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## Skeletal evidence of brucellosis in a medicolegal context: A report of two cases

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**Abstract:** Brucellosis is a zoonosis caused by gram-negative bacteria of the genus *Brucella*. Osteoarticular complications are the most frequent symptoms of brucellosis occurring in up to 40% of the cases. Two brucellosis cases submitted to the Department of Forensic Medicine and Toxicology at the National and Kapodistrian University of Athens Medical School for forensic anthropological evaluation are reported. The value of the contribution of the brucellar lesions to the location of the most likely geographic origin of two missing individuals is presented. The presence of a pathologic condition, such as brucellosis, in skeletal remains can give significant information about the biological profile of an unidentified victim and greatly enhances the chances of obtaining a positive identification.

**Key Words:** legal medicine, forensic anthropology, positive identification, brucellosis.

Brucellosis is the most prevalent zoonosis worldwide with more than 500.000 new cases annually, caused by gram-negative bacteria of the genus *Brucella* [1,2]. The genus includes three species pathogenic to domestic animals and through them to humans: *B. abortus*, *B. melitensis*, and *B. suis* [3]. *B. canis* is also a human pathogen but of lesser importance [4]. The bacteria are transmitted to humans through consumption of unpasteurized dairy products and infected meat. Other modes of transmission include skin contact, the respiratory tract and transplacental [5]. Direct person-to-person spread is extremely rare [6]. An incubation period ranging from three weeks to several months is required before the onset of the disease [7].

Brucellosis is divided according to its duration into acute (less than 2 months), subacute (2-12 months), and chronic form (more than 12 months) [8]. Primary infection typically manifests as chronic respiratory

illness, fever and splenomegaly [7]. Secondary infection of skeletal tissue can occur when the bacteria become systemic and spread to cancellous bone [7].

The aim of this study is to report two brucellosis cases submitted to the Department of Forensic Medicine and Toxicology at the National and Kapodistrian University of Athens Medical School for forensic anthropological evaluation. Additionally, the value of the contribution of the brucellar lesions to the location of the most likely geographic origin of two missing individuals is presented.

### CASE REPORTS

#### Case 1

A partially skeletonized body was discovered in an irrigation canal of a rural reed-covered area. The body was missing the head and was wrapped in a blanket. A

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**Figure 1.** Skeletal lesions in L4 vertebral body due to brucellosis: (a) Case 1, (b) Case 2.

day after the recovery, the head of a young woman was found by the police in a plastic bag 120m away from the rest of the body, following information given by the perpetrator.

Forensic anthropological analysis revealed that the remains belonged to a European female, 30-40 years old. European ancestry traits included narrow nasal aperture, sharp nasal sill, and parabolic palate [9]. The age was based upon the morphology of the sternal 4<sup>th</sup> rib end [10]. In order to examine the remains, the skeletal elements were prepared by a chemical simmering method for removing all the remaining soft tissues from bone. During the macroscopic examination of the skull two perimortem depressed fractures caused by a blunt instrument were observed on the left temporal and parietal bone. In addition, cervical vertebrae 2 to 5 presented four knife marks probably caused during the removal of the head. This also reflects the desire of the perpetrator to conceal the identity of the victim. Thus, the manner of death for this victim has been ruled a homicide.

Dental examination indicated congenital absence and several caries on the upper and lower dentition. During the examination of vertebrae, the anterior-superior surface of the vertebral body of L4 showed evidence of slight erosion and reactive bone. The lumbar vertebra presented a lytic lesion in the antero-superior angle of the body (vertebral epiphysitis), adjacent to the annulus fibrosus. No other articular or discal surfaces, nor posterior facets, were affected. In particular, the L4 shows an epiphysitis of the anterior and superior rim, in which the loss of tissue is quite clear and is characterized by a granulomatous appearance (Fig. 1a). This lumbar vertebra also showed a well-developed bony protrusion which extended through the entire superior margin of the vertebral body. These pathological alterations led to the conclusion that the person was affected by brucellosis. No other brucellar lesions were observed on the rest of the skeleton.

After the perpetrator was arrested and confessed, the verification of the person's identity became feasible.



**Figure 2.** Human skeletal remains recovered from a septic tank (Case 2).

Forensic anthropological analysis led to a match to a missing persons' police file. The remains, which had been exposed for roughly 6 months, belonged to a 33 year-old female from Romania.

### **Case 2**

Human skeletal remains were discovered in a domestic septic tank with a depth of 4.5m of a relatively new apartment building by a worker who was called to remove the wastewater. The new apartment block had been built 10 years ago but it was never inhabited. The remains were covered in a greyish building material with a thick liquid consistency (Fig. 2). Following recovery by the police, the remains and associated evidence were submitted to the laboratory for forensic anthropological evaluation.

