PATHOLOGIC FINDINGS IN REINTRODUCED PRZEWALSKI'S HORSES (EQUUS CABALLUS PRZEWALSKI) IN SOUTHWESTERN MONGOLIA

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Abstract: The Przewalski's horse (Equus caballus przewalskii) was extinct in the wild by the mid 1960s. The species has survived because of captive breeding only. The Takhin Tal reintroduction project is run by the International Takhi Group; it is one of two projects reintroducing horses to the wild in Mongolia. In 1997 the first harem group was released. The first foals were successfully raised in the wild in 1999. Currently, 63 Przewalski's horses live in Takhin Tal. Little information exists on causes of mortality before the implementation of a disease-monitoring program in 1998. Since 1999, all dead horses recovered (n = 28) have been examined and samples collected and submitted for further investigation. Equine piroplasmosis, a tick-transmitted disease caused by Babesia caballi or Theileria equi, is endemic in Takhin Tal and was identified as the cause of death of four stallions and one stillborn foal. In December 2000, wolf predation was implicated in the loss of several Przewalski's horses. However, thorough clinical, pathologic, and bacteriologic investigations performed on dead and surviving horses of this group revealed lesions compatible with strangles. The extreme Mongolian winter of 2000–2001 is thought to have most probably weakened the horses, making them more susceptible to opportunistic infection and subsequent wolf predation. Other occasional causes of death since 1999 were trauma, exhaustion, wasting, urolithiasis, pneumonia, abortion, and stillbirth. The pathologic examination of the Przewalski's horses did not result in a definitive diagnosis in each case. Several disease factors were found to be important in the initial phase of the reintroduction, which could potentially jeopardize the establishment of a self-sustaining population.

Key words: Przewalski's horse, Equus caballus przewalskii, reintroduction, strangles, predation, piroplasmosis.

INTRODUCTION

The last recorded sightings of Przewalski's horse (Equus caballus przewalskii), or takhi in Mongolian, in the wild occurred in the Dzungarian Gobi Desert in southwestern Mongolia.1,2,3 The species has only survived because of captive breeding based on 13 founder animals. At present, six Przewalski's horse reintroduction projects exist: two in Mongolia (Hustain Nuruu and Takhin Tal [www.takhi.org]); two in China (Jimsar and Gansu), and one each in Kazakhstan and in Ukraine.4,5,6 Currently, only the two projects in Mongolia have released horses into the wild.

The first group of captive born Przewalski's horses arrived at the Takhin Tal site (45°53.80N, 93°65.22E) on the edge of the 12,500-km² Gobi-B National Park and International Biosphere Reserve in 1992. To date, a total of 73 horses have been transported to the site. The first harem group was released from the adaptation enclosures in 1997, and in 1999, the first foals were successfully raised in the wild. At present 63 Przewalski's horses live in Takhin Tal, 52 are free ranging, belonging to four harems and one bachelor group.7

Horses were transported to Mongolia from various captive breeding institutions in Europe and Australia. They were submitted to a thorough clinical examination under anesthesia to fulfill International Union for Conservation of Nature (IUCN) reintroduction requirements and Mongolian veterinary health protocols.4,8 After examination, they were placed in a 30-day preshipment quarantine. Serologic examinations were carried out for the following equine diseases: equine infectious anemia (EIA), equine viral arteritis, vesicular stomatitis, glanders, and contagious equine metritis. A complement fixation test was conducted for Trypanosoma equiperdum. The horses received two doses of an anthelmintic (Ivomec-P, Merck, Sharpe & Dohme, Haarlem, NL-2031, The Netherlands), and they were vaccinated against equine influenza and tetanus (Prevacun FT, Hoechst Roussel Vet, Vienna A-1232, Austria).
There are only a few published reports on diseases of captive Przewalski’s horses and from the other reintroduction projects. No information is available on diseases affecting Przewalski’s horses before extinction in the wild. Ataxia caused by a degenerative myelopathy associated with vitamin E deficiency has been observed in different European and North American institutions.13,23 Laminitis has been diagnosed in several Przewalski’s mares held in a semireserve.33 Coccidioidomycosis has been reported to be the most important cause of mortality in the Przewalski’s horse population at the Wild Animal Park in San Diego.40 Equine herpesvirus types 2 and 5 (EHV-2 and EHV-5) were isolated from Przewalski’s horses without clinical signs in several German zoos,4 and the neurologic form of EHV-1 was diagnosed once at the Berlin Zoo.13 A comprehensive parasitologic survey carried out at Takhin Tal showed seasonally variable infestation, predominantly with Strongyloides sp.10 A report from Hustain Nuruu gave statistical data concerning the mortality of reintroduced Przewalski’s horses but no information on specific diseases or causes of death.3

