Reproduction and Development of the Released Przewalski's Horses (*Equus przewalskii*) in Xinjiang, China

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In China, the first Przewalski's horse (Equus przewalskii) group was released in Kalamaili Ungulate Protected Area in Xinjiang, in August 2001. The objective of this study was to investigate reproduction and development of released Przewalski's horses in Xinjiang, China from 2002 to 2006. Twenty-four descendants were naturally born, average reproduction rate was 38.7%, and average survival rate of foals was 69.1% in this interim. Frequent alternation of the leading stallion and infertility in female horses due to environmental factors were main causes for the low reproduction rate. The infant mortality rate of released Przewalski's horses was 25.0%, and 83.3% of death in infants was due to the leading stallion infanticide. The released Przewalski's horses exhibited seasonal breeding, 70.8% of infants were born in May and June. The fertility rates were 8.3% in April and 37.5% in May, which were lower compared to those of the captive Przewalski's horse groups (18.3% and 44.3%, respectively). Furthermore, the fertility rate in June was 33.3% and higher than the captive groups (18.3%). These findings showed that the breeding peak of the released Przewalski's horse groups was later than the captive groups, and suggested that the altered survival environment and food supplies were the main reasons contributing to the delayed breeding peak.

J. Equine Sci. Vol. 19, No. 1 pp. 1–7, 2008

Key words: fertility rate, infanticide, mortality rate, Przewalski's horse, seasonal breeding

Ancestors of the endangered Asian wild horse or Przewalski's horse (*Equus przewalskii*) once roamed throughout Central Asia, China and Europe [14]. The Przewalski's horse was discovered by a Russian officer, N. von Przewalski, 1879/80 in the Dsungarian Gobi (Mongolia) and was first scientifically documented by a zoology professor A. Poliakov in 1881 [12]. Przewalski's horse was extinct in the wild by mid 1960s [12, 15], and remnant populations exist only in small captive breeding herds. Although evolutionary divergence of

the Przewalski's horse and domestic horse (equus caballus) occurred ~ 250,000 years ago, these 2 species are capable of interbreeding and producing fertile offspring [17]. Interspecies embryo transfer has also been successfully performed [1, 5]. All living Przewalski's horses are descended from 13 founder individuals, and the last of which was captured in Mongolia in 1947 [3]. Although classified as endangered, the Przewalski's horse reproduces successfully in captivity, and current population exceeds 1,000 individuals.

Eighteen Przewalski's horses (eight males and ten females) were reintroduced into China from June 1985 to December 1991. Przewalski's Horse Breeding Institute was set up in Jimsar County Xinjiang, China [22]. With increasing size of the captive population, Przewalski's horses were released into the wild around

This article was accepted October 9, 2007

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the Kalamaili Ungulate Protected Area in Xinjiang, in August 2001. Chinese researchers have investigated reproduction of captive Przewalski's horses. Although there have been several reproductive observations of the captive Przewalski's horse in Xinjiang, China [19, 22], similar studies have not been done since Przewalski's horses were released to the wild in 2001. The aims of this study were to investigate reproduction and development of the released Przewalski's horses in Xinjiang during the period of 2002 to 2006, and to evaluate key factors that affect reproduction of the released Przewalski's horse in the wild.

Materials and Methods

Research area

The location of released Przewalski's horses in August 2001 was near the monitoring station (covering the area $89^{0}14'E-89^{0}36'E$, $45^{0}49'N-46^{0}4'N$) which was set up in the northern Kalamaili Ungulate Protected Area in Xinjiang, China. The climate of the released area is characterized as a cold winter weather with the lowest temperature of -38°C, and an extremely hot summer with the highest temperature of 50°C. Spring and autumn are short, and the averages temperature is 2.4°C. The annual average rainfall is 159 mm per year, which falls mainly in autumn. The vegetal traits of the area are desert grassland and desert vegetation, and mainly dominated by the Ceratoides lateens and Stipa glareosa. Besides Przewalski's horse, the large and medium size ungulates are Wild Ass (Equus hemionus) and Tibetan Antelope (Gazella subgutturose). The consumer is Wolf (Canis Lupus), the only predator to the ungulates. The climatic damages include drought, cold wave, low temperature, dry-hot wind and snowstorm. The Protected Area also serves as a winter pasture of the Kazakhstan. Since lots of domestic livestock are pastured in the area, such as sheep, goats, domestic horses and camels from November to next May, the wild animals face the cruel environment in this period.

