Intestinal Microbiota – A Modulator of the *Trypanosoma cruzi*-Vector-Host Triad

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Abstract. Chagas disease affects millions of people, and it is a major cause of death in Latin America. Prevention and development of an effective treatment for this infection can be favored by a more thorough understanding of *T. cruzi* interaction with the microbiome of vectors and hosts. Next-generation sequencing technology vastly broadened the knowledge about intestinal bacteria composition, showing that microbiota within each host (triatomines and mammals) is composed by high diversity of species, although few dominant phyla. This fact may represent an ecological balance that was acquired during the evolutionary process of the microbiome-host complex, and that serves to perpetuate this system. In this context, commensal microbiota is also essential to protect hosts, conferring them resistance to pathogens colonization. However, in some situations, the microbiota is not able to prevent infection but only modulate it. Here we will review the role of the microbiota on the parasite-vector-host triad with a focus on the kinetoplastida of medical importance *Trypanosoma cruzi*. Novel strategies to control Chagas disease based on intestinal microbiome will also be discussed.

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1. Introduction

The intestinal ecosystem is an environment in which biological and biochemical interactions occur at various hierarchical levels, connecting microbial communities and their hosts. [1, 2] Studies of fecal samples revealed that the microbiota from a wholesome intestine is an intricated ecological community composed of trillions of

43   microorganisms, from viruses to unicellular eukaryotes.[3] However, in this article, we

44   will use the term microbiota to refer only to the population of bacteria of an organism.

45   The intestinal microbiota is highly dynamic, it varies over time and is modulated

46   by environmental conditions (use of antibiotics, lifestyle, diet and hygiene preferences,

47   metabolic dysfunction, immunodeficiency and hyper immunity).[4]The application of

48   new high-performance methodologies for analysis of bacterial species, such as the new

49   generation sequencing (NGS) of 16S rRNA, revolutionized the knowledge about the

50   intestinal microbiome. [5] It is now known that about 1000 bacterial species inhabit the

51   human adult intestine; however, the predominant genera are *Lactobacillus,*

52   *Bifidobacterium, Bacteroides, Eubacterium, Clostridium, Ruminococcus,*

53   *Peptostreptococcus, and Peptococcus.*[5] Despite the large number of distinct species,

54   they belong to a relatively small number of phyla, especially Bacteroidetes and

55   Firmicutes. [6]

56   In healthy hosts, the presence of this microbiota contributes to the prevention of

57   pathogen colonization.[7] Additionally, it has an important impact on various aspects of

58   the hosts physiology and metabolism; such as, protection of intestinal epithelium,

59   digestion of host nutrients, production of vitamins and hormones, and regulation of

60   immune responses, modulating the expression of immunological mediators and the

61   recruitment of certain cell populations.[8, 9]

62   Changes in microbiota composition usually have a direct effect on parasitic

63   infection, in part because parasites and bacteria metabolize substrates interactively and

64   secrete products that affect each other, interfering with the survival and physiology of

65   both. [10]Likewise, the microbial community constitution is an extremely important

66   factor for host immune responses: imbalance between the microbiota and the immune

67 system may alter the host's homeostasis and lead to greater disease susceptibility, and

68 therefore dictate the success of the intestinal pathogens.

69   Published data demonstrate that the intestinal microbiota usually has a deep

70 influence on the parasite-host relationship.[11] It is well known that intestinal microbiota

71 composition is determinant for some parasites pathogenicity, as described for

72 *Entamoeba histolytica*,[12] *Trichuris* muris,[13] *Schistosoma mansoni*,[14] *Eimeria*

73 *falciformis*,[15] *Eimeria ovinoidalis*,[16] *Ascaris lumbricoides*,[17] and *Giardia lamblia*.

74 [18]

75   On the other hand, this microbiota can reduce the damages of other infectious

76 agents, such as *Cryptococcus neoformans*,[19] *Strongyloides venezuelensis*,[20] and

77 almost all enteropathogenic bacteria (*Clostridium difficile*, *Clostridium perfringens*,

78 *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella xexneri*

79 and *Vibrio cholerae*). [21, 22, 23] In few reported cases - *Raillietina cesticillus,*[24] *Isopora*

80 suis,[25] and *Trichuris trichiura* [17]- the microbiota composition appear not to influence

81 the outcome of the disease.