Necropsy information for Takhin Tal before 1998 is scant. Local pathologists attributed more than 40% of the deaths between 1992 and 1998 to “lung problems” and the “effects of sand storm.” A significant peak in deaths was noted in April and May. A basic necropsy protocol was established to improve data collection and identify the causes of death. Formalin-fixed organ tissues were examined from most dead horses since 1999.44,45 The aim of this study was to provide qualitative information on the mortality of reintroduced Przewalski’s horse in Takhin Tal and to evaluate the importance of the different causes of mortality to facilitate the adaptation of management procedures as necessary.

**MATERIALS AND METHODS**

Tissues from 24 Przewalski’s horses (11 males and 13 females, aged 6 mo to 16 yr), one aborted fetus, and three stillborn foals, all of which died during the winter and spring seasons (December to early July), were fixed in 10% neutral-buffered formalin and submitted for histopathologic examination. The number of submitted organs per individual was variable, but lung, heart, liver, and kidney were generally available. Four carcasses were frozen, and they were necropsied by chain saw (Fig. 1). Only skeletal remains were found in three cases of presumed wolf predation (Table 1).
Formalin-fixed organ samples were embedded in paraffin, sectioned at 4–5 μm, and stained with hematoxylin and eosin (H&E). Selected sections were stained with periodic acid–Schiff (PAS), Gram stain, Prussian Blue, and/or lipofuscin stain.

Using prestained slides (Testsimples®, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland), blood smears from the May 2000 stillborn foal were examined by light microscopy. Subsequently, ultrastructural investigations on organs of this foal were performed. Formalin-fixed lung and spleen samples were postfixxed in 2% osmium tetroxide, embedded in 100% Spurr’s resin, and ultrathin sections were stained with saturated uranyl acetate and 0.5% lead citrate and examined with a Zeiss EM 902 electron microscope. Frozen nasal mucosa and enlarged lymph nodes of suspected stranglings cases from winter 2000–2001 were submitted for bacteriologic examination.

Immunohistochemical analysis for African horse sickness (AHS) was performed on lung samples from the two stallions that died in 1999. Immunohistochemistry using avidin–biotin complex method was applied to the lung of horse 25 using antibodies against Toxoplasma gondii, Neospora sp., and Leishmania sp. Polymerase chain reaction (PCR) for Leishmania sp. was carried out using established protocols.

RESULTS

A summary of Przewalski’s horse mortalities, 1999–2003, including age, length of time at Takhin Tal, main pathologic lesions, and suspected cause of death, is presented in Table 1.

