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PARASITOLOGICAL ANALYSIS OF LEONESE ROYALTY FROM COLLEGIATE-BASILICA OF ST. ISIDORO, LEÓN (SPAIN): HELMINTHS, PROTOZOA, AND MITES

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ABSTRACT: The royal burial chamber of what is today the Collegiate-Basilica of St. Isidoro in León, Spain, built and remodeled between the 10th and 13th centuries and in the 20th century renamed the Kings' Pantheon, has 13 royal tombs that were opened in the presence of the Abbot-Prior of the Collegiate to enable a group of researchers to obtain all possible information from the royal remains. Several samples were sent to the Parasitology Unit of the Animal Pathology (Animal Health) Department at the Veterinary Faculty of León (Spain). In all the tombs, eggs and remains of nonparasitic mites were observed. In a piece of linen cloth from the bottom of 1 tomb, an *Anoplocephala perfoliata* egg was found. Furthermore, 4 mummified bodies were found. In 2 of these, those belonging to Infantes María and Fernando, *Ascaris lumbricoides* eggs were found and in the latter *Trichuris trichiura* eggs. We have not found in the literature reviewed any records of studies of this kind carried out in Spain.

The royal burial chamber of what is today the Collegiate-Basilica of St. Isidoro in León was originally built on the orders of King Alfonso V (999–1027) of the Astur-Leonese dynasty, following the destruction of the city by Almanzor at the end of the 10th century. This king rebuilt in bricks and mortar a nunnery dedicated to St. Pelayo, to which a relic of St. John the Baptist was entrusted. Inside the convent, he founded a cemetery in a separate chapel, at the front of which the bodies of bishops and members of the Leonese dynasty from bygone centuries were buried in an ossuary: at the back he laid to rest the bodies of his parents, King Vermudo II, "the Gouty," and Queen Elvira. King Alfonso V himself was also buried there, together with his son Vermudo III, "the Young one." Although this cemetery had an altar dedicated to St. Martin, it was never considered a church but rather a simple cemetery (Pérez Llamazares, 1923).

When his daughter Sancha (1037–1067) and her husband Fernando I of Castile (1037–1065), of the House of Navarre, a marriage which united the kingdom of León with Castile for the first time, inherited the kingdom of León, they transformed the royal brick cemetery of Alfonso V into a sumptuous stone-masonry basilica. It was consecrated in honor of St. John the Baptist and St. Pelayo, whose relics were moved into the new temple. Some years later, the body of St. Isidoro, the Archbishop of Seville, was taken to León, and in his honor the temple was consecrated once more and dedicated to him (Pérez Llamazares, 1927).

Sancha and Fernando's church was rather small. It had 3 very narrow naves and was bordered to the west by a square, 2-storey building. The upper part of this building was a royal gallery connected to the temple, and the lower floor, acting as a portico, was a Kings' cemetery, which in the 20th century had become known as the Kings' Pantheon (Fig. 1). It is in this intermediate area, neither holy as a church, nor used for human passage like a cloister, where the royal bodies from the St. Pelayo Convent were taken and where its founders, 3 of their sons, Sancho "The Elder," King of Navarre and Fernando I's father, the last Count of Castile, as well as princes and nobles of the Leonese court, were buried.

This church was remodeled to make way for the Romanic monument that has stood until the present. Of the old building, besides the Pantheon, only the north and west walls have survived, incorporated in the porticos of the new building. Moreover, another 2 porticos were added to the original, 1 of which had a half-barreled vault; it was later walled in to form an enclosure that became known as the Arched Chapel, used as an ossuary and known today as the Infantes' Pantheon because it was here that the tombs of some descendants of royal and noble Leonese families were taken (Viñayo González, 1995).

In the Royal Pantheon, 33 tombs were found in 3 rows; in the first 2 rows, closest to the altar, lay the bodies of kings, queens, and heirs to the throne, whereas in the third row were nobles of the Court, counts, and countesses. The last member of royalty to be buried here was Princess María, daughter of Fernando III, "the Saint," in 1235 (Lucas de Tuy, 1926). Ambrosio de Morales (1572) describes it as a dark place, always closed up, where Mass could never be celebrated for lack of space because the tombs were so close to each other.

