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A comparison of horn quality of the white line in the domestic horse (Equus caballus) and the Przewalski horse (Equus przewalskii)

K.-D. Budras and *Christina Schiel*

Freie Universität Berlin, Institut für Veterinär-Anatomie, Berlin

Summary

Using scanning and transmission electron microscopical techniques impressive structural variations were observed in the two species. In the domestic horse the terminal horn tubules and cap horn tubules situated between the horny leaflets were empty of contents in their centres, so that actual tubular structures occurred. Bacterially decayed horn can be identified with the naked eye as black or chalky white discolourations. It should be removed during hoof care and horn cavities should be disinfected with iodoform ether. Stable hygiene is of crucial significance. Urea, or a mixture of urine and dung, causes a decrease of horn quality.

In Przewalski horse the white line is significantly smaller, the percentage of stabilizing leaflet horn is correspondingly larger and the amount of terminal horn is relatively less. These terminal horn "tubules" are not centrally empty but are filled by reticular or stony medullary cells. In this case the term "tubule" is misleading. The intact medullary cells are important for the maintenance of a barrier against ascending bacterial invasion. These barriers are better developed in Przewalski horse than in the domestic horse.

Keywords: hoof, white line, Equus caballus, Equus przewalskii

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Ein Vergleich der Hornqualität der weißen Linie beim Hauspferd (Equus caballus) und beim Przewalski Pferd (Equus przewalskii)

Für die Untersuchung standen Hornproben von Warmblut- und Przewalski-Pferden zur Verfügung, die mit Raster- und Transmissionselektronenmikroskopischen Methoden erforscht wurden. Dabei traten eindrucksvolle Unterschiede zwischen den beiden Spezies hervor.

Beim Hauspferd sind die zwischen den Hornblättchen gelegen terminalen Hornröhrchen und die Kappenhornröhrchen in ihrem Zentrum leer, so daß hier tatsächlich eine Röhrchenstruktur vorliegt. Bakteriell zersetztes Horn kann mit dem bloßem Auge als schwarze oder kalkig weiße Verfärbung erkannt werden. Es sollte bei der Hufpflege entfernt werden, kraterförmige Horndefekte sollten mit Jodoformäther desinfiziert werden. Die Stallhygiene ist hierbei von entscheidender Bedeutung. Harnstoff oder eine Mischung von Urin und Mist führen zu einer Minderung der Hornqualität.

Bei den Przewalski-Pferden ist die weiße Linie signifikant schmaler, der Anteil an stabilisierendem Blättchenhorn ist entsprechend größer und die Menge an terminalem Röhrchenhorn relativ geringer. Diese terminalen Horn"röhrchen" sind zentral nicht leer, sondern mit soliden (steinigen) Markzellen gefüllt. Deshalb ist der Begriff "Röhrchen" irreführend. Die intakten Markzellen sind für die Aufrechterhalung einer Barriere gegen eine aufsteigende bakterielle Keimbesiedlung wichtig. Diese Barriere ist beim Przewalski-Pferd besser entwickelt als beim Hauspferd.

Schlüsselwörter: Huf, weiße Linie, Equus caballus, Equus przewalskii

Introduction

Przewalski horses (Equus przewalskii) are prehistoric wild horses the living ancestors of the domestic horse. During previous centuries these cinnamon coloured horses with a shoulder height of approximately 140 cm were spread over large areas of Eurasia. The prehistoric wild horses were named after the Russian general and Asian scientist Przewalski who published a description of these animals in the eighties of the last century.

In the wilderness Przewalski horses were last observed in the late sixties of this century, in the Mongolian Desert Gobi. Later they were also extirpated in this area. During the turn of the century wild horses were caught and brought to Europe. Approximately 1000 descendants of these prehistoric wild horses are still living in zoological gardens and game preserves. The living Przewalski horses are now strictly protected.

As the Przewalski horse is the direct ancestor of the domestic horse (Equus caballus) a comparison seems interesting, to determine the extent to which breeding and maintenance have influenced the hoof structure. Our structural investigation was focused on the white line (zona alba) as it plays a central role in hoof diseases and consists of horn showing a variation of origin, structure and quality. Laminitis causes an expansion of the white line due to the new formation of cap horn. The width and structure of the white line are indicative of the functional condition of the suspensory apparatus and the degree of recovery from a previous episode of laminitis (*Budras* and *Huskamp*, 1995).