Equine piroplasmosis was diagnosed in four stallions (horses 3, 4, 28, and 31) and one stillborn foal (horse 7). The stallions died after several days of apathy, decreased appetite, fever, heavy infestation with ticks (Dermacentor nuttalli), and bilateral hemorrhagic nasal frothy discharge terminally. In addition, horse 3 had hoof trauma and horse 4 had several wounds because of intraspecies aggression. The stallions died during their first “tick season” (March–June). At necropsy, the trachea and bronchi were filled with froth and the lungs were congested and edematous. The spleen was massively enlarged and congested. Histopathologic lesions were consistent with acute piroplasmosis. Severe diffuse pulmonary edema and hemorrhages filled the alveolar spaces and markedly expanded the subpleural, interlobular, peribronchial, and perivascular interstitium (Figs. 2, 3). Numerous macrophages in the alveolar spaces, interstitium, and blood vessels contained hemosiderin. Immunohistochemical examination of the lungs for AHS was negative. In the heart, there was multifocal, moderate to severe, subendocardial and perivascular edema and hemorrhage. Mild periporal infiltration with mononuclear cells and rare neutrophils were seen in the liver. Kupffer cells were swollen and often contained hemosiderin. Centrilobular sinusoids were dilated, the hepatocytes contained small lipid vacuoles, and there was occasional necrosis of liver cells. Severe congestion, hemosiderosis, and lymphoid depletion were observed in the spleen. Horses 4, 28, and 31 had moderate to severe renal lesions, characterized by extensive necrosis of the tubular epithelium, distended tubules containing hyaline and hemoglobin casts (Fig. 4), and multifocal interstitial hemorrhages. Intracytoplasmic protozoa in varying developmental stages (schizonts, gamonts, and budding sporonts), consistent with Klosiella equi, were present in the epithelial cells of the medullary tubules (Fig. 4, inset) in two stallions (horses 3 and 4).

Horse 7 was a stillborn foal from horse 16. All examined organs were severely congested. Many erythrocytes in blood smears were parasitized with one or two small, rounded organisms (2–4 μm in diameter) compatible with Babesia caballi or Theileria equi (Fig. 5). No “Maltese cross” typical of T. equi was observed. Besides congestion, the lungs showed severe diffuse alveolar and interstitial edema, increased mononuclear infiltration in the alveolar septa, and numerous alveoli contained pale eosinophilic membranes (amniotic fluid) and macrophages. In the heart, there was mild interstitial and perivascular edema with some mononuclear cells. Periportal infiltration in the liver, with macrophages mainly, was associated with slight bile duct hyperplasia and loose fibrovascular tissue forming portoporal bridges. Periportal sinuses were dilated and congested, and some hepatocytes were necrotic.

There was diffuse renal cortical tubulonephrosis characterized by hydropic swelling of the tubular epithelial cells, pyknosis or fading of the nucleus, and deposition of an unidentified yellow-beige, coarse granular pigment, which did not stain for iron or lipofuscin. The spleen was highly congested, and lymphoid follicles were prominent. Ultrastructural examination of the spleen revealed one or two rounded protozoa in numerous swollen erythrocytes (Fig. 5, inset). The intraerythrocytic trophozoites were in parasitophorous vacuoles and 2–3 μm in diameter. Because of the poor quality of the samples, only a nucleus, cytoplasmic organelles, and globules interpreted as host cytoplasm were visible.