Thus the Pantheon remained until 1809, when Napoleon's troops billeted a squadron of Dragons in St. Isidoro's basilica, turning the upper story into barracks, the temple into a military store and barn, and the Royal Pantheon and Chapel in the cloister into stables. Most of the royal urns and those in the adjoining chapels were desecrated in their search for booty, the bones were piled up in a corner, and the stone coffins were used as drinking troughs for the horses. The clergy of the Basilica took the royal remains, some of which were mummified, to a nearby church (Pérez Llamazares, 1976). When this French invasion was over, the Pantheon was reestablished, as it had been in the 13th century the remains were returned to the tombs that had not been destroyed and here most of them remained, mixed and confused, as if these tombs were ossuaries (Fig. 2). Later came the seizure by the State, the sale of Church lands, and the suppression of the monastic community.

The building was occupied again as military barracks in 1836 during the Carlist Wars and in 1868 as a result of the Gloriosa revolution, which led to the First Republic, after both occupations restorations were begun.

Presently, the Pantheon has 13 royal tombs, which were opened in the presence of the Abbot of the Collegiate, so that a group of researchers could investigate and ascertain as much as possible about the royal remains, bearing in mind that the identification of these royal persons, with very few exceptions, was virtually impossible (Prada Marcos, 1998, 2000).

The group included archaeologists, historians, textile restor-

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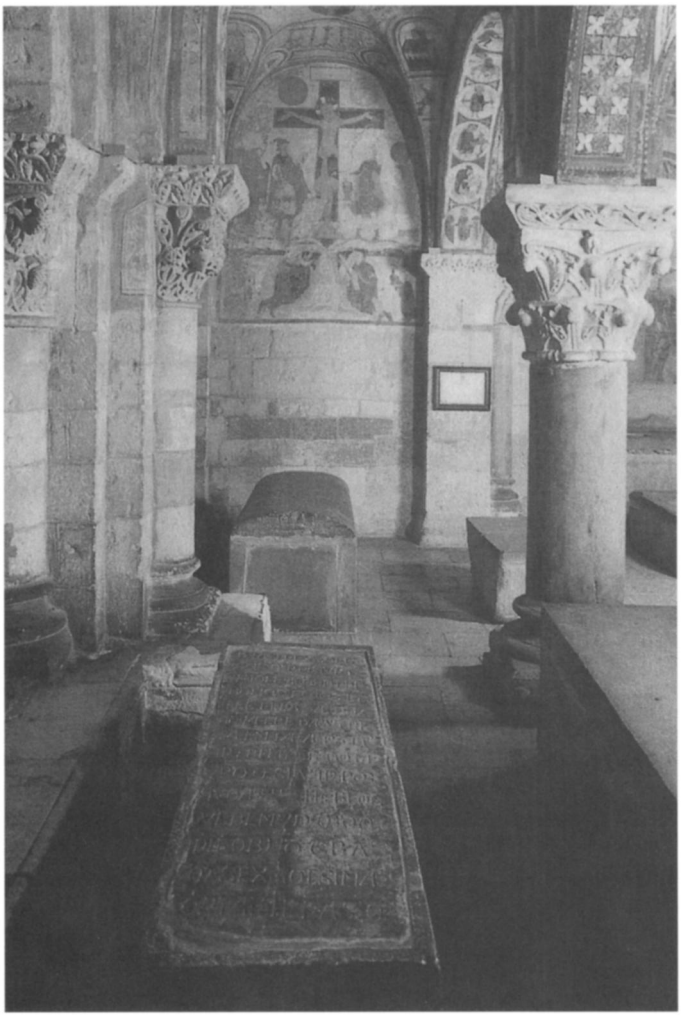

FIGURE 1. Northern nave of the Royal Pantheon of St. Isidoro, which is headed by the Crucifixion of Jesus Christ. On the floor, in the foreground is the tomb of King Alfonso V and in the background, that of Infanta-Queen Sancha. Collegiata-Basilica of St. Isidoro. León, Spain. (Imagen MAS)

