

**HOST SUSCEPTIBILITY, BIOCHEMICAL AND PHYSICAL CHANGES IN
TOXOPLASMA GONDII INFECTION: A FOCUS ON ABO BLOOD
GROUPS AND HEPATIC INDICATORS**

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HOST SUSCEPTIBILITY TO TOXOPLASMA: ROLE OF ABO AND HEPATIC INDICATORS

ВОСПРИИМЧИВОСТЬ К ИНФИЦИРОВАНИЮ TOXOPLASMA GONDII: ГРУППЫ КРОВИ АВО И ПОКАЗАТЕЛЕЙ ФУНКЦИИ ПЕЧЕНИ

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ВОСПРИИМЧИВОСТЬ, БИОХИМИЧЕСКИЕ И ФИЗИЧЕСКИЕ ИЗМЕНЕНИЯ ПРИ ИНФЕКЦИИ TOXOPLASMA GONDII: РОЛЬ ГРУПП КРОВИ АВО И ПОКАЗАТЕЛЕЙ ФУНКЦИИ ПЕЧЕНИ

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Abstract

Background *Toxoplasma gondii* is an obligate intracellular protozoan parasite that infects almost all warm-blooded animals, including humans. **Objectives:** This study investigated the association between toxoplasmosis infection and potential risk factors such as animal contact and blood type, in addition to analyzing selected serum biochemical parameters. **Methods:** between **December 2022 and May 2023** in **Baghdad, Iraq**, and involved **pregnant women aged between 21 and 25 years**. A total of **134 venous blood samples** were collected from participants attending medical centers and maternity units in the city. **Results:** demonstrated that there was **no statistically significant association** between toxoplasmosis and interaction with animals ($p > 0.05$), indicating that animal contact alone may not be a sufficient risk factor in the studied population. Interestingly, **blood type emerged as the only variable showing a statistically significant positive association** with *T. gondii* infection, suggesting a possible genetic or immunological predisposition related to certain blood groups. Furthermore, although not statistically significant, infected individuals displayed **slightly lower mean serum levels of liver enzymes** — aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) compared to non-infected controls. **Conclusion:** This finding can indirectly suggest the possible slight, hepatic involvement in latent or chronic toxoplasmosis and justifies further research. These results help us further understand the pathogenesis and epidemiology of toxoplasmosis and why there must be more information about its prevention on the population level, particularly among receptive aged people. These factors should be addressed to avoid negative effects and secure the health of next generations who can contribute to the country development.

Keywords: *Toxoplasma gondii*, Blood Group, Abortion, Liver Function, pregnant women, Physical changes.

Аннотация

Введение. *Toxoplasma gondii* — облигатный внутриклеточный простейший паразит, инфицирующий почти всех теплокровных животных, включая человека. **Цели:** В настоящем исследовании была изучена связь между токсоплазмозом и потенциальными факторами риска, такими как контакт с животными и группами крови человека, а также проанализирован ряд биохимических параметров сыворотки крови. **Методы:** Исследование беременных женщин в возрасте от 21 до 25 лет проводилось в период с декабря 2022 по май 2023 года в Багдаде, Ирак. Всего было собрано 134 образца венозной крови женщин, посещавших медицинские центры и родильные отделения города. **Результаты:** Было показано, что между токсоплазмозом и контактом с животными не обнаружено ($p > 0,05$) статистически значимой связи, что указывает на то, что для оценки риска в исследуемой популяции одного лишь контакта с животными может быть недостаточно. Важно, что группа крови человека оказалась единственным параметром со статистически значимой положительной связью с инфицированием *T. gondii*, что предполагает возможную генетическую или иммунологическую предрасположенность с определенными группами крови. Кроме того, хотя статистически значимых различий не выявлено, у инфицированных лиц наблюдались более низкие средние уровни сывороточных ферментов печени — аспаратаминотрансферазы (АСТ), аланинаминотрансферазы (АЛТ) и щелочной фосфатазы (ЩФ) — по сравнению с неинфицированными лицами контрольной группы. **Вывод:** Полученные данные косвенно указывают на возможное умеренное поражение печени при латентном или хроническом токсоплазмозе и обосновывают необходимость проведения дальнейших исследований. Эти результаты помогают лучше понять патогенез и эпидемиологию токсоплазмоза и обосновывают необходимость улучшения понимания по профилактике токсоплазмоза на популяционном уровне, особенно среди восприимчивых пожилых людей. Указанные факторы следует

учитывать, чтобы избежать негативные последствия и обеспечивать здоровье будущих поколений, которые могут внести свой вклад в развитие страны.

