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Research Article

Preliminary Results of the Analysis of Coprolite Material of a Dog Unearthed from the Van-Yoncatepe Necropolis in Eastern Anatolia

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Abstract: This study presents the results of the analyses of the coprolite material found among the skeletal remains in M6, a burial chamber discovered in the necropolis of the Van-Yoncatepe Castle, which dates back to the beginning of the 1st millennium BC (Early Iron Age). For this purpose, microbiological and parasitological examinations, as well as biochemical analyses have been carried out. Because the crystallisation values were so high, the parasite evidences could not been revealed. As a result of the microbiological examination, no micro-organisms were isolated. As for the biochemical analyses performed, the material was tested for glucose, uric acid, cholesterol, triglyceride, total protein, albumin, calcium (Ca), phosphorus (P) and magnesium (Mg). Attempts were then made to ascertain the feeding habits of the Yoncatepe dogs.

Key Words: Coprolite, analysis, dog, necropolis, Van-Yoncatepe

Doğu Anadolu Van-Yoncatepe nekropol'ünde Ortaya Çıkarılan Köpek Koprolit Materyalinin Ön Analiz Sonuçları

Özet: Bu çalışmada, M.Ö. 1. binyılı başlarına (erken demir çağı) tarihlendirilen Van-Yoncatepe kalesi nekropol'ünde ortaya çıkarılan M6 mezar odasında köpek iskelet kemikleri içerisinde bulunan koprolit materyali analiz sonuçları sunuldu. Bu amaçla mikrobiyolojik ve parazitolojik muayeneler ile biyokimyasal analizler yapıldı. Parazitolojik muayenede, kristalize olmuş parazit kanıtlarına rastlandı. Kristalizasyonun fazla olması nedeniyle paraziter kanıtların tam olarak ayrımı yapılamadı. Mikrobiyolojik incelemeler sonucunda her hangi bir etken izole edilmedi. Biokimyasal analiz olarak, glukoz, ürik asit, kolesterol, trigliserit, total protein, albumin, kalsiyum (Ca), fosfor (P), magnezyum (Mg) değerlerine bakıldı. Tüm bu işlemlerle, Yoncatepe köpeklerinin beslenme alışkanlıklarının belirlenmesine çalışıldı.

Anahtar Sözcükler: Koprolit, analiz, köpek, nekropol, Van-Yoncatepe

Introduction

Dogs and cats are among the earliest of domesticated animals. In ancient Egypt, cats were so prized that they were raised to deity status. Exporting or killing a cat was punishable by death. Cats were also highly valued for their abilities to control vermin (1).

In the Early Iron Age, however, dogs would have assumed significant roles within the socio-cultural frame

of the period (2). It is in support of this view that some skeletal remains belonging to dogs buried together with humans were unearthed from the Van-Yoncatepe necropolis, which is situated in a region culturally close to Egypt.

Skeletal remains discovered in various archaeological sites have so far aroused the interest of many researchers. It is thanks to this interest that such remains have been examined and valuable data has been obtained (3-5).

To date, the examinations performed on the archaeological bones of dogs have made it possible to estimate both the morphological appearance (6,7) and body weight of dogs (8-12). Dental and mandibular (13-15), skull (15), and long-bone measurements (10,12,16-19) were widely used in these examinations.

Apart from skeletal remains, some coprolite material both belonging to dogs and humans was discovered in various burial environments (5,20,21). Examinations on human coprolite material revealed the presence of certain micro-organisms and bacteria in the intestinal tract (22). Some viable bacteria spores were reported in the Roman site of Vindolanda in Northumberland (23). It has been stated that spores could survive for a long period of time depending on the suitability of the conditions in a certain burial environment, and that it is possible to come across some parasitic remains, as well as micro-organisms, in such places. To illustrate, eggs belonging to some types of parasites have been found in various early sites, increasing the interest in this field of archaeology (5). It was this interest that led to the discovery of ancient helminth species in various sites (23-26).