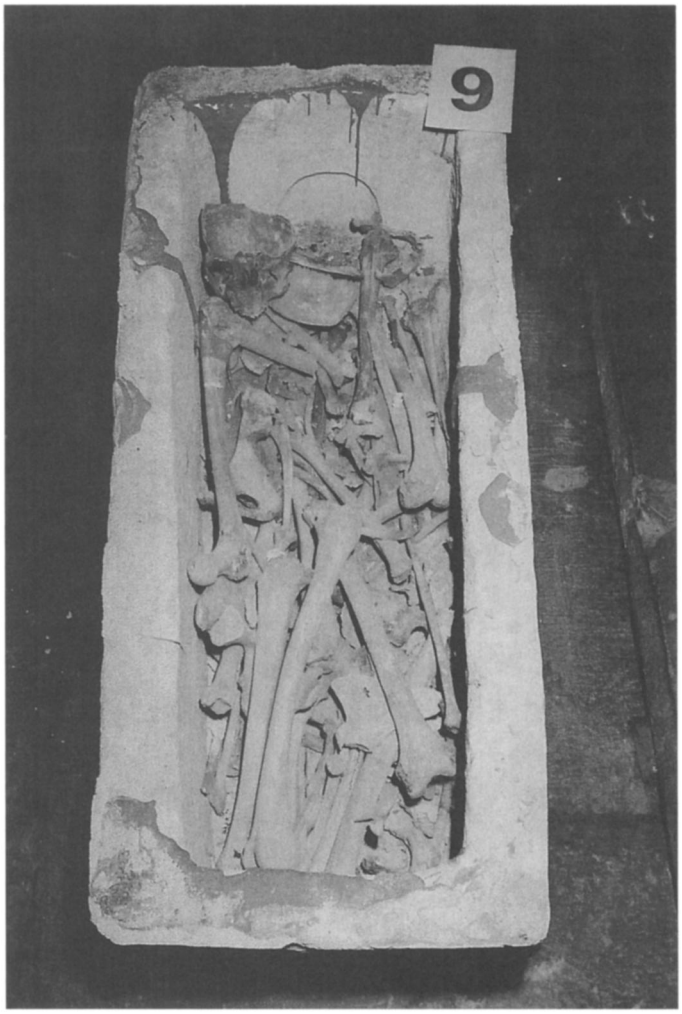

FIGURE 2. Tomb no. 9. Ossuary. Collegiata-Basilica of St. Isidoro, León, Spain. (Imagen MAS)

ers, biologists, anthropologists, forensic scientists, paleopathologists, odontologists, histologists, photographers, etc. The in-depth analysis by the team was aimed at establishing the cause of death, illnesses, diet, clothing, vital statistics, etc. and also at helping to understand our origins.

In the context of this interdisciplinary project, our department received several samples taken in situ for parasitological study during the investigation.

MATERIALS AND METHODS

When the tombs were opened, each was numbered and each item removed was given a reference. Confirming historical documentation, 10 were ossuaries and the number of skeletal remains in each varied between 32 and 910 bones. In the other 3 tombs, 4 uncorrupted bodies were found, belonging to Infanta-Queen Sancha (Fig. 3), sister of King Alfonso VII "the Emperor," and 3 children, attributable to Infante Fernando, son of King Fernando II and grandson of Alfonso VII, Infanta Leonor, daughter of King Alfonso IX and sister of Fernando III "the Saint," who shared the same tomb as the body of Infanta María, daughter of Fernando III "the Saint," the last person to be buried in the Pantheon. The remains were in good condition because of the spontaneous mummification caused by the atmospheric conditions, i.e., ventilation, low humidity, and low temperature, together with the speed with which this process occurs in young children.

The samples came from different tombs and included hair, bones, some mummified remains of muscles from the lower limbs and quadriceps, stones, and remains of clothing from the bottom of the tombs; from the mummified bodies, both head and pubic hairs were collected, together with samples of the scalp, thoracic and abdominal cavities, bone fragments, and remains of clothing.

Each sample was examined, firstly macroscopically and then using a stereomicroscope. They were scraped; any scabs they had were removed and were again examined using the stereomicroscope. Afterward, the samples were hydrated for 2–3 days in distilled water with 10% glycerine. After this time, each sample was filtered through a 150 μm mesh and the liquid obtained was poured into a conical sedimentation flask. The larger particles from the samples (little bone fragments, little stones or remains of clothing) were washed with pressurized distilled water and the homogeneous liquid was added to the flask. It was left to settle for 20 min, after which the supernatant was decanted by hand and again distilled water was added to the conical flask. This process was repeated several times to obtain the cleanest possible sediment. After the final wash, the supernatant was removed with a vacuum pump, leaving approximately 50 ml of sediment in the flask. This was homogenized and using a pasteur pipette, 5 McMaster chambers were filled to observe the sediment of the whole chamber under a microscope using a magnification of ×40.