82   *T.* cruzi is the etiological agent of Chagas disease, the most important parasitic

83 disease in the Americas, affecting approximately 6 to 8 million people and causing

84 around 12,000 deaths per year. [26]Little is known about the modulation of *T. cruzi*

85 infections by the intestinal microbiota, in insects or vertebrate hosts. Approximately

86 30% of infected individuals will develop cardiac, digestive or neurological changes

87 during the chronic phase. Chagas disease pathogenesis has not been fully elucidated,

88 and different theories try to explain it, such as parasite persistence and

89 autoimmunity.[27]This fact contributes to the difficulty in developing an effective

90 treatment. In this review, we will summarize the current knowledge on microbiome of

91   *T. cruzi* invertebrate and vertebrate hosts, highlighting new approaches and research

92   gaps in this field (Figure 1).

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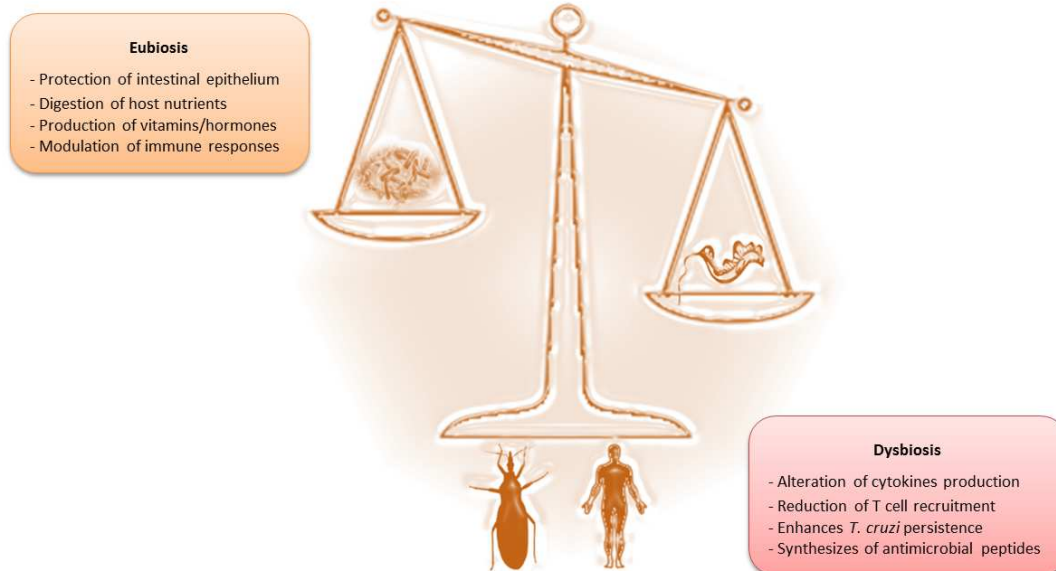
Figure 1. Multi-effects of the intestinal microbiota on the vector-parasite-host triad. In healthy, non-infected, vectors and hosts, the resident microbiota will play an important role in the maintenance of homeostasis (eubiosis). During *T. cruzi* infection, the parasite and bacteria metabolize substrates interactively and secrete products that affect each other and interfere in the survival and physiology of the host (dysbiosis).

2. Gut microbiota in parasite-vector interface

101   Hemiptera insects began to inhabit our planet about 400 million years ago, being

102   favored by the emergence of vascular plants, whose phloem served as their food source.

103   Throughout the evolutionary process, adaptations of the oral apparatus of these

104   arthropods allowed the acquisition of new feeding habits, such as hematophagy. [28,

105   29]Triatomines (Hemiptera: Reduviidae), popularly known as kissing bugs, are life-long

106   obligatorily hematophagous arthropods which feed on various animals, mainly

107   mammals. During hematophagy, several microorganisms can reach triatomines

108   alimentary tract and begin its colonization.