Furthermore, the white line is of great importance as the site of "white line disease" (*Redden*, 1993). A further reason for the selection of the white line relates to animal walfare. One can obtain sufficient horn samples from the weight bearing surface (margo solearis) during hoof trimming by the blacksmith, without having to perform painful interventions or animal experiments.

The goal of our comparative hoof research on the domestic horse and the przewalski horse was to investigate whether or to what extent the hoof form, structure and quality, as well as the function of the white line, were influenced by horse maintenance and breeding.

Materials and methods

Samples from over 70 warm-blooded horses were available from previous investigations. The research on Przewalski horses was carried out on 4 hooves that were provided by the Zoological Park of Berlin.

Additionally, we received 15 horn rings which the blacksmith had removed from the hooves of narcotized Przewalski horses.

Furthermore, we obtained the opportunity to include in our investigation a herd of 12 Przewalski horses from a semireserve close to Berlin (Schorfheide), where they are being prepared for reintegration into Mongolia. Although hoof abrasion of these wild horses is limited due to reduced motility, they manage without a vet or blacksmith. Horn chips of approximately 6 x 4 cm and a thickness of 2 cm break out of the horn wall of these Przewalski horses (Fig. 1). Altogether we collected 10 horn chips in the semireserve for use in our investigation. The examination of all horn samples was performed by scanning electron microscopical methods. The specimens were glued to aluminium object plates and were sputtered with gold to a thickness of 5 nm. They were then examined by scanning electron microscope. In order to verify our results we performed transmission electron microscope investigations (routine method, *Budras* et al., 1989).

Results

I. Gross anatomical investigation

a) In the domestic horse all hooves were judged healthy. None had signs of present or past laminitis. All hooves were well worn and would not have required trimming. Horn clefts were not visible.
b) The hooves of the przewalski horse showed various longitudinal horn clefts, which ran parallel to each other, a few cm apart (Fig. 1). In some cases the horn clefts began proximally at the level of the coronary segment approximately 2 cm distal to the hairline. In the majority of hooves, however, the horn clefts began halfway between the coronary segment and the margo solearis. All horn clefts reached the distal margo solearis. Due to the elongation of the hoof plate a horizontal fracture occurred at the level of the solar horn running parallel to the white line. This is probably the consequence of insufficient horn abrasion due to reduced motility. Between the longitudinal and horizontal horn clefts, approximately 2 cm thick horn chips with a length of 6 cm and a breadth of 4 cm broke out of the hoof plate. (We collected these horn chips for the scanning electron microscopical investigations.) In this way several successive horn chips had broken out of the hoof plate. They began at the hoof dorsum and continued to the heels.
In the late autumn when the animals reach their greatest body weight of approx. 400 kg the loss of horn chips almost ceases. The hooves then look as though they were trimmed by a blacksmith. Due to the horn growth and abrasion the areas of broken-out horn were hardly visible. Apparently the horn clefts were only superficial and did not reach the dermis since bleeding and lameness seldom occurred.