In Anatolia, skeletal remains of dogs dating back to 7,000 BC have been discovered in one of the very early sites in Çayönü (27-29). It has been reported that skeletal remains belonging to domesticated dogs were scarce in Çayönü (29), whereas there were plenty of them in the Van-Yoncatepe necropolis, where this study was carried out (15).

There are various analyses carried out on the skeletal remains from various archaeological sites in Anatolia (15,30); however, to the authors' knowledge, no studies have been performed on the coprolite material of dogs.

In our previous studies, we presented the data on typological classification, age and sex of, and anatomico-pathologic deformations on the skeletal remains of these dogs (15), as well as their estimated shoulder height (15) and body weight (31). Further to these studies on the Van-Yoncatepe dogs, we here aimed to analyse the coprolite material found among the skeletal remains of the same dogs and determining their feeding habits and social life.

Materials and Methods

In this study, we used the coprolite material found intact among the skeletal remains of dogs in burial chamber M6 (Figure 1). This was the chamber discovered in the necropolis of the Van-Yoncatepe castle, dating back to the beginning of the 1st millennium BC (Early Iron Age) (2). The Van-Yoncatepe castle (Eastern Anatolia) is 15 km south-east of Tushpa, the capital of the ancient Urartian Kingdom. The excavations carried out here from 1997 to 2002 revealed that there existed various burial chambers in the necropolis situated to the north of the castle. Plenty of bones scattered in three burial layers were found in M6, one of these burial chambers where the coprolite material used in this study was discovered.

Our study aimed to indicate the nutritional habits and caring conditions by microbiological, parasitological and biochemical analysis of the coprolite material sample. It was aimed to perform the analysis that is discussed above.

Microbiological examinations

The coprolite material found intact was slit in a petri dish under sterile conditions. The sample taken from the centre was left to soak in sterile distilled water at + 4 ºC

Figure 1. The coprolite unearthed from burial chamber M6.

for 12 h. The suspension obtained was used for microbiological examinations. Smears were prepared for microscopic examinations. Gram-Stain, Ziehl-Neelsen and Malachite Green-dyed slides were used for bacterioscopy. As to the fungal examinations, smears were stained with Lactophenol Cotton Blue Stain. For the bacteriological culture, the suspension was inoculated onto blood agar plates, and incubated at 37 ºC for 72 h under both aerobic and microaerobic conditions. The same suspension was inoculated to Sabouraud Dextrose Agar for the mycological culture and it was incubated at + 25 ºC for 3 weeks (32).

Parasitological examinations

Native smears were prepared (33). The material was then examined under 20 and 40 magnifying phase-contrast microscope (Nikon).

Biochemical analysis: 3.8 g of coprolite material was shaken for 24 h in a shaker containing 6 ml of sterile distilled water. The mixture obtained was centrifuged (10 min 3000 x g), and a sample of 2 ml was taken from the supernatant. This sample was tested for glucose, uric acid, cholesterol, triglyceride, total protein, albumin, calcium (Ca), phosphorus (P) and magnesium (Mg). Glucose, uric acid, cholesterol, triglyceride, total protein, albumin (Human Gesellschaft für biochemical und Diagnostica mbH, Max-Planck-Ring 21.D-65205. Germany), calcium (Ca), phosphorus (P) and magnesium (Mg) (Spinreact, S.A.Ctra.Sarita Coloma, 17176 Sant Esteve De Bas Gi., Espana) were measured spectrophotometrically by means of the kits mentioned above.

Results

Macroscopic examinations

The macroscopic examinations carried out on the coprolite material found among the skeletal remains of dogs in burial chamber M6 revealed small pieces of bones.

Microbiological findings

No kind of bacteria, fungi or spores were found in the stained preparations for bacteriological examinations, nor were any micro-organisms detected in the cultures.

Parasitological findings

Microscopic examination of the smears revealed crystallised remains of parasites and leukocytes. Although over-crystallisation made it almost impossible to accurately classify these parasites, they were still likened to the eggs of tapeworm ring (Figure 2/A, B), oxyurid (Figure 2/C) and ascarid (Figure 2/D). In order to accurately identify the parasitic bodies, a further study was planned in which genetic material could be analysed by polymerase chain reaction.

