Evaluation of primary epidermal lamellar density in the forefeet of near-term fetal Australian feral and domesticated horses

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Objective—To investigate the density of the primary epidermal lamellae (PEL) around the solar circumference of the forefeet of near-term fetal feral and nonferal (ie, domesticated) horses.

Sample—Left forefeet from near-term Australian feral (n = 14) and domesticated (4) horse fetuses.

Procedures—Near-term feral horse fetuses were obtained from culled mares within 10 minutes of death; fetuses that had died in utero 2 weeks prior to anticipated birth date and were delivered from live Thoroughbred mares were also obtained. Following disarticulation at the carpus, the left forefoot of each fetus was frozen during dissection and data collection. In a standard section of each hoof, the stratum internum PEL density was calculated at the midline center (12 o'clock) and the medial and lateral break-over points (11 and 1 o'clock), toe quarters (10 and 2 o'clock), and quarters (4 and 6 o'clock). Values for matching lateral and medial zones were averaged and expressed as 1 density. Density differences at the 4 locations between the feral and domesticated horse feet were assessed by use of imaging software analysis.

Results—In fetal domesticated horse feet, PEL density did not differ among the 4 locations. In fetal feral horse feet, PEL density differed significantly among locations, with a pattern of gradual reduction from the dorsal to the palmar aspect of the foot. The PEL density distribution differed significantly between fetal domesticated and feral horse feet.

Conclusions and Clinical Relevance—Results indicated that PEL density distribution differs between fetal feral and domesticated horse feet, suggestive of an adaptation of feral horses to environment challenges. (*Am J Vet Res* 2011;72:871–876)

Musculoskeletal tissues such as bone, muscle, articular cartilage, and tendon adapt to the biomechanical environment in which they function.[1–3] When stresses are relieved from a biological structure, such as when an astronaut enters the weightless environment of space, locomotory structures adapt by reducing material density and the subsequent strength of that structure.[4] Conversely, when a young racehorse enters a training program, the new stimulus of high limb loading combined with high training speed induces deposition of new bone.[1]

Whereas the adaptation of some biological tissues to loading has been investigated, less is known about the adaptive capabilities of the structures that make up an important inner layer of the hoof wall, the stratum internum. It has not been conclusively established whether the suspensory components of the hoof wall are capable of adapting to the challenges of the environment. The basic anatomic features of the hoof wall and its attachment to the distal phalanx by the suspensory lamellae in mature horses has been well described.[5] Leaf-like primary dermal lamellae attached to the distal phalanx via the dermis interconnect with PEL that are attached to the inner hoof wall, thereby suspending the distal phalanx within the protective hoof capsule. Several studies[6–9] have investigated the shape, orientation, and spacing of the PEL of domesticated horses at the fetal stage through adulthood. The PEL distribution around the solar circumference of the foot of adult horses has a predictable pattern of distribution; in the dorsal aspect of the hoof, the PEL are closely spaced and oriented at right angles to the hoof wall. Primary epidermal lamellae at the quarters in the feet of adult horses are more widely spaced than are those at the toe; they are not perpendicular to the hoof wall and angle back in a caudal direction toward the heel. Primary epidermal lamellae appear to be organized into well-delineated zones, and PEL density correlates well with stress.[6–9]

Abbreviations

MDC	Midline dead center
PEL	Primary epidermal lamellae

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It has been suggested that the distribution of PEL around the hoof wall perimeter may be a result of stress exerted on the foot over time and is not predetermined at birth.[6,9] In the dorsal aspect of the hoof of fetal and neonatal nonferal (ie, domesticated) foals, the PEL are arranged homogeneously, and there is no significant difference in PEL distribution between the MDC and quarter regions of the hoof.[6,9] It has been hypothesized that the architectural arrangement of the structures of the stratum internum is quite fluid and can adapt during growth and during times of variations in stress to accommodate the weight-bearing and support requirements of the digit.[2]

Many domesticated horses are typically forced into a program of intermittent exercise interspersed with long periods of confinement. It is common for horse breeders to confine mares and foals after birth to allow the foal to gradually become prepared for the challenges of the outside environment and safeguard it from injury to unprepared immature anatomic structures. Newborn feral horse foals are not confined. Shortly after birth, feral horse foals must generally begin to travel long distances to maintain contact with the dam, which must in turn keep up with the rest of the social band. The postnatal physiologic and biomechanical demands on newborn feral horse foals exceed those on newborn domesticated foals and may promote developmental adaptations in feral horse fetuses that favor survival in a more extreme environment. Australian feral and domesticated horses have been separated genetically for at least 50 years,[a] are exposed to vastly different environments, and have dissimilar travel patterns. For example, it is generally assumed that feral horses travel much greater distances than do their domesticated counterparts.[10–12,a] These factors may contribute to fetal hoof adaptation.