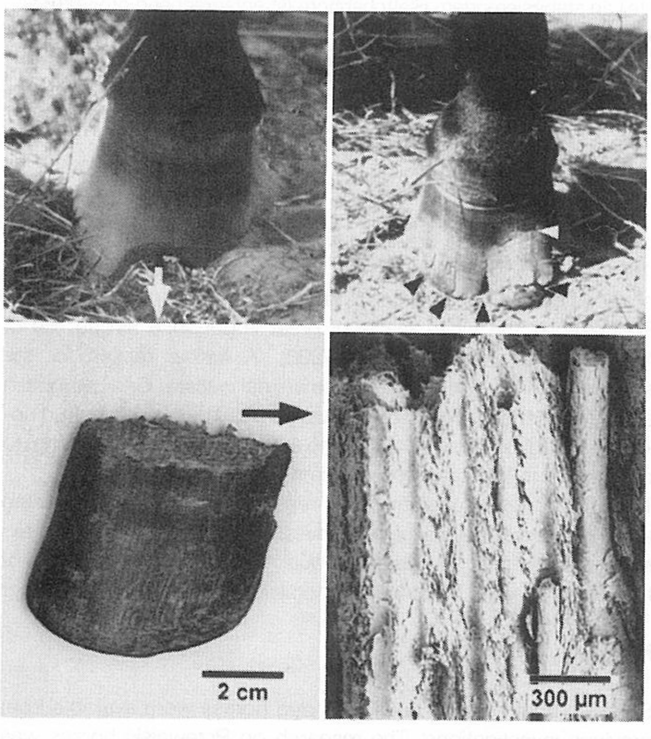

Fig. 1: top: Hoof of the Przewalski horse. Between the longitudinal clefts (Δ) and horizontal clefts (◁), hornchips break out of the hoof capsule (bottom left),
bottom right: Scanning electron microscopic picture of a longitudinal fractured surface.

II. Scanning and transmission electron microscopical investigations
a) The white line in the domestic horse
The white line, situated between the hoof plate and the solar horn (Fig. 2, top) consisted of two parts: The outer part was approximately 1 mm broad and striking white. It was composed of the basal portions of the horny laminae and the intermediate cap horn consisting of cap horn arcades and small cap horn tubules (Fig. 2, centre) which were produced over the crests of dermal laminae of the wall segment. The inner part of the white line was approximately 2.5 mm broad and was composed of the apical portions of horny laminae alternating with interlaminar terminal horn tubules. Considering both portions, the white line's breadth added up to 3.5 mm. The size of the terminal horn tubules and cap horn tubules varied widely; the diameters ranged from 50 to 300 μm. Distally at the margo solearis, the terminal horn tubuli consisted of peripheral scale-shaped horn cells of the cortex and were centrally empty or contained small amorphous, crumbly medullary remains, so that actual tubular structures occurred (Fig. 2, bottom). Approximately 1 cm proximally, where the terminal horn tubuli are produced around the terminal dermal papillae, the central horny medullary cells appear as large, scaly solid cells (Fig. 3, top). To explain how solid horn cylinders develop into tubular structures, we must briefly discuss the process of keratinization. In this process the terminal tubulary horn, the cortex, develops around the terminal dermal papillae. Protruding distally, the keratinizing cells glide along the terminal dermal papillae through a distance of 500 μm. They are nourished by diffusion from the dermal blood capillaries. A tubular cortex of solid, concentrically arranged horn cells of good quality, comparable to the brickwork of a factory chimney, develops.
The medulla is of suprapapillar origin. It develops above the peak of a terminal dermal papilla. Due to the continuous new horn supply, the keratinizing epidermal cells quickly move away from the nutrifying dermal blood capillaries. As a consequence of insufficient nutrition a superficial, incomplete and precipitated keratinization of the medullary cells occurs. The newly formed horn masses of the solid horn tubule's medulla can be compared to a brittle brickwork of bad quality. The medullary cells (equivalent to the bricks) contain a comparatively small amount of keratin filaments and keratin associated proteins (homogeneous keratin). The cells appeared to be speckled since the cell organelles were not completely disintegrated. Paraplasmatic enclosures in the form of lipid droplets and glycogenous accumulations frequently occurred. The intercellular cementing substance (comparable to the mortar) was produced in excess. This process broadened the intercellular spaces forming vesicular enlargements between the remains of desmosomes. The scanning electron microscopic picture shows that the cementing substance bulges out of the inter-

cellular space as in a badly built wall. Disintegration occurs between the cementing substances and the horn cells forming a potential path for bacterial invasion.