In December 2000, wolves were reported to have killed eight Przewalski’s horses from one free-rang-
<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Time at TT</th>
<th>Date of death</th>
<th>State at necropsy</th>
<th>Histo-pathologic findings</th>
<th>Main pathological findings</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>2.5 yr</td>
<td>6 mo</td>
<td>Dec 1998</td>
<td>Fresh body</td>
<td>Stones in urethra</td>
<td>Urolithiasis</td>
<td>Urolithiasis</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>8 yr</td>
<td>7 mo</td>
<td>Jan 1999</td>
<td>Fresh body</td>
<td>Generalised congestion</td>
<td>Urolithiasis</td>
<td>Urolithiasis</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>6 yr</td>
<td>1 yr</td>
<td>May 1999</td>
<td>Fresh body</td>
<td>Hoof trauma, pulmonary edema, hemosiderosis, splenic congestion, <em>Klossiella equi</em></td>
<td>Piroplasmosis, trauma</td>
<td>Piroplasmosis</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>4 yr</td>
<td>1 yr</td>
<td>Jun 1999</td>
<td>Fresh body</td>
<td>Pulmonary edema, tubulonephrosis, hemosiderosis, splenic congestion, <em>Klossiella equi</em></td>
<td>Piroplasmosis</td>
<td>Piroplasmosis</td>
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<tr>
<td>5</td>
<td>Fetus</td>
<td>16 yr</td>
<td>5 yr</td>
<td>Jan 2000</td>
<td>Aborted fetus</td>
<td>Autolysis</td>
<td>Abortion</td>
<td>Abortion</td>
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<tr>
<td>6</td>
<td>F</td>
<td>11 yr</td>
<td>7.5 yr</td>
<td>Dec 2000</td>
<td>Bones and skin</td>
<td>Rhinitis</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>7 yr</td>
<td>8.5 yr</td>
<td>Dec 2000</td>
<td>Frozen body</td>
<td>Hyperplasia, lymphadenitis, metastatic strangles, wasting</td>
<td>Strangles, trauma</td>
<td>Strangles</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>5.5 yr</td>
<td>4.5 yr</td>
<td>Dec 2000</td>
<td>Frozen body</td>
<td>Hyperplasia, lymphadenitis, metastatic strangles, wasting</td>
<td>Strangles, wasting</td>
<td>Strangles</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>7 mo</td>
<td>*</td>
<td>Dec 2000</td>
<td>Frozen body</td>
<td>Rhinitis</td>
<td>Rhinitis</td>
<td>Rhinitis</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>6 yr</td>
<td>6.5 yr</td>
<td>Feb 2001</td>
<td>Frozen body</td>
<td>Mandibular lymphadenitis, metastatic strangles, wasting</td>
<td>Strangles, trauma</td>
<td>Strangles</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>6 mo</td>
<td>*</td>
<td>Dec 2000</td>
<td>Frozen body</td>
<td>Pericarditis, pneumonia, hemosiderosis</td>
<td>Pericarditis, pneumonia, wasting</td>
<td>Pericarditis, pneumonia, wasting</td>
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<tr>
<td>12</td>
<td>F</td>
<td>7 mo</td>
<td>*</td>
<td>Jan 2001</td>
<td>Frozen body</td>
<td>Mandibular lymphadenitis, metastatic strangles, wasting</td>
<td>Strangles, wasting</td>
<td>Strangles</td>
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<tr>
<td>13</td>
<td>M</td>
<td>7 mo</td>
<td>*</td>
<td>Feb 2001</td>
<td>Frozen body</td>
<td>Mandibular and mesenteric lymphadenitis, metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
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<td>14</td>
<td>F</td>
<td>7 yr</td>
<td>2.5 yr</td>
<td>Feb 2001</td>
<td>Frozen body</td>
<td>Mandibular and mesenteric lymphadenitis, metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
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<tr>
<td>15</td>
<td>F</td>
<td>4 yr</td>
<td>2.5 yr</td>
<td>Feb 2001</td>
<td>Frozen body</td>
<td>Mandibular and mesenteric lymphadenitis, metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
