



ATTI
DELLA
SOCIETÀ TOSCANA
DI
SCIENZE NATURALI

MEMORIE • SERIE A • VOLUME CXXXI • ANNO 2024



Edizioni ETS

ATTI DELLA SOCIETÀ TOSCANA DI SCIENZE NATURALI

MEMORIE

Via Santa Maria, 53 - 56126 Pisa

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Atti are published yearly in two Issues (Serie A - Abiological, ISSN 0365-7655; Serie B - Biological, ISSN 0365-7450) in December. Some monographic volumes may be published as Supplementi.

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GINO FORNACIARI ⁽¹⁾

THE POISONING OF THE GRAND DUKE OF TUSCANY FRANCESCO I (1541-1587) AND GRAND DUCHESS BIANCA CAPPELLO (1548-1587): NEW FINDINGS

Abstract - G. FORNACIARI, *The Poisoning of the Grand Duke of Tuscany Francesco I (1541-1587) and Grand Duchess Bianca Cappello (1548-1587): New Findings*.

The hypothesis of the death by poisoning of the Grand Duke of Tuscany Francesco I and the Grand Duchess Bianca Cappello has sparked a debate that has lasted for more than four centuries. A few years ago, a study by toxicologists at the University of Florence had revived the hypothesis of arsenic poisoning. The results of current research, revealing the presence of *Plasmodium falciparum* proteins in the spongy bone tissue and immature forms of *P. falciparum* in Francesco I's erythrocytes, confirmed the historical sources, according to which the Grand Duke died of tertian malaria.

Key words - Tertian malaria, *Plasmodium falciparum*, arsenic poisoning, Medici, Renaissance, Postmedieval archaeology, Florence, Italy

Riassunto - G. FORNACIARI, *L'avvelenamento del Granduca di Toscana Francesco I (1541-1587) e della Granduchessa Bianca Cappello (1548-1587): nuove acquisizioni*.

L'ipotesi della morte per avvelenamento del Granduca di Toscana Francesco I e della Granduchessa Bianca Cappello ha suscitato un dibattito che dura da oltre quattro secoli. Alcuni anni orsono lo studio effettuato dai tossicologi dell'Università di Firenze aveva rilanciato l'ipotesi dell'avvelenamento da arsenico. I risultati delle ricerche attuali, rivelando la presenza di proteine di *Plasmodium falciparum* nel tessuto osseo spugnoso e di forme immature di *P. falciparum* negli eritrociti di Francesco I, hanno confermato le fonti storiche, secondo le quali il Granduca morì per malaria terzana.

Parole chiave - avvelenamento da arsenico, malaria pernicioso, *Plasmodium falciparum*, Medici, Rinascimento, archeologia postmedievale, Firenze

INTRODUCTION

Sometimes even authoritative scientific journals publish articles that are unreliable or insufficiently documented. Unfortunately, even palaeopathology, i.e. the study of diseases directly in human bodies in the past, has been subject to this misuse.

The hypothesis of the arsenic poisoning of Francesco I de' Medici (1541-1587) (Fig. 1) and his wife Bianca Cappello (Fig. 2) by his brother Ferdinando, a cardinal and successor in the Grand Duchy of Tuscany (Fig. 3), was put forward some years ago in a

scientific article (Mari *et al.*, 2006) and subsequently in two popular volumes (Mari *et al.*, 2007; Ferri & Lippi, 2007).

SUPPOSED EVIDENCE OF ARSENIC POISONING

The authors claimed to have evidence of arsenic poisoning based on analyses conducted on some hair formations found on the jawbone of Francesco I, whose skeletal remains were exhumed in 2004 as part of the 'Medici Project' (Fornaciari, 2009) and on two biological samples found in a crypt of the church of Santa Maria Assunta in Bonistallo, the parish church of the Medici villa in Poggio a Caiano, where, according to an archive document, the vases containing the viscera of Francesco I and Bianca Cappello were taken after the autopsy and embalming (Mari *et al.*, 2006). Remember that the body of Francesco I was buried in the Basilica of San Lorenzo in Florence, while Bianca's corpse has never been found.

The biological finds from Bonistallo, interpreted as fragments of human liver belonging to individuals of the opposite sex, were also attributed to the couple thanks to the presence in the crypt of ceramic fragments and two cast bronze crucifixes believed to be late 16th century, but found to date back to the 18th-19th century (Fornaciari, 2009).