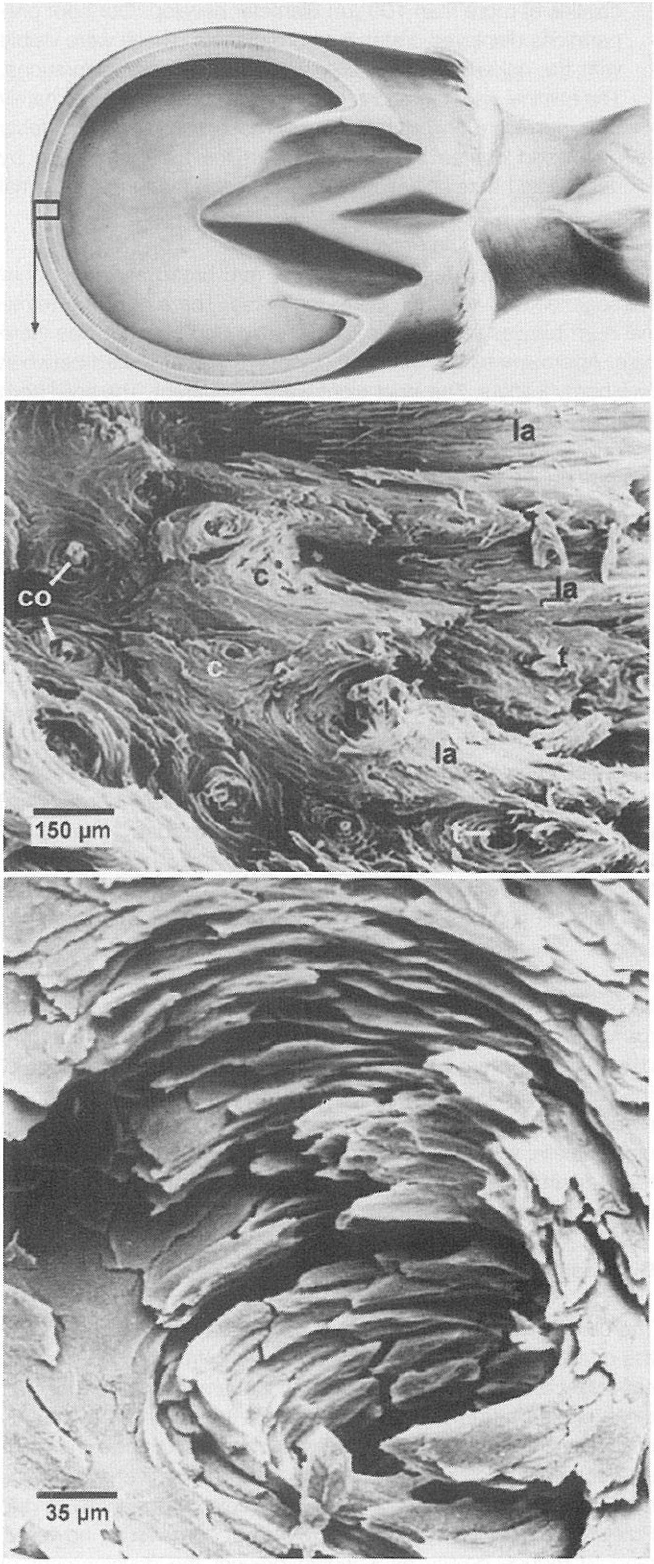

Fig. 2: Domestic horse: top illustration: outer part of the white line with leaflet horn (1a) and intermediate terminal horn tubuli (t); cap horn (c),
center: on the outside the white line touches the coronary horn (co),
bottom: diagonally broken terminal horn tubule.

During the process of cellular distal protrusion three different procedures are responsible for the development of empty or almost empty horn tubules, deriving from the originally solid medullary cells:

1. The medullary cells shrink markedly and transform into crumbly, amorphous cell detritus.
2. Between the medullary cells the cementing substance is of such poor quality that single plate-shaped cells or cell groups

