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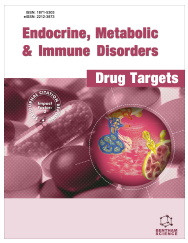
CASE REPORT

A Unique Case of Heart Transplant and *Toxoplasma gondii* Infection in a Parasite-Seronegative Recipient: A Case Report



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Abstract: ***Introduction*:** The protozoan Toxoplasma (*T.*) gondii is responsible for toxoplasmosis, and this parasitosis represents a high risk in solid organ transplant procedures. In heart transplant patients, *T. gondii* infection is usually fatal since immunosuppressive drug therapy is administered to recipients.

***Case presentation*:** A 62-year-old woman with severe biventricular dysfunction and seronegative for *T. gondii* underwent a cardiac transplantation from a donor seropositive for anti-*T. gondii* IgG antibody. The recipient exhibited post-transplant complications, including acute renal failure and difficulty in weaning from mechanical ventilation, ultimately requiring tracheotomy. The recipient underwent immunosuppressive pharmacological prophylaxis to prevent organ rejection. From a virological point of view, the recipient was monitored, and analyses of blood and serum revealed the presence of T. gondii DNA. In addition, other viral and bacterial infections were observed. Afterward, molecular and anatomopathological investigations on cardiac biopsies were performed, and neither test revealed the presence of *T. gondii* DNA.

***Conclusion*:** On the one hand,prompt infection management and continuous monitoring are crucial to control viral and bacterial loads and, on the other hand, to optimise antimicrobial treatment, thus ensuring the gradual clinical stabilisation of the patient. Finally, it is important to highlight the need to review diagnostic screening protocols for organ donors to detect potential reactivation of microorganisms, viruses, and parasites that could pose a fatal risk to recipients.

Keywords: Case report, heart transplantation, Infection, *Toxoplasma gondii*.

1. INTRODUCTION

Toxoplasma (*T*). *gondii* is a protozoan parasiteresponsible for toxoplasmosis, an infection that can affect individuals of any age or sex, transmitted through food contaminated with cysts or oocysts of the parasite [1]. Furthermore, it is important to note that transmission of T. gondii has been observed in patients undergoing solid-organ and haematopoietic stem cell transplantation [2, 3].

According to recent studies [4-6], the overall prevalence of donor-derived toxoplasmosis in heart transplant recipients remains low, although it is clinically significant due to its high morbidity and potential for fatality in seronegative recipients. Moreover, heart transplant recipients are highly at risk because *T. gondii* has a strong tropism to myocardial tissue. The reported clinical case highlights a rare but critical event, underlining the gaps in current screening and prophylaxis protocols. The clinical course of *T. gondii* infection in cardiac transplant recipients is generally favourable. However, in some cases, it can be fatal, especially in immunosuppressed patients, as described by Wołyniec *et al.* [7] and Mastrobuoni *et al.* [[8]], who have documented the sporadic detection of tissue cysts in myocardial biopsies. Finally, Hermanns *et al.* [9] demonstrated that cardiac transplantation from a donor seropositive for *T. gondii* into a seronegative recipient was fatal, since the recipient died after three weeks.Reactivation of latent toxoplasmosis in such patients can be triggered by immunosuppression or surgical stress, as reported by Akella *et al.* [3].

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Although the parasite can localise in most tissues, serological analysis and Polymerase Chain Reaction (PCR) remain the cornerstones for accurate diagnosis [10]. This case report describes a rare case of detection of T. gondii DNA in the recipient's blood. The recipient is seronegative to anti-*T. gondii* antibodies (both IgM and IgG), whereas the donor was seropositive to anti-*T. gondii* IgG antibodies.

2. CASE PRESENTATION

We report the case of a 62-year-old woman who was followed up for severe aortic insufficiency (EROA: 0,30 cm^2) with initial signs of Left Ventricular (LV) dilatation (LV diastolic diameter 58 mm) and underwent elective cardiac surgery.

