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Research Article

Serological Investigation of *Toxoplasma gondii* Infection in Livestock and Humans in Bahawalpur Cholistan Region

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Abstract

Toxoplasmosis is a zoonotic disease caused by the *Toxoplasma gondii* (*T. gondii)* apicomplexan parasite. Due to significant populations of cattle and buffaloes, the Cholistan region is a major hub for livestock production. Exposure to contaminated food, soil, or feces infects livestock, including cattle, buffaloes, and their handlers. The purpose of this study was to investigate the seroprevalence of *T. gondii* in livestock and their handlers in Bahawalpur cholistan region. This study used a cross-sectional approach, incorporating collecting data samples from cattle, buffalo and their handlers at the same point of time. The study was conducted in the Cholistan region of Bahawalpur. Total 170 blood samples of butchers (N=50), farmers (N=35), cattle (N=35), and buffaloes (N=50) were randomly collected from Bahawalpur abattoir and buffalo and cattle farms. Latex agglutination kit test (LAT) was used to detect the presence of IgG antibodies against *T. gondii.* Pearson's Chi-square test was used to evaluate the seropositivity among various age groups by using WINPEPI software for Windows (Version 11.39). The overall seroprevalence of *T. gondii* infection in butchers, farmers, cattle and buffaloes Bahawalpur were 41.17%, 22.85%, 22.85% and 22.72% respectively by using Latex Agglutination test (LAT) to detect IgG *T. gondii* antibodies. Thirteen butchers were seropositive at 1:256 dilution suggesting recent contact; four butchers and three farmers were seropositive at 1:128 due to acquired immunity, while four butchers and five farmers were seropositive at dilution 1:16, indicating non-specific immunity. Six cattle and two buffalo were seropositive at 1:256, indicating recent infection, two cattle and two buffalo were seropositive at 1:128 suggesting acquired immunity, and five buffaloes were found seropositive at 1:16 dilution showing residual immunity. Chi square test revealed non-significant results among different age groups of butchers, farmers, cattle, and buffalos。 The seropositivity was not significantly different among the various age groups of butchers, farmers, cattle, and buffaloes.

Keywords: Seroprevalence, Toxoplasmosis, Zoonosis, livestock infection, handlers exposure, one health

1. Introduction

Toxoplasma gondii (T. gondii) is a ubiquitous protozoan parasite described by Charles Nicolle and Louis Manceaux Africa. They were discovered within the tissue of African rodent called the gundi (*Ctenodactylus gundi)* [1,2]. Later, Alfonso Splendore was discovered it in rabbits (South America). Its genus name was derived from the Greek word *toxon*, meaning "bow" on the basis of its shape [3]. *T. gondii* increases the risk of toxoplasmosis in animal and humans [4].

Undercooked or raw meat consumption of infected animal is a significant source of zoonotic infection in humans. The disease is transmitted to livestock and humans by infected feces via definitive host (cats) [5–7]. Mutton and pork are more common sources because livestock are intermediate hosts [8].