Ключевые слова: *Toxoplasma gondii*, группа крови, аборт, функция печени, беременные женщины, физические изменения.

1 **1 Introduction**

2 Toxoplasma gondii is an obligate intracellular protozoan parasite, widely
3 distributed across the globe; it naturally infects almost all warm blooded animals
4 including people with the cat, and especially the domestic cat family, being its
5 definitive host. The parasite is highly adaptive as it has modified itself to harbor
6 various environmental conditions and many intermediate hosts which include
7 livestock, rodents, birds and humans [1]. Human beings become infected after taking
8 food or water that contains oocysts that are shed in cat feces, eating undercooked or
9 raw meat that may contain tissue cysts, vertical transmission in which the mother
10 infects the unborn baby or rarely through organ transplantation or blood transfusion
11 [2]. Seroprevalence of T. Gondii infection is different across the globe depending on
12 geographical locations, culture, climate, and socioeconomic status [2]. The varying
13 diet (e.g., uncooked or poorly cooked meat), sanitation and also the exposure to
14 domestic animals are other considerable risk factors leading to the differences in the
15 infected rates [3]. Epidemiological reports in the recent past show that there is a
16 seroprevalence of up to 60 percent to as low as 10 percent depending on the area. As
17 an example, a seroprevalence of T. gondii in female undergraduate students of 66.5
18 percent in Jordan [4] and 100 percent positivity of IgG but a small proportion (9.4
19 percent) of IgM of university students in Saudi Arabia [5]. Research done locally in
20 Thi-Qar province indicated the prevalence rate of toxoplasmosis to be 21.94 percent
21 among the university students, whereas an almost equal proportion of the IgM and
22 IgG seropositivity was reported at Kirkuk University resulting in the total of 21.5
23 percent prevalence rate [6,7]. All these numbers indicate the current public health
24 importance of T. gondii, particularly when considering populations at risk of
25 increased exposure. On a biological level, the parasite has a sexual and an asexual
26 phase of its lifecycle. Tissue cysts may last through the life of the host in
27 intermediate hosts. In case of immune deficiency, there is a potential reactivation
28 that may result in severe clinical manifestation such as ocular toxoplasmosis,

29 encephalitis, and congenital infection [8]. Strain of parasites, exposure dosage, mode
30 of transmission, and host genetic and immunological status are factors that
31 determine the level of severity of infections [9]. Recently, it has been highlighted that
32 extensive efforts are needed to determine the local epidemiology and host risk
33 factors so that effective prevention and awareness measures are developed especially
34 among young adults and women of childbearing age. It is important to note that
35 latent toxoplasmosis can affect not only maternal-foetal health, but also be related
36 to certain alterations in the biochemical parameters, which could have an influence
37 on the liver functions and overall metabolic balance [10,11].

38 **Study Design and Sample Collection**

39 This cross-sectional study was conducted between December 2022 and May
40 2023 in Baghdad, Iraq, and involved pregnant women aged between 21 and 25 years.
41 A total of 134 venous blood samples were collected from participants attending
42 medical centers and maternity units in the city. The mean age of the participants was
43 21.25 years, with the majority falling within the broader age group of 20 to 40 years.

44 **Serological Testing for *Toxoplasma gondii***

45 To detect prior and recent infections with *T. gondii*, Enzyme-Linked
46 Immunosorbent Assay (ELISA) was employed. A commercially available ELISA
47 kit (ACON Laboratories, San Diego, CA, USA) was used to measure anti-
48 Toxoplasma IgG and IgM antibodies in serum samples. All procedures were carried
49 out according to the manufacturer's instructions.

50 **ABO Blood Group Typing**

51 ABO blood group typing was performed using monoclonal antisera for anti-
52 A and anti-B antigens (Atlas Medical, UK). A standard slide agglutination technique
53 was applied. One drop of whole blood was mixed with one drop of antisera on a
54 clean glass slide and gently stirred.

55 **Liver Function Tests (LFTs)**

56 To evaluate the potential impact of *T. gondii* infection on hepatic function,
57 serum liver enzyme levels were assessed for all participants. Testing was conducted
58 using an automated biochemical analyzer and standard enzymatic colorimetric assay
59 kits obtained from a German manufacturer (Biotechnologies, Germany). All
60 procedures were performed under strict adherence to the manufacturer's protocols,
61 ensuring accuracy and reproducibility of results.

62 **Ethical Considerations**

63 All participants provided informed consent prior to sample collection. The
64 study protocol was reviewed and approved by the Ethical Committee of the College
65 of Pharmacy, University of Baghdad. Confidentiality of participant data was strictly
66 maintained throughout the study.