To further investigate the relationship between the architecture of the PEL and growth and development, the study of this report evaluated the density of the PEL around the solar circumference of near-term fetal feral and domesticated horse feet. We hypothesized that differences in PEL architecture between feet from fetal feral and domesticated horses would provide evidence of preadaptation of the foot to the environmental challenges encountered by feral horses.

Materials and Methods

The study was approved by the University of Queensland Animal Ethics Committee that monitors compliance with the Animal Welfare Act and The Code of Practice for the care and use of animals for scientific purposes. Four domesticated horse fetuses and 14 feral horse fetuses were used in the investigation.

Domesticated horse fetuses—Four Thoroughbred fetuses were obtained from the same veterinary hospital following the death of each fetus in utero; gestational age of the 4 fetuses was 2 weeks less than full term (ie, expected foaling date minus 2 weeks). The deaths of the foals were unrelated to musculoskeletal disease and were not related to the present study.

Feral horse fetuses—Fourteen feral horse fetuses were obtained from 2 locations in Australia. The fetuses were obtained during necropsy of pregnant, lactating mares following controlled feral horse culling operations. Fourteen mares were euthanized via shooting, and a near-term fetus was removed from the uterus of each mare within 10 minutes of death.

The feral mares used in the study were obtained from Lawn Hill (an unfenced 31,000-km^2 [12,000–square mile] area in remote Northern Queensland, Australia [latitude, 18.67°S; longitude, 138.35°E]; n = 5) and King's Canyon (a semiarid desert environment in central Australia [latitude, 24.50°S; longitude, 132.10°E]; n = 9). At the Lawn Hill location, water holes were typically 19 to 32 km (12 to 20 miles) apart, and feed was scarce during the annual dry season. It was assumed that Lawn Hill horses travelled long distances to obtain food and water. We assessed the horses' phenotype as stock horse type. Horses have been feral in this area since the early 1900s[13] and, according to local stockmen, have had minimal domestication for the past 50 years. The substrate on which horses travelled to access feed was hard rocky shale. The dams' feet had short walls and were symmetrical with minimal wall flaring and appeared grossly free of pathological changes in the external aspect of the hoof.

At the King's Canyon location, the desert area consisted of a valley system bordered by high, prominent escarpments. There was high competition for feed resources in this area between feral cattle, horses, and camels. Vegetation was scarce in the areas that were > 16 km (10 miles) from the single permanent water hole; by use of a global positioning system, King's Canyon horses seeking food had been tracked as far as 55 km (35 miles) away from the single water hole.[12] Substrate was rocky on both the horse trails and feeding grounds. Feet of the adult King's Canyon horses were similar to the feet of the Lawn Hill horses. Feral horses have inhabited this area for > 140 years[13,a] and are under very little pressure from human intervention. The breed origins of the horses were similar to those of the Lawn Hill horses, with evidence of early influences from Thoroughbred, Arabian, and station hack horses.[10,13]

Foot collection—For each fetus, the left forelimb was disarticulated at the carpus immediately following euthanasia of a feral mare or delivery from a domesticated mare, placed in a sealed plastic bag, and chilled on ice until placed at –20°C within 4 hours of collection. Freezing in remote locations was made possible by the use of a small deep freezer powered by a portable generator. Feet remained frozen for the duration of dissection and data collection.