The remaining sediment was gathered in 2 15-ml tubes and cen-

trifuged for 3 min at 1,500 rpm, and the supernatant was discarded. In 1 of the tubes, a saturated saline solution was added; it was homogenized, and another 5 McMaster chambers were examined by flotation under the microscope. In addition, the bottoms of all the McMaster chambers were examined. Afterward, this same tube was filled with a saturated saline solution to the top and a slide cover was placed over it, it was left to float for 5 min and then studied under a microscope using a magnification of ×40. The other tube was used to carry out sucrose flotations, which were also then observed under a microscope using a magnification of ×40.

The muscle tissue samples were studied, once they were hydrated, with a trichineloscope to determine the presence or absence of muscular cysts.

The hairs were observed under stereomicroscope to check for the possible presence of parasites. Afterward, 1 part of the samples was placed on several slides and a few drops of potash and dyed potash were added; they were left to stand for 20 min, after which they were observed under a microscope using a magnification of ×100.

Another part of the samples taken was treated by diluting in 7% potassium hydroxide (KOH) and maintained under agitation at 37 C for 12 hr. Then they were distributed into tubes and centrifuged for 10 min at 3,000 rpm; the supernatant was discarded, and a solution of sucrose (density = 1.27 g/L) was added and mixed with the sediment and the samples centrifuged (1,500 rpm for 2 min) a second time. After centrifugation, a sucrose solution was added to the tube until it reached the upper part of the glass edge. A coverslip was placed on the tube for 5 min to recover all parasites present in the solution, and it was observed under the microscope using a magnification of ×100.

The left-overs of the hair samples were examined using a fluorescent microscope and a culture of agar to observe the presence or absence of dermophytes (ringworm).

RESULTS

Among the samples taken from the bottom of the tombs with remains (bones, clothing, hairs, and teeth as well as stones, earth, and dust), eggs and remains of mites, i.e., heads, legs, tarsi, and nails, were observed, and only on rare occasions have whole mites been found. Nonparasitic mites belonging to O. Oribatei and the family Aphelacanidae (*Aphelacarus acarinus*) were found. They are semicosmopolitan mites from more or less arid areas. Others belong to the superfamily Tarsonemoidea and the family Podaplipidae, exclusively associated with insects, like those belonging to the superfamily Pyemotoidea and the family Acarophenacidae, although these are also associated with cereal grain, and finally, other mites belonging to the family Cheyletidae, which are predators of the aforementioned.

In the abdominal cavity of the mummified bodies, a large number of *Ascaris lumbricoides* eggs were found in the mummies of Infantes Fernando and María, both of whom died as children. The eggs were oval, 64–76 μm long by 42–46 μm wide, grayish in color, and with an outer mammillated shell. In the abdominal cavity of Infante Fernando, *Trichuris trichiura* eggs with their typical lemon shape were found, although the polar ends had been lost; the eggs measured 50–60 × 26–32 μm (Fig. 4).

Among the samples of the thoracic cavities of the Infantes, no parasites was found, and none was observed in the remains of the other 2 mummies, belonging to Sancha and Leonor.

The egg of a cestode, measuring 90 × 80 μm, was found on examination of a piece of linen; the egg had a thick wall, inside which a pear-shaped structure was observed. Although it was deformed, it is thought to belong to the Anoplocephalidae, more exactly to *Anoplocephala perfoliata* (Fig. 5**a**).

From tomb no. 4, a sample from a quadriceps muscle was taken. Of all the sections made (approximately 200), one possessed what appeared to be a cyst of *Sarcocystis* sp. In another, in a small rounded area, what seemed to be a transversal cut of another cyst was observed. This could not be confirmed because the sample had deteriorated greatly (Fig. 5**b**).

FIGURE 3. Mummified body of Infanta-Queen Sancha. Collegiata-Basilica of St. Isidoro, León, Spain. (Imagen MAS)

DISCUSSION

The study of human diseases in ancient civilizations began in 1910 with Ruffer, who proved the presence of calcified *Schistosoma hematobium* eggs in the kidneys of 2 Egyptian mummies from the 20th dynasty (1250–1000 BC) (Contis and David, 1996).

Since then, the vast number of studies carried out on human remains in different parts of the world have generated much information on parasitic diseases from ancient times to the present day (Grove, 1990; Kliks, 1990; Contis and David, 1996; Aspöck et al., 1999; De Araújo and Ferreira, 2000), which, together with historical data, completes the information necessary for the understanding of our past.