Crawling children have a higher incidence of infection due to direct exposure to contaminated soil [9]. Headache, fever lethargy, and convulsions are early symptoms of *toxoplasmic* encephalitis [10]. *T. gondii* also induces infertility, abortion, mummification, early embryonic death and stillbirth in animal and humans [11,12]. Ocular manifestations are rare in postnatally acquired infections [2]. Felines are definitive hosts exhibit a predator-prey type life cycle. Pet cats have a lower incidence of infection than feral and stray cats [13]. *T. gondii* life cycle consists of intestinal and tissue phases furthermore three infectious stages including tachyzoites (rapidly multiplying form), bradyzoites (tissue cyst form), and sporozoites (in oocysts) [14]. In felines intestinal phase of infection consist of merogony and gamogony [5,15]. Various studies have been conducted to evaluate the seroprevalence of *T. gondii* in cattle [16], buffaloes, and their owners in many regions of the world. The existing literature reveals that incidence rate was 44.8% in cattle in Sudan and 22.2% was in domestic ruminants, including cattle and buffaloes in Ethiopia [17]. In Egypt, the incidence rate seroprevalence of cattle was 5.3% [2,18] and in Italy, the seroprevalence rate was 14.8% in water buffaloes [19]. In comparison to other studies findings the incidence rates were 12.2% and 0%, respectively, in cattle and buffaloes [20], while another study from North areas of Pakistan reported that the seroprevalence of *T. gondii* of 19.75 % in cattle, and 15.17 % in buffaloes. Systematic and meta-analysis revealed the prevalence of *T. gondii* infection was 16.94% in cattle and 22.26% in buffaloes [21]. It was found that 20.37 % human population in northern areas of Pakistan was seropositive for *T. gondii* infection (Ahmad, 2014); however, the seropositivity rate was lower in men than in women [22]. The literature reveals incidence of toxoplasmosis in Europe was up to 54% [23] and 22.06% of 60 million people in the USA [24]. Approximately, 25% of the cattle population was seropositive for anti-*T. gondii* antibodies in South-West Pakistan [25] however, the seroprevalence was 10% and 12% in District Lahore, Punjab-Pakistan [26]. Recent studies have shown the seroprevalence of anti-*T. gondii* antibodies in animal handlers was 44.7% in Bahawalpur, Pakistan [27]. In 1980, toxoplasmic encephalitis associated with acquired immunodeficiency syndrome (AIDS) [28]. Toxoplasmosis is risk for people with immune system dysfunction, HIV, cancer, chemotherapy, diabetes [29]. It diagnosed by using biological, serological, or histological methods or combination of these methods while clinical signs are nonspecific. Serological tests including indirect hemagglutination (IHA), indirect immunofluorescence (IFAT), enzyme-linked immunosorbent assay (ELISA), and latex agglutination Test (LAT), are used to diagnose *T. gondii* [30–33]. Additionally, microscopic examination detect tachyzoites or bradyzoites [34]. The work on hematological and biochemical on camels and prevalence of Toxoplasmosis in sheep, cattle and farms and their handlers had been done in division Bahawalpur, Punjab-Pakistan. Cholistan region is the major hub of livestock. Due to highly significant health concerns and continuous exposure, the current study was designed to evaluate the seroprevalence of *T. gondii* in Bahawalpur abattoir and local buffalo and cattle farms. This study evaluates the association between age of livestock and their handlers and risk of infection, determine which age group at higher risk.

T. gondii is an obligate parasite with a complex life cycle that includes sexual (in cats) and asexual reproduction (intermediate hosts). In sexual reproduction, definitive hosts are infected by consuming tissue cysts that containing bradyzoites, which are excreted in the small intestine. They undergo sexual reproduction, forming microgametes and macrogametes. After fertilization, the zygote develops into unsporulated oocysts, which are excreted through the feces of definitive host. In asexual reproduction, livestock and humans are infected by ingesting sporulated oocysts through contaminated soil, water, and undercooked food source. In the small intestine,

sporozoites or bradyzoites multiply into tachyzoites, which invade the muscles and brain of the intermediate host. Later in life, the cysts weaken the host's immunity. When a definitive host eats the intermediate host, the cycle restarts [35].

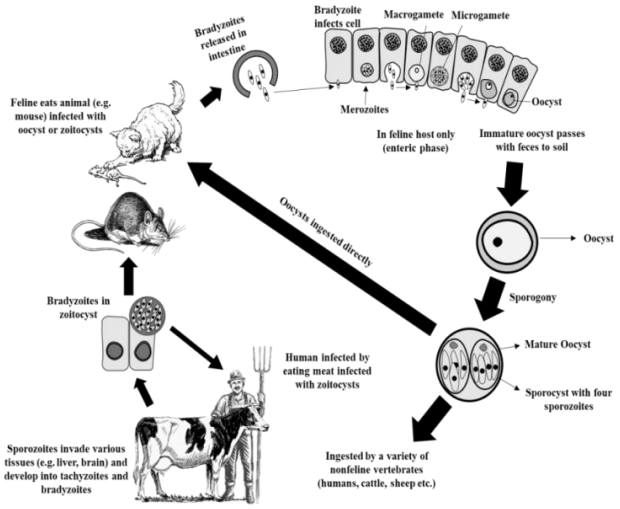

Figure 1. Life cycle of *T. gondii.*

2. Materials and methods

2.1. Study area

The current study was conducted in the Department of Parasitology, University of Veterinary and Animal Sciences, Lahore Pakistan, as well as in local dairy farms and slaughterhouses in Bahawalpur.