Assessment of PEL around the hoof wall perimeter—Foot sectioning and measurement were conducted as described by Bidwell and Bowker.[6] Each frozen foot was cut parallel to the ground surface to expose all lamellae including the bars. The foot was held on its side on the band saw base plate, firmly abutting a 100-mm^2-thick timber block to ensure that the cut was made parallel to the ground. The first cut was made 10 mm proximal to the weight-bearing surface. Consecutive 5-mm-thick slices were removed sequentially until the first slice proximal to the white line, in which all lamellae around the hoof wall perimeter were vis-

ible, was obtained. This section was used for all quantitative PEL measurements. The slice was cleaned with fresh water and patted dry with a paper towel. A pen mark was made on the hoof wall at the MDC (12 o'clock position) and at the medial and lateral break-over points, medial and lateral toe quarter points, and medial and lateral quarters to allow identification of these landmarks following photography. The medial and lateral break-over points were defined as the points on the outer hoof wall at 11 and 1 o'clock. The medial and lateral toe quarter points were located at 10 and 2 o'clock. The quarters were defined (as for Bidwell and Bowker[6]) as the points over the palmar processes of the distal phalanx at the more palmar point of the lateral cartilages. These points were typically located at 4 and 6 o'clock. The slice was then positioned on a custom-made photographic jig. A camera[b] was screw mounted on the photographic jig at a distance of 400 mm from the foot slice. A 100-mm-long ruler was placed in the subject plane to calibrate photographs for digital measurement. External lighting was provided to produce high-quality photographs. The hoof slice was photographed in the transverse plane **(Figure 1)** to allow calibrated digital measurement of PEL density by use of image-processing software.[c] The software allowed image enhancement and magnification and maintained the calibrated scale during magnification.

PEL density calculation—Primary epidermal lamellar density measurements were calculated from calibrated photographs by use of image-processing software.[c] Primary epidermal lamellar density was measured as described by Bidwell and Bowker[6] to allow direct comparison to their PEL density data for domesticated horse fetuses. Individual PEL were marked by use of a colored counting marker so that no PEL would be overlooked or counted more than once. Primary epidermal lamellar marking started at each hoof wall marker point and progressed for 25 PEL on either side of each marker. The last PEL at either extreme of a grouping of 50 PEL was marked with an arrow supplied by the image-processing software.[c] Once this process was completed, the straight-line distance between the bases of the first and last PEL of each grouping of 50 PEL was measured by use of the image-processing software.[c] Primary epidermal lamellar density values for matching medial and lateral zones were averaged and expressed as 1 measurement. Primary epidermal lamellar density (number of PEL per millimeter) was calculated for each point by use of a formula as follows:

$$\text{PEL density} = 50 / \text{straight-line distance (mm) spanning the grouping of 50 PEL}$$

Statistical analysis—For feet from feral or domesticated horse fetuses, PEL densities at the 4 locations were compared among locations by use of multiple regression analysis. Comparison of PEL density at each landmark location between feral and domesticated horse fetuses was performed by use of a nonparametric Wilcoxon *t* test. Significance was set at a value of $P < 0.05$. All data are reported as mean ± SE. Statistical analyses were performed by use of a software program[d] for statistical computing and graphics.

Results

The PEL density distribution in feet of fetuses collected from the 2 feral horse populations did not differ significantly. Thus, the data from the Lawn Hill and King's Canyon horse fetuses were combined to represent a single feral fetal group (n = 14). A pattern in PEL distribution was identified in the feet of feral fetuses; there was a significant ($P < 0.05$) reduction in PEL density from the dorsal aspect to the palmar aspect of the foot **(Figure 2)**. In feral horse fetuses, the mean difference between PEL densities at the MDC and the quarters was 25%; this difference was significant ($P < 0.05$). The pattern of PEL density around the hoof wall perimeter in feet of domesticated horse fetuses was homogenous; there was no significant ($P > 0.05$) difference in PEL densities among the 4 locations.

