Palaeoparasitological study of gastro-intestinal content in horses at a Scythian kurgan (3rd century BC) found in Kazakhstan

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ABSTRACT
As part of the excavations of the Scythian kurgan of Berel' (3rd century BC), twenty samples were studied, taken from nine of thirteen horses inhumed with the corps of a prince and a woman discovered under the stone tumulus. The palaeoparasitological diagnosis of the samples, based on the identification of preserved helminth eggs, revealed a well known equine parasite, *Oxyuris equi*, and another nematode belonging to the Strongylidea order. The identi-

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fication of parasites provides information on the health status of the horses, and the results are compared with palaeoparasitological diagnosis conducted on the two bodies recovered in the princely tomb. This original work has established the first paleoparasitological record of horse remains from ancient Kazakhstan.

MOTS CLÉS
Paléoparasitologie,
helminthes,
œufs,
Scythes,
Kourgane,
Kazakhstan.

RÉSUMÉ
Étude paléoparasitologique des contenus gastro-intestinaux des chevaux d'un kourgane scythe (III^e siècle av. J.-C.) retrouvé au Kazakhstan
Dans le cadre des fouilles d'un kourgane scythe retrouvé à Berel' (III^e siècle av. J.-C.), vingt échantillons sont étudiés, provenant de neuf des treize chevaux inhumés avec les corps d'un prince et d'une femme découverts dans le tumulus de pierre. Le diagnostic parasitologique, basé sur l'identification des œufs d'helminthes conservés, révèle la présence d'un parasite équin bien connu, *Oxyuris equi*, ainsi qu'un autre nématode appartenant à l'ordre des Strongylidea. L'identification des parasites fournit des indications sur l'état de santé des animaux, et les résultats sont confrontés à ceux obtenus sur les deux corps humains retrouvés dans la tombe princière. Cette étude est unique en paléoparasitologie et débute le référentiel des parasites anciens de chevaux pour le Kazakhstan.

INTRODUCTION

The Altai Scythian frozen tombs of Pazyryk culture have been known in central Asia since 1927, when a Soviet expedition discovered a group of royal burials on the eponym site of Pazyryk, southern Siberia (Rudenko 1970). A large amount of organic artefacts, such as clothes, ornaments, sacrificed horses, or tattooed mummies are often recovered from these frozen tombs.

In 1998-1999, a new frozen tomb, dating from the beginning of the 3rd century BC, was excavated in the Kazakhstan eastern Altay mountains by an archaeological team directed by Zainullah Samashev, from the Kazakh Institute of Archaeology and Henri-Paul Francfort from the CNRS, France (Francfort *et al.* 1998). The tomb, 23 m in diameter and 2.40 m high, was located in a Scythian cemetery by the Bukhtarma River, near the modern-day village of Berel'. Four meters below the surface, the preservation of the remains from the burial pit was excellent. Despite having been looted, the burial, containing two human bodies, was associated with important organic remains, among which were thirteen sacrificed horses with full trappings. The study of the human bodies was coordinated by E. Crubézy, A. Ismagulova and E. Ljudvikova, while the horses were first studied on site, then in the laboratory by S. Lepetz. The analysis showed that the horses were exclusively males aged between eight and nineteen years of age. The horses were positioned in two layers, seven in the lower part, six above, according to an overall South-western/North-eastern axis, the head toward North East. All seemed to lie on their bellies.

All bodies were collected, taken to Almaty in a refrigerated truck and then stored in a refrigerated room. In 2000, samples were taken by S. Lepetz and Cl. Puyalto-Moussu for various analyses, among which palaeoparasitology.

A palaeoparasitological diagnosis was established based on the morphological and morphometrical characteristics of the intestinal parasite helminth eggs found in the samples after extraction. The aim of the study was to assess the parasitic inten-

sity and the origin of the horses (breeding area or geographical origin) (Francfort *et al.* 2000). The archaeologists raised the hypothesis that the horses may have been offered as a sacrifice by neighbouring allied populations. This theory was based on the discovery of many different artefacts in the tomb, some of them from the Achaemenid culture that indicate contacts between local Scythian culture of Altai and the Persian Achaemenid Empire.

MATERIAL AND METHOD

Twenty samples from the content of the gastro-intestinal tract were retrieved for Paleoparasitological analysis, coming from nine of the thirteen horses from the frozen tumulus (Table 1). The samples were composed of well preserved digestive tract contents, which may evade the issue of contamination. The samples were constantly stored in a cold chamber or freezer from the setting to the Palaeoparasitology lab to keep their integrity.

