

Review articles

Parasites in Forensic Science: a historic perspective

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ABSTRACT. Parasites show a great potential to Forensic Science. Forensic Science is the application of any science and methodology to the legal system. The forensic scientist collects and analyses the physical evidence and produce a report of the results to the court. A parasite is an organism that lives at the expense of another and they exist in any ecosystem. Parasites are the cause of many important diseases. The forensic scientists can use the parasites to identify a crime scene, to determine the murder weapon or simply identify an individual. The applications for parasites in the Forensic Science can be many and more studies should be made in Forensic Parasitology. The most important parasites in Forensic Science are helminths specifically schistosomes. Through history there are many cases where schistosomes were described in autopsies and it was related to the cause of death. Here we review the applications of parasites in Forensic Science and its importance to the forensic scientist.

Key words: Forensic Science, parasites, human disease, human history

Forensic Science

It was never easy to define Forensic Science. Over the years the definition of Forensic Science has been varying depending on the circumstances in which it has been used. The broader definitions are those that show greater variation but are nevertheless accepted and implicit as the "application of science pertaining to the law"[1]. The forensic word originates from the Latin "forensis" which means the art or the study of the public [2]. Forensics is the application of science to the legal system [3]. For example, in a clinical setting a physician uses the results to treat and care a patient, while in a forensic setting, the physician or a nonmedical professional such as a lawyer uses the results to employ them into a legal case.

The role of a forensic scientist is to assist the court, through science, to determine whether evidence has scientific relevance to a legal case. The forensic scientist, when faced with a case, has to determine which evidence is relevant to the investigation. After gathering all the information

and evidence pertinent to the case, the forensic scientist has to decide which examinations, tests or analyses are most appropriate to study the evidence in question. After interpreting the results, the forensic scientist must produce a clear and concise report of his results and refer him to the court.

Parasites as a cause of human disease

An organism that lives at cost of another it is a parasite. Actually, parasites are subdivided into protozoa, helminths (worms) and ectoparasites. They are present everywhere in the living world [4]. Parasites are commonly accepted as vectors of death and disease, and it is acknowledged that they are an important part of ecosystems so they have one of the most important roles on biological evolution [5–8].

Parasites certainly represent more than half of the global diversity of species and include protozoa and macro parasites [9,10]. The ecology of hosts largely determines the diversity of parasites. The great diversity of parasites can also be predicted by environmental factors such as proximity to the

equator and warmer temperatures [11]. Often more than one species of parasite infects the host. Several clinical and experimental studies have been carried out on the host and have revealed that the interaction between parasite species has an impact on parasite dynamics, host health and disease management [4]. The initial immune response of a host has been shown to be critical in the ability of a parasite to successfully infect the host [12,13]. Protozoa are unicellular parasites. They are classified into Sarcomastigophora (e.g., *Amoeba* and *Trypanosoma*), Ciliophora (e.g., *Balantidium coli*) and Apicomplexa (e.g., *Toxoplasma* and *Plasmodium*). Helminths are multicellular parasites and have successfully adapted to a parasitic lifestyle. They are classified into three taxonomic groups: cestodes, such as the pork tapeworm that causes cysticercosis (e.g., *Taenia solium*), nematodes that include the major intestinal worms (e.g., *Ascaris lumbricoides*) and trematodes that include the flukes, such as the schistosomes (e.g., *Schistosoma mansoni*) [14,15].

The World Health Organization estimated that in 2015, 212 million people were affected by malaria and that 429 000 people die from this infection yearly. Most of deadly infections (92%) take place in sub-Saharan Africa. Children under 5 years are at a especially high risk, 70% of all fatalities regard this patient group. The most widespread plasmodial species is *Plasmodium falciparum*. The large majority of fatal cases are due to this species. Malaria contributed in 2013 to 7% of fatalities among children under five years of age worldwide, and 15% in sub-Saharan Africa [16].