The distribution of PEL around the hoof wall perimeter varied between feral and domesticated horse fetuses. Primary epidermal lamellar density was significantly ($P = 0.01$) higher at the MDC in the feral horse fetuses (5.1 PEL/mm), compared with the PEL density at that location in the domesticated horse fetuses (4.4 PEL/mm). The MDC-to-quarter difference was also greater ($P = 0.03$) in feral horse fetuses than it was in domesticated horse fetuses. However, the PEL density at the break-over, toe quarter, and quarter sites did not vary significantly ($P > 0.05$) between groups (Figure 2). Another comparison of primary interest in this study was the difference in PEL densities at the MDC and the quarters. In the present study, the PEL densities at the MDC and quarter locations were 4.4 ± 0.3 PEL/mm and 3.9 ± 0.3 PEL/mm, respectively, in domesticated horse fetuses and 5.1 ± 0.5 PEL/mm and 3.8 ± 0.7 PEL/mm, respectively, in feral horse fetuses. In 2 previous studies, the PEL density at the MDC and quarter locations were 4.0 PEL/mm and 4.2 PEL/mm,[6] respectively, in domesticated horse fetuses and 4.2 PEL/mm and 4.3 PEL/mm,[9] respectively, in domesticated horse fetuses. The PEL density pattern for domesticated horses, in all 3 studies, is very similar. However, the PEL density in the MDC of hooves from feral horse fetuses was numeri-

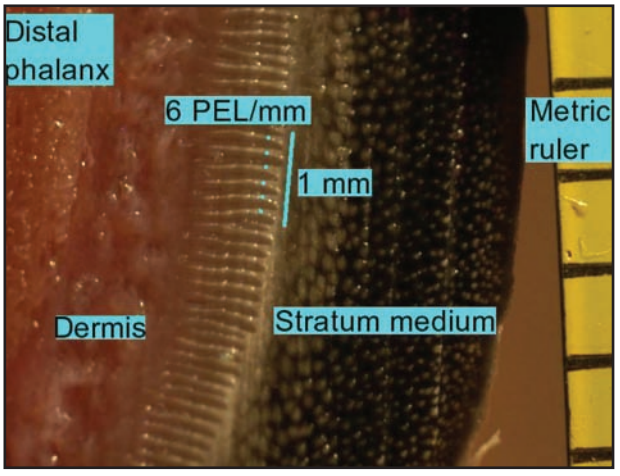

Figure 1—Digital photograph of a transverse section of the left forelimb hoof of a feral horse fetus illustrating the distribution and density of the PEL at the MDC location. The PEL density at the location marked is 6 PEL/mm.

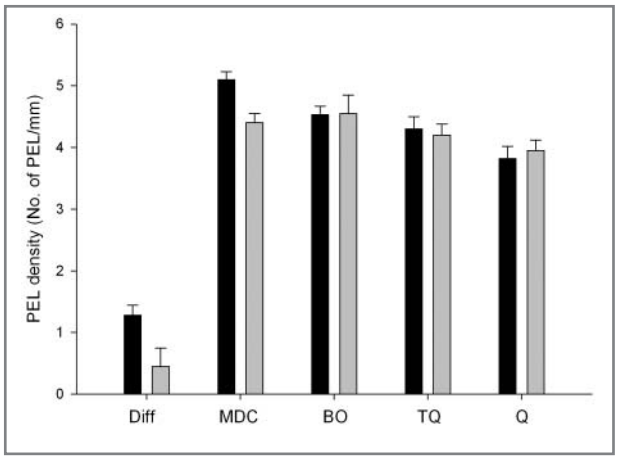

Figure 2—Mean ± SE PEL density in the left forefoot hooves of near-term fetuses obtained from 14 feral mares (black bars) culled at 2 locations (King's Canyon and Lawn Hill) in Australia and near-term fetuses obtained from 4 domesticated mares (gray bars). One fetus was obtained from each mare; domesticated horse fetuses were stillborn. Primary epidermal lamellar density (number of PEL per millimeter) was measured at the MDC (12 o'clock position) and at the medial and lateral break-over points, medial and lateral toe quarter points, and medial and lateral quarters. The medial and lateral break-over points were defined as the points on the outer hoof wall at 11 and 1 o'clock. The medial and lateral toe quarter points were located at 10 and 2 o'clock. The medial and lateral quarters were defined (as for Bidwell and Bowker[6]) as the points over the palmar processes of the distal phalanx at the most palmar point of the lateral cartilages; these points were typically located at 4 and 6 o'clock. For matching medial and lateral locations, densities were averaged to provide 1 density for that location (ie, 1 break-over [BO] value, 1 toe quarter [TQ] value, and 1 quarter [Q] value). The difference between mean MDC and mean Q values (Diff) was also calculated. The PEL density at the MDC differs significantly ($P < 0.05$) between the groups. The difference between TQ and Q values in feral horse fetuses was significantly ($P < 0.05$) larger than the calculated difference in domesticated horse fetuses.

cally greater than findings in the domesticated horse fetuses.