The anthropological analysis revealed that the remains originated from a 35-45 year old European male, with a living stature of 166-173 cm. European ancestry traits included narrow nasal aperture, sharp nasal sill, and parabolic palate [9]. The morphological assessment of the pelvis and skull indicated that the remains belonged to a male. The age was based upon the morphology of the pubic symphysis [11], the auricular surface [12], and the

sternal 4<sup>th</sup> rib end [10]. In addition, the Lamendin's aging technique was applied using teeth 15, 34, 35, and 45 [13]. During dental examination, it was observed that all the teeth that were found presented severe dental calculus.

Antemortem pathologies included a healed fracture of the left 5<sup>th</sup> rib and some pathological lesions in the vertebrae. In particular, Schmorl's nodes were observed on the inferior surface of the vertebral bodies of T5 and T9. Syndesmophytes were present on the anterior surface of vertebral body of T10. In addition, a lytic lesion was observed in the antero-superior angle of the body of L4 (Fig. 1b). The alterations of the anterior face are coarse ossifications of the ligaments that present as large angular osteophytes. Moreover, a sub-periosteal reaction in a form of finely porous microtrabecular neoformed bone to the antero-superior surface of the vertebral body was observed in this region of the vertebra. The observed lesions on the lumbar vertebra appear consistent with brucellosis. Brucellar alterations were not found on any other bone of the skeleton.

Forensic anthropological analysis and identity documents which were found in the victim's clothing provided the necessary information to establish a positive identification. The skeletal remains belonged to a 37 year-old male from Pakistan whose remains were in the septic tank for approximately 7 years after his death but he had never been reported missing.

## DISCUSSION

### *Skeletal pathology and differential diagnosis*

According to the World Health Organization (WHO), osteoarticular complications are the most frequent symptoms of brucellosis occurring in up to 40% of the cases [14]. Sometimes, bone lesions are extensive and they are observed in different regions of the body at the same time [15]. The spine is the most commonly affected structure, especially the lumbar vertebral bodies. Long bones are less frequently affected, while flat bones are the least often involved [16]. It has been reported that apart from the spine, brucellar lesions may appear on the femoral head, hip, humerus, tibia, knee, sacroiliac joint, shoulder, and carpals [17-24]. The evidence of the above studies was based on X-rays, CT scans and MRI imaging. In the early stages of the disease, radiographs and bone scintigrams may appear normal [14]. In the chronic phase the disease is characterized by various organ-related manifestations, bones and nerves [1]. It has been reported that skeletal manifestations are more frequent and more evident during the chronic and subacute stages [8, 22, 25, 26]. According to Capasso in the initial phase there is a characteristic brucellar polyserositis with a pleural, peritoneal or pericardial seat [1]. Brucella infection can cause spondylitis in the lumbar and thoracic spine, osteomyelitis in long bones and the pelvis, and arthritis [27, 28]. The articular or osteoarticular localizations of

brucella are actually more frequent, and some authors consider them necessary to diagnose brucellosis [1]. The most common form is a serosal arthritis of the major joints, primarily the knee, and the small joints of the hands, especially the interphalangeal joints [1]. However, the most typical localization is at the vertebral column. Today about 30% of brucellar infections in humans develop the typical spondylitis brucellaris known as anterior epiphysitis [1]. Adult males are affected much more frequently than females [3]. The spinal lesions are usually located in the vertebral bodies, especially of the lower thoracic, lumbar, and lumbosacral areas, often involving more than one vertebra [3]. The lumbar section of the spine is affected in 80% of the cases [1]. The inflammatory brucellar course in the vertebral column initially affects the intervertebral disc tissues and the bone of the antero-superior corner of the body immediately below the affected disc [1, 7, 29]. Subsequently the fibres of the anterior longitudinal ligament are affected, as is the periosteum of the anterior face of the vertebral body [1]. The inflammatory activity involves only the vertebral body below the disc and only the region in contact with the annulus fibrosus, producing an osteolysis of the antero-superior vertebral corner. This is usually followed by an osteolysis of the anterior superior somatic angles caused by the continued spread of the bony infiltration on the part of the brucellar granuloma, beginning with the anterior part of the vertebral disc [30]. This initial invasive osteolytic phase is followed by a phase of osteogenic reaction [31].

The vertebral lesions observed in the two described forensic cases are attributable to the so-called anterior vertebral epiphysitis. Apart from brucellosis, several infectious diseases can produce similar lesions like haematogenous osteomyelitis, staphylococcal spondylitis, salmonellosis, and tuberculosis [1]. Haematogenous osteomyelitis produces localizations on the anterior part of the vertebral body [32]. The localization of haematogenous osteomyelitis on the vertebral column generally occurs at the level of the dorso-lumbar hinge. Staphylococcal spondylitis is characterized by the destruction of the superior anterior somatic angle, while the plates of the affected vertebra and the one above it are unaffected. The osteolytic lesions are not limited to the area below the imprint of the annulus fibrosus, but rather spread, generally affecting all of the anterior half of the upper part of the vertebral body. In addition, the sclerotic reactions are quite limited [1, 33]. Salmonellosis can also cause spondylitis with osteolytic lesions of the antero-superior somatic angle, contrary to what occurs in brucellosis where this is followed by new bone formation [34]. In tuberculosis, the part of the vertebra involved is almost exclusively the vertebral body and definitely the anterior portion of the body [35]. The involvement of posterior elements of vertebrae is very uncommon [3]. Vertebral lesions are mostly localized in the lower spine, in the inferior part of the thoracic and lumbar