After signing the informed consent, an aortic valve replacement was performed with a biological prosthesis No. 23 through a ministernotomy at the fourth intercostal space, followed by the administration of blood cardioplegia directly into the coronary ostium. During weaning from extracorporeal circulation, hypokinesia of the right ventricle was observed on the transesophageal echocardiogram, with the ECG showing ST-segment elevation at aVF, D-II, and D-III. Extracorporeal membrane oxygenation (ECMO), using an arterial cannula in the ascending aorta, a vent in the cardiac apex, and a venous cannula in the left femoral vein, was placed. A coronary angiography was performed and showed a long spiral dissection from the proximal tract up to the posterior interventricular (IVP) branch. The spiral dissection extended to the distal section of the right marginal branch and the posterior descending artery. An intra-aortic balloon pump (IABP) was placed in the left femoral artery. Over the following days, the attempt to wean from mechanical assistance [ECMO and IABP 1:1] was ineffective, and treatment with continuous renal replacement therapy for oliguria was initiated.

The patient was transferred to the Department of Cardiac Surgery Ward of the Polyclinic Hospital of Bari to evaluate the potential inclusion in the emergency heart transplant list. Upon arrival, the patient was intubated in synchronized intermittent mandatory ventilation at 40%, responsive to simple orders, in V-A ECMO at 4.5 L/min and IABP 1:1, with dobutamine infusion 5 μm/kg/min and adrenaline 0.07 μm/kg/min. Chest radiography showed lung field congestion with a right pleural effusion. Transthoracic echocardiography revealed severe biventricular dysfunction (LVEF, 15%; TAPSE, 10 mm). Instrumental and laboratory tests and the patient's clinical conditions did not contraindicate admittance to the national emergency heart transplant list. The patient was transplanted (February 6th) seven days after the inclusion on the emergency heart transplant list. No noteworthy complications occurred. The ischemic time was 3 hours. The patient presented complications, including acute renal failure treated with haemodialysis and difficulty in weaning from mechanical ventilation, for which it was decided to wean *via* tracheotomy.

2.1. Clinical Microbiology Report at Admission and Therapeutic Procedures

Serological pre-transplant evaluation of the recipient, conducted at the Microbiology and Virology Unit of Polyclinic Hospital of Bari, revealed seronegativity for anti-*T. gondii* (IgM and IgG) (Table **1**).

Post-transplant immunosuppressive therapy included mycophenolate mofetil and methylprednisolone. Tacrolimus was excluded due to acute renal dysfunction. Anti-thymocyte globulin was administered on postoperative days 1 and 4, respectively. In addition, prophylaxis included the administration of Valganciclovir plus Trimethoprim-Sulphamethoxazole (TMP-SMX), with a daily dose of 1 tablet containing TMP (80 mg) and SMX (400 mg) for the first 3 months following transplantation. However, this therapy was initiated but early discontinued due to acute cardiac damage, with a plan to resume this therapy during follow-up.

On day 48 post-transplant, *T. gondii*DNAwasdetected in peripheral blood *via* PCR using the ELITeInGenius® platform and ELITe MGB® kit (ELITechGroup, A Bruker Company, Turin, Italy).This test amplifies a highly repeated genomic region (200-300 copies) specific to *T. gondii*.

The recipient's condition rapidly and severely worsened, with symptoms including headache, general malaise, fever, and respiratory difficulties. Moreover, 2 days after transplantation, Cytomegalovirus (CMV) DNA was detected in peripheral blood, with a viral load of 937 International Units (I-U). CMV DNA was detected and quantified by means of PCR using CMV ELITE MGB® kit (ELITechGroup), according to the manufacturer's instructions.Subsequently, high DNA CMV viral loads were detected until March 9th, and on that day, a high DNA CMV viral load (1923501 gv/ml) was also detected in the bronchoalveolar lavage. On March 25th, a decreased viral load of CMV DNA was detected, whereas until this date, both Epstein-Barr Virus (EBV) DNA and *T. gondii* DNA were undetectable.On April 2nd, *T. gondii* DNA was detected in peripheral blood with a Cycle Threshold (Ct) of 30.22, but on April 7th its Ct value was 40.64, indicating a reduced parasitaemia.Subsequent PCR tests for T. gondii DNA in peripheral blood, conducted on June 13th and 17th,respectively, were negative. In addition, serological tests with Chemiluminescence methods (CLIA) were conducted on June 17th using the LIASON® instrument (DiaSorin® S.p.A., Saluggia, Italy) to assess the possible presence of antibodies directed against *T. gondii*. It is important to highlight the absence of seroconversion during follow-up, a phenomenon likely due to the severe immunosuppression. A post-transplant clinical timeline (Fig.**1**) summarizes both microbiological andvirology events.