67 **Sample Collection**

68 A total of 5 mL of venous blood was collected from each participant using
69 sterile disposable syringes under aseptic conditions. Blood was drawn from the
70 antecubital vein and transferred immediately into plain (non-anticoagulant)
71 vacutainer tubes, designated for both blood group testing and serological assays.
72 Following collection, the blood samples were allowed to clot at room temperature
73 (approximately 22–25 °C) for 15–30 minutes to facilitate serum separation. The
74 clotted samples were then centrifuged at 3000 revolutions per minute (rpm) for 15
75 minutes using a bench-top centrifuge. This process ensured effective separation of
76 the clear serum from the cellular components. After centrifugation, the sera were
77 carefully aliquoted into labeled sterile Eppendorf tubes using micropipettes. These
78 aliquots were prepared for the anti-Toxoplasma IgM and IgG ELISA assays. To
79 maintain the integrity and stability of the antibodies, serum samples were promptly
80 stored at –20 °C in a dedicated medical-grade freezer until laboratory analysis. All
81 sample processing steps were conducted under controlled laboratory conditions
82 using personal protective equipment (PPE) to minimize contamination and ensure
83 biosafety compliance.

84 **Statistical Analysis**

85 Percentages and other results were created using Microsoft Office Excel
86 2016. The SAS (2012) program determined how various factors affected the
87 study's parameters. This study used the chi-square test to compare percentages
88 meaningfully; a meaningful link had a P value of 0.001 or lower.

89 **2 Result and discussion**

90 In this study, a total of 150 schoolchildren were examined to assess the
91 relationship between *Toxoplasma gondii* infection and contact with cats. As shown
92 in(Figure 1), there was a notable variation in the number of positive and negative
93 cases according to the level of interaction with cats. Among pupils reporting direct
94 and frequent contact with cats, 10 out of 30 tested positive, indicating a relatively
95 high infection rate of 33.3%. In contrast, none of the 10 individuals reporting limited
96 or occasional contact tested positive. Meanwhile, 23 of the 110 pupils who stated
97 they had no contact with cats were seropositive, representing a 20.9% infection rate.
98 Statistical analysis using the chi-square test revealed a significant association
99 between cat contact and toxoplasmosis seropositivity ($\chi^2 = 11.99$, degrees of
100 freedom = 2, $P = 0.0054$), confirming that this relationship is statistically
101 significant. These findings align with recent research emphasizing the pivotal role of
102 cats in the transmission of *T. gondii*. Li et al. (12) highlighted that direct or indirect
103 exposure to cat feces, which contain infectious oocysts, remains one of the primary
104 routes of human infection, especially among children who are prone to close
105 interaction with cats and contaminated environments [1]. Similarly, Khalil et al. (13)
106 described cats as definitive hosts that shed environmentally resistant oocysts,
107 contributing to prolonged contamination of soil and surfaces, thereby increasing
108 infection risk in populations with frequent cat exposure [2]. But the idea that 20.9
109 percent of the seropositive denied any contact with cats implies other ways of
110 transmission. In a study by Nguyen et al. (14) it was revealed that undercooked meat,
111 consumption of fresh, non-washed fruits and vegetables and contaminated water are

112 also major contributions of *T. gondii* infection as non-feline sources particularly in
113 areas that do not boast of a good sanitation and food safety systems [3]. These
114 alternative tracks have also been emphasized by Li et al. (12), especially in low
115 economic regions since the hygiene activities might not be high to stop the
116 environmental transmission [1]. In conclusion, the analysis demonstrates a
117 statistically significant association between *T. gondii* infection and contact with cats
118 among schoolchildren, highlighting the importance of public health interventions.
119 Raising awareness about the risks of toxoplasmosis and promoting preventive
120 behaviors—such as regular handwashing, proper food handling, and minimizing
121 direct exposure to cat feces—are crucial, particularly in environments frequented by
122 children.

123 The present study demonstrated a statistically significant association between
124 *Toxoplasma gondii* infection and ABO blood group types among a cohort of 150
125 schoolchildren. The prevalence of toxoplasmosis was highest in individuals with
126 blood group A (41.2%), followed by blood groups B (26.5%), AB (14.3%), and O
127 (11.5%). The chi-square analysis revealed a significant difference ($\chi^2 = 12.348$, $df =$
128 3 , $p = 0.006$), suggesting that individuals with certain blood groups—particularly A
129 and B—are more susceptible to *T. gondii* infection compared to those with blood
130 groups O and AB. These findings align with previous reports proposing an
131 immunogenetic basis for susceptibility to parasitic infections, including
132 toxoplasmosis. ABO antigens are known to influence host–pathogen interactions by
133 serving as receptors or modulating immune responses. Blood group A, characterized
134 by the presence of N-acetylgalactosamine antigen, may facilitate parasite adhesion
135 or immune evasion, enhancing vulnerability to infection (15,16). Dardé (17)
136 emphasized the role of host genetics, including blood group antigens, in determining
137 susceptibility to *T. gondii*, proposing that immune responses against the parasite may
138 vary based on antigenic expression (17). Mangi et al. (18) found that in a Pakistani
139 population, there was a higher prevalence of toxoplasmosis in the blood group A and