2.2. Blood collection and serum separation

A total of 170 serum samples (50 butchers, 35 farmers, 35 cattle, and 50 buffaloes) were randomly collected from the Bahawalpur abattoir and local buffalo and cattle farms. Under aseptic measures, 9-10 mL of blood was collected by vein puncture using of syringes and transferred into a screw-capped sterile clean test tube to avoid hemolysis [36]. All blood samples were labeled accurately with the name or number of the butchers, farmers, cattle, and buffalo and the date of collection. The blood samples were allowed to clot at room temperature for half an hour. Blood sample test tubes were centrifuged at 3500 rpm for at least 5 min. Purified serum were collected into Eppendrof tubes labeled with code numbers and stored at -20^{o}C until further processing [37].

2.3. Serum analysis

All serum samples were analyzed by using commercial test kit, "Toxoplasma Latex" to detect specific IgG *Toxoplasma* antibodies, manufactured by Quimica Clinica Apelicada, SA Amosta, and Sapin.

2.4. Reagents and control

The commercial test kits (LAT) were contained 1 x 4.0 mL latex reagent, 1 x 0.5 mL positive controls, 1 x 0.5 mL negative controls, slides and disposable stirrers. All reagents were preserved within 0.1% sodium azide.

2.5. Storage and Stability of "Toxoplasma Latex" Kit

All kit reagents were stored at 2-8 ^{o}C to maintain stability in accordance with the manufacturer's recommendations (Quimica Clinica Apelicada, SA Amosta, and Sapin).

2.6. Procedure

The "*Toxoplasma gondii* Latex" was used according to the manufacturer's instructions. Before running the assay, all test reagents were brought to room temperature, and sera were thawed. Twenty-five microliters of physiological saline (0.9% NaCl) solution was added to each well of a 96-well microtiter plate with the help of a micropipette or micro dispenser. Twenty-five microliters of the test sera were added to the first well. This was mixed with normal saline, and 25 microliters was then transferred to the adjacent well and so on. Twenty-five microliters of the mixture were discarded from the last well. In this way, serial dilutions were made by the two-fold dilution technique to the 8th well, 1:256. One drop of diluted serum each from 1:16, 1:128 and 1:256 dilutions were placed onto a slide, in the beach area. The latex reagent was mixed well, and one drop of the latex reagent was added over each serum drop. Both drops were mixed well with the help of a disposable stirrer, and the slide was tilted slightly. The presence of antibodies determined by agglutination was observed within five minutes. The antibody titer was shown by the highest dilution of the test serum where there was significant agglutination.

2.7. Interpretation

Positive: 1:16 sera dilutions showed a low level of antibodies. Positive titers of 1:128 indicate a acquired infection or the

presence of moderate levels antibodies. A titer of 1:256 or higher than 1:256 indicates the high level of antibodies which indicate, stronger immune response or may indicates more recent or repeated exposure to the parasite.

2.8. Statistical analysis

T. gondii seroprevalence among different age groups of butchers, farmers, cattle, and buffaloes were explored by Pearson's Chi-square test by using the WINPEPI software for Windows (Version 11.39).

3. Results and discussion

3.1. Seroprevalence in butchers and farmers

Samples were collected to evaluate the IgG *Toxoplasma* antibody positivity at different dilutions and illustrated any potential relationship between age groups and antibody titers. By collecting samples from different age groups, we aimed to investigate seropositivity rates and antibody titers in age based groups. Of the 170, 50 were collected from butchers and 35 from farmers to detect anti-*T. gondii* antibodies using "Toxoplasmosis Latex" kit. The literature reveals many studies conducted on sheep, cattle, and goat species [38] [39] [22] [35]. The butchers and farmers' ages ranged from 15 to above 50 years. The participants' ages were categorized into five groups i.e.; 15-20 years (G1), 21-30 years (G2), 31-40 years (G3), 41-50 years (G4), and <50 years (G5). G5 had the highest seropositive percentage of 41.17% and 35.71% among butchers and farmers, respectively as per the samples test, while G4 had a seroprevalence percentage of 30% among butchers and 27.27% among farmers; G3 had 30% and 0% among butchers and farmers and no positive cases were reported in the rest of the groups.