In this picture, the detection times for *T. gondii*, CMV, EBV, *K. pneumoniae*, and *C. albicans* are illustrated.

Microbiological tests performed on March 21st, including urine and blood cultures, were positive for *Klebsiella (K.) pneumoniae* and *Candida* (*C.*) *albicans,* respectively. Moreover, microbiological investigations showed that the isolated *K. pneumoniae* is carbapenemase-resistant (KPC),

Table 1. Recipient's serological status before transplant.

	Values	Reference Intervals		Results
T. gondii IgG Antibody	<3.00 IU/ml	<7.2 IU/ml	Negative	Negative
		≥7.2-8.8 IU/ml	Doubt	
		>8.8 IU/ml	Positive	
T. gondii IgM Antibody	<3.00 IU/ml	<6.0 IU/ml	Negative	Negative
		≥6.8.8 IU/ml	Doubt	
		>8.8 IU/ml	Positive	
CMV IgG Antibody	60.2 U/ml	<12 U/ml	Negative	Positive
		≥12-14 U/ml	Doubt	
		>14 U/ml	Positive	
CMV IgM Antibody	11.5 U/ml	<18 U/ml	Negative	Negative
		≥18-22 U/ml	Doubt	
		>22 U/ml	Negative	
EBV VCA IgG Antibody	483.0 U/ml	<20.0 U/ml	Negative	Positive
		≥20.0 U/ml	Positive	
EBNA IgG Antibody	83.4 U/ml	<5.0 U/ml	Negative	Positive
		≥5.0-20.0 U/ml	Doubt	
		>20.0 U/ml	Positive	
EBV VCA IgM Antibody	<10.0 U/ml	<20 U/ml	Negative	Negative
		20-40 U/ml	Doubt	
		>40 U/ml	Positive	
HSV1/2 IgG Antibody	>30.0 Index	<0.9 Index:	Negative	Positive
		≥0.9-1.1 Index	Doubt	
		>1.1 Index	Positive	
HSV1/2 IgM Antibody	<0.5 Index	<0.9 Index	Negative	Negative
		≥0.9-1.1 Index	Doubt	
		>1.1 Index	Positive	
VZV IgG Antibody	1935.0 mIU/ml	<135 mIU/ml	Negative	Positive
		≥135-165 mIU/ml	Doubt	
		>165 mIU/ml	Positive	
VZV IgM Antibody	7.1 Index	<9.0 Index	Negative	Negative
		≥0.9-1.1 Index	Doubt	
		>1.1 Index	Positive	

Abbreviations: CMV: Cytomegalovirus, EBNA: Epstein-Barr Nuclear Antigen 1, EBV: Epstein-Barr virus, HSV: Herpes Simplex virus, *T. gondii: Toxoplasma gondii*, VCA: Viral--Capsid Antigen, VZV: Varicella Zoster virus.