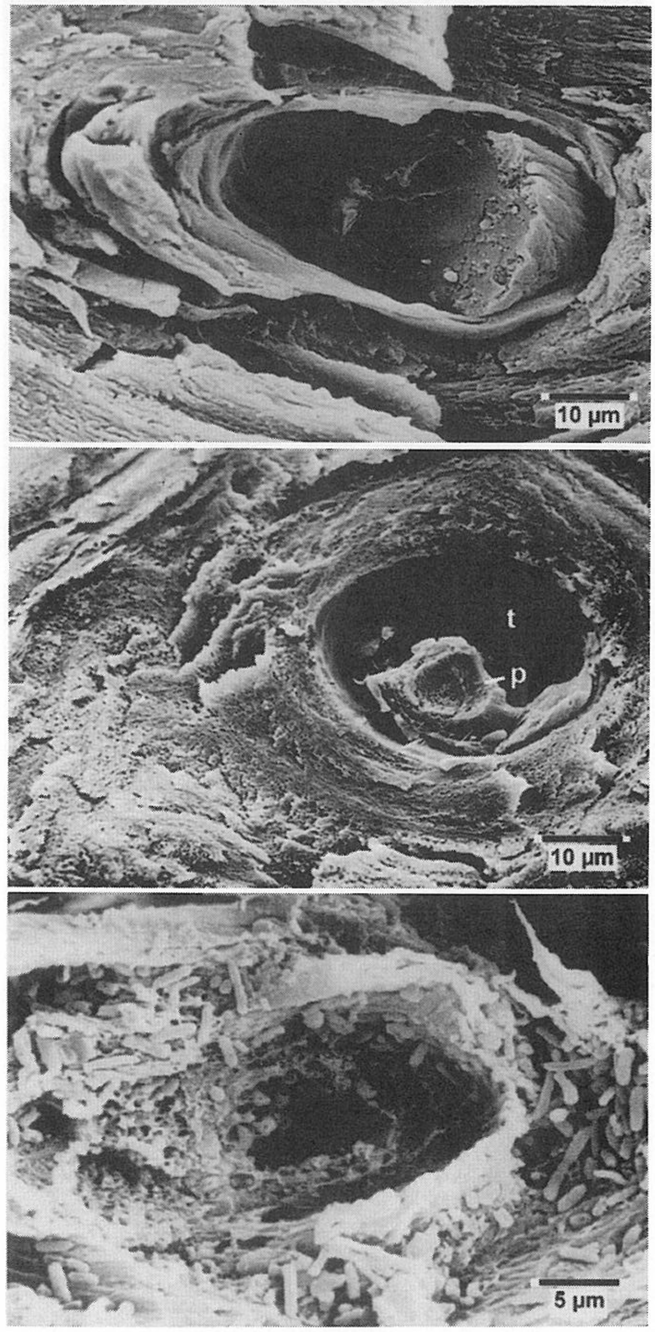

Fig. 3: Terminal horn tubuli of the white line in the domestic horse: Suprapapillar solid horn tubules develop at the sole to wall border. Between the medulla and cortex horn clefts occur (top illustration).
Horn tubules (t) develop due to the loss of plate-shaped medullary cells (p) (centre).
The bacterial destruction of the medullary cells and subsequently of the cortical cells creates broad, hollow tubuli (bottom).

fall out of the centre of the horn tubuli as single plates or entire stocks of plates (Fig. 3, centre).

3. Corresponding to process 1. and 2. hollow horn tubules develop due to bacterial decay of medullary cells (Fig. 3, bottom).