Discussion

The study reported here revealed a significant difference in the PEL distribution patterns in feet of feral and domesticated horse fetuses. The PEL density of feet of feral horse fetuses at the MDC was greater (by 16%) than that of feet of domesticated horse fetuses measured at the same location. Previous studies[6,9] reported a homogenous pattern of lamellar distribution around the hoof wall perimeter in domesticated horse fetuses. The feral horse fetuses in the present study had a higher PEL density in the dorsal aspect of the foot, compared with that in the palmar aspect of the foot, which is the pattern of lamellar density in adult domesticated horses.[6–9] This pattern of distribution appears to be laid down according to the discriminate biomechanical loading of the equine hoof during locomotion.[14] It is known[15] that the MDC is an important focal point of physiologic stress and strain. The presence of a mature PEL distribution pattern at birth may represent preadaptation of the feral horse neonate to the extreme conditions encountered by its genetic predecessors.

The pattern of lamellar density has been investigated in domesticated mixed-breed horses.[6–8,9] In domesticated adult horses, the PEL density distribution matches known patterns of biomechanical loading in the foot during locomotion. The distribution of PEL around the hoof perimeter in adults is most dense at the toe, slightly less in the toe quarter region, and further reduced at the quarters and heels.[6–8,9] In contrast, feet of domesticated horse fetuses and neonates have a homogeneous distribution of PEL with no difference in lamellar density between the dorsal and palmar aspects of the foot.[6,9]

A limitation of the present study was the low number of domesticated horse fetuses used for comparison with feral horse fetuses. However, the PEL distribution pattern in 33 domesticated mixed-breed horse fetuses and neonates has been reported previously.[6,9] In the present study, the methods used were the same as those used by Bidwell and Bowker,[6] and this fact permitted direct comparison of data. The difference between the present study's methods (and those applied by Bidwell and Bowker[6]) and the methods used by Douglas and Thomason[9] was that the section cut to expose the lamellae was made parallel to the ground in the former and parallel to the coronet in the latter. However, the angle of cut did not appear to alter the outcome because the PEL densities determined by Bidwell and Bowker[6] and Douglas and Thomason[9] and values determined for domesticated horses in the present study were similar. In the previous studies by Bidwell and Bowker[6] and Douglas and Thomason,[9] a homogeneous lamellar distribution pattern was detected in domesticated horses at birth. Those researchers concluded that biomechanical forces applied during weight bearing early after birth were responsible for directing the adaptation response of the tissues, which results in a differential distribution of PEL around the hoof wall perimeter in mature horses. The similarities of the PEL density patterns in the domesticated horse fetuses in the present study and in domesticated horse fetuses in previous studies strengthen the findings of our study.

The feral horses from which fetuses were obtained for use in the present study lived in extreme environmental conditions that necessitated long-distance travel to obtain food and water. Horses from the King's Canyon area were often seen 19 km (12 miles) from the nearest water source and have been sighted 55 km (34 miles)[12] to 65 km (40 miles)[a] away from water. Feral horses have been present in the 2 study locations for > 100 years[10,13] and, according to local stockmen, have been closed populations for at least 50 years. The genetic interval of the feral horses has not been accurately determined, but this period could represent as many as 20 generations of breeding. If differences in foot characteristics and PEL architecture were to exist between feral and domesticated horse populations, then the size of the feral horse sample in the present study would be likely to identify them.

There are at least 3 explanations for the difference between the feral horse fetus observations in the present studies and the domesticated horse fetus data obtained in the present and previous studies. It is possible that the small number of domesticated horse fetuses in the present study skewed the results. This is, however, unlikely because, although the sample size for domestic horse fetuses was low (n = 4), the findings for those

4 fetuses were highly similar and the data obtained in the present study were supported by results from previous studies.[6,9] Another explanation is the possibility of feedback from the very active feral dam to its fetus causing the foal to be born with adult-like PEL distribution patterns. The constant remodeling of the hooves of feral dams may liberate growth factors and cytokines in sufficient concentrations to hematogenously influence fetal foot development.