Biochemical analysis findings

The data obtained by means of spectrophotometric analyses are presented in the Table.

Discussion

The location of the coprolite material found intact among the skeletal remains in burial chamber M6 gave the impression that it was a mass of hard faecal matter from inside the rectum of the dog. Pieces of bones observed in the faeces during the macroscopic examinations were important evidence showing that the dog had lived on a diet of other animals, especially foodstuff containing plenty of bones.

Upon the examination of the stained preparations, it was observed that there were no bacteria, fungi or spores, nor were any micro-organisms detected in the cultures. This showed that no bacteria in the burial environment contaminated the coprolite material, or those bacteria, fungi or spores that were present in the coprolite material might have been crystallised over time.

Parasitological examinations revealed parasitic remains. However, it was not possible to accurately identify these remains because the piece of faeces that we analysed had toughened due to the congestion in the burial ground and crystallised in time. Nevertheless, tania ring, oxyuris and ascarid eggs were detected during the examinations. These findings were important in the sense that they were evidence of parasitic infestation. Moreover, they gave us the impression that the Van-Yoncatepe dogs were fed with raw food. In order to accurately identify the parasitic bodies, a further study was planned in which genetic material could be analysed by polymerase chain reaction.

Glucose, uric acid, cholesterol, triglyceride, total protein, albumin, calcium (Ca), phosphorus (P) and magnesium (Mg) levels in the coprolite material suggested that these dogs usually lived on a diet of animal meat and bones. Pieces of bones observed in the coprolite material were in support of this view.

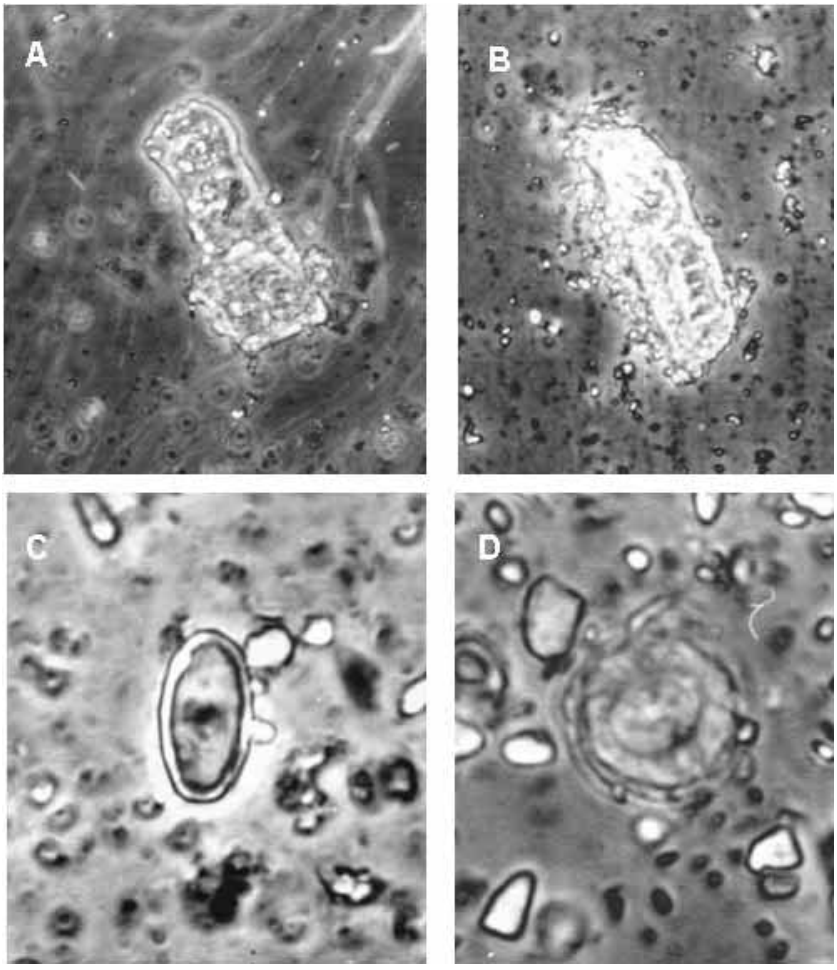

Figure 2. Remains of parasites.
A, B: The eggs of tapeworm ring, x20, C: The eggs of oxyurid, x40, D: The eggs of ascarid, x20.