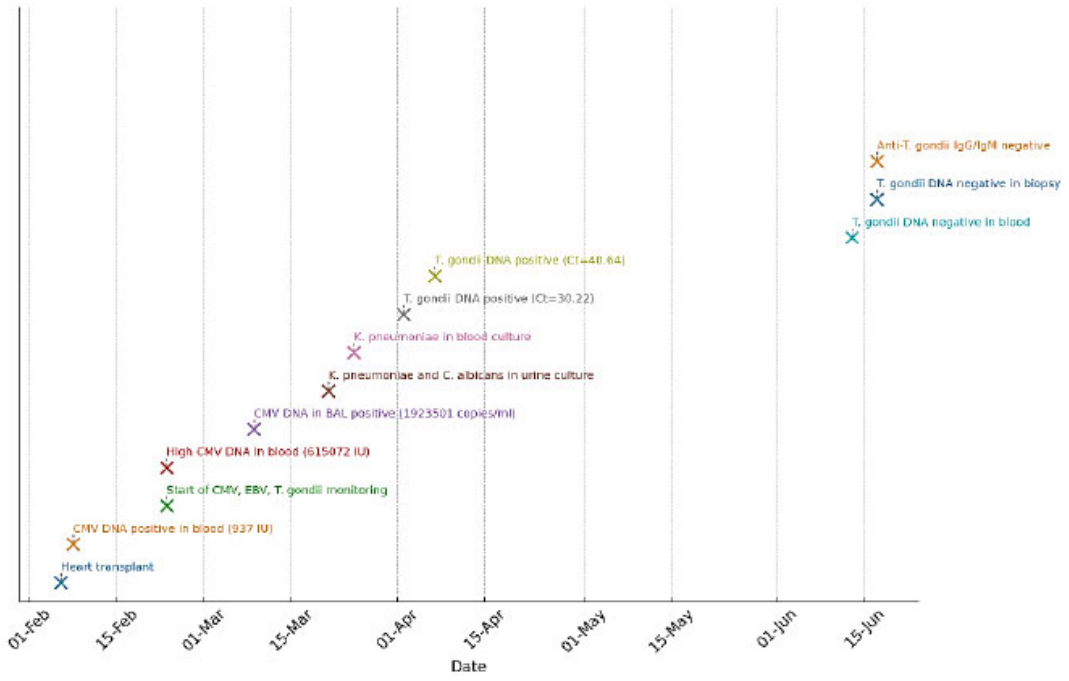

Fig. (1). Clinical timeline post-transplant *T. gondii* infection. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

characterised by resistance to all antibiotics except Ceftazidime with Avibactam and aminoglycosides. On March 31st, the haematological picture showed anaemia, leukocytopenia, thrombocytopenia, and anisocytosis (Table **2**), likely due to systemic infection. Therefore, the recipient was transferred to the intensive care unit (ICU), where she underwent thoracotomy surgery and was discharged at the end of May due to overall clinical improvement.

On June 17th, after the ICU, dismissed recipient's heart biopsy was performed, and the ventricular endomyocardial fragments showed a mild and focal interstitial lymphocytic infiltrate in the absence of myocyte damage. Finally, 1 month later, another heart biopsy was performed, and the result showed no changes related to acute cellular rejection, whereas diffuse hypertrophic-regressive changes in myocardiocytes were observed. In Figs. (**2A** and **B**), the myocardial sections are depicted.

In Fig. (**2A**), the myocardial biopsy performed on June 17th, stained with haematoxylin and eosin (H&E) at 10X magnification (original magnification: 400X), is illustrated. The ventricular endomyocardial fragments show a mild focal interstitial lymphocytic infiltrate in the absence of myocyte damage. The DNA PCR for the detection of *T. gondii* in myocardial tissue was negative. Heart muscle samples were fixed in 10% buffered formalin, embedded in paraffin, and stained with H&E.

In Fig. (**2B**), a histological section of an endomyocardial biopsy performed on July 17th, stained with immunohistochemistry for CD68, a macrophage/monocyte-specific marker, is shown at a magnification of 10X (original magnification: 400X). The biopsy revealed no evidence of acute cellular rejection; however, widespread hypertrophic-regressive changes of myocardiocytes were observed. The density of CD68-positive monocytes/macrophages is low, and the myocardial tissue appeared relatively preserved. The section was stained with H&E. CD68 monoclonal antibodies (Millipore®) were used at a dilution of 1:200 following a routine protocol (deparaffinisation, rehydration, antigen retrieval, inactivation of endogenous peroxidase, and blocking of non-specific reactions). The section was incubated with the primary antibodies for 12 hours at 4°C, followed by application of the labelled streptavidin-biotin-peroxidase complex (LSAB, Dako Corporation®) and visualised with diaminobenzidine (DAB kit; Dako Corporation®).