A first consideration regarding these findings in the church of S. Maria a Buonistallo is that the crypt, where hundreds of bodies have been placed over the centuries, was not excavated using archaeological techniques; moreover, as the authors of the study themselves state, the recovery of the fragments of vases and organic material was carried out directly by the masons (Fig. 4).

Nevertheless, the authors argued that the poisoning hypothesis could not only be plausible, but also provable. Chemical analysis revealed the presence, in these remains, of arsenic in toxic doses. At the same time, the Florentine researchers claimed, without disclosing either the methodology or the molecular data obtained,

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Figure 1. Scipione Pulzone, *Portrait of Francesco I de' Medici* (1541-1587), 1590 © Gabinetto Fotografico delle Gallerie degli Uffizi.



Figure 2. Workshop of Alessandro Allori, *Portrait of Grand Duchess Bianca Cappello* (1548-1587), c. 1560-1585 ©Gabinetto Fotografico delle Gallerie degli Uffizi.



Figure 3. Scipione Pulzone, *Portrait of Cardinal Ferdinando de' Medici* (1549-1609), 1598, Kunsthistorisches Museum, Vienna).

that the DNA of one of the two organic samples would be compatible with that of the piliferous formations found in Francesco I.'s jawbone, in the zinc box where the Grand Duke's bones had been placed in 1956 at the end of the anthropological study carried out by Gaetano Pieraccini.

It is an incontrovertible fact, however, that in the zinc box of Francis I, reopened in 2004, there was no trace of organic material, neither skin nor beard, but only the remains of the textiles that wrapped the bones (Fig. 5), which were, moreover, carefully cleaned by the anthropologist Giuseppe Genna in the 1950s to carry out the anthropological study and to make a plaster cast of the Grand Duke's skull (Fig. 6).

Thus, it seems highly plausible that the DNA found, and compared with that of the organic remains in the Buonistallo church, is not the original DNA of Francis I, but is due, as was often the case in non-DNA free laboratories not dedicated to the study of ancient DNA, to pollution by modern DNA.

As for the presence of arsenic, it was customary after the autopsy to treat the removed viscera with arsenical compounds to aid preservation (Marinozzi, 2013).

In conclusion, even at the time of the publication of the work on the poisoning of Francis I and Bianca Cappello, strong doubts remained as to the reliability of the results (Fornaciari, 2006).



Figure 4. The works, not archaeologically correct, carried out by the masons in the church of Santa Maria Assunta in Bonistallo.



Figure 5. The zinc box of Francis I, reopened in 2004, revealed no trace of organic material, neither skin nor beard, but only the remains of the textiles that wrapped the bones.

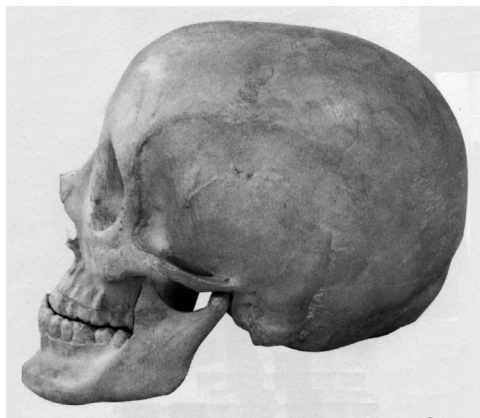


Figure 6. Plaster cast of the skull of Francesco I made by Giuseppe Genna in 1948. Courtesy of the Museum of Anthropology and Ethnology, University of Florence.

THE SYMPTOMATOLOGY OF FRANCESCO AND BIANCA

The documentation of the court physicians Pietro Cappelli, Giulio Cini and Baccio Baldini, who treated Francesco I and Bianca Cappello, details the course of the grand ducal couple's sudden illness (Florence State Archives, in Pieraccini, 1924).

On 6, 7 and 8 October 1587, the Grand Duke took great pains to hunt in the surrounding area of his villa in Poggio a Caiano, an agricultural area cultivated with rice paddies, a malarial environment *par excellence*.

8 October: Francis I is ill, with violent fever accompanied by incoercible vomiting, followed by insomnia and restlessness.

9 October: the fever persists all day, rising towards evening.

10 October: the doctors diagnosed a tertian malarial fever, so Francis underwent an initial bloodletting.

Night between 10 and 11 October: the Grand Duke is better and resumes his activities.

