

UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI
CAMPUS UNAÍ
INSTITUTO DE CIÊNCIAS AGRÁRIAS
BACHARELADO EM CIÊNCIAS AGRÁRIAS

**Risk factors and seroprevalence of anti-*Toxoplasma gondii*
antibodies in cattle in the state of Minas Gerais, Brazil**

Bruno Montijo da Silva

Unaí
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Trabalho de Conclusão de Curso apresentado
ao Curso de Ciências Agrárias, como parte
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APROVADO em 07 /02 /2018

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29 **Risk factors and seroprevalence of anti-*Toxoplasma***
30 ***gondii* antibodies in cattle in the state of Minas Gerais,**
31 **Brazil.**

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39 **Abstract**

40 **Background**

41 *Toxoplasma gondii* is an obligate intracellular protozoan parasite responsible for
42 infections in animals and humans worldwide. In cattle, clinical cases of the infection
43 have not been reported, but the real problem of toxoplasmosis in lies in the fact that
44 the tissues of infected animals may contain *T. gondii* tissue cysts. The aim of the
45 present work was to determine seroprevalence and risk factors for *T. gondii* in cattle
46 of Unaí, Minas Gerais State, Brazil.

47 **Results**

48 Obtained a count of anti-T positive animals through the analysis of samples collected
49 (n = 612) from cattle in the city of Unaí, state of Minas Gerais, Brazil. *T. gondii*
50 antibodies from 369 animals, with a prevalence of 60.29% (95% CI: 56.35% -
51 64.10%).

52 **Methods**

53 Was conducted in the Unaí city. A total of 612 bovine blood samples (10 mL) were
54 collected from 31 herds. A comprehensive questionnaire that investigates the
55 epidemiological aspects of toxoplasmosis were used in the analysis of risk factors
56 associated with the animal-level and herd-level prevalence. The reseoreh for anti-T.
57 *gondii* antibodies was performed IFAT.

58 **Conclusion**

59 The results of the present indicated that infection of dairy cattle with *T. gondii* is
60 widespread in Unaí, Minas Gerais State, which is of public health concern and has

61 implications for prevention and control of toxoplasmosis in this region. Therefore,
62 integrated control strategies and measures are recommended to prevent and control *T.*
63 *gondii* infection in dairy cattle.

64 **Key words**

65 Protozoan, parasite, seroprevalence, epidemiological, prevalence.

66 **Background**

67 *Toxoplasma gondii* is an obligate intracellular protozoan parasite responsible for
68 infections in animals and humans worldwide. Domestic cats and other felids are
69 definitive hosts, and almost all homeothermic animals, including domestic animals
70 and humans, are intermediate hosts [1].

71 In cattle, clinical cases of the infection have not been reported, but the real problem of
72 toxoplasmosis in cattle lies in the fact that the tissues of infected animals may contain
73 *T. gondii* tissue cysts. Insufficiently cooked meat and poor personal hygiene
74 principles during cooking may cause latent or even clinical infections in humans [2].

75 Therefore, interest in *T. gondii* in cattle stems mainly from a public health
76 perspective; if cattle carry infectious tissue cysts they may be an important source of
77 human infections since beef is often consumed undercooked [3].

78 The role of beef in human *T. gondii* infection is unclear. There is evidence that
79 suggests an important role for beef as a source of human infection. Eating raw beef
80 has been reported as one of the risk factors that predicts acute infection [4-5-6], and in
81 four out of 26 human toxoplasmosis outbreaks between 1965 and 2001 the most
82 probable source was the consumption of raw or undercooked beef [7].

83 Information about the seroprevalence of *T. gondii* in domestic ruminants is of interest
84 to implement future strategies on public health programs and to define the cycle of
85 this protozoan parasite in countries.

86 The aim of the present work was to determine seroprevalence of and risk factors for *T.*
87 *gondii* infection in cattle of Unaí, Minas Gerais State, Brazil.