Since the beginning of humanity that humans are being affected by a numerous of parasitic infections. Over the years, it has been described over 300 species of parasitic helminths and more than 70 species of parasitic protozoa in humans [17]. Most of these parasites are rare but there are about 90 species affecting humans that are rather common. Most of the important parasitic diseases in the World are caused by a small number of those parasites and they occur mostly in the tropics [18]. The most important parasitic infection worldwide is malaria. Several of the human parasitic diseases that cause death and considerable socioeconomic problems are listed as the main neglected tropical diseases (NTD). Such as ascariasis, hookworm infection, schistosomiasis, Chagas' disease, trichuriasis, lymphatic filariasis, onchocercosis, dracunculosis, human African trypanosomiasis, leishmaniasis and not parasitic: Buruli ulcer,

leprosy, and trachoma [19]. Most of the common infectious diseases that affect humans are parasitic infections [20]. Of all biological species that inhabit the earth more than half take a parasitic lifestyle [21]. On average, for every promising host species there is at least one parasite species [22].

More than two billion people in the world are infected with intestinal helminths. In developing countries, they are common because in those countries the sanitation facilities are inadequate [23,24]. Other parasites are vector-borne, and therefore particularly prevalent in tropical regions. Countries where the parasite that causes malaria is also endemic, infections by helminths remain prevalent [25]. It can often happen that both parasites affect the same host at the same time [26,27].

The application of parasites in Forensic Science

Protozoa

Malaria

Malaria can be proven post-mortem by microscopy of stained thin and thick blood films at least before autolysis occurs. Even later, malaria may be diagnosed by the detection of plasmodial antigens such as histidine-rich protein 2 used for malaria rapid diagnostic tests and by PCR [28].

Trypanosoma

Chagas disease is a public health problem in Latin America and has a high mortality rate. The parasite that causes this disease is *Trypanosoma cruzi*. *Trypanosoma cruzi* is a flagellate protozoan and its form of transmission is through insects of the subfamily Triatominae to humans and other mammals [29]. During the acute phase of Chagas disease the symptoms are usually: fever, splenomegaly, progressive cardiac dilatation and generalized lymphadenopathy [30]. Satoh et al. [30] reported a case of a 48-year-old Brazilian man that collapsed unexpectedly at work. The autopsy revealed that he was infected with *Trypanosoma* parasite. They determined that the cause of death was chronic cardiomyopathy caused by Chagas disease [30].

Amoeba

Testate amoebae are a polyphyletic group of protozoa and they are shelled. Testate amoebae are commonly found living in aquatic or moist habitats.

Such as estuaries, lakes, rivers, wetlands, soils, litter, damp sediments and moss habitats [31,32].

Swindles et al. [31] investigated the value of using testate amoebae for the discrimination of soils from wet ground and puddles. They demonstrated this application with a practical case where they analysed soil residues in trousers from a 10-year-old case and were able to determine where the sediment had come from.

Helminths

Since the beginning of human history helminths have been infecting humans. And as described by Hippocrates and in Egyptian medical papyri we can recognize many of the clinical features of helminth infections [18,33,34]. Through history we have seen many applications of parasitology in cases related to Forensic Science.

Schistosoma

In medico-legal context parasites can be found in cadavers and can be related to the cause of death or simply having no context to the death. Motomura et al. [35] unexpectedly detected in an autopsy case *S. japonicum* eggs in the liver tissue where the presence of the eggs was not related to the cause of death [28,36]. Schistosomiasis is an infection caused by one of the three main schistosomes: *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum* [35]. When a host is infected with schistosome its immune system can be suppressed or circumvented by the parasite. As soon as the worms enter the circulatory system an immune response is triggered in the host directed against the migratory larvae of schistosome but not against the adult worms [36].

The first autopsy where *Schistosoma japonicum* was described for the first time was reported by Majima in 1888 [37]. Nakashima et al. [38] reviewed 349 cases autopsied between 1929 and 1986 at Kurume University with the view to clarify the profile of schistosomiasis japonica in the Chikugo river basin where the disease was highly endemic. They described the different findings during autopsy related to the disease. They observed different types of parasitic nodules in the liver and blockage on the blood circulation in the portal vein system [38].

Echinococcus

The tapeworm of the genus *Echinococcus* is responsible for a parasitic infestation called hydatid disease; this disease is common worldwide [39].