140 a low prevalence in the blood group O (18), thus confirming this hypothesis.
141 Likewise, our findings indicated that the blood group O, although dominant in the
142 sampling, was the least infected whereby there is a form of protection with patients
143 of this blood group due to the protection presented by the lack of A or B antigens
144 from the parasite to attach or infect the host cell. Further on, Moln r et al. (19)
145 postulated that a larger innate immune response in people with blood type O could
146 be much more active, per especially with regard to neutrophil signaling that is
147 relevant to controlling intracellular pathogens including *T. gondii* (19). Recent
148 evidence has endorsed the idea that ABO blood group polymorphism may contribute
149 to the susceptibility to not only *T. gondii* but other pathogens, altering cytokine
150 profiles, antibody production, and cellular immune system activity (9, By way of
151 example, a 2022 overview by Ahmadpour et al. demonstrated that the disparity in
152 patterning of glycosylation between blood group antigens could alter the immune
153 milieu remodelling the pathogenesis or chronic of toxoplasmosis (20). Moreover,
154 research by Lin et al. (21) showed that host surface glycoproteins, influenced by
155 ABO gene expression, could either facilitate or impede *T. gondii* adhesion and
156 invasion (22).

157 This study investigated the distribution of *Toxoplasma gondii* seropositivity
158 across different age groups among 150 individuals. As shown in figure 3, the number
159 of positive cases was highest in individuals under 20 years of age (11 cases),
160 followed by those over 40 years (10 cases). The 20–30 age group accounted for 8
161 cases, while the lowest number was reported in the 30–40 age group with only 4
162 positive cases. In total, 33 individuals were seropositive for *T. gondii*. Chi-square
163 analysis revealed a value of 3.485 with 3 degrees of freedom and a p-value of 0.323,
164 indicating no statistically significant association between age group and the
165 prevalence of toxoplasmosis ($P > 0.05$). Although the observed differences suggest
166 that younger (<20) and older (>40) individuals may carry a higher burden of
167 infection, the variation was not sufficient to demonstrate a significant trend within

168 the studied population. Despite the lack of statistical significance, these age-related
169 patterns are in line with several recent reports that describe higher *T. gondii*
170 seroprevalence in both younger and older populations. Children and adolescents,
171 especially those under 20 years, are often exposed through poor hand hygiene, soil
172 contact, and interaction with cats or contaminated food, which facilitates infection.
173 According to Fereig et al. (23), school-aged children in rural and peri-urban areas
174 showed elevated rates of infection due to environmental exposure and insufficient
175 awareness of transmission risks.

176 Conversely, increased infection rates in individuals above 40 years may
177 reflect cumulative lifetime exposure. Older individuals are more likely to have had
178 prolonged contact with sources of infection, such as contaminated food or
179 undercooked meat, which is supported by findings from Foroutan et al. (24) and
180 Bigna et al. (25), who reported a consistent age-related increase in seroprevalence
181 of toxoplasmosis in various regions worldwide. However, other studies emphasize
182 that age alone may not be an independent risk factor, but rather a proxy for lifestyle
183 behaviors and environmental exposure. Dardé and Mercier (26) argue that variations
184 in infection rates across age groups are largely influenced by dietary habits, hygiene,
185 socioeconomic status, and cultural practices rather than intrinsic age-related immune
186 differences. Therefore, while this study did not find a statistically significant age
187 effect, the trend aligns with broader epidemiological data suggesting that both the
188 very young and the elderly remain important target groups for prevention and public
189 health education regarding toxoplasmosis transmission.