2.2. Donor

According to the guidelines for organ donors, only the IgG assay is required [11], and in this case, the donor was found to be seropositive.The donor's medical history and condition showed a rather peculiar condition. She was a 76-year-old woman, a smoker (10 cigarettes per day), who suffered from arterial vasculopathy and had undergone clipping of a cerebral aneurysm in 2016.Skin findings included keloids, petechiae, and ecchymoses. Cardiac evaluation revealed mitral and tricuspid regurgitation. Laboratory testing showed positive anti-Hepatitis B Virus (HBV) core IgG and anti-HBe IgG antibodies, with negative HBsAg antibodies and undetectable HBV DNA (<1 IU/mL). The donor was seropositive for CMV and *T. gondii* (Table **3**).

Table 2. Recipient's report before, during, and post-transplantation.

Serological Tests as of January 26th	
Vaccination coverage:	Rubella, Chickenpox, and Measles.
Contact immunization:	CMV, HSV1/2, EBV, VZV, Adenovirus, and Parvovirus B19.
Lack of antibody coverage:	*T. gondii.*
Surveillance Results from February 8th	
CMV DNA in blood samples:	From February 8th to March 23rd: Positive. From March 4th to March 25th: Positive.
CMV DNA in BAL:	March 9th: Positive.
T. gondii DNA in blood samples:	April 2nd: Positive with Ct= 30.22. April 7th: Positive with Ct= 40.64. From June 13th to June 17th: Negative.
T. gondii DNA in biopsy specimen:	June 17th: Negative. July 17th: Negative.
EBV DNA in blood sample:	Negative until May 7th.
IgG and IgM antibodies against *T. gondii*	June 17th: Negative.
Microbiological Tests	
Urine culture:	Positive for *K. pneumoniae* and *C. albicans*.
Blood culture:	March 25th and March 26th: Positive for *K. pneumoniae*.
CVC:	March 27th: Positive for *K. pneumoniae*
Haematological picture as of March 31st	
Haemoglobin (Hb): 9.4 g/dl. Leukocytopenia: 2.840/µl. Thrombocytopenia: 166.000/µl. Anisocytosis: 17.2%.	

Abbreviations: *C. albicans:Candida albicans*, CMV: Cytomegalovirus, EBNA: Epstein-Barr Nuclear Antigen 1, EBV: Epstein-Barr virus, HSV: Herpes Simplex virus, *K. pneumoniae: Klebsiella pneumonia*, *T. gondii: Toxoplasma gondii*, VCA: Viral-Capsid Antigen, VZV: Varicella Zoster virus.

Table 3. Donor serological status.

-	Values	Results
T. gondii IgG Antibody	85.3 IU/ml	Positive
CMV IgG Antibody	72.6 IU/ml	Positive
EBV VCA IgG Antibody	483.0 U/ml	Positive
EBNA IgG Antibody	83.4 U/ml	Positive
HSV1/2 IgG Antibody	>30.0 Index	Positive
VZV IgG Antibody	1935.0 mIU/ml	Positive
Specific *Treponema pallidum* total antibodies	<0.10 AU/ml	Negative
Strongyloides stercoralis IgG	7.6 U	Negative

Abbreviations: EBNA: Epstein-Barr Nuclear Antigen 1, EBV: Epstein-Barr virus, VCA: Viral-Capsid Antigen, HSV: Herpes Simplex virus, *T. gondii: Toxoplasma gondii*, VZV: Varicella Zoster virus.