The larval stages of *Echinococcus granulosus* cause cystic echinococcosis and results in the presence of one or more massive cysts or hydatids. It can involve multiple organs such as the heart, liver, brain, kidneys, long bones and lungs [40].

Demirci et al. [41] described a case of sudden death of a 10-year-old girl due to cardiac hydatid cyst rupture located in the right ventricle.

Büyük et al. [39] reported the case of a 17-year-old man who died after he was admitted to a public hospital with the following symptoms: nausea, vomiting and fainting. A forensic autopsy was realized at Forensic Council of Turkey. On the autopsy they observed a macroscopically non-ruptured hydatid cyst in the liver. They attributed the cause of death to anaphylactic shock caused by intravascular spread of the cyst contents.

Ectoparasites

Ozdemir et al. [42] studied the rate of occurrence and vitality of *Demodex* in forensic autopsies. *Demodex* is an ectoparasite that lives on the skin and is transmitted from human to human by skin contact. It can be a vector for pathogenic organisms and can stay alive in dead bodies for a long time. The authors concluded that if the necessary measures are not taken, the personnel performing the autopsies may be at risk [42]. This study demonstrates a case where a parasite may have forensic application without being directly related to the cause of death or even to the criminal investigation.

Forensic historical interest of parasites

Pre-History

Phylogenetic investigations show that precursors of actual plasmodial species existed long before hominids evolved. For instance, dinosaurs were already affected by plasmodia. *P. vivax*, *P. ovale* and *P. malariae* the oldest plasmodia affecting humans already existed long before *Homo sapiens* developed and affected *Homo erectus* [43]. Ascariasis, amoebiasis and fasciolosis have been proven in fossilized Neolithic human faeces [44–46].

Ancient Egypt

The oldest human malaria infections, so far, were proven in 4000 year old Egyptian mummies [47]. In the paper titled “Autopsy of an Egyptian mummy (Nakht-ROM I)” an autopsy of a 3200-year-old