A third explanation is that natural selection in the Australian wilderness environment favors foals that are more able to travel long distances shortly after birth. Presumably foals born with weak, painful hooves and angular limb deformities are eliminated. We hypothesized that in feral horses, a fetal PEL density pattern that more closely resembled that of the adult horse foot was a preadaptation, aiding feral horse survival in wilderness environments. With the exception of culling programs, horses in Australia have no predators. However, horses in remote areas are susceptible to drought and often die in large numbers in dry seasons.[10] This raises the question of whether exposure to a wilderness habitat for a period of 100 years provides sufficient genetic pressure to select for this trait in a relatively closed breeding population. Foot type is not generally a trait selectively bred for in the domesticated horse population.[16] However, in some breeds (eg, halter-bred Quarter Horse), a small-sized and upright foot phenotype is selectively bred for, possibly resulting in reversal of any selection process for a more ready-to-travel-from-birth phenotype.

Results of a recent study[17] of > 100,000 Dutch Warmbloods indicated that limb conformation had a moderate genetic relationship to conformation grade and that foot conformation traits had a genetic relationship to sporting performance. Additionally, the prevalence of uneven feet in the population increased from 5.3% to > 8% during the 12-year period of the study.[17] It is feasible that the feet of feral horses have slowly adapted over the last 100 years, whereas the feet of typical domesticated horses have remained unchanged or even developed in a reverse direction, enhancing the hoof differences between the 2 groups. The gross morphological differences between the feet of feral adult horses in other studies[18–21] and the feet of domesticated horses[17,21–23] support this theory. The central and northern Australian feral horse populations, which are subjected to the extreme selection pressure of semiarid habitats, may have inherited a trait that set them apart from their domestically managed counterparts.

Horses have previously been categorized as adapted to wet temperate zones.[24] However, results of a water homeostasis study[25] involving desert-dwelling horses in the Namib Desert, Namibia, indicated that desert horses have water conservation capabilities similar to those of desert sheep and asses. It was concluded that Namib Desert horses were able to cope well with short-term dehydration (3 days' duration) by reducing their water turnover rate to an extent that compares well with the water turnover rate reduction achieved by other mammals adapted to semiarid environments.[25] If desert horses have developed physiologic mechanisms to allow habitation of a desert environment, then it is feasible that the integument may also have adapted to allow desert horses to pursue travel and feeding behaviors necessary for survival.

Although a difference in PEL density patterns in near-term feral and domesticated horse fetuses was detected in the present study, the duration of that difference after birth is not known. The domesticated horse neonate may develop a more adult-like PEL density pattern soon after it is ambulatory. A homogenous PEL density pattern may be associated with less robust or even painful hooves that render a newborn unfit to survive a feral life. A survival advantage may be conferred to a foal born with an adult-like PEL distribution pattern because the neonate can successfully travel over hard terrain soon after birth. Among the feral King's Canyon horses, 2 of the authors have observed newborn foals completing the same 12- to 15-mile journeys to food and water as the adults in their band. High activity early after birth coupled with the anatomic advantage of the adult-like PEL density pattern may have long-lasting effects and result in development of adult feet that are able to withstand extreme environmental challenges.

If, in fact, there is a major anatomic advantage of the feral horse foot phenotype over the domesticated horse foot phenotype, this difference may be exploited in better preparing the tissues of the foot for the challenges of an athletic environment. It is known from the human medical literature[26,27] and more recently from the equine medical literature[1] that structures such as bones, articular cartilage, and tendons are responsive to timely controlled exercise loading, particularly in the early growth and development periods of organs. It is possible that we may take a lesson from the environment and activity levels of feral horses and modify the feet of young domesticated horses to better accommodate athletic pursuits later in their careers. If the qualities of the feral horse foot are heritable, then horse breeders may be able to select and breed a more robust foot in domesticated horses. Greater quantity and better quality of tissues of the equine hoof may reduce training time lost because of lameness due to weak, poorly conformed feet.

The mean daily distances traveled and intervals between water intake by Australian feral horses are greater than those recorded for other wild equidae.[12] The study reported here has provided evidence that an anatomic variation exists between the feet of Australian feral and domesticated horse fetuses, which may be long lasting after birth. The unique environments of Australian feral horses and these physiologic and anatomic foot adaptations warrant further study.

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