190 The analysis of liver enzyme concentrations among individuals infected and
191 not infected with *Toxoplasma gondii* revealed interesting patterns. The mean serum
192 AST (aspartate aminotransferase) levels were notably lower in the toxoplasmosis-
193 positive group (23.2 ± 10.5 U/L) compared to the negative group (32.1 ± 1.48 U/L).
194 ALT (alanine aminotransferase) values were similar between both groups, with
195 means around 21 U/L, while ALP (alkaline phosphatase) levels showed only a slight

196 decrease in the positive group (149.1 ± 8.44 U/L) compared to the negative group
197 (153.2 ± 49.5 U/L). These findings suggest that *T. gondii* infection might not be
198 associated with elevated liver enzymes indicative of acute hepatic injury in this
199 cohort. This aligns with recent studies indicating that while *T. gondii* can affect the
200 liver, especially in immunocompromised patients, many infected individuals
201 maintain normal or even slightly decreased liver enzyme levels, possibly reflecting
202 subclinical or chronic infection phases (27,28). The wider standard error of the mean
203 (SEM) observed for AST levels in the toxoplasmosis-positive group suggests
204 notable variability among individuals. This variation may stem from differences in
205 the phase of infection (acute vs. chronic), individual immune responses, or the
206 presence of comorbid conditions influencing hepatic function. In contrast, the
207 relatively stable ALT and ALP values across both seropositive and seronegative
208 groups support the notion that *Toxoplasma gondii* infection does not typically lead
209 to overt hepatocellular or cholestatic injury detectable through standard liver
210 function testing. However, liver involvement can become more apparent in
211 vulnerable populations, particularly immunocompromised individuals such as those
212 undergoing chemotherapy, organ transplant recipients, or patients with advanced
213 HIV. In these cases, clinical presentations may include hepatomegaly, elevated
214 transaminases, or granulomatous hepatitis, which have been increasingly
215 documented in recent literature (29,30,31). Thus, although routine liver enzyme
216 screening may not be essential for all patients, it remains a valuable diagnostic tool
217 for monitoring hepatic complications in at-risk groups.

218 Based on the ROC curve extracted from the provided image, the ALP marker
219 demonstrated an approximate area under the curve (AUC) of 0.613, indicating a
220 weak-to-moderate discriminatory ability, while ALT showed an AUC of 0.377,
221 suggesting performance worse than random guessing (AUC = 0.5). Such a low value
222 for ALT may reflect reversed threshold labeling, class misassignment, or artifacts
223 from image-based data extraction rather than true clinical performance. No reliable

224 AUC estimate could be obtained for AST due to insufficient point extraction.
225 Clinically, ALP is typically elevated in cholestatic or obstructive liver injury
226 patterns, whereas ALT (and AST) is more specific to hepatocellular injury; however,
227 the diagnostic utility derived from ROC curves depends on the clinical context and
228 the reference standard used. Given that AUC values below 0.7 are generally
229 considered suboptimal for clinical decision-making (32,33,34), these findings
230 suggest limited standalone diagnostic value. Confirmatory analysis using the
231 original dataset, with statistical comparison of ROC curves (e.g., DeLong's test) and
232 calculation of 95% confidence intervals, shown figure 5.

233 **3 Conclusion**

234 The findings of this study indicate that, within the investigated population,
235 animal contact alone does not constitute a significant risk factor for *Toxoplasma*
236 *gondii* infection, whereas blood type shows a statistically significant association,
237 suggesting a potential genetic or immunological predisposition linked to certain
238 blood groups. Although the observed reduction in mean serum levels of AST, ALT,
239 and ALP among infected individuals was not statistically significant, it may reflect
240 mild hepatic involvement during latent or chronic stages of the disease. These results
241 emphasize the importance of considering both host-related factors (such as blood
242 type) and subtle biochemical changes in understanding the epidemiology and
243 pathogenesis of toxoplasmosis.

244 **Recommendations**

245 It is recommended to develop targeted public health campaigns focusing on
246 high-risk blood groups to raise awareness about prevention and early detection of
247 toxoplasmosis. Further large-scale studies should be conducted with broader
248 demographic coverage to confirm the association between blood type and *T. gondii*
249 infection and to explore the underlying mechanisms. Routine screening for
250 toxoplasmosis should include liver enzyme assessments, particularly for individuals
251 with chronic or latent infections, to detect subtle hepatic involvement. Preventive

252 education programs on safe food handling, hygiene, and general preventive
253 measures must be enhanced, especially among reproductive-age individuals, to
254 reduce the risk of congenital transmission. Additionally, toxoplasmosis screening
255 and preventive measures should be integrated into maternal and reproductive health
256 programs to safeguard the health of future generations and contribute to national
257 development.