The materials were treated following the standard protocol used in our laboratory (Le Bailly 2005). A portion of each samples were first rehydrated for a week using 0.5% trisodium phosphate and 5% glycerinated solution. The material was then crushed in a mortar. The suspension was submitted to an ultrasound device for 1 minute at 50°C, on a frequency of 35 kHz (Sonorex, RK 103 H), and the suspension strained through 315-µm, 160-µm, 50-µm, and 25-µm meshes, under a constant ultrapure water flux obtained with a purification system (Millipore, Direct-Q^{TM} 5). A portion of each sample was used for microscopic examination. The material was placed between slides and cover slides, and examined for evidence of parasites. Twenty slides of each sample were examined at magnification of × 100 and × 400. Observations were recorded using a numeric camera linked to a computer with SAISAM software (Microvision instrument).

TABLE 1. – List of the samples studied in Reims (M. Le Bailly).

Samples	Horses	Description
37	C	Stomach or intestine content of C.
43	C	Alimentary content of C (colon?). Rectum axis.
47	B	Intestinal wall containing SL 46
55	F	Rectum content of F.
69	G	Content of G.
74	H	Content of H.
79	L	Content of L (rectum?).
82	L	Caecum content of L.
83	L	Digestive content (ventral part) near hip bone of L.
84	L	Digestive content of L.
88	L	Digestive content of L.
96	K	Large intestine content of K.
98	K	Digestive content lower part 3 and 4 of K.
100	K	Dorsal intestine content right side (large intestine?) of K.
127	K	Rectum content of K.
129	K	Intestine wall with hemorrhagic zones K.
135	N	Digestive content of N.
141	N	Digestive content with large intestine wall of N.
161	I	Large intestine content + wall of horse I.

RESULTS

Two different egg morphotypes were found in the samples.

– The eggs of the first group were ovoid with a smooth, thick and operculated eggshell, measuring between 63-80 µm in length and 39-45 µm in width (Fig. 1). The typical characteristics of these eggs indicate that they belong to the genera *Oxyuris* sp. The species *Oxyuris equi*, the horse pinworm, is a direct-cycle parasite of equine intestines, responsible of limited pathogenic significance. Gravid females migrate down the rectum and out through the anus to cement their eggs, perpetrating anal pruritus (Georgi 1980, Kassai 1999).

– The eggs from the second group were ovoid. They appeared as thin and smooth eggshell, and measuring between 63 and 80 µm in length, for 28 and 43 µm in width (Fig. 2). The morphological and morphometrical data of these eggs were not sufficient to determine the taxa beyond the

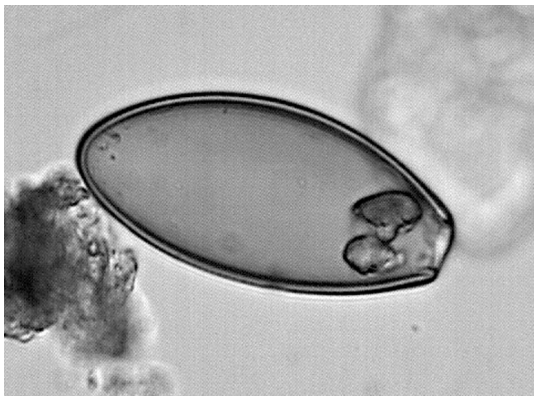

FIG. 1. – Egg of *Oxyuris equi* found in horse F from Berel' (84 × 40 µm). Photographer: M. Le Bailly.

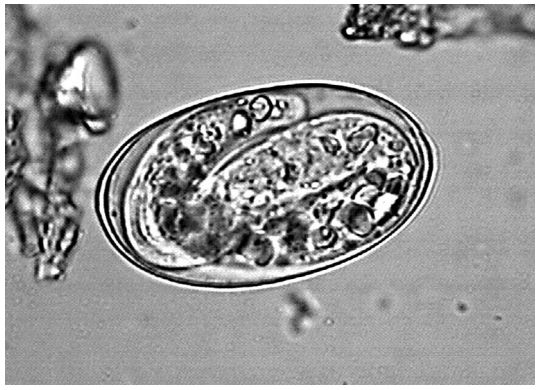

FIG. 2. – Strongylidea egg found in horse K from Berel' (67 × 39 µm). Photographer: M. Le Bailly.

TABLE 2. – Results of the paleoparasitological analyses (presence/absence) (M. Le Bailly).

Samples	Horses	*Oxyuris equi*	*Strongylidea*
37	C	No	No
43	C	No	No
47	B	No	No
55	F	Yes	No
69	G	No	Yes
74	H	No	Yes
79	L	No	Yes
82	L	No	Yes
83	L	No	No
84	L	No	Yes
88	L	No	No
96	K	No	No
98	K	Yes	No
100	K	Yes	No
127	K	Yes	Yes
129	K	No	No
135	N	No	No
141	N	Yes	No
161	I	Yes	No

Strongylidea order, which include many families such as Strongylidae, Ancylostomidae, Trichostrongylidae, or Haemoncinae. The average size of these eggs (70.72 µm in length and 38.91 in width) provides a possible additional evidence for the nematode families Trichostrongylidae or Strongylidae, responsible for mucosal infections of horse gastro-intestinal tract (Thienpont *et al*. 1979, Soulsby 1982). General results are presented in table 2.