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<tr>
<td>16</td>
<td>F</td>
<td>3.5 yr</td>
<td>2.5 yr</td>
<td>Feb 2001</td>
<td>Frozen body</td>
<td>Mediastinal and mesenteric lymphadenitis, metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
<td>Metastatic strangles, wasting</td>
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*Table 1.* Background information, main pathologic findings, and suspected cause of death for Przewalski’s horses examined at Takhin Tal (TT) from Dec 1998 to Jun 2003.
Table 1. Continued.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Time at TT</th>
<th>Date of death</th>
<th>State at necropsy</th>
<th>Histo-pathologic findings</th>
<th>Main pathological findings</th>
<th>Diagnosis</th>
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<tr>
<td>18</td>
<td>M</td>
<td>7.5 yr</td>
<td>5.5 yr</td>
<td>Feb 2001</td>
<td>Fresh body</td>
<td>x</td>
<td>Hydrothorax, hydropericardium, pulmonary edema, tubulonephrosis</td>
<td>Shock (unknown etiology)</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>3 yr</td>
<td>* Mar 2001</td>
<td>Frozen body</td>
<td>x</td>
<td>Hemosiderosis</td>
<td>Wasting, exhaustion</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>4 yr</td>
<td>* Mar 2001</td>
<td>Head</td>
<td>x</td>
<td><em>Gasterophilus</em> sp. bots in pharynx</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>5 yr</td>
<td>4 yr Mar 2001</td>
<td>Frozen body</td>
<td>x</td>
<td>Subcutaneous hemorrhages, hemosiderosis, <em>Gasterophilus</em> sp. bots in stomach and pharynx</td>
<td>Stress, exhaustion, wasting, trauma</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>9 mo</td>
<td>* Apr 2001</td>
<td>Fresh body</td>
<td>x</td>
<td>Purulent rhinitis, bronchopneumonia, septicemia</td>
<td>Purulent rhinitis, bronchopneumonia, septicemia</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>3 yr</td>
<td>* May 2001</td>
<td>Bones and skin</td>
<td>NS</td>
<td>Necrotizing pneumonia</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>2 yr</td>
<td>* May 2001</td>
<td>Bones and skin</td>
<td>NS</td>
<td>Necrotizing pneumonia</td>
<td>Unknown</td>
<td></td>
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<tr>
<td>25</td>
<td>F</td>
<td>11 yr</td>
<td>8 yr May 2001</td>
<td>Fresh body</td>
<td>x</td>
<td>Necrotizing pneumonia</td>
<td>Pneumonia</td>
<td></td>
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<tr>
<td>26</td>
<td>F</td>
<td>5 yr</td>
<td>2 yr May 2002</td>
<td>Fresh body</td>
<td>x</td>
<td>Necrotizing pneumonia</td>
<td>Death during parturition</td>
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<tr>
<td>27</td>
<td>F</td>
<td>Stillborn</td>
<td>* May 2002</td>
<td>Fresh body</td>
<td>x</td>
<td>Fetal pulmonary atelectasis</td>
<td>Stillborn</td>
<td></td>
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<tr>
<td>28</td>
<td>M</td>
<td>4 yr</td>
<td>1 mo Jul 2002</td>
<td>Fresh body</td>
<td>x</td>
<td>Pulmonary edema, tubulonephrosis, hemosiderosis, splenic congestion</td>
<td>Piroplasmosis</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>Stillborn</td>
<td>* May 2003</td>
<td>Fresh body</td>
<td>x</td>
<td>Fetal pulmonary atelectasis, aspiration of amniotic fluid, liver necrosis</td>
<td>Stillborn</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>3 yr</td>
<td>* May 2003</td>
<td>Fresh body</td>
<td>x</td>
<td>Fracture of femur</td>
<td>Trauma</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>4 yr</td>
<td>1 yr Jun 2003</td>
<td>Fresh body</td>
<td>x</td>
<td>Pulmonary edema, tubulonephrosis, hemosiderosis, splenic congestion</td>
<td>Piroplasmosis</td>
<td></td>
</tr>
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</table>