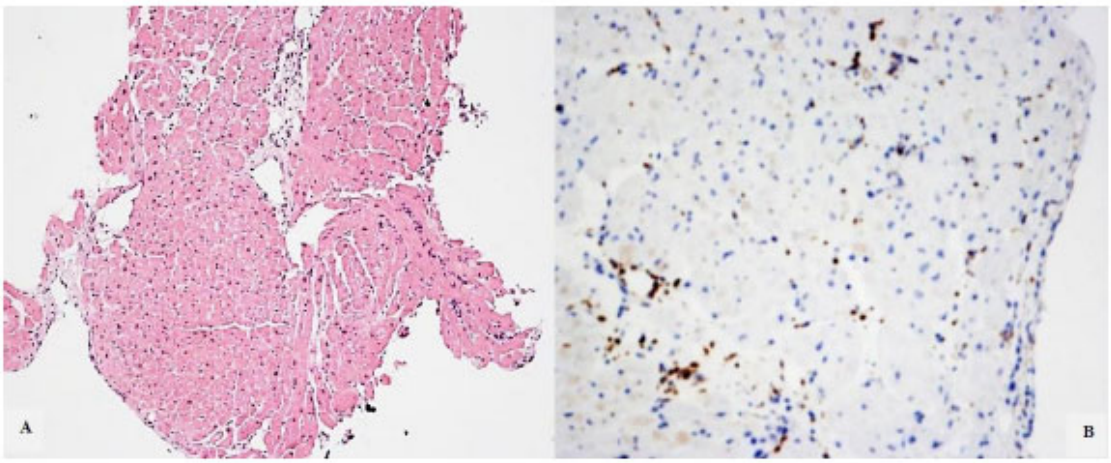

Fig. (2). Cardiac muscle tissue from an endomyocardial biopsy. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

3. DISCUSSION

Toxoplasmosis is an infection that can lead to serious complications in immunocompromised individuals, including transplant recipients [12]. Transmission of *T. gondii* in solid organ transplant recipients is a rare but potentially life-threatening event, especially when a seronegative recipient receives an organ from a seropositive donor [2, 7, 9].

In this case, the donor was seropositive for *anti-T. gondii* IgG, whereas the recipient was seronegative for both IgG and IgM, an immune profile that significantly increased the risk of donor-derived toxoplasmosis [9]. As previously stated, since we do not have information on the presence of anti-*T. gondii* IgM donor antibodies, two hypotheses can be formulated. The first assumes that the donor was positive for both IgG and IgM antibodies directed against *T. gondii*. In this circumstance, an IgG avidity test [13] would be essential to clarify whether the infection occurred before or after donation.

The second hypothesis is that the donor possesses only anti-*T. gondii* IgG antibodies, whereas IgM antibodies were negative. Current guidelines for donor screening do not recommend the determination of anti-IgM antibodies to *T. gondii* [11], and this is a limitation. On the other hand, a high IgG avidity would suggest a past infection with a lower risk of transmission, whereas a low IgG avidity would indicate a more recent infection with possible active transmission [13]. However, this test was not performed on the donor, and therefore, parasitic reactivation cannot be ruled out entirely in this scenario. Furthermore, in both circumstances, transmission of tachyzoites *via* the transplanted organ can be considered plausible, especially in conditions of immunosuppression. [14].

Therefore, this case highlights a clear need for careful consideration before discontinuing prophylactic regimens in high-risk recipients, along with the potential role of therapeutic administration alternatives when standard therapy is contraindicated. The diagnosis of toxoplasmosis in this patient was further complicated by the absence of a detectable serological response, probably attributable to severe immunosuppression due also to the post-transplant immunosuppressive therapeutic regimen.

As Nori and Ali stated in a previous study [[15]], this clinical picture exhibits unique features that indicate the immune system's inability, weakened by immunosuppression, to mount an adequate antibody response, even when the parasite is actively replicating and detectable by molecular methods such as PCR. Clinically, this case highlights the potential for serious consequences when this system is compromised and the crucial role of the immune system, particularly the production of antibodies, in controlling and eliminating *T. gondii* infections. Regarding the prophylaxis initially administered, this was discontinued due to acute cardiac dysfunction. Given the timing of a PCR positivefor *T. gondii*, discontinuation of TMP-SMX could have created a therapeutic window for the proliferation of the protozoan, particularly in an immunocompromised host. Literature supports that TMP-SMX is highly effective in preventing both reactivation of toxoplasmosis and primary infections, and its absence likely contributed to the outcome of this clinical case [16, 17]. This highlights the importance of balancing infection prevention with organ-specific complications and suggests that alternative prophylaxis or resumption should be implemented as soon as clinically feasible.