DISCUSSION

The eggs identified in this study are common parasites of horse gastro-intestinal tract, and the discovery of such parasites is not surprising. However, some points deserve to be discussed.

OXYURIS EQUI

The discovery of *Oxyuris equi* eggs is not new in Palaeoparasitology. Jansen and Over (1966) identified of eggs of the horse pinworm in samples coming from Valkenburg-on-Rhine, a Holland Roman site dated from the 1st century AD. In 1988, Jones *et al.* published the identification of the equine nematode in a British Roman site dated from 80-90 years AD. Finally in 1997 Bouchet and Bentrad identified eggs of *Oxyuris equi* in samples coming from the lakeside settlement of Charavines, France, dating from the 11th century AD. The discovery of horse pinworm in the Berel' samples is another archaeological example of a horse parasite, and pushes back the historical limit of the horse pinworm presence.

In modern horses, to the best of our knowledge, there is no published data about horse pinworm in Kazakhstan. From all the neighbouring regions, only Iran and Ukraine give respective prevalence of this parasite in around 1.7% (Eslami *et al.* 2005) and 36% (Kuzmina *et al.* 2005).

STRONGYLIDEA ORDER

The presence of Strongylidea eggs in animal samples had not previously been established by palaeoparasitological studies (Bouchet *et al.* 2003, Gonçalves *et al.* 2003). The present work provides first evidence and a chronogeographical context for these animal parasites. The discovery of Strongylidea eggs in Berel' dating from the 3rd century BC indicates the presence of these animal parasites in ancient Central Asia.

Current veterinarian studies in Kazakhstan and neighbouring regions have identified the presence of Trichostrongylidae and Strongylidae families. The prevalence of infection in herbivores varies between 0.1% to 0.6% in sheep and goats from Kazakhstan (Morgan *et al* 2006), 7.7% to 19.5% in goats from Mongolia (Sharkhuu 2001), 28.3% in horses from Iran (Eslami *et al.* 2005), and 13.6% to 27.3% in horses from Ukraine (Kuzmina *et al.* 2005).

The difference of parasitic charges observed in the nine horses of our study is not significant, and the pathologies found are frequent in horses (Dunn 1978). As a consequence, it is not possible to determine differences in the geographical origin or in horse rearing. The hypothesis raised by the archaeologists regarding the possible horse offering from different allied neighbouring tribes cannot be confirmed by palaeoparasitological evidence.

However, the common parasites found are not responsible for serious infections in adult horses and therefore not lethal. For that reason, the health status of the animals could not have been the cause of sacrificing. Finally, the animal offering as a sacrifice is a more probable cause of death than a bad health status. This result is additional evidence to support a strong bonding between Scythians and their horses, and the importance of these animals during Scythian life and death.

The palaeoparasitological analysis carried on the two human bodies found in the tumulus (Crubezy *et al.* 2006) revealed the presence of ovoid eggs identified as Ancylostomidae, with size and form similar to the eggs found in the horse samples. The identification of eggs belonging to the Strongylidea order, in both human and animal samples, is very interesting and raises the issue of a possible parasitic disease common to both horse and human, such as Trichostrongyliasis, which is quite prevalent in rural regions. The prevalence of these parasites is not known in Central Asia, as opposed to neighbouring region such as Iran, where it varies between 7.5% and 85% by human in Ispahan (Acha & Szyfres 2005). The palaeoparasitological results obtained in Berel' may represent a human disease transmitted by animals, which is a very rare observation in archaeology (Dittmar & Teegen 2003). However, the determination of these eggs solely based on their morphology and size observations is too uncertain (Thibert *et al.* 2006). The definitive answer to the real nature of these eggs would probably be revealed using molecular methods, which is becoming frequently applied to Palaeoparasitology (Iniguez *et al.* 2006; Loreille *et al.* 2001, 2003).

CONCLUSION

The palaeoparasitological analysis conducted on the Berel' horses are unique and represent a first record of animal Palaeoparasitology for this period, in a region never studied before. The presence but also the absence of parasites shed light on the chronogeographical migration of parasites, and also on animal palaeopathology, often neglected in archaeological studies.

Furthermore, the analyses demonstrate the excellent preservation of helminth eggs in cold climate and permanent frozen soil (permafrost), as already observed with the Alps iceman (Aspöck *et al.* 1996).

In terms of cultural anthropological aspects, this study enforces the real sacrificial nature of the animal deposit in the human burial. However,

like horse ancient mitochondrial DNA analysis (Keyser-Tracqui *et al*. 2005), this study does not allow the identification of different geographic origins or rearing types of the horses inhumed with the human bodies. Finally, the absence of real differences in horse analysis, both in parasitological and molecular ones, could be an additional evidence for a unique horse group coming from the same breeding, and offered as a sacrifice to the prince.

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