109   In recent years, triatomines microbiota has been evaluated by NGS, showing that

110   the ecological diversity of its microbiome is low but dynamic, changing according to

111   genera and gender, development stages origin, and blood sources.[30, 31, 32] The

112   assessment of *T. brasiliensis* and *T. pseudomaculata* microbiome by denaturing gradient

113   gel bands sequencing revealed their microbiota was mostly composed by Proteo- and

114   Actinobacteria; being *Serratia* the predominant genus. [33]Analyzes of the 16S rRNA

115   gene of the intestinal microbiota of *Triatoma maculata* and *Rhodnius pallescens*

116   captured in the same locality of Colombia, showed the distinct composition of bacteria

117   community. In *R. pallescens*, *Williamsia* and *Kocuria* (orders Corynebacteriales and

118   Actinomycetales, respectively) were the most prevalent genera, while in *T. maculate,*

119   *Dietzia*, *Aeromonas* and *Pelomonas* (orders Actinomycetales, Aeromonadales and

120   Burkholderiales, respectively) were predominant.[30]Another study confirmed that 70%

121   of *Triatoma diminiata* microbiome was composed by bacteria from orders Bacillales,

122   Actinomycetales, Enterobacteriales and Burkholderiales. However, the predominating

123   bacteria in bugs fed on dogs was Burkholderiales, in those fed on humans was

124   Bacillales, and for those fed on porcupine was Enterobacteriales. [31]Interestingly,

125   Rodríguez-Ruano et al., [34] showed that the microbiome composition is particularly

126   determined by host species, receiving less influence of locality and environment.

127   Following a blood meal, kissing bugs can also ingest the protozoa *T. cruzi*. Once

128   inside the insect gut, *T. cruzi* have to invade surrounding tissues of the vector and

129   transform to epimastigote forms and later, in infective metacyclic forms, which are

130   eliminated with excreta and can achieve the host bloodstream through the bite site.

131   During this journey, *T. cruzi* and the resident microbiota maintain an intimate

132   interaction looking for a balance for the establishment of both.

133   Independently of gut microbiota composition, most of *T. cruzi* is destructed in

134 the first hours of vector infection. [35]After that, parasite-microbiota interaction is

135 essential to control *T. cruzi* amount. *In vitro* experiments showed that bacterial clusters

136 can adhere to *T. cruzi* surface through D-mannose recognizing fimbriae and lead to

137 parasite lysis. [35]Furthermore, a control of parasite replication is also orchestrated by

138 the local bacteria.[36]Thus, to provide continuity to its life cycle in the digestive tract of

139 triatomines and increase their chances of reaching a new host, *T. cruzi* needs to

140 overcome the microbiota trypanolytic activity. The interaction between parasite and

141 microbiota could vary among different vectors. As an example, *T. cruzi* Dm28c strain

142 when stimulated induce antibacterial activities in *Rhodnius prolixus*, resulting in fewer

143 bacteria and higher parasitemia. However, the *T. cruzi* Y strain is not able to produce

144 the same effects, being inefficient in the establishment of the infection in the vector.[37]

145   Vectors infected with *T. cruzi* synthesize antimicrobial peptides, such as

146 defensins and prolixicin, to control the expansion of the new invader, in a strain-

147 dependent manner. [38]These bioactive molecules may also affect the resident

148 microbiota richness,[34]and consequently, benefit or impair parasite survival. For

149 example, the use of a selective inhibitor of NF-κb in *R. prolixus* modulated the gene

150 expression of defensins, increasing the microbiota and reducing *T. cruzi* population.

151 [39]Furthermore, the knockdown of the antimicrobial product from *Triatoma infestans*

152 midgut (TiAP) increased by 600 times the amount of gut bacteria and, consequently,

153 reduced the number of *T. cruzi* epimastigotes.[40]So, TiAP controls microbiota growth,

154 contributing to *T*. cruzi establishment in the vector. Similarly, a Kazal-type inhibitor

155 from the midgut of *R. prolixus* (RpTI) is involved in microbiota regulation and its

156 silencing with RNA interference technology resulted in higher bacterial loads.[41]In

157 contrast, Díaz et al.[42]reported that triatomines challenged with *T. cruzi* have their

158   microbiome altered in a species-specific manner; harboring a more diverse bacterial

159   community than the negative controls. The significance of this increase in diversity

160   must be better investigated.