vertebrae [3, 36, 37]. The destruction of the vertebral body is usually purely lytic leading to cavitation. Tuberculosis is characterized by lack of new bone formation [38]. In brucellosis the osteolytic lesions are characteristically confined to the lower dorsal and upper lumbar sections [1]. The lytic lesions affect the antero-superior angle of the vertebral body and are followed by a sclerotic response [3]. Hence according to the above and taking into consideration possible differential diagnoses, the observed lesions on the two lumbar vertebrae of the two forensic cases examined seem attributable to brucellosis. Lumbar vertebrae from both individuals exhibit lytic lesions in the antero-superior portion of the body that display sclerotic margins due to granulomatous reaction. It is therefore necessary to differentiate brucellosis from other infectious diseases which can cause vertebral lesions, so it can be considered as a supplementary factor in the elaboration of hypotheses relating to an unidentified individual.

### ***Epidemiology and distribution***

Brucellosis is a public health problem in many developing countries [39]. In many brucellosis-endemic countries, health systems are not well-organized and the cases are considerably under-reported. It is likely that the official data underestimate the true disease burden [40, 41]. Moreover, as brucellosis is one of the great imitators of infectious diseases, it can be frequently misdiagnosed [42]. The correct diagnosis and the specific treatment are often not made until the disease is at an advanced stage, thus leading to bone destruction [15]. It is evident that a highly knowledgeable medical staff and an adequately functional notification system are required to control brucellosis.

Brucellosis is a mandatory notifiable infectious disease in Greece. The Department of Epidemiological Surveillance and Intervention of the Hellenic Center for Disease Control and Prevention (HCDCP) collects data regarding brucellosis cases from all areas of the country through the established mandatory notification surveillance system. According to HCDCP, in the period 2005-2011, 1.410 incidents of brucellosis were reported, i.e. the mean annual report was 18 cases/1.000.000 of population. The rate is constantly declining since 1998, when the vaccination program in sheep and goats was re-established, substituting the "test and slaughter" policy which had been implemented for 5 years. An isolated peak of the disease in the human population occurred in 2008 (31/1.000.000) because of an outbreak in the island of Thassos, which reached 104 cases. The incidence was attributed to the consumption of non-pasteurized dairy products during the period of the Easter holidays, when dairy products are traditionally produced and distributed [43].

Greece is located in southern Europe, on the eastern edge of the Mediterranean Sea, sharing boundaries with Albania, Bulgaria, Turkey and the former Yugoslav Republic of Macedonia (FYROM). Brucellosis is endemic

in all the countries surrounding Greece and the rates of incidence there are considerably higher. More specifically, the annual cases per million of population in Albania are 63.6, in the FYROM 148 and in Turkey 262.2 [2]. Bulgaria was considered to be brucellosis-free, but during 2005-2007 a re-emergence was recorded. Among 2.054 people tested due to occupational risk, a total of 105 human cases of brucellosis were diagnosed [44].

In addition, due to its geographical location and EU membership Greece is a destination for immigrants originating from the Eastern Bloc (i.e., former communist states of Eastern and Central Europe), the Middle East, Asia, and North Africa. In all regions brucellosis is endemic. More specifically, Romania is bovine-brucellosis free since 1969. But like most former communist countries, after 1989 when the transition from governmental to private owned properties was made, there were several outbreaks of sheep and swine brucellosis. During the transition the number of animals decreased and so did the brucellosis-control measures. Vaccination against brucellosis is prohibited in Romania but the incidents are gradually decreasing [45]. In Pakistan, the veterinary care of domesticated animals is limited and many physicians are unaware of the disease [46]. The incidence of human brucellosis is unknown because of the lack of reporting and notification system. However, the disease is endemic and it is most frequently diagnosed in women, as it is common practice for the females to tend the cattle at home in rural areas.

### **CONCLUSION**

The presence of pathological conditions in unidentified skeletal remains can contribute to forensic investigations in a decisive manner. Brucellar lesions can provide clues to how and where people lived and ultimately can lead to their identification. As the percentage of human brucellosis in Greece was considerably lower in comparison to other countries in the region, the presence of brucellosis in lumbar vertebrae was an indication that the human remains in both cases belonged to foreigners. This helped direct the investigative process to potential victims.

Another element that led us to this conclusion was that the lesions in the bones are characteristic of the chronic phase of the disease. It is known that brucellosis is a mandatory notifiable infectious disease in Greece and the infection of bones is rare as it is usually treated while being in the initial phase. The presence of a pathologic condition, such as brucellosis, in skeletal remains can therefore give significant information about the biological profile of an unidentified victim and greatly enhances the chances of obtaining a positive identification.

**Conflict of interest.** The authors declare that they have no conflict of interest concerning this article.

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