Trichuris trichiura is the oldest parasite found in human remains. Aspöck et al. (1996) found it in the intestinal contents of a Neolithic glacial mummy that was 5,200–5,300 yr old,

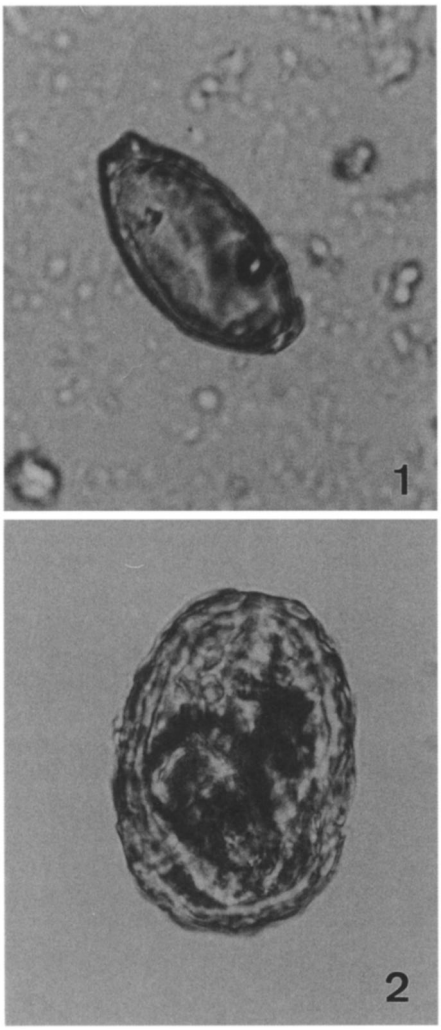

FIGURE 4. **a.** *Trichuris trichiura* egg (50 × 26 μm) in the abdominal cavity of the Infante Fernando. **b.** Egg of *Ascaris lumbricoides* (64 × 44 μm) in the mummies of Infantes Fernando and Maria.

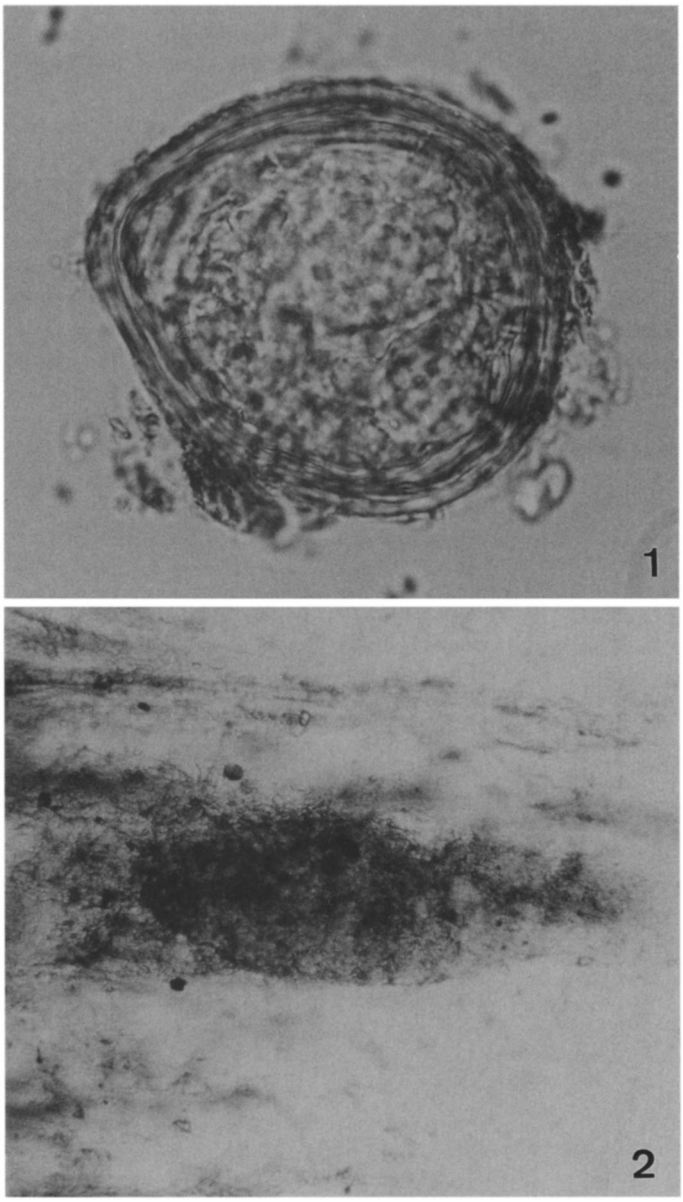

FIGURE 5. **a.** Egg of *Anoplocephala perfoliata* (98 × 82 μm) from the bottom of a tomb. **b.** Possible cyst of *Sarcocystis* sp. (416 × 128 μm) from a sample of the quadriceps muscle.

