	4.7	4.5	2.8	5.4	4.5	4.3	4.3	4.3	4.3	4.7	5.4	5.4		94.7	93.9	94.7	94.7	94.7	18
19	0.6	0.8	1.1	1.3	1.5	1.5	1.3	2.1	1.7	1.0	0.2	0.0	0.0	0.0	1.3	1.5	1.3	4.3		98.4	100.0	100.0	100.0	19
20	0.0	0.0	0.2	0.4	0.6	0.8	0.8	2.3	0.4	1.1	0.8	0.6	0.6	0.6	1.3	0.2	0.8	4.6	0.6		98.4	98.4	98.4	20
21	0.6	0.8	1.1	1.3	1.5	1.5	1.3	2.1	1.7	1.0	0.2	0.0	0.0	0.0	1.3	1.5	1.3	4.3	0.0	0.6		100.0	100.0	21
22	0.6	0.8	1.1	1.3	1.5	1.5	1.3	2.1	1.7	1.0	0.2	0.0	0.0	0.0	1.3	1.5	1.3	4.3	0.0	0.6	0.0		100.0	22
23	0.6	0.8	1.1	1.3	1.5	1.5	1.3	2.1	1.7	1.0	0.2	0.0	0.0	0.0	1.3	1.5	1.3	4.3	0.0	0.6	0.0	0.0		23
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	

Divergence

1 KR186119 S. fusiformis
2 AF176927 S. fusiformis
3 U03071 S. fusiformis
4 KU247911 S. buffalonis
5 KT901162 S. hirsuta
6 MK420020 S. gigantea
7 KT901151 S. bovini
8 KX545381 S. bertrami
9 MN334328 S. silva
10 KT901136 S. bovifelis
11 MK420018 S. tenella
12 KT901167 S. cruzi
13 LC171830 S. cruzi
14 AF176935 S. cruzi
15 JX679466 S. sinensis
16 GQ251020 S. elongata
17 MT792481 S. hominis
18 LC171833 S. fayeri
19 MZ197784 S. cruzi Sc1-DRC-Eg
20 MZ197780 S. fusiformis St2-DRC-Eg
21 MZ197785 S. cruzi Sc3-DRC-Eg
22 MZ197786 S. cruzi Sc4-DRC-Eg
23 MZ197787 S. cruzi Sc5-DRC-Eg

F. 5