161

162   **3. Gastrointestinal microbiota in the parasite-mammal interface**

163   Novel bioluminescence imaging systems have evidenced the persistence of *T.*

164   *cruzi* infection in the GIT (gastrointestinal tract) during the acute and chronic Chagas

165   disease.[43, 44]The persistence of *T. cruzi* in the gut could contribute to the development

166   of GIT disorders, notably megacolon and/or megaesophagus, resulting in altered

167   peristaltic movements, dysphagia and pain. It is believed that the chronic

168   gastrointestinal symptoms of Chagas disease are a consequence of the destruction of the

169   myenteric neurons by the parasite. [45]Furthermore, continuous migration of *T. cruzi*

170   from the GIT to other organs such as the heart has been suggested, indicating that the

171   intermittent traffic of parasites can be involved in chronic Chagas cardiomyopathy.[44,

172   46]

173   In the gut, *T. cruzi* may interact with thousands of commensal bacteria, but little

174   is known about this ecological relationship. Apparently, an indirect contact should occur

175   between parasite-bacteria, since *T. cruzi* is preferentially found in the muscularis

176   externae of GIT.[47]The impact of this protozoa on microbiota profile and metabolome

177   were characterized in an immunocompetent murine model, [48]in which *T. cruzi*

178   disrupted fecal microbiome and caused biochemical alterations in a synchronized

179   manner. For example, variations in linoleic acid metabolism could be observed. [48]

180   Linoleic acid metabolism has been associated with an important immune-modulating

181   response, affecting T cell recruitment and cytokines production in the colon, [49]which

182   could favor *T. cruzi* persistence.

183   Researches on germ-free mice infected with T. *cruzi* have been performed to

184   characterize immunoregulation and clinical evolution of Chagas disease in this

185   experimental model. Silva et al., [50]showed that the lack of the natural microbiome

186   negatively influenced parasitemia intensity, mortality rate, spleen size, and cardiac

187   damage. However, the same findings were not obtained by Duarte et al.,[51]in whose

188   study the infection outcome did not alter significantly between control and germ-free

189   mice, despite a higher production of inflammatory cytokines in the first group.

190   The role of specific species of bacteria on Chagas disease immunomodulation

191   was also evaluated in germ-free mice.[52]Mono-association of *T. cruzi* with *E. coli, E.*

192   *faecalis, B. vulgatus* or *Peptostreptococcus* sp produced a Th1 immune response, higher

193   levels of IgGs and increased survival rate. Interestingly, these tested bacteria are

194   predominant in the indigenous microbiota, but there is no evidence that this population

195   group is more resistant to the development of Chagas disease clinical manifestations.[53

196   ]In this respect, characterization of the microbiome in coprolites and colon of a chagasic

197   pre-Columbian Andean mummy revealed that paleofeces were constituted

198   predominantly by Firmicutes, with *Clostridium* spp. and *Turicibacter* spp. representing

199   the most abundant bacterial genera. [54]

200   Since gut microbiome depends on intestinal health, it is expected its impairment

201   during Chagas disease, regardless of ancestry. Quantitative and qualitative analysis of

202   the microbiota in chagasic megaesophagus and health esophagus showed a more

203   elevated bacterial concentration and variability in chagasic patients, with a

204   predominance of the aerobic gram-positive bacteria *Streptococcus* sp and the anaerobic

205   *Veillonella*. [55]In the proximal jejunum of patients with megacolon, it was observed an

206   overgrowth of facultative and strict anaerobes microorganisms, which returns to

207   normality after surgical treatment.[56]

208   Dysbiosis in Chagas disease may also be associated with the emergence of

209 secondary diseases. The proliferation of certain bacteria in the esophageal lumen can

210 cause pulmonary infections, dysplasia of the esophageal mucosa and

211 cancer.[55]Individuals with a more advanced stage of esophageal dilation have elevated

212 concentrations of *Staphylococcus* sp, *Corynebacterium* sp, *Peptostreptococcus* sp and

213 *Veillonella* sp, bacteria that are capable of reducing nitrate into nitrites, which have been

214 associated with the formation of proven esophageal carcinogens nitrosamines. [57, 58]

215

216 **4. Novel approaches based on intestinal microbiota to control chagas disease**

217   In triatomines, obligate bacterial symbionts are essential to obtain some nutrients

218 from the blood-diet, without which several aspects of insect physiology would be