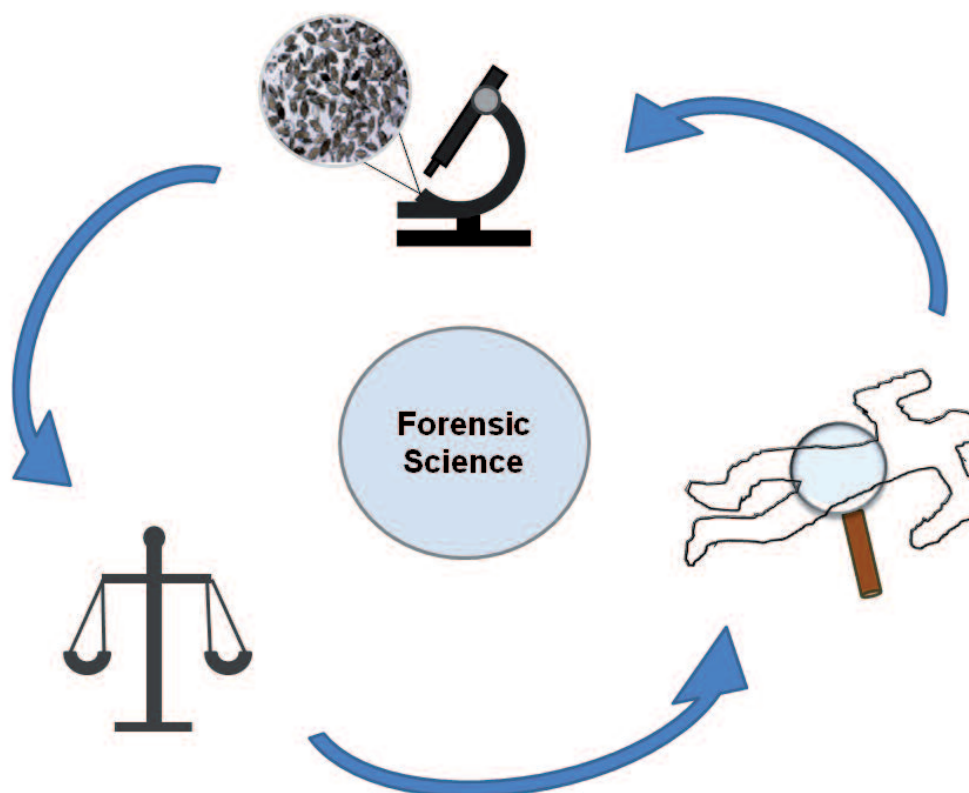


Fig. 1. Schematic representation of the use of parasites in Forensic Science

Egyptian mummy made by Iles [48] is described. In this autopsy they detected the presence of calcified eggs of *Schistosoma* sp. in the liver, kidney and in the small and large intestines. In the intercostal muscle they saw a cyst of *Trichinella spiralis*. Also in the small and large intestines they found calcified eggs of *Taenia* spp. [48].

Miller et al. [49] studied 23 mummies recovered from the North Argin X-group cemetery (AD 350-550) in order to determine the presence of schistosome circulating anodic antigen. They found the schistosome antigen in 15 mummies. With this study the authors have demonstrated the application of an immunological assay for schistosomiasis to mummies [49]. The same study could be applied to a mummy with forensic relevance and help to reveal the cause of death or even help to identify the individual.

Saint-Louis

Louis IX was the King of France between 1226 and 1270 AD. He died when he was 56 years-old in the city of Tunis, Northern Africa. In 1297 Louis IX was officially canonized and was recognized as "Saint-Louis" [50]. Charlier et al. used 2 grams of viscera taken from the King to make a complete and full medical and forensic anthropological analysis

[50]. A Scanning Electron Microscope (SEM) examination revealed numerous parasitic formations. They identified these parasitic formations as adult male *Schistosoma*. This identification was possible because of the morphology and size of the worms. The worms were semi-circular with a gynecophoral canal and their maximal length was 28 mm. They concluded that Louis IX was infected by schistosomiasis. Probably the French King contracted the disease during the 8th Crusade, when he was in Damietta (1250) that is next to the delta of the Nile or during his presence in the Near East (Accra, Cesarea and Jaffa). The authors believe that the cause of death for the King was not directly related with *Schistosoma* and his cause of death remains unknown [50].

Napoleon Bonaparte

Between 1798 and 1799 Napoleon Bonaparte was in Egypt and there he took baths with water from the Nile. After this time in Egypt, Napoleon Bonaparte began to develop symptoms: pain on the right side, urinary pain, enlarged liver, swollen ankles and rashes. As the water from the river Nile was probably infected with *Schistosoma* there is the possibility that Napoleon may have contracted this

infection and this has contributed to his cause of death [51].

First World War

Le Bailly et al. [52] observed samples taken from the abdominal cavities of two German soldiers who fought in the First World War. The samples were positive for intestinal helminths. They recovered eggs from roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), tapeworm (*Taenia* sp.), and eggs from the Capillariidae family were identified. As in the First World War the sanitary conditions in the trenches were poor and there was the presence of commensal animals, this was probably the cause of these infections on the two soldiers [52].

Cold War

In the twentieth century, a case of helminthosis changed the course of History. In China during the Cold War schistosomes fell ill Mao's troops and he had to abort his attack on Taiwan (historically known as Formosa) allowing American ships to have time to enter the Taiwan Strait [53].

Future Perspectives

Forensic Science is the application of any science to civil and criminal laws. Parasitology can be resourceful for a forensic scientist during an investigation, when he is faced with a parasite that can help solve the case (Fig. 1). In the literature studies that are directly related to the study of Forensic Parasitology are rare. Since parasites account for more than half of the diversity of species on the planet we think it would be important to further develop the study of parasites in the Forensic Science. A parasite may be important in identifying an individual as well as assisting in determining where a crime was committed or even helping to identify the murder weapon. From our point of view it must be given greater importance to the parasites since they demonstrate a great potential for Forensic Science. Parasites have a promising role in Forensic Science either in identifying the cause of death, helping to determine where the crime occurred or even having an impact on other Forensic Science related actions.

In the present paper, we demonstrate different applications of parasites in Forensic Science. Here we show, according to scientific literature, that the most important parasites in Forensic Science are

helminths more specifically schistosomes. Since these parasites are in great abundance in the ecosystem we believe that it should be given more importance in its application to the Forensic Science.

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