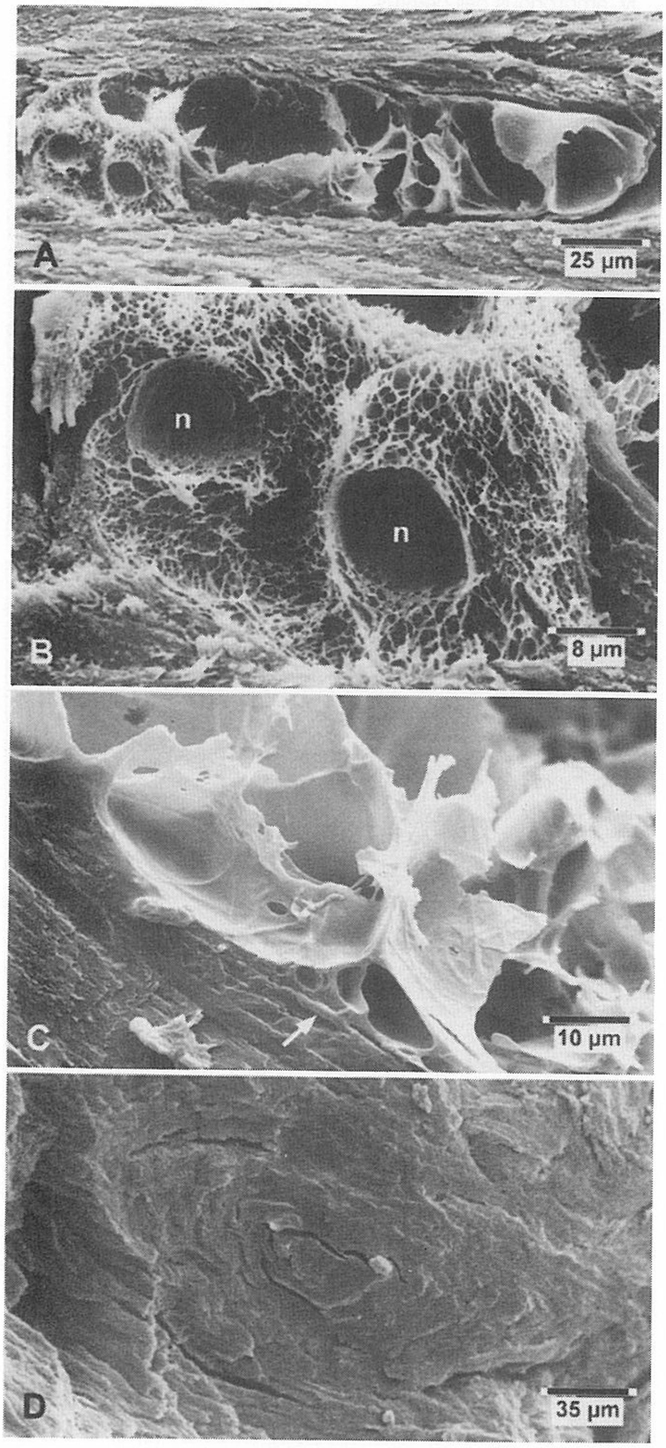

Fig. 4: Terminal horn tubuli of the white line in the Przewalski horse:
A: longitudinal fracture of a terminal horn tubule with reticular and lamellar medullary cells.
B: reticular medullary cell: position of the disintegrated nucleus (n).
C: lamellar medullary cell and cementing substance (↑) between cortical cells.
D: "stony" horn tubule.

The cortical cells on the other hand are more resistant and are therefore not destroyed at first. Eventually, a complete destruction of the cortical cells occurs, so that extremely large cavities of more than 100 μm diameter develop. Our hoof preparations displayed crater-like horn defects. These were visible with the naked eye as chalky white or black discolourations. The hairline discolourations having a diameter of approximately 0.01 mm were only detectable microscopically. These defects developed due to the destruction of the tubular medulla by ascending bacteria, which can easily reach the terminal dermal papillae.

b) The white line in the Przewalski horse

The white line was approximately 2.2 mm broad and was more homogeneous than in the domestic horse. The size of the terminal horn tubules reached up to 100 μm. Cap horn tubules were rare. Approximately sixteen horn cylinders were situated between two horny leaflets. The inner eight were inapparent. The solid horn cylinder consisted of scale-shaped cortical cells and cuboidal medullary cells of stony consistency (Fig. 4, D), that creates an efficient barrier against bacterial decay and ascending bacterial invasion. The outer eight horny cylinders consist of solid or reticular medullary cells (Fig. 4, A). Development of a trabecular or lamellar inner structure of the medullary cells can be observed during the process of distal protrusion. Above the peak of the terminal dermal papillae suprapapillar solid medullary horn cells developed. The newly formed medullary cells of the Przewalski horse were, in comparison to the domestic horse, remarkably homogeneous since the cell organelles were largely disintegrated and contained few glycogenous accumulations and lipid droplets. The content of keratin filaments and keratin associated proteins was comparatively high. The intercellular spaces were mainly narrow and connected by cementing substance of good quality (Fig. 4, C). During the process of cellular protrusion distally, large cavities, a few microns in diameter, developed in place of the disintegrated cell nucleus (Fig. 4, B). Between the bundles of keratin filaments smaller cavities of less than 1 μm were created. These cavities, which enlarge distally, were enclosed by horn trabeculae (Fig. 4, B) or horn lamellae (Fig. 4, C). The horn trabeculae and lamellae formed a three dimensional reticular structure. The cell surroundings were bordered by solid horny lamellae, the surroundings of the disintegrated nucleus by perforated horny lamellae. The entire reticular medullary cells did not separate from each other or from cortical cells, so that no empty medullary cavities developed. Bacterial destruction of the horn masses could not be observed at any place.