3.2. Antibody titers and age-based exposure

According to antibody titration measurements, four samples from the butchers showed 1:16 and 1:128 while five samples showed 1:256 screening dilutions as shown in Tables. Titration measurements with respect to age showed five samples of farmers showing 1:16, and three samples showing 1:128 screening dilutions. No samples were positive at 1:256 screening dilution. In contrast to earlier studies, seroprevalence was not age-dependent in cattle, buffalo and equids [5]. The seropositivity percentages were 26% and 22.85% in butchers and farmers. Seropositivity was not significantly different among the different age groups of butchers and farmers. In group 5, the non-significant seropositivity was 41.17 % in butchers and 35.71 % in farmers. The chi-square test and Extended Mantel-Haenszel test showed no significant association between seroprevalence and various age groups of butchers (Table 1). Globally, the seropositivity was high in various regions. The incidence vary according to geographic factors including climate, route, and age [40]. The chi-square test and Extended Mantel-Haenszel test showed no significant association between seroprevalence and various age groups of farmers (Table 2). An antibody titer of 1:16 indicates residual or non-specific immunity. In comparison to earlier findings, a low titer indicated past exposure and probable immunity, and a high titer 1:256 suggested a present infection [41]. The highest seropositive percentage in G5 was followed by 41.17% in butchers and 35.71% in farmers and, in G-4, 30% and 27.27% respectively due to continuous exposure at abattoirs and livestock farms meet the findings of earlier studies [42]. In comparison to earlier studies older butchers and farmers had higher seropositive cases for anti-*T. gondii* antibodies than younger ones [9]. Present study reveals The present study revealed that, 80% of all primary infections are asymptomatic due to the immune systems effectiveness [43]. It is transmitted to the definitive or intermediate host [44]. Current study reveals seroprevalence of anti-*T. gondii* antibodies in butchers and farmers was recorded as 26% and 22.85%, respectively although the seroprevalence was not significantly different among different age groups of butchers and farmers. In contrast to earlier findings anti-*T. gondii* antibodies were detected by using LAT [45]. Various ages of 20-50% females in Karachi and Khyber Pakhtunkhwa (KP) were seropositive for anti-*T. gondii* antibodies [46]. In Tanzania 50.0% of females of different ages were seropositive for anti-*Toxoplasma* antibodies [47]. The variation in seropositivity results may due to environmental and geographical conditions, and close association of females with pet [48]. A total 50 butchers and 35 farmers were examined in

the current study. Five butchers had an antibody titer of 1:256 which indicates possible recent contact. Four butchers and three farmers showed an antibody titer of 1:128 which may due to acquired or evolving immunity. However, four butchers and five farmers showed butchers and farmers who had been in close and prolonged contact with the animals in abattoirs and farms showed the highest seropositivity of *T. gondii*. A study conducted in Southern Italy, reported highest incidence (87.0 %) among the slaughterhouse staff [49]. The literature reveals that seroprevalence in butchers and farmers was 26% and 22.85%, respectively, and it increased with age. It was more prevalent in warm, moist areas than in cold areas of different African countries [50], and seropositive percentage in butchers was 21.42% and in farmers, it was 13.63% among different age groups of both butchers and farmers [9]. A total of 50 buffaloes and 35 of cattle samples were analyzed for anti-*T. gondii* antibodies at screening dilutions of 1:16, 1:128, and 1:256 using the commercial kit "Toxoplasmosis Latex" based on the principle of LAT. The cattle and buffaloes' ages ranged from 1 to 15 years and above. These are categorized into three age groups: G1, G2, and G3. The G1 ranges from 1-5 years, G2 from 6-10 year, and G3 from above 11 year. The G3 cattle and buffaloes show non-significant lowest seropositive percentage as compared to G1 and G2.

The results show G2 cattle and buffaloes had the highest seropositive percentage, 50% and 46.66% respectively. According to antibody titration measurement, five buffaloes showed positive antibody titer at 1:16 dilution two cattle and two buffalo sample showed positive antibody titer at 1:128 and six cattle and two buffalo samples showed positive antibody titer at of 1:256 dilution.