discovered in the Italo-Austrian alpine valley of Ötztal. The findings of Bouchet (1997) in Chalain (Jura, France), are also from the Neolithic period, but in this case, *Trichuris* was found in human coprolites (3200–2980 BC). Furthermore, *A. lumbricoides* eggs were found in human coprolites from the Paleolithic period in the Grande Grotte at Arcy-Sur-Cure (Yonne, France).

Sebela et al. (1990) found both parasites in samples from the pelvic region of Bronze Age skeletons from Moravia (1600–1500 BC). Aspöck et al. (1974) found eggs of both *A. lumbricoides* and *T. trichiura* in Iron Age human excrement preserved in mineral salts from the salt mines in Hallstat and Hallein (800–200 BC). Szidat (1944) found eggs of both species in bog mummies in eastern Prussia (660 BC), and Jansen and Over (1962) found them in human feces in Germany (100 BC–500 AD). Wei (1973) and Wu et al. (1996) recovered *T. trichiura* eggs in Chinese mummies belonging to the Western Han Dynasty (100 BC) and to the Warring States Period (300 BC), respectively.

Likewise, *T. trichiura* has been reported from human coprolites, sediments and coprolites from latrines, cesspools, tombs of archaeological materials from the Roman Era (753 BC–476 AD) in Jerusalem (Cahill et al., 1991); together with *A. lumbricoides*, it has been found in Holland (Kuijper and Turner, 1992) and in the Medieaval period (476–1500 AD) in England (Taylor, 1955; Moore, 1981) and France (Bouchet, 1995).

Both parasites have also been found in North America in coprolites from latrines in Elden Pueblo (Arizona) dated at 1070–1250 (Reinhard et al., 1987) and from Ferryland, Canada, from the 17th century (Horne and Tuck, 1996). Faulkner et al. (2000) found *T. trichiura* eggs in the 19th century city of La Fayette, Michigan. This parasite is much more common in South America. Ferreira et al. (1980, 1983) reported its presence in coprolites and human mummies (3600–360 BP) in Brazil, as did De Araújo et al. (1981), whereas Pizzi and Schenone (1954) found it in the intestinal contents of the body of an Inca

child in Chile. None of these studies mentions *A. lumbricoides*, though its presence is illustrated in the Florentine Codex of Sahagún, in which a man and a dog are shown defecating round worms or *tzoncooalt*. In addition, there are references to plants that the Aztecs used mainly as seasoning such as *epazot* (*Chenopodium ambrosioides*), *picietl* (tobacco, *Nicotiana tabacum*), or the herb *memeya* (Cordero del Campillo, 2001) but with certain laxative, purgative, and antihelminthic effects.

Ascaris lumbricoides has been reported in Egyptian mummies by Cockburn et al. (1975) and David (1997). Faulkner et al. (1989) found it in desiccated human feces that were dated to be 2,177 ± 145 years old, and Loreille et al. (2001) reported the parasite in Namur (Belgium), in a latrine from the 16th century.

As observed, *T. trichiura* and *A. lumbricoides* have been among the most prevalent parasites in humans throughout history. Those affected normally suffer abdominal pains and certain sensitivity (rashes, insomnia, anxiety), anemia, and sometimes chronic diarrhea and stunted growth (Schmidt and Roberts, 1984; Cooper and Bundy, 1988). Their presence is linked with a low social class and lack of hygiene, which affected, in ancient times, kings, noblemen, workers, and slaves alike.

The *A. perfoliata* egg, whose natural hosts are equines, was found on the remains of a linen cloth from the bottom of 1 of the tombs. Owing to the pillaging of the tombs and the fact that they were used as drinking troughs for horses when Napoleon's troops invaded the city in the 19th century, the egg must have been introduced by chance contamination and then remained in the bottom of the tomb until it was found on the piece of cloth.

We have not found any reference in the literature to the presence of *Sarcocystis* sp. in mummies, but the presence of protozoan parasites should not be surprising at all.

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