* Presence or absence of samples for histopathologic investigation.
* x, histologic samples available.
* *, born in Takhin Tal.
* NS, no histologic samples collected.
Figure 2. Photomicrograph of the lung of horse 3, which died of piroplasmosis. There is massive edema that fills the alveolar lumen (*) and expands the interlobular (short arrows) and perivascular interstitium (long arrows). H&E, ×10.

ing harem group during the night. The remaining free-ranging groups were driven back to the adaptation enclosures by the local staff. During and after this procedure, further deaths occurred. At clinical examination 3 wk later, 14 horses exhibited very poor body condition and inappetance. Purulent discharge from the mandibular region, consistent with strangles caused by Streptococcus equi infection, was observed in most of the horses younger than 5 yr (Fig. 6). Several horses were immobilized and treated with systemic, long-acting antibiotics. In total, 15 Przewalski’s horses died within a few months.

Three fresh carcasses (horses 15, 16, and 17) were emaciated, had enlarged lymph nodes and fistulas in the mandibular region, serous atrophy of the coronary fat, hydropericardium, and abscesses (up to 15 cm in diameter) of the mesenteric or mediastinal lymph nodes (or both) compatible with metastatic strangles (Fig. 7). Of the frozen carcasses of four further Przewalski’s horses (11, 12, 13, and 14), three had fistulating mandibular lymph nodes and hydropericardium and the fourth had acute pneumonia. Horse 11 had a metacarpal fracture, which reportedly occurred during the wolf attack.

Skeletal remains from three further horses were recovered at the site of the wolf kills (horses 8, 9, and 10). Only feeding of wolves on carcasses could be confirmed. Old fistulas of the mandibular lymph nodes were observed on one head (horse 8). Marked hyperemia and swelling of the nasal mucosa were seen in another head (horse 9). Samples were collected from both horses for bacteriologic and histologic investigation. Histologic examination of the tissue samples from the examined carcasses and frozen remains revealed suppurative lymphadenitis of the mesenteric, mediastinal and/or mandibular lymph nodes, mucopurulent rhinitis, acute exudative pneumonia, and pulmonary edema. The clinical diagnosis of strangles was confirmed by culture of β-hemolytic Streptococcus equi from mandibular and mesenteric lymph node abscesses, and/or frozen nasal mucosa of two horses.

Wasting, characterized by hepatocellular atrophy, lipofuscinosis, hepatic and splenic hemosiderosis, and splenic lymphoid depletion, were observed in six carcasses during the strangles episode and in
three other cases (horses 6, 19, and 21) that died at the end of the winter. Further infectious diseases were diagnosed in two horses. In horse 22, gross lesions were emaciation, hydrothorax, hydropericardium, ascites, purulent rhinitis, and pulmonary congestion, and edema. Histopathologic examination revealed acute purulent bronchopneumonia and numerous intravascular gram-negative bacterial colonies in several organs, indicating terminal septicemia. An older mare (horse 25) died without previous clinical signs; the lungs were dark red on necropsy. Histopathologic examination revealed severe, acute, multifocal, fibrinohemorrhagic, and necrotizing bronchopneumonia with a few syncytial cells and numerous colonies of gram-negative bacteria. Occasionally, protozoan-like structures resembling *Toxoplasma* sp. cysts were seen within the alveolar spaces or septa. Immunohistochemically, these structures did not stain for *Toxoplasma gondii*, *Neospora* sp., or *Leishmania* sp. antibodies. *Leishmania* sp. were not identified by PCR. The cause of this fatal pneumonia is still under investigation.

Three full-term fetuses were stillborn. Piroplasmosis was diagnosed in one foal (horse 7 as described). Presentation of another foal was correct, but it was stuck in the birth canal (horse 27); the mare (horse 26) died during parturition. The last stillborn probably died intrapartum because of hypoxia (horse 29). The lungs were atelectatic, and the airways were filled with amniotic fluid. There were hypoxic changes in the liver, characterized by marked, centrilobular, hepatocellular necrosis and congestion. The cause of death in two horses was trauma (horses 21 and 30).

**DISCUSSION**

The reintroduction of captive born Przewalski’s horses into their former natural range exposes them to climate extremes, potential water and food shortage, and predators (particularly the grey wolf [*Canis lupus*]). Introduced species may be naïve to local infectious agents and parasites. Prospective veterinary evaluation and the investigation of mortalities have been recommended for monitoring of reintroduced populations.\(^\text{16}\) Mortality data are used to adapt management procedures to improve reintroduction success and ensure the establishment of a self-sustaining population.