Moreover, the recipient experienced CMV reactivation and severe nosocomial infections caused by *K. pneumoniae* (carbapenemase-resistant strain) and *C. albicans*. These complications were not unusual in immunocompromised patients and required aggressive, targeted therapy [18, 19]. However, these infections were also successfully resolved. Finally, another important feature of this case was the detection of *T. gondii* DNA by PCR on peripheral blood, but not

on myocardial biopsies. Several mechanisms could explain these discrepancies: i) the uneven distribution of the parasite in the tissues, ii) the timing of sample collection, since PCR on blood is performed during the active phase of infection, whereas biopsies are performed later, during the recovery phase, and iii) the lower sensitivity of PCR in myocardial tissue compared to blood, possibly due to DNA degradation or sampling variability.

Based on the scientific literature and the case we observed, we suggest that positive IgG detection in donors should be carefully evaluated, indicating the need to promptly prepare and administer prophylactic therapy for recipients [20, 21].In our opinion, despite the complexity of post-transplant outcomes, early molecular diagnosis and timely clinical intervention were fundamental strengths in achieving appropriate management to definitively stabilise the patient.Therefore, it is clear that accurate monitoring of reactivation of the infection in asymptomatic *T. gondii* recipients would be of great importance for the safety of organ donation. This clinical case highlights the importance of vigilant surveillance and flexible treatment strategies in high-risk transplant recipients.

CONCLUSION

In conclusion, this case highlights the diagnostic and therapeutic challenges of donor-derived *T. gondii* infection in the heart transplant recipient, particularly in the context of a mismatch between a seropositive donor and a seronegative recipient. Detection of T. gondii DNA in PCR of blood, despite negative serology and tissue biopsies, underscores the importance of molecular diagnostics in immunocompromised patients.

AUTHORS' CONTRIBUTIONS

The authors confirm their contributions to this paper as follows. L.G., G.F., and D.P. were responsible for the study design. T.M. conducted the analysis and interpretation of results. A.T. and A.M.C. were responsible for data acquisition. C.I.G. prepared the original draft of the manuscript, and T.B. and L.S. provided supervision. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

C. Albicans	=	*Candida Albicans*
CT	=	Cycle Threshold
CMV	=	Cytomegalovirus
EBNA	=	Epstein-Barr Nuclear Antigen 1
EBV	=	Epstein-Barr Virus
ECMO	=	Extracorporeal Membrane Oxygenation
HBV	=	Hepatitis B Virus
H&E	=	Haematoxylin And Eosin
HSV	=	Herpes Simplex Virus
Iabp	=	Intra-Aortic Balloon Pump
ICU	=	Intensive Care Unit
IU	=	International Units
LV	=	Left Ventricular
K. Pneumoniae	=	Klebsiella Pneumoniae
PCR	=	Polymerase Chain Reaction
T. Gondii	=	Toxoplasma Gondii
TMP-SMX	=	Trimethoprim-Sulfamethoxazole
VCA	=	Viral-Capsid Antigen

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethical Committee of the Polyclinic Hospital (Protocol 0026792/25, no. 7882), Bari, Italy.

HUMAN AND ANIMAL RIGHTS

All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1975 Helsinki Declaration, as revised in 2013.

CONSENT FOR PUBLICATION

The patient undersigned the informed consent according to Italian laws and regulations.

STANDARDS OF REPORTING

CARE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

Luigi Santacroce is the Senior Editor of the journal EMID-DT; Thea Magrone is an Associate Editorial Board Member of EMID-DT. All other authors declare no conflict of interest.

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Declared none.

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