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259 None

260 **.Conflict of interest :**

261 There are no conflicts of interest , according to the author

262 **.Funding :**

263 None

РИСУНКИ

Figure 1. Disease relationship with contact with animals

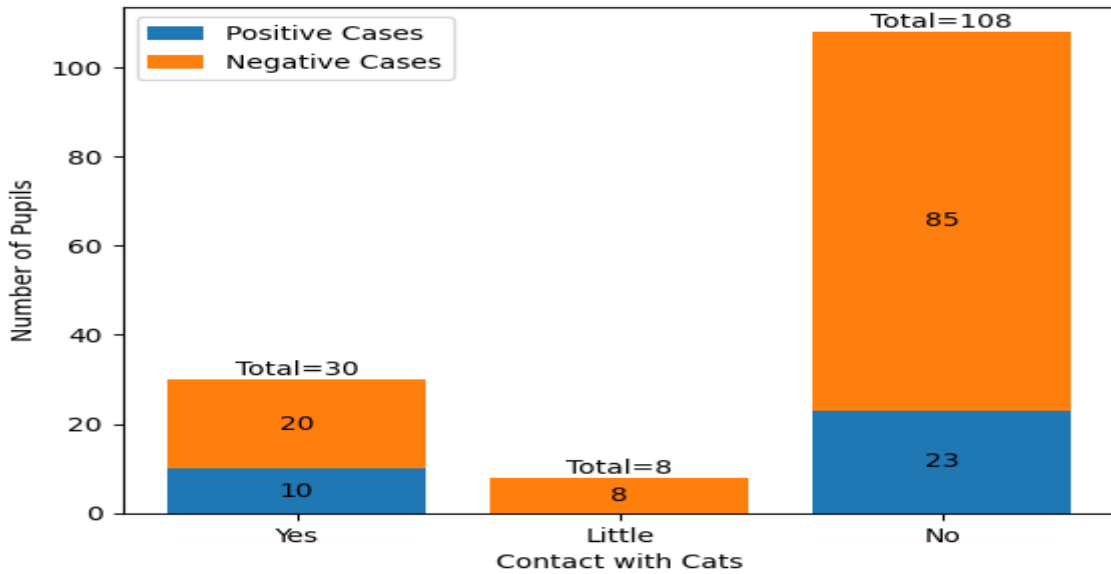


Figure 2. The relationship of blood groups with disease

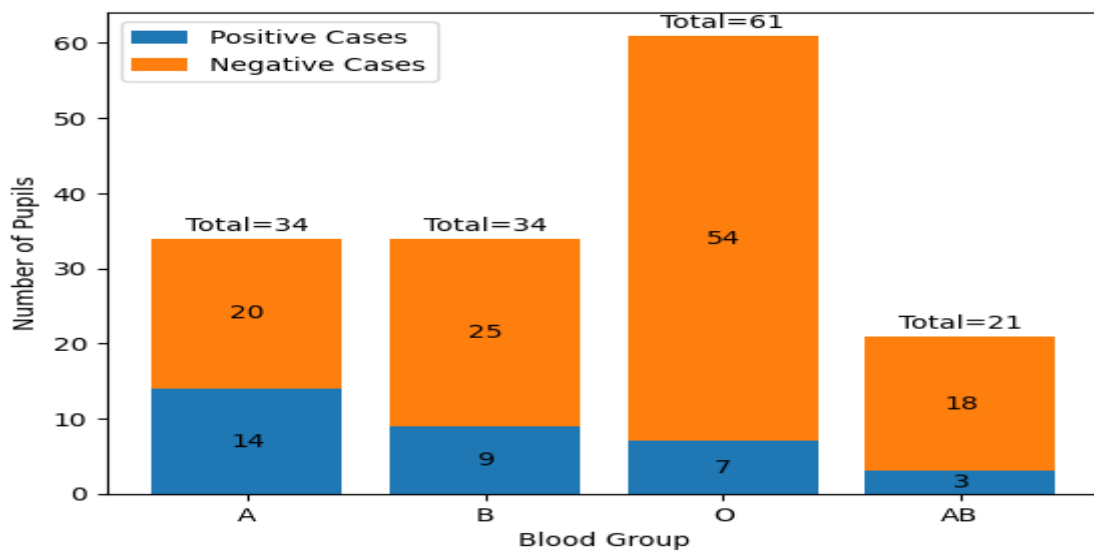


Figure 3. The relationship of age groups with disease

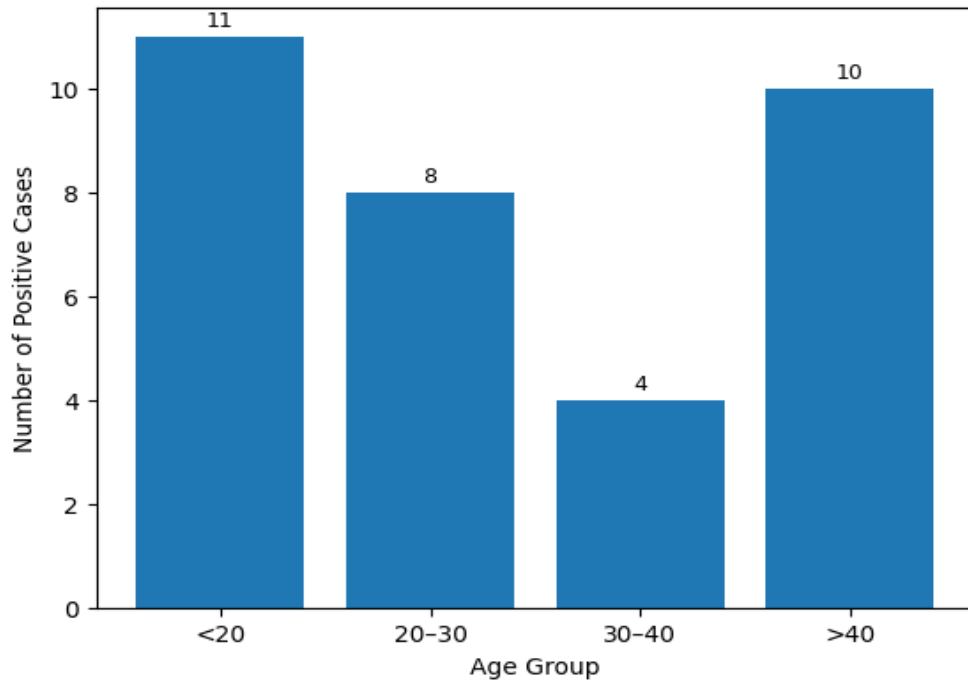


Figure 4. Average of AST, ALT and ALP concentration in persons infected with toxoplasmosis.