12, 13 and 14 October: Francis again presents violent chills caused by high fever, accompanied by intense sweating that lasts all night.

15, 16, and 17 October: the Grand Duke's condition improved slightly, but worsened again in the following days, again with high and intermittent fever, profuse sweating, incoercible vomiting, dry mouth, constipation and increasing restlessness.

18 October: François I seems to be improving again and two bloodlettings are performed on him.

19 October: Francis I confesses and dictates his last will. In the afternoon, his fever rises again, first accompanied by great restlessness, which is followed by severe asthenia and loss of consciousness two hours before his death, which occurs at the age of 46.

Almost at the same time, Bianca Cappello also fell ill and the court physicians, albeit in less detail, described her illness as very similar to that of her husband.

On 19 October, the Grand Duchess felt ill, seized by an extremely violent attack of fever, and from then on her symptoms were characterised by intermittent attacks of fever, with a symptomatology similar to that of Francis I.

The death occurred on 20 October, at the age of 39.

VERY QUESTIONABLE INDICATIONS OF POISONING

The Florentine toxicologists argued that the symptomatology manifested by François I, characterised by incoercible vomiting, dry mouth, pains and heartburn, continuous restlessness, enlarged liver, pulmonary lesions and diffuse oedemas, would be typical of arsenic poisoning and quite different from that of malarial infection.

In this regard, it should be emphasised that, in populations of countries where malaria persists in an endemic form, the clinical picture of Francis I disease appears typical of acute infection with *P. falciparum*. Indeed, one of the apparatuses most affected during an acute malarial attack is the gastro-intestinal tract. Incoercible vomiting, always accompanied by a febrile state, is the main symptom, which usually occurs with a high frequency (one to seven times a day) at the onset of the disease (White & Breman, 2017). The resulting loss of fluid and electrolytes causes dehydration accompanied by dry mouth and eventually leads to cardiovascular collapse.

Acute tertian malaria, whether or not accompanied by gastro-enteric symptoms, also includes pulmonary oedema as well as neurological disorders such as agitation, behavioural disturbances and loss of consciousness.

Oral ingestion of arsenic trioxide in high concentrations may be associated with gastro-intestinal symptoms, such as acute gastralgias, profuse salivation, vomiting, dry mouth, thirst, slurred speech, diarrhoea, tenesmus, and neurological symptoms, such as convulsions, behavioural disturbances and coma, but is never accompanied by fever (Ratnaike, 2003).

Apart from incoercible vomiting and unquenchable thirst, Francis I did not show any other symptoms of acute arsenic poisoning during his eleven-day agony.

At Cardinal Ferdinando's request, the bodies of Francesco and Bianca were subjected to an autopsy and the doctors confirmed that a form of pernicious malaria, or malignant tertian fever, had been the cause of the sudden and simultaneous death of the Grand Ducal couple.

The legend of arsenic poisoning

However, soon after their deaths, persistent rumours began to spread that Ferdinand had murdered his brother and sister-in-law with arsenic. But there was no lack of other, rather fictional versions of the incident. It was even said that Bianca had prepared a poisoned cake to offer to her brother-in-law Ferdinand but, by mistake, Francesco tasted it too and the Grand Duchess, in despair, ate it herself so as not to outlive her beloved (Galluzzi, 1841).

All these fanciful reports about the death of Francis I and Bianca Cappello were already strongly contested by Guglielmo Saltini in 1863 (Saltini, 1863).

EVIDENCE OF PERNICIOUS *PLASMODIUM FALCIPARUM* MALARIA

In the Laboratory of Parasitology of the Faculty of Veterinary Medicine of the University of Turin, extracts of spongy bone samples from Francis I were tested for the presence of two proteins typical of *P. falciparum*, the histidine-rich type 2 protein (P.f. HRP-2) and lactate dehydrogenase (pLDH), using two highly sensitive qualitative antibody detection methods: the Malaria Antigen RAPIIDTEST® and the MalariaDetect™ RAPIIDTEST® (DiaSys, Connecticut, USA) (Fig. 7). The latter test is used for the differential diagnosis between *P. falciparum* and the other three *Plasmodium* species (*P. vivax*, *P. ovale* and *P. malariae*).