Mortality and survivorship

Like captive females, breasts of the released Przewalski's horses began to swell the week before parturition. Dripping milk occurred and the vulva swelled one or two days before labor. Animals were in an upset mood during this period, and it was observable that the parturition was coming. As the birth was usually unproblematic and takes place overnight or early in the morning, it was difficult to investigate the process of parturition. If the foal had died from some diseases or by the leading stallion infanticide, the foster mother wouldn't leave the carcass that day, and other Przewalski's horses will stay close by her almost half day as well. This phenomenon was administered to find the carcass. The cause and the time of the death were judged by examining the dead body. If there were bloodstains on the leading stallion, infant was killed from the leading stallion infanticide.

Reproduction rate and survival rate

Reproduction rate describes the percentage of the female horses that gave birth among all female population that were capable of giving birth. Reproduction rate (%)=(the number of female horses that gave birth/total number of female horses that should produce offspring) \times 100. Survival rate is the percentage of offspring survived after birth. Survival rate (%)=(Survived foals/Total foals) × 100. Annual growth rate is the change in the number of horses in a population per year. The number of newly released Przewalski's horses is also included when calculating annual growth rate. Annual growth rate (%)=[Number of the horses at the end of the year-(Number of the horses at the beginning of the year + Number of newly released horses)/Number of the horses at the beginning of the year] \times 100.

Results

Figure 1a shows the first Przewalski's horse (*Equus przewalskii*) group was released in Kalamaili Ungulate Protected Area in Xinjiang, China in August 2001. A breeding group was composing of twenty seven Przewalski's horses (Fig. 1b, c). Based on experience of the first release, seven additional adult stallions were released to join the first released bachelor herd in May 2002. The expanding bachelor herd could supply new leading stallion for the breeding group, and it can also house the retired leading stallion and ejected small stallions.

Ten Przewalski's horses died in the wild in 2002, and there were no new born horses, making the annual growth rate to be –41.7% (Table 1). The first foal of the released Przewalski's horse was born in April 2003 (Fig. 1e), but died from the leading stallion infanticide

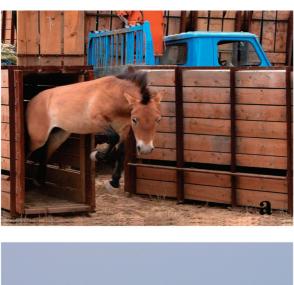












Fig. 1. The released Przewalski's horses (*Equus przewalskii*) in Xinjiang, China. The first Przewalski's horse was being released into the wild on August, 2001 (a). A group of released Przewalski's horses in summer (b) and winter (c) in the wild condition. Mating behavior was observed in the breeding season (from May to September) (d). A Przewalski's horse gave birth to her baby (e). A foal was killed by the leading stallion on her birthday (f).

(Fig. 1f). Nine adult mares gave birth to seven foals (3 male/4 female) during this year, and three foals were killed by the leading stallion, and one died from malnutrition at five month of age. Three female foals

were successfully raised in the wild. Overall, the reproduction rate was 77.8%, survivor rate was 42.8% and the annual growth rate was 4.8% during this year.

Another ten Przewalski's horses were released in the

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Year	Harems	Breeding mares	Foals	Reprodution rate (%)	Survivor rate (%)	Annual increasing rate (%)	
2002	1	12	0	0		-41.7	
2003	1	9	7	77.8	42.8	4.8	
2004	2	9	3	33.3	66.7	0	
2005	3	14	3	21.4	66.7	3.0	
2006	4	18	11	61.1	100	32.4	

Table 1. The breeding record of the released Przewalski's horses (Equus przewalskii) from 2002 to 2006

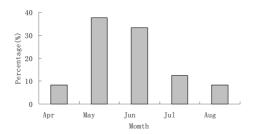


Fig. 2. Seasonal births in released Przewalski's horses (*Equus przewalskii*) in Xinjiang, China. The released Przewalski's horses gave birth to 24 foals in the wild from 2002 to 2006, and they were born in April (n=2, 8.3%), May (n=9, 37.5%), June (n=8, 33.3%), July (n=3, 12.5%) and August (n=2, 8.3%).

same released area as the second breeding group in August 2004. The breeding mares of this year were from nine original mares released into the wild in 2001, they gave birth to 3 foals (1 male/2 female). One female foal was killed by the leading stallion and the other two foals survived. Reproduction rate was 33.3%, survivor rate was 66.7% and the annual growth rate was 0 in 2004.