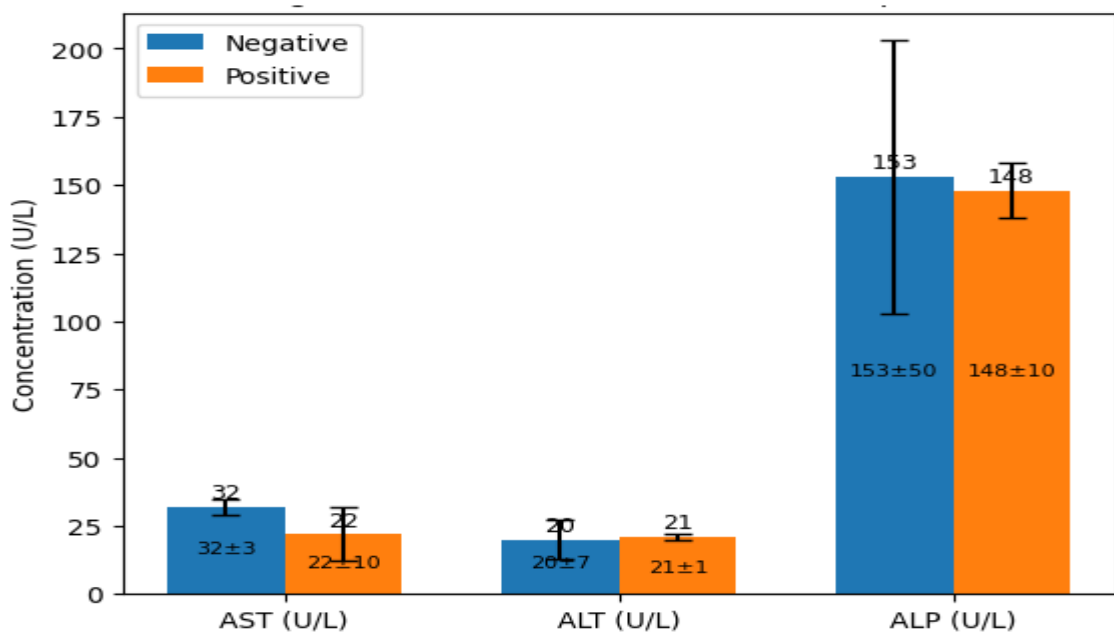
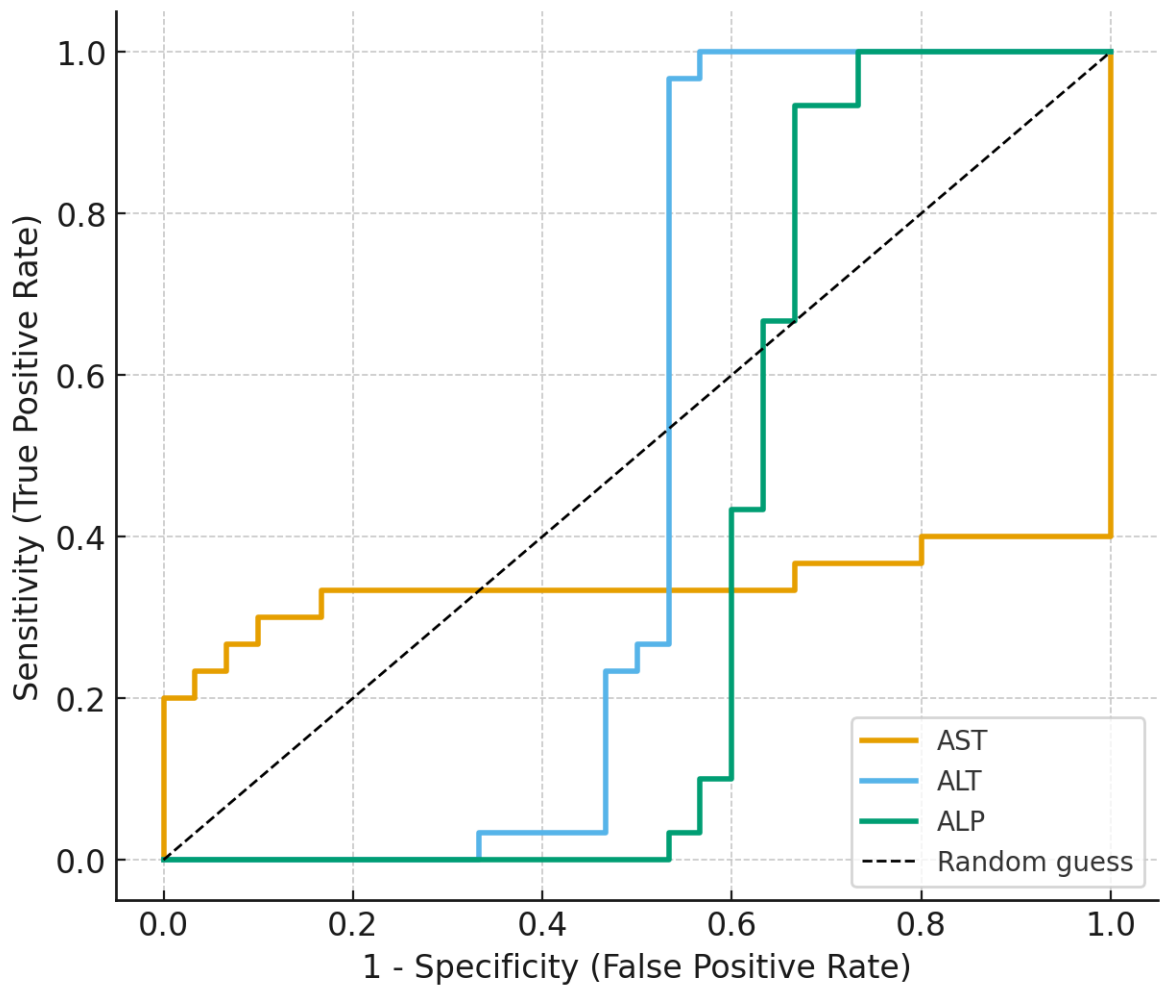


Figure 5. ROC Analysis Comparing the Predictive Performance of AST, ALT, and ALP



ТИТУЛЬНЫЙ ЛИСТ_МЕТАДААННЫЕ

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Блок 3. Метаданные статьи

HOST SUSCEPTIBILITY, BIOCHEMICAL AND PHYSICAL CHANGES IN
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ВОСПРИИМЧИВОСТЬ, БИОХИМИЧЕСКИЕ И ФИЗИЧЕСКИЕ
ИЗМЕНЕНИЯ ПРИ ИНФЕКЦИИ TOXOPLASMA GONDII: РОЛЬ ГРУПП
КРОВИ АВО И ПОКАЗАТЕЛЕЙ ФУНКЦИИ ПЕЧЕНИ

Сокращенное название статьи для верхнего колонтитула:

HOST SUSCEPTIBILITY TO TOXOPLASMA: ROLE OF ABO AND HEPATIC
INDICATORS

ВОСПРИИМЧИВОСТЬ К ИНФИЦИРОВАНИЮ TOXOPLASMA GONDII:
ГРУППЫ КРОВИ АВО И ПОКАЗАТЕЛЕЙ ФУНКЦИИ ПЕЧЕНИ

Keywords: Toxoplasma gondii, Blood Group, Abortion, Liver Function, pregnant
women, Physical changes.

Ключевые слова: Toxoplasma gondii, группа крови, аборт, функция печени,
беременные женщины, физические изменения.

Оригинальные статьи.

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