The histopathologic lesions in the four stallions that died in the spring of 1999, 2002, and 2003, particularly the severe pulmonary edema, splenic...
congestion, renal tubular necrosis with hemoglobin casts, and the presence of hemosiderin-laden macrophages in several organs, were consistent with changes described in cases of equine piroplasmosis. In addition, the horses had severe tick infestations. Equine piroplasmosis is a tick-borne disease caused by two intraerythrocytic protozoans, Babesia caballi and Theileria equi; it is characterized by fever, anemia, icterus, and other nonspecific clinical signs, and T. equi appears to be more pathogenic. The incubation period is 5–21 days. The pathogenesis includes hemolysis, kallikrein shock, and overproduction of proinflammatory cytokines.

The main differential diagnoses for equine piroplasmosis are AHS and EIA. AHS is not known to occur in Mongolia, and it was excluded by immunohistochemical investigation. The horses tested negative for EIA before shipment, and, histologically, the lymphoid infiltrates and reticuloendothelial hyperplasia typical of EIA were not observed. Equine piroplasmosis is endemic to Mongolia and northern China. Serologic examination of serum samples confirmed the presence of piroplasmosis as the etiologic agent. The tick season in southwestern Mongolia is limited and lasts from March to June, with a small second peak in September (Rüegg, pers. comm.). Consequently, naive horses are potentially infected with piroplasms during their first spring in Takhin Tal. A serologic study being conducted at Takhin Tal indicates a high prevalence of piroplasmosis in domestic horses and Przewalski’s horses that have spent one or more tick seasons in southwestern Mongolia (Rüegg, pers. comm.). The tick, Dermacentor nuttalli, found in high numbers on the horses between April and June, may carry the agents of equine piroplasmosis in Mongolia. Piroplasmosis could explain the increased mortality during April and May observed before 1999 because “lung problem” was the most common pathologic finding reported. This includes pulmonary congestion and edema as seen in piroplasmosis. Clinical signs of piroplasmosis are mostly inapparent or mild in horses born in endemic areas because of the development of both innate and acquired immunity. However, clinical piroplasmosis and high mortality rates have been reported from fully susceptible
Figure 5. Blood smear from foal 7. Two erythrocytes, each containing two piroplasma organisms. Prestained slide, ×100. Insert: electronmicrograph of the spleen showing an erythrocyte containing two piroplasma organisms. ×7,000.

horses introduced into endemic areas. Four stallions (horses 3, 4, 28, and 31) died of piroplasmosis in their first spring “tick season” in Takhin Tal, during the mating season, which is very stressful for the stallions. Piroplasmosis is potentially an important mortality factor in combination with exertion and stress. Pyrexia may be important in the development of clinical cases of piroplasmosis. A lower relative resistance to piroplasmosis has been observed in stallions in South Africa. In mammals, males generally have a greater parasite burden and prevalence, and they are significantly more vulnerable to death due to parasitism. The influence of immunodepressive androgens and variable exposure to pathogens because of sex-specific behavior are suggested causes.

Foals born in an endemic area are protected by maternal antibodies to piroplasmosis, but naive pregnant mares are susceptible to piroplasmosis. Transplacental infection by piroplasms results in abortion, especially in the last trimester. Aborted fetuses often have a very high parasite load, whereas intraerythrocytic parasites are rare in infected adult horses, probably because of the transient parasitemia. The stillborn foal (horse 7) had a marked parasitemia. Pulmonary edema, severe splenic congestion, and renal tubulonephrosis have been described previously in aborted fetuses in the presence of intrauterine babesiosis.

Piroplasmosis was identified as an important mortality factor in newly introduced Przewalski’s horses, and management procedures can be applied to decrease the incidence of further cases. Elimination of piroplasmosis in the reintroduction area is not possible, but several measures have been implemented to diminish the risk of an outbreak, e.g., transport of horses from Europe to Mongolia after the tick season (late June), remote dart application of Imidocarb (Carbesia®, Schering-Plough, F-92307 Levallois, France) at a subtherapeutic dose during tick season, transmission of B. caballi and T. equi while the horses are under control in the adaptation enclosures. Furthermore, the provision of certain local plants (Artemisia spp.), known to have medicinal antiparasitic properties, has been proposed (Rüegg, pers. comm).