88 **Methods**

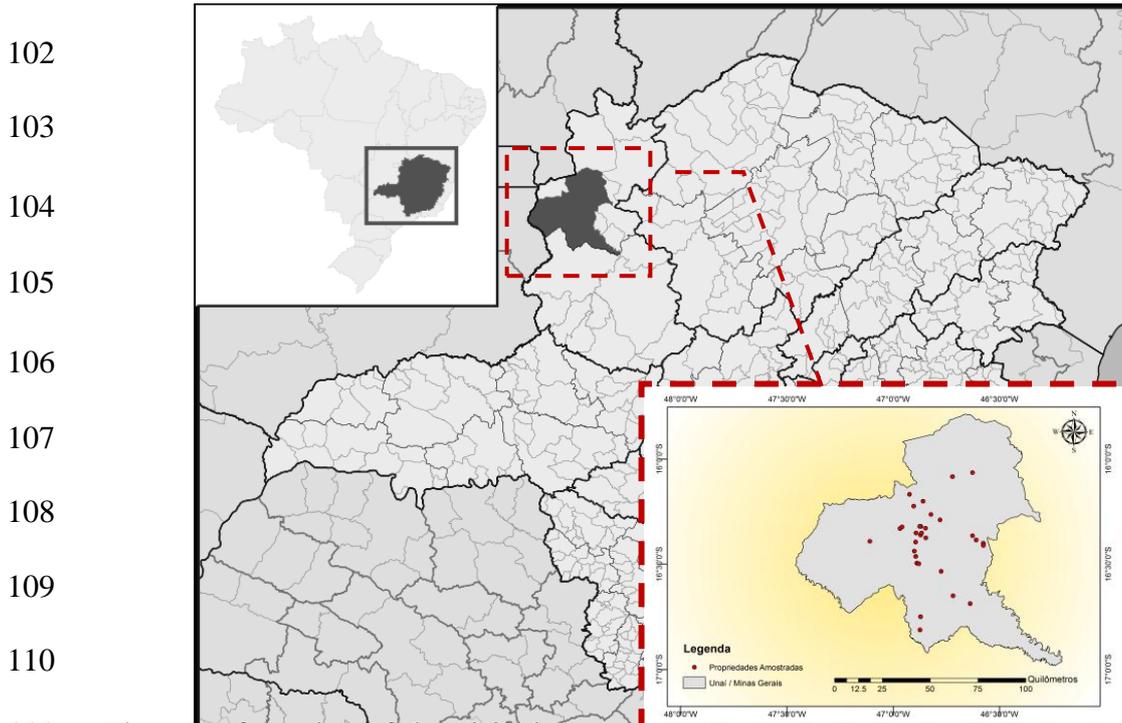
89 **Ethics statement**

90 In this study, all procedures using animals complied with the Ethical Principles in
91 Animal Research adopted by the College of Animal Experimentation (COBEA) and
92 were approved by the Ethical Committee for Animal Welfare, IPESA, Minas Gerais
93 State, Brazil.

94 Study areas and Spatial analysis

95 The survey was conducted in the Unaí city (16°21'50"S and 46°54'15"O), which
 96 extends over 8 447.107 km² in Minas Gerais State, in Southeast Brazil [8]. The region
 97 is mainly hilly and is 640m above sea level. The average annual temperature is 27°C.
 98 According to the Köppen classification, the climate is of Aw type (i.e., tropical wet
 99 and dry or savanna climate; with the driest month having precipitation less than 60
 100 mm (2.4 in) and less than 4% of the total annual precipitation).

101 **Figure 1.** Point locations of *T. gondii* dairy herds in the studied subpopulation.



111 The georeferencing of the visited properties was carried out with a GPS device. The
 112 data were then transported to the ArcGIS 10.1, Geographic Information System (GIS),
 113 where they were placed on the Unaí/MG map. The database modeling and chart
 114 plotting stages were carried out at the Institute of Agrarian Sciences, Federal
 115 University of the Jequitinhonha and Mucuri Valleys.

116 Sample size

117 The number of samples was calculated assuming that the prevalence of *T. gondii* is
 118 approximately 50% in order to maximize the sample size, obtain a minimal
 119 confidence interval of 95.0%, and maintain the statistical error under 5%. Calculations
 120 were executed using an EpiInfo program (CDC, version 7.2.0.1), resulting in a sample
 121 size of 384 bovines.

122 Study animals and Blood collection

123 A total of 612 bovine blood samples (10 mL) from 31 herds were collected by
 124 venocentesis and centrifuged at 1000 g for 10 min. Cattle from each farm were

Seroprevalence of anti-*Toxoplasma gondii* antibodies in cattle in Brazil.

125 selected randomly using a table of random digits. In cattle, approximately 10% on
126 each farm were sampled. All of the animals sampled were clinically healthy. The
127 serum samples were stored at -20°C for further analysis.

128 **Serological examination**

129 To search for anti-*T. gondii* antibodies, IFAT was performed [9]. To obtain the
130 antigens, the “RH” strain was used, as described by Camargo [10]. The sera were
131 diluted in a saline buffer solution containing 0.1M phosphate, pH 7.2 (PBS), with cut-
132 off points 64 for bovine species. A commercial conjugate (Sigma Chemical - F7887)
133 was used for species, and positive and negative controls were used for each slide.
134 Reactions in which the tachyzoites presented total peripheral fluorescence were
135 defined as positive.

136 **Questionnaire**

137 A comprehensive questionnaire that investigates the epidemiological aspects of
138 toxoplasmosis were used in the analysis of risk factors associated with the animal-
139 level and herd-level prevalence. The analysed variables and respective categories
140 were as follows: management system, herd size, technical monitoring, stock up
141 facility for food, surface type of milking shed, methods of cleaning, water source,
142 feeder, dogs access locations, stage of gestation that abortions occurred.