In May 2005, the amount of the released Przewalski's horse group had changed, and two primary breeding groups were reassigned into three groups. There were 14 breeding mares and 3 new born female foals during this year. One foal was killed by the leading stallion on the day she was born and the other two survived. Reproductive rate was 21.4%, survivor rate was 66.7% and the annual growth rate was 3.0% this year (Table 1).

The amount of the released Przewalski's horse groups changed again in July 2006. The released breeding groups were naturally divided into four groups in the wild. Breeding mares were eighteen, and newborn foals were eleven (7 male/4 female) during this year. Reproduction rate was 61.1%, survivor rate was 100% and growth rate was 32.4%. Among the 11 foals, two were the offspring of two mares born in the wild in 2003, and they were the second generation of the first released Przewalski's horses in the wild (Table 1).

Mating behavior of the released Przewalski's horse was observed from May to September (Fig. 1d). The released Przewalski's horses gave birth to 24 foals in the

wild from 2002 to 2006, and these foals were born in April (8.3%), May (37.5%), June (33.3%), July (12.5%) and August (8.3%). The number of newborn foals in May and June accounted for 70.8% of all the foals, and the main birth season of the released Przewalski's horse was confirmed to be in May and June (Fig. 2). The average reproduction rate was 38.7%, and the survival rate of foals was 69.1% from 2002 to 2006. Comparing the birth data of captive groups with the released Przewalski's horses in Xinjiang, China, the birth season of the released Przewalski's horses is later than those of the captive groups in Xinjiang, China (Fig. 3).

Discussion

This study could be the first one to describe reproduction and development of the released Przewalski's horses in Xinjiang, China. The present study showed that the average reproduction rate was 38.72%, and the average survival rate was 69.05% from 2002 to 2006. The morality rate for infants of the released Przewalski's horses was 25%, and 83.3% of the cause of death was due to the leading stallion infanticide. In addition, the released Przewalski's horses showed patterns of seasonal breeding with 70.8% infant horses born in May and June. These findings suggested that the breeding peak of the released Przewalski's horses were later than those of the captive groups. Most likely, the altered survival environment and food supplies were the main causes

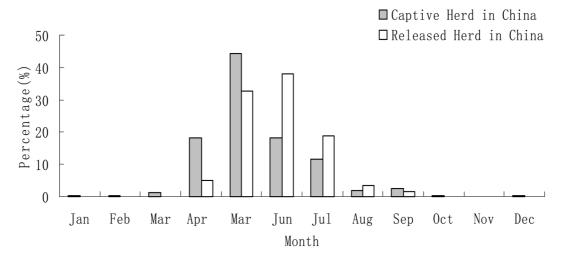


Fig. 3. Seasonal births in captive and released Przewalski's horses (Equus przewalskii) in Xinjiang, China.

contributing to the delayed breeding peak.

Factors that determine the reproduction rates of mares are infertility, abortion and sterility in Przewalski's horses [4, 22]. In generally, infertility arises from mismatch between stallions and mares, congenital sterility, altered environment and diseases [22]. The average non-fertilized rate of the released Przewalski's horse was 32% from 2002 to 2006, but the non-fertilized rate was 100% in the first year after released in the wild suggesting an environmental cause. In 2003 and 2004, non-fertilized rate was 66.7% and 78.6%, respectively. This was because the released Przewalski's horses changed from assigned group to self-divided group, leading to the frequently alternation of the leading stallion. In addition, due to the conflict among stallions, the leading stallion exhausted in physical strength, as well as a lack of experience in mating and leading, which led to low success rate of mating. Along with adapting to grouping modes, and increasing reproductive population, the alternate of the leading stallion decreased prominently in the reproduction population, and the leading stallion have become experienced in leading group, as a result, the reproduction rate of the released Przewalski's horse got back to stable correspond in 2005 and 2006.