219 compromised, notably its development. [59, 60]It is noteworthy to note that the

220 availability of nutrients affects the vector, the *T. cruzi* population density and the

221 number of metacyclic tripomastigotes in the rectum. [61] Therefore, bacterial

222 communities in the insect gut are essential for *T. cruzi* survival.[62]Interestingly, new

223 methodologies are being developed to facilitate the characterization of triatomines gut

224 ecosystem: RADseq-based analysis was used to disclose mixed DNA from vectors

225 abdomens, enabling the determination of *T. cruzi* DTUs, microbial diversity, and blood

226 meal source. [63]

227   In this sense, it is quite plausible to think about novel strategies of *T. cruzi*

228 transmission blocking and vector control based on its microbiota (Figure 2), since the

229 traditional strategies seem to be ineffective, such as the use of insecticides. [64]Studies

230 employing antibiotic treatment, specific antibodies or rearing gnotobiotic lines has

231 brought important information about the role of intestinal bacteria on

232 parasites.[65]Triatomine engineering aiming antimicrobial peptides reduction results in

233   increased bacterial load in the midgut and decreased *T. cruzi* parasitemia, influencing

234   vector competence. [40] It is noted that production of genetically-modified vectors that

235   interferes in microbial colonization is an advantageous strategy because it can be

236   applied to all species of triatomines and impairs *T. cruzi* survival. Intestinal microbiota

237   can also be modified by RNA interference-based technologies to control vectors: *E. coli*

238   expressing specific dsRNAs for *Rhodnius prolixus* heme-binding protein and catalase

239   affected mortality, molting and oviposition rates.[66]Other examples of promising

240   alternatives to control vector infection are the use of bacteria with trypanocidal activity,

241   such as *Serratia*, a commensal of triatomine guts that deregulates *T. cruzi* mitochondrial

242   activity.[67] and the treatment of *R. prolixus* with physalin B, a natural secsteroid that

243   promotes an increase in gut bacterial microbiota and significantly decreases the number

244   of *T. cruzi*. [68]

245   In mammals, commensal microbes interact with parasites that cohabitate and

246   change the progression of the infection. Recent discoveries, [44; 43]show the intestine as

247   a preferential site of *T. cruzi*, where local bacteria can act directly on the parasite and

248   determine its infectivity. Furthermore, infection can also be modulated at distance. In

249   both cases, the mutualism developed between parasites and microbiota seems to be

250   associated with subclinical manifestations. [69]Therefore, the administration of

251   prebiotics and probiotics to replace the resident microbiota can be promising, since the

252   newly introduced bacteria will compete with the parasites for nutrients and space as

253   well as stimulate the host's immune system to react against infection.[70]Identification

254   of which prebiotics/probiotics can boost protective immune responses can contribute to

255   the success of future treatments. In this respect, oral and intraperitoneal inoculation of *L.*

256   *casei* in NIH mice resulted in reduction of circulating parasites.[71]

257   Associated to this, specific diets may contribute to the growth of the microbiota

258   species of interest that diminish the virulence and survival of the parasites.[69]High fat

259   diet and protein deficiency seems to increase parasitemia and leucocyte infiltration in

260   cardiac tissue. [72, 73] Such aspects become more evident when analyzed in germ-free

261   mice. Cintra et al. [74]showed that protein deficiency resulted in a more severe Chagas

262   disease in germ-free mice than the controls. Santos et al. [75]observed that the effect of a

263   deficient fatty acid diet on a germ-free *T. cruzi*-infected model resulted in a larger

264   amount of tripomastogotes per ml of blood and a lower survival rate.