143 **Statistical and spatial analysis**

144 The animal and herd prevalences were estimated by defining the specific weight of
145 each sample unit in the universe of herds and animals included in the study [11-12].
146 Risk factors associated with the presence of anti-*T. gondii* antibodies were estimated
147 by applying a structured questionnaire on each farm. The questions addressed
148 economic aspects, animal management, and biosecurity practices. A dataset was built
149 and then analysed using the statistical software STATA® (version 11). It was adopted
150 a Poisson regression model to run the analysis due the high animal and herd
151 prevalence. The regression uses the count of seropositive animals as the outcome to
152 estimate the Incidence Rate Ratio (IRR). Initially, the univariate model selected
153 variables with a significance level below 0.15, and later, in the multivariate model, the
154 significance level was 0.05. Thus, the statistically significant variables should be
155 understood as herd risk factors that elevate the number of seropositive animals inside
156 the herd.

157 **Results**

158 The analysis of the samples (n=612) of cattle collected from the municipality of Unai,
159 Minas Gerais State, showed a count of animals positive for anti-*T. gondii* antibodies.
160 of 369 animals, with a prevalence of 60.29% (95% CI: 56.35% - 64.10%). The results
161 of this study are presented in Table 1.

162 **Table 1.** Detection of anti-*T. gondii* antibodies (IFAT-IgG) in bovine sera from the
 163 Unaí municipality, Minas Gerais state, Brazil.

Species	IFAT		Total
	Reagent n/%	Non-reagent n/%	
Bovine	369 (60.29)	243 (39.71)	612

164 A total of 63 variables were analyzed, using the STATA 11 program, based on the
 165 questionnaires applied to the properties.

166 All the farms analyzed presented seropositive animals, but there was no association
 167 ($P \geq 0.05$) between the different types of exploitation, management and facilities used
 168 in the properties. There was no significant association ($P \geq 0.05$) between the observed
 169 reproductive factors. There was a significant association ($P < 0.05$) for the properties of
 170 11 to 100 hectares, being a protection factor, decreasing the count of positive animals.

171 Discussion

172 Out of 612 animals examined 369 (60.29%, 95% CI: 56.35% - 64.10%) were
 173 seropositive for *T. gondii* antibody. Different studies revealed that the prevalence from
 174 0 to 100% was recorded in different areas of the world [13]. This difference in
 175 prevalence is depending up on cat density, climate condition, age of the animals,
 176 species, sex, altitude and management of animal production [14-15].

177 There was a significant association ($P < 0.05$) for the properties of 11 to 100 hectares,
 178 being a protection factor, decreasing the count of positive animals. This fact may have
 179 occurred due to the greater sanitary control in the management of these properties.

180 The high prevalence of *T. gondii* in large [16-17-18] and small [19-20-21-22]
 181 ruminants poses a direct human health risk because encysted bradyzoites in the
 182 muscle retain infectivity across a wide temperature range. Therefore, beef that has
 183 been inappropriately frozen or cooked might cause infection in a consumer. Cases of
 184 human toxoplasmosis from these areas confirm that the risk of toxoplasmosis
 185 suggested by high seropositivity in food animals is reflected in the presence of human
 186 disease [23-24]. The present study, although based on a modest sample size, is
 187 important because a significant and widely distributed threat to human health in food
 188 animals has been detected.

189 A major reason for the control of *T. gondii* infection in meat animals is reduction of
 190 the reservoir of human infection. Cattle are generally thought not to be significant in
 191 this context [25]. However, beef is often consumed undercooked ('rare' beef steaks,
 192 roast beef, steak tartar), and at least one outbreak of toxoplasmosis whose source was
 193 raw beef has been documented [7]. In addition, one out of four beef samples randomly
 194 chosen from UK retail outlets tested positive for *T. gondii* by PCR [26]. These facts,

Seroprevalence of anti-*Toxoplasma gondii* antibodies in cattle in Brazil.

195 along with the circumstantial evidence provided by the above-mentioned data on the
 196 high prevalence of cattle infection in Brazil, countries in which human infection is
 197 highly prevalent as well, all suggest that the role for cattle as a *T. gondii* reservoir for
 198 human infection should be reconsidered.

199

200 **Conclusions**

201 The results of the present survey indicated that infection of dairy cattle with *T. gondii*
 202 is widespread in Unaí, Minas Gerais State, which is of public health concern and has
 203 implications for prevention and control of toxoplasmosis in this region. Therefore,
 204 integrated control strategies and measures are recommended to prevent and control *T.*
 205 *gondii* infection in dairy cattle.

206 **Authors' contributions**

207 TRS conceived and designed the study, drafted the manuscript. RRN critically revised
 208 the manuscript. BMS, WCCQ, MOM conducted studies which generated serum
 209 samples used in the present study, and also revised the manuscript. BMS, WCCQ
 210 performed all serological screening and data analysis, and drafted the manuscript. All
 211 authors read and approved the final manuscript.

212 **Acknowledgements**

213 Institution that released the PIBC/UFVJM stock exchange.

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297 **Figures**

298 **Figure 1.** Point locations of *T. gondii* dairy herds in the studied subpopulation

299 **Tables**

300 **Table 1.** Detection of anti-*T. gondii* antibodies (IFAT-IgG) in bovine sera from the
 301 Unaí municipality, Minas Gerais state, Brazil.

302 **Additional files**

303 "Not applicable"

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