Predation by wolves is a potential threat to the reintroduction project. Several attacks by wolves on Przewalski’s horses have been observed in past years. The horses had apparently adapted to their...
new environment and were able to defend themselves against wolves (Simmayr, pers. comm.). The high mortality caused by wolf predation reported in the winter 2000–2001 was assumed to have been promoted by other factors. Clinical examination and pathologic investigation of the carcasses, as well as bacteriologic cultures, demonstrated lesions typical of strangles. Purulent pharyngitis and lymphadenitis of the upper respiratory tract caused by S. equi are seen in equine strangles. It is a highly contagious disease, particularly with overcrowding, poor housing, inadequate nutrition, or adverse climate, causing severe depression in affected horses. The infection was likely acquired by the first Przewalski’s horse group through contact with domestic horses; strangles appears to be endemic within domestic horses of the region (Suchebataar, pers. comm.). Spreading of infection to the entire group probably occurred when the infected horses were driven back to the adaptation enclosures to avoid wolf attacks. These horses and those that died later in the winter showed emaciation and wasting. The climate in 2000–2001, characterized by a catastrophic winter with extremely low temperatures (less than −50°C), high snow drifts and storms (“dzud”), a local 30-yr cyclic weather condition, and preceded by a dry summer, is likely to be the primary cause for the weakening of the horses. Poor body condition and environmental stress likely primed the horses for a S. equi infection and subsequent predation by wolves. Little can be done to prevent losses due to climate, especially during a dzud. Horses surviving strangles and environmental stress likely would have been highly susceptible to predation by wolves. The infection was likely acquired by the first Przewalski’s horse group through contact with domestic horses; strangles appears to be endemic within domestic horses of the region (Suchebataar, pers. comm.). Spreading of infection to the entire group probably occurred when the infected horses were driven back to the adaptation enclosures to avoid wolf attacks. These horses and those that died later in the winter showed emaciation and wasting. The climate in 2000–2001, characterized by a catastrophic winter with extremely low temperatures (less than −50°C), high snow drifts and storms (“dzud”), a local 30-yr cyclic weather condition, and preceded by a dry summer, is likely to be the primary cause for the weakening of the horses. Poor body condition and environmental stress likely primed the horses for a S. equi infection and subsequent predation by wolves. Little can be done to prevent losses due to climate, especially during a dzud. Horses surviving strangles and environmental stress likely would have been highly susceptible to predation by wolves.

Infectious diseases were diagnosed in two further Przewalski’s horses, but no etiologic agent was isolated. A yearling died of bacterial bronchopneumonia and septicemia (horse 22) and an older mare (horse 25) had pneumonia of suspected viral and/or parasitic origin. Horse 25 requires further investigation, especially with respect to equine influenza viruses, which have caused severe respiratory disease epidemics in horses from Inner Mongolia and China.

In conclusion, considering the dramatic hardships due to climate and feeding habits, and the presence of potential predators, the newly introduced captive and wild-born Przewalski’s horses have adapted successfully to their new environment. Although the pathologic examinations of the Przewalski’s horses in Takhin Tal did not indicate a definitive diagnosis in each case, they revealed the importance of several disease patterns during
the initial phase of the project, which could potentially jeopardize the establishment of a self-sustaining population. Even examination of frozen horses produced adequate samples for an exact diagnosis in many cases. Bacteriologic investigation was possible from tissues that had been frozen for 4–6 wk. *Streptococcus* is very resistant to freezing, although there may be considerable variation between bacterial species and environmental conditions. Pathologic and other investigations in remote field projects are often difficult to perform, but important information can be gained from the diligent examination of the collected samples. Reintroducing species extinct-in-the-wild to their natural habitat and reestablishment of self-sustaining populations from captive-born specimens is a very challenging task requiring a multidisciplinary approach and the cooperation of various health and conservation professionals. Thorough pathologic examinations should be an integral part of any reintroduction project because they provide valuable background information.

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