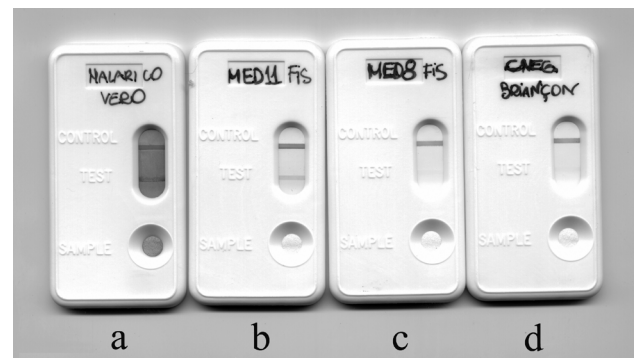


Figure 7. Immunological response to *P. falciparum* malaria by the Malaria Antigen RAPIIDTEST®: from left to right, positive line in a modern *falciparum* malaria (a), serum and positive identification of P.f. HRP-2 in the skeletal remains of Francesco I (b), negative results from Joan of Austria [MED8] and from the Briançon control sample (c).

The same test established the presence of *P. falciparum* histidine-rich type 2 protein and *P. falciparum* lactate dehydrogenase not only in the spongy bone tissue of Francesco I de' Medici (Fornaciari *et al.*, 2010), but also in three other members of the Medici family, i.e. Cardinal Giovanni, Don Garzia and the Grand Duchess Eleonora di Toledo, who in 1562 died of "febbre

terzana”, evidently pernicious malaria, after an autumnal state visit to the malarian Maremma region of Grosseto (Fornaciari *et al.*, 2010), and Detect™ RPYDTEST® showed no evidence of *non-falciparum* or mixed infections.

Recently, biological samples found by Donatella Lippi in 2011 in a vase of XVI century containing the viscera of an unknown member of the Medici family in the “Sarcostia Vecchia” of the Basilica of S. Lorenzo in Florence were studied with modern biomedical technology by the Institute for Mummy Studies of Bolzano (EURAC), directed by Armand Zink. This important research not only revealed the presence of immature forms of *P. falciparum* in the erythrocytes (Fig. 8), but also calculated the tissue parasitemia at 38%, a very high level (Maixner *et al.*, 2023). Finally, the fact that another member of the Medici family, although of unknown identity, certainly died from acute pernicious malaria caused by *P. falciparum*, constitutes a clear, further confirmation of the previous immunological studies.

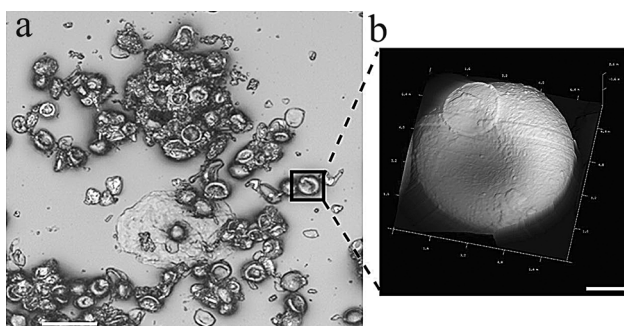


Figure 8. Atomic force microscopy (AFM) of blood vessel section: Sample area at with numerous red cells at light microscope for AFM imaging (scale bar: 20 μ) [a]; enlarged area showing a ring stage of *P. falciparum* in erythrocyte (scale bar: 2 μ) [b]. Courtesy of Marek Janko and Frank Maixner (Maixner *et al.*, 2023).

THE DEFINITIVE ACQUITTAL OF HIS BROTHER FERDINAND, CARDINAL AND FUTURE GRAND DUKE

The hypothesis of arsenic poisoning of Francis I and Bianca Cappello has sparked a debate that has lasted for more than four centuries. A few years ago, a study of the Professor Francesco Mari and colleagues, toxicologists of Florence university, had revived the hypothesis.

The results of the current researches, providing conclusive proof of the presence of *P. falciparum* proteins in the skeletal remains of François I, confirm the historical sources, according to which the Grand Duke died of acute pernicious malaria.

Now the poisoning theory will have to be put back among the many legends that have surrounded the Medici grand ducal dynasty, while Cardinal Ferdinand, the future Grand Duke, is finally acquitted of an infamous accusation.

CONFLICT OF INTEREST STATEMENT

The Author declares that he has no conflict of interest neither known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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(ms. pres. 15 novembre 2024, ult. bozze 15 dicembre 2024)