Table 1. Detection of IgG *Toxoplasma* Antibodies among Butchers Using LAT

Groups	Age	No. tested	Antibody Titer Reciprocal			Seropositive	Seropositive (%)	*p-value*
			16	128	256			
G_1	15-20	10	0	0	0	0	0	-
G_2	21-30	03	0	0	0	0	0	-
G_3	31-40	10	02	01	0	03	30[a]	*p>0.05*
G_4	41-50	10	0	01	02	03	30[a]	*p>0.05*
G_5	<51	17	02	02	03	07	41.17[a]	*p>0.05*
Total		50	04	04	05	13	26	

[a] significant between age groups ($p < 0.05$), [b]not significant ($P > 0.05$)

Table 2. Detection of IgG Toxoplasma Antibodies among Farmers Using LAT

Groups	Age	No. tested	Antibody Titer Reciprocal			Seropositive	Seropositive (%)	p-value
			16	128	256			
G_1	15-20	02	0	0	0	0	0	-
G_2	21-30	03	0	0	0	0	0	-
G^3	31-40	05	0	0	0	0	0	-
G_4	41-50	11	02	01	0	03	27.27	*p>0.05*[b]
G_5	<50	14	03	02	0	05	35.71	*p>0.05*[b]
Total		35	05	03	0	08	22.85	

[a] significant between age groups ($p < 0.05$), [b]not significant ($p > 0.05$)

Table 3. Detection of IgG *Toxoplasma* Antibodies among Cattle Using LAT.

Groups	Age	No. Tested	Antibody Titer Reciprocal			Seropositive	Seropositive (%)	*p-value*
			16	28	256			
G_1	1-5	10	0	0	0	0	0	-
G_2	6-10	15	0	0	03	03	20	$p>0.05^b$
G_3	11-15 and above	10	0	02	03	05	50	$p>0.05^b$
Total		35	0	02	06	08	22.85	

[a] significant between age groups ($p< 0.05$), [b]not significant ($p > 0.05$)

Table 4. Detection of IgG *Toxoplasma* Antibodies among Buffaloes Using LAT.

Groups	Age	No. Tested	Antibody Titer Reciprocal			Seropositive	Seropositive (%)	*p-value*
			16	128	256			
G_1	1-5	18	0	0	0	0	0	-
G_2	6-10	17	02	0	0	02	11.76	$p>0.05^b$
G_3	11-15 and above	15	03	02	02	07	25	$p>0.05^b$
Total		50	05	02	02	09	22.72	

[a] significant between age groups ($p < 0.05$), [b]not significant ($P > 0.05$)

The seropositive percentage in cattle and buffaloes was 22.85% and 18% (Table). Existing literature reveals the seroprevalence was not age-dependent in cattle and buffalo [6]. Chi-square test and Extended Mantel-Haenszel test reveals there is no significant association was found between seroprevalence and age groups in cattle (Table). In other words, seroprevalence was not significant between the different age groups of cattle. In cattle, G3 were showed non-significant higher seropositive rate rather than other groups.

In ruminants, reports of clinical toxoplasmosis have been observed associated with fever, dyspnea, nervous signs, and abortion.

Serious toxoplasmosis is usually seen in immunosuppressed animals and human beings. Toxoplasmosis in cattle and buffaloes is important because of its zoonotic importance. Contamination of feed by feces of cats, flies, and cockroaches carries oocysts [51]. Intimate association with ruminants and pets is an additional responsible factor in positive serology [52].

Chi-square test and Extended Mantel-Haenszel test showed there is no significant association was found among seroprevalence and various age groups in buffaloes (Table). The G3 showed non-significant higher rate of seropositivity among the buffaloes. Current study reveals seroprevalence in cattle and buffaloes were as 22.85% and 18%, respectively however the incidence rate was not significantly different among the various age groups of cattle and buffaloes. The study reveals *T. gondii* antibodies are widespread in the animal population [53]. Different serological tests, including LAT, is used to detect IgG *Toxoplasma* antibodies in ruminants [54] its positive percentage is 20% in Egypt and 25% in Pakistan [55]. The seroprevalence of toxoplasmosis is different in different regions of the world due to different management and geographical conditions [56]. The present study shows that seropositivity was high in the G-3 group of cattle and buffaloes, recorded at 50% and 25%, while in G-2 it was 20% and 11.76%. There was no seropositive case was reported in G-1(1-5 years) of cattle and buffaloes. The present study revealed that the

prevalence of antibodies varied with the age of the animals. These findings are agreed with the results of Dámek et al. [5], Celi et al. [55], Khan et al. [56] and Guesmi et al. [57], who reported that infection incidence was higher in old animals rather than younger ones.