The sterility rate of Przewalski's horses was not high in the released groups [4], only one mare showed sterility prominently, and no the leading stallion exists in Xinjiang, China. It is suggested that sterility has no significant influence on the reproduction rate of released population. The abortion rate of captive Przewalski's horses is about 6% in Xinjiang, China [22]. Infection caused by bacteria, epiphyte and virus, trauma, diseases and embryo deformation were possible factors that lead to the abortion of Przewalski's horse [4]. Since Przewalski's horses released into the wild had augmented living space, and complex living environment, the accurate data of abortion could not be obtained; therefore, it was hard to quantify the influence of abortion on the reproduction rate of the released Przewalski's horses.

In Hustai National Park, Mongolia, the average survival rate of the released Przewalski's horses is 56.4%, and 51.95% of foals death was caused by wolf predation, 18.9% by inborn diseases and abortion, 8.8% by injury, 5% by insufficient milk intake, and 12.6% by unknown reasons [2]. For captive Przewalski's horses in Xinjiang, the average survival rate is 85%. Fifty two percent of foal death was from inborn diseases, 32% was from leading stallion infanticide [4]. The present data showed that the average survival rate of the released Przewalski's horses is 69.1% from 2002 to 2006. Eight three point three percent of the dead foals was caused by the leading stallion infanticide, and 16.7% by some diseases. These results showed that the lethality caused by inborn disease decreases when Przewalski's horses were released into to wild, and primary causes that influence the survival rate of Przewalski's horses have become the leading stallion infanticide and wolf predation from farmed condition to the wild.

Mammals in temperate regions exhibit a seasonal cycle in reproduction and birth that limited to a specific time of the year [21]. Animals form relatively steady reproductive cycle in the evolution process is resulted from the adaptation to the environment [16]. Usually, domestic horses can be estrous all seasons, and can produce foal every season, though almost all foaling of them are occurred in spring, and they are classified as seasonal breeding animals [20]. It has been reported that Przewalski's horses are born in all months of the year, but more than 75% of them were born from April to July in the northern hemisphere [18]. In Xinjiang, the births of captive Przewalski's horse have been recorded in all months of the year except December from 1988 to 2005, the birth rate in May is the highest (44.3%), and April is similar to June, accounted for 18.3% [22]. The present data showed that 70.8% infants of released Przewalski's horse were born in May and June from 2003 to 2006 in Xinjiang, suggesting that the released Przewalski's horses showed seasonal breeding. These findings are similar to those found in Mongolia, the reproduction rate of released Przewalski's horses was higher in June and July from 2002 to 2005 [9-11]. The reproduction of wildlife can be influenced by temperature, humidity, illumination and other environmental factors [6, 8].

Przewalski's horses are like domestic horses polyestrous and have a gestation period of 330-340 days [12]. In the present study, the birth peak of the released Przewalski's horses groups were later than the captive groups, this differences were under different living environment condition. This phenomenon has direct relationship with the conditions of their habitat. The released regions of Przewalski's horses in Xinjiang $(89^{0}14'E-89^{0}36'E, 45^{0}49'N-46^{0}4'N)$ were in the north of Przewalski's Horses Breeding and Research Center of Xinjiang $(88^{0}45'E-88^{0}50'E, 44^{0}10'N-44^{0}15'N)$. The latitude difference affects phenophase, and the different phenophase directly affect seasonal rhythm such as environment temperature, humidity, illumination and other ecological factors, accordingly also influenced seasonal reproduction. In addition, the greater the net energy that the animal obtains from food is, the greater the potential success of their reproduction rate is [7].

In a farmed condition, nutrition allowance has been steadily supplied all year round, and the birth of Przewalski's horse has not been limited due to abundance of the food resource. In the wild, nutrition availability is greatly fluctuated during different

seasons, and is the most abundant at the beginning of June in the released regions; therefore, high reproduction rate were observed in May and June.

In summery, frequent alternation of the leading stallion and the infertility female horses were the causes to the low reproduction rate in the released Przewalski's horse; the leading stallion infanticide mainly caused low survival rate of Przewalski's horse; altered survival environment and food availability were the main reasons attributed to the delayed breeding peak comparing to farmed Przewalski's horse groups in Xinjiang, China.

Acknowledgments

This study is supported in part by a Grant-in-Aid for National Natural Science Foundation of China (NSFC) (No. 30570239 and No. 30670261) and Scientific Research (Basic Research B-18310044, P06445) from the Japan Society for the Promotion of Science.

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