Discussion

The comparison between the hooves of the Przewalski horse and the domesticated horse portrayed obvious structural differences, especially of the white line which is of fundamental importance for the horn quality. In several publications the mechanical stability dependent on the water content was considered to be the decisive characteristic of horn quality. For the protective layer of the hoof plate this property is of indisputable importance. In the Przewalski horse an increased hardness of horn would, however, tend to reduce quality as it would prevent the development of longitudinal horn clefts and therefore the removal of horn chips. Through this mechanism the Przewalski horse maintains a healthy hoof structure despite an unnatural maintenance in the semi-reserve. Whether this process also occurred in the natural environment of the horses could however not be examined as the

Przewalski horse has been extirpated in the wilderness. For the various hoof segments several other quality features besides the mechanical stability must be considered.

The leaflet horn's resistance against tensile forces in the wall segment is of fundamental importance for the suspension of the third phalanx (*Pellmann*, 1995). This feature as well as the water-binding capacity of the perioplic horn serve as decisive characteristics of horn quality. Furthermore, the desquamation capacity of the solar horn, allowing maintenance of a natural curvature of the sole, is of fundamental importance, as is the elasticity of the frog segment for the maintenance of the hoof mechanism (*Knezevic*, 1962).

The White line consisting of relatively soft and elastic horn has a hinge-like function, as it is situated between the rather rigid hoof plate and solar horn. It has clinical importance in the correct shoeing of horses (*Ruthe*, 1955) and in the process of laminitis (*Marks*, 1984; *Marks* and *Budras*, 1987) which is associated with a broadening of the white line (*Budras* and *Huskamp*, 1995).

Compared to the domesticated horses the white line in the Przewalski horse was significantly narrower, the percentage of stabilizing leaflet horn was correspondingly larger, and the amount of soft tubular horn was relatively less. In this connection the following correlations must be considered: The broader the white line, the greater is the share of soft terminal tubular horn and the more horn defects occur, predisposing to bacterial invasion. The tubular horn of the white line of domesticated horses resembles the economical structure of an empty cylinder, which combines the greatest stability with the least possible material consumption. The exclusive consideration of this aspect would leave the medullary cells without function (*Habermehl*, 1984). It only focusses on the mechanical stability of the horn masses and ignores further important functions such as the suppression of ascending bacterial colonization. Yet this particular function is of immense importance as functional damage of the white line demonstrates (*Redden*, 1990). The medullary cells of horn tubules occupy the central areas and, therefore, form a significant component of the barrier against ascending bacterial invasion. The medullary cells represent the weak point of this barrier, because, compared to other horn components, they are most susceptible to bacterial decomposition. In domesticated horses they easily fall out of the centre of terminal horn tubules leaving behind a hollow tubular structure. In Przewalski horses the medullary cells, which are stony or spongy, are kept together by intercellular cementing substances (membrane-coated material) of good quality. Entire medullary cells do not separate from each other or from cortical cells. The term "tubule" is misleading in this case because they are not hollow. The role as a barrier against lytic (*Mölling*, 1993) and bacterial decomposition is rather underdeveloped in the horse. Under natural circumstances the wild horse is not confronted with the bacterial or lytic effects of urea, so that in the course of evolution no effective barrier has been developed. This is generally different in stable maintenance of the domesticated horses. The stablefloor conditions, with high concentrations of urea within mixtures of urine and dung, as well as a dense germ concentration and the intervention through shoeing can overstrain the barrier causing white line diseases (*Redden*, 1990; *Mölling*, 1993; *Budras* et al., 1995). According to the morphological criteria, this barrier against bacterial decomposition is more efficient in the Przewalski horse than in the domesticated horse.

Last but not least, we would like to discuss the question concerning the prophylaxis and therapy of horn defects. Bacterially decayed horn should be removed during hoof care and horn cavities should be disinfected with iodoform ether.

Prophylaxis should include the observance of hygenic requirements during hoof trimming. Tools, such as the hoof rasp, ought to be disinfected. Stable hygene is of crucial significance. Urea, or a mixture of urine and dung, causes a reduction of the resistance against tensile forces (*Kueng*, 1991) and, therefore, a decrease of horn quality (*Zenker*, 1991). During an investigation on the hoof horn of cattle (*Mölling*, 1993) it was shown that a mixture of urine and dung caused large amounts of intercellular cementing substance to be removed. Urea causes a removal of keratin proteins from horn cells.

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Klaus-Dieter Budras
Christina Schiel

Freie Universität Berlin
Institut für Veterinär-Anatomie
Koserstraße 20
14195 Berlin