3.3. Seroprevalence in cattle and buffaloes

In the present study, among 35 cattle and 50 buffaloes, five buffaloes gave 1:16 antibody titer, indicated residual or nonspecific immunity, two cattle, and two buffalo showed 1:128 titer indicated that the infection was due to acquired or evolving immunity furthermore, six cattle and two buffalo were positive at 1:256 antibody titer reveals that there was no recent contact with the parasite. Low titer indicated past exposure and probable immunity, and a high titer of 1:256 strongly suggested present infection indicated by findings of Varada et al. [58] & Maheswari et al. [59]. Current study concluded that the seroprevalence of IgG *Toxoplasma antibodies* in cattle and buffaloes was 22.85% and 18%, respectively. The incidence were not significantly different among various age groups of cattle and buffaloes [57]. Regarding with age, the highest seropositive percentage was in G3. In G-3 seropositive percentage of cattle and buffaloes was 25%, 50%respectively. The relationship between age and seroprevalence was consistent with previous studies, which also reported higher infection rates in older animals compared to younger ones. Our study agreed with previous study indicated that age is a contributing factor to *T. gondii* infection [58]. Antibody titer measurement shows that five buffaloes had a titer of 1:16, indicating residual nonspecific immunity. Two cattle and two buffalo showed the 1:128 antibody titer indicate acquired immunity. Additionally, six cattle and two buffalo exhibited 1:256 antibody titer indicated no recent contact with the parasite.

These results are agreed with findings of Dámek et al. [5], Celi et al. [55], Khan et al. [56] and Guesmi et al. [57] reported that low titers indicate past exposure and probable immunity, while high titers (1:256) reveals recent exposure or ongoing infection. Findings reveals the incidence of anti-Toxoplasma antibodies in cattle and buffaloes was different across the different regions of Egypt and Pakistan [20]. These differences highlighted the management practices and geographical factors, and local conditions. Toxoplasmosis is significant in ruminants due to its zoonotic importance. Contamination of feed by cat feces, flies, and cockroaches carrying cysts, as well as close contact with ruminants and pets, contribute to positive serology [59]. Intermediate host are infected when they consume contaminated water, food and undercooked meat [60].

This study highlights the seroprevalence of anti-Toxoplasma antibodies in cattle and buffaloes and their handlers. The overall seroprevalence rates were 22.85%, 18%, 26%, and 22.8% in cattle buffaloes, butcher and farmers. There was no significant difference of seropositivity among various age groups. However, the highest seropositive percentages were observed in older animals. Future research should focus on further understanding the factors influencing seroprevalence and conducting precise assessments and implementing standardized protocols to determine regular serological surveillance, biosecurity intervention, and standardized diagnostic testing to mitigate the zoonotic disease risk.

4. Conclusion

This study reveals a high seroprevalence of Toxoplasma gondii in farmers, butchers, buffaloes, and cattle in the Cholistan region of Bahawalpur, highlighting significant public and animal health risks due to close contact and exposure. Although no significant differences were observed across age groups, the findings emphasize the urgent need for a One Health approach that integrates human, animal, and environmental strategies. Preventive measures such as improved farm biosecurity, proper hygiene practices, safe handling and cooking of meat, controlling cat populations, and raising awareness among high-risk groups are critical. Implementing surveillance programs, public education, and promoting food safety and hygiene can significantly reduce the transmission of T. gondii and protect both human and animal health.

Ethical approval

This study was approved by the Animal Ethical Committee of the Faculty of Veterinary Science, University of Veterinary and

Animal Sciences, Lahore Pakistan. Written informed consent was obtained from all participants.

Conflicts of Interest

The authors report no conflicts of interest.

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Authors Contribution

AK: methodology and writing original draft. KUR: supervision of analysis. HIH, ZS and AIA: analysis. AK, WR, ZS and AI: writing review and editing. KUR: visualization. AIA and KUR: conceptualization, and resources. HIH and AIA: Final Editing and Reviewing. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Data availability statement

The data presented in this study are available on request from the corresponding author.

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