265   New insights about which mechanisms are involved in parasite-microbiota

266   interaction are also needed. For example, the role of inflammasome should be better

267   elucidated, since its activation controls microbial dysbiosis, protecting the organisms

268   from autoinflammatory responses. However, parasites can reduce inflammasome

269   activation, promoting dysbiosis.[76]

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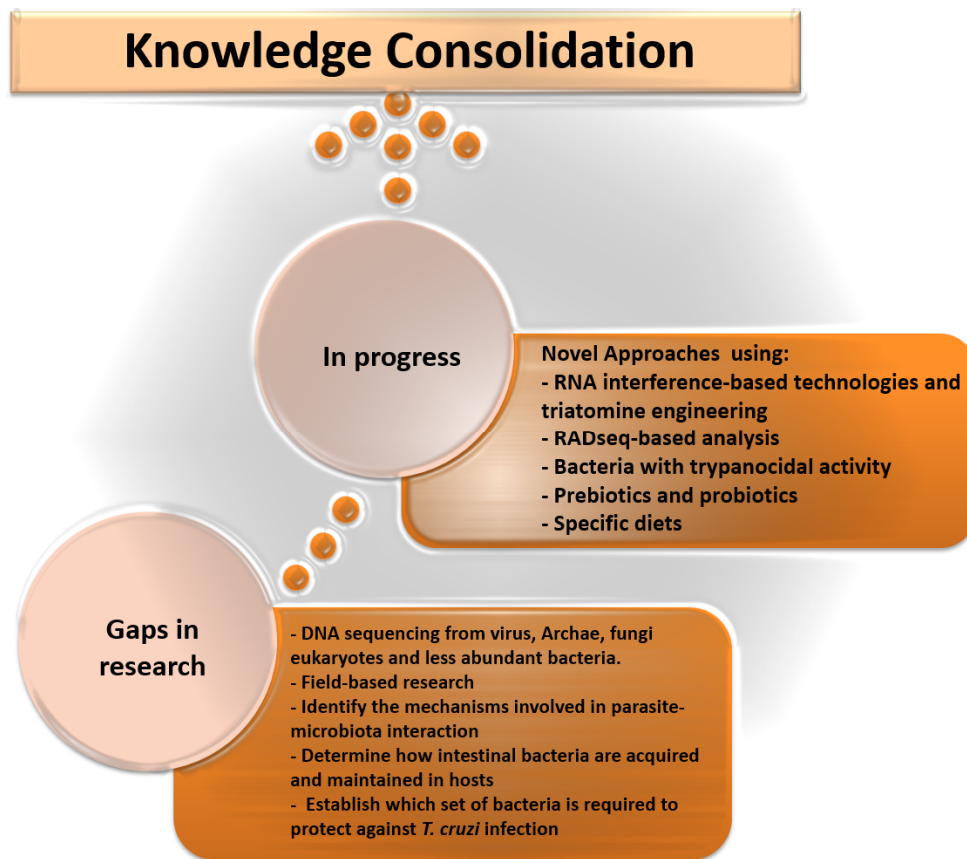
Figure 2. Challenges to consolidate the knowledge about the role of the intestinal microbiota on the vector-parasite-host triad. Gaps in research need to be fulfilled to determine the real importance of the intestinal microbiota on *T. cruzi* infection. Novel approaches are essential to elucidate crucial issues.

5. Conclusions

Reports on parasites and microbiota interaction have become extremely common because of next-generation sequencing technology. However, a bias may have been created because of the possibility of lack of DNA sequencing from less abundant, but of pathological importance, bacteria populations. Furthermore, expanding knowledge about Archae diversity [77]and its interaction with the microbiota can evidence new aspects of the complex GIT ecosystem. Also, the inclusion of virus, fungi and, eukaryotes should be considered in the next studies.

Importantly, some results may be valid for certain ecological conditions, but not to others. So, field-based research can bring to light information that could not be

286 obtained in controlled lab-models. Another research line that should be further explored

287 in order to address how intestinal bacteria are acquired and maintained in hosts and

288 which combination of bacteria could be required to protect against *T. cruzi* infection.

289 [78]Understanding the mechanisms that interfere in infection progression is essential.

290 Experiments with *T*. cruzi infected animals treated with antibiotics and recolonized with

291 specific bacteria can provide important information of how these microorganisms

292 modulate the infection. Gene exchange among microbiome-parasite-hosts is a

293 possibility that should be considered in this intimate relationship.

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295 There is no conflict of interest to be discussed.

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Highlights

- Intestinal microbiota has a deep influence on the parasite-host relationship
- In triatomines, gut microbiota can benefit or impair *T. cruzi* survival
- In mammals, *T.* cruzi-associated dysbiosis affects immune responses
- Novel approaches based on gut microbiota can be proposed to control Chagas disease