Table 1. Biochemical analysis of the dog coprolite material

Glucose mg/g	Uric acid mg/g	Cholesterol mg/g	Triglyceride mg/g	Total protein mg/g	Albumin mg/g	Calcium (Ca) mg/g	Phosphorus (P) mg/g	Magnesium (Mg) mg/g
0.078	0.09	0.268	0.521	42	6	0.156	0.017	0.024

Intake of minerals and their ratios in the faeces vary depending on the daily food consumption and feeding habits of dogs. Likewise, the amount of calcium (Ca), potassium (K) and magnesium (Mg) in the faeces varies according to animal species and the type of food consumed (34,35). To the authors' knowledge, as far as the ratios of minerals and above-mentioned elements are concerned, the only information available is that of the parameters pertaining to the mean levels of such substances found in the faeces of ruminants and poultry (36). However, our present knowledge about the feeding habits of dog races does not allow us to determine such mean values for this animal.

A close examination of each biochemical parameter obtained from the coprolite material revealed that biochemical substances could be preserved for years. It might be thought that such substances disintegrate in a natural environment and lose their properties through the years. Yet, under certain conditions, they might still remain intact and can bear invaluable information, as in our case, about how ancient dogs fed or were fed.

Since short-chain triglycerides dissolve in water and can easily be absorbed, it is probable that the ones observed in the coprolite material were long-chain triglycerides. Of note, it has been reported that long-chain triglycerides are found abundantly in the faeces in the case of a pancreatic disease (37).

It has been asserted that high levels of glucose in the faeces might also be a sign of pancreatic disease or old age (38). This assertion is based on the fact that glucose is much more easily absorbed in young and healthy animals. It passes rapidly through their intestines during the period between birth and maturity and is rarely detected in faeces. In the case of diseases affecting the liver, pancreas or intestines, however, the rate of absorption decreases (34,39). Especially in patients with cancer, lactate is reconverted into glucose during the Cori cycle, increasing gluconeogenesis (34).

High levels of triglyceride, Ca and P in the faeces suggest that an animal most probably feeds or is fed with meat and/or bones (40), and the extra amount not absorbed is discharged as faeces (33,41). High levels of triglyceride, Ca, P and glucose may also be a reminder of malnutrition or osteological disorders. This might explain the deformations observed on the skeletal remains of the Van-Yoncatepe dogs (15) and the high levels of such biochemical substances as triglycerides, Ca and P detected in the coprolite material found among the skeletal remains of these dogs.

To the authors' knowledge, no studies have so far dealt in detail with the analyses of coprolite material or faeces of present dog races. There are some studies performed on the feeding habits of dogs (34,35,42), but they lack information about breakdown of matter, making it impossible for us to compare our findings.

It is possible to conclude that the Van-Yoncatepe dogs were in a good physical condition and were used as hunting or working partners considering their large format (15). This was probably due to the intake of a considerable amount of such substances as magnesium, which is known to accumulate in the muscles. It is in support of this view that a high level of magnesium was detected in the coprolite material examined in this study. It is significant that the level of magnesium, which is normally high in herbivores, was also found to be high in carnivores (the Van-Yoncatepe dogs) probably feeding on herbivores. The levels of total protein and albumin detected in the faeces are another sign showing that the diet of the Van-Yoncatepe dogs was mainly based on protein.

In conclusion, the analysis of the coprolite material brings to mind the following possibilities:

-A diet rich in meat and bones,

-Consumption of raw animal food,

-Diseases/Disorders of the liver, pancreas, intestines or skeletal system,

-Old age,

